Genetics of mineral accumulation in potato tubers

Nithya Subramanian

A Thesis Submitted for the Degree of
Doctor of Philosophy

University of Nottingham

September 2011

THESIS CONTAINS

CD

ABSTRACT

As a major food source potato delivers significant levels of minerals to the human diet. The aim of this study was to understand the control over the mineral concentrations found in tubers. The three-dimensional patterns of mineral distribution in tubers give clues to the processes leading to storage in the tuber. Within the tuber flesh, calcium and phosphorus content decreased towards the centre of the tuber (on FW basis). The elements iron, magnesium, zinc, manganese, sulphur and chlorine were higher at the stem end, while potassium was higher at the bud end. Remobilisation of minerals within the tuber was evident after six months of cold storage. Mineral variation was explored in potato germplasm. Three diverse germplasm collections, the Commonwealth Potato Collection, the Phureja and Tuberosum Core Collection and the Neotuberosum Population demonstrated wide variation for tuber mineral concentrations, an interaction with tuber yield and, on multivariate analysis, consistent parallels between some minerals suggesting unsuspected shared processes affecting their concentrations. The 12601ab1 x Stirling tetraploid mapping population was used to identify QTLs for tuber mineral concentration using REML analysis to account for local field variation. Transgressive segregation for tuber mineral concentrations was detected. The genetic map for this population was extended using DArT markers and QTLs were identified on all 12 linkage groups for all minerals studied. Two bulk segregant analyses were performed to add precision to the QTL analysis. One approach identified candidate genes on the potato genome sequence and used nearby SSRs to seek association in the tetraploid mapping population. A second approach used the variation present in the highly diverse Neotuberosum Population to identify DArT markers which were associated with the tails of the distribution of minerals. Using the latter approach, single superscaffolds containing candidate loci and trait-associated DArT markers could be aligned with a small part of mapping population QTLs, providing additional resolution.

ACKNOWLEDGMENTS

I would like to convey my sincere gratitude to my major advisor Dr. Gavin Ramsay for his advice, guidance and care shown throughout the course of the project. I am grateful to my co-advisors Prof. Philip White and Dr. Martin Broadley for their valuable discussions and expert guidance, which made this a very successful project. I profusely thank Dr. Christine Hackett for her help with statistical analysis and QTL mapping, without which a comprehensive thesis was not possible. I extend my gratitude to Dr. Linda Cardle for helping with bioinformatic analysis. I am very appreciative of my lab manager Gaynor McKenzie for her assistance with laboratory tools and techniques, which facilitated the successful completion of various experiments in a timely manner.

I would like to extend my sincere thanks to Drs. Glenn Bryan, Finlay Dale, John Bradshaw, Sanjeev Kumar, Ankush Prashar and Karen McLean for their valuable contributions during different stages of this work. I am thankful to my scientific assessors Drs. Alison Karley (JHI), Sean Mayes (Nottingham), and liaison officer Dr. Craig Simpson (JHI) for their guidance and support. My special thanks to Mr. Ralph Wilson and his associates for help with potato planting and harvesting, and Mr. Euan Caldwell and his colleagues for field plot maintenance. I am very grateful to Ms. Jacqueline Thompson and Dr. Alison Karley for their assistance with acid-digestion and ICP-MS analysis, and Dr. Louise Shepherd and her group, Ms. Jacqueline Thompson and Mrs Gladys Wright for helping with potato sample preparation. I am also thankful to Dr. John Hammond at Warwick HRI for ICP-mineral analysis of tuber samples during 2008 and 2009. I am grateful to Mr. Philip Smith for his help in proofreading my thesis.

My special appreciation to all the staff, student friends and officemates for their support and for providing an excellent working environment. I particularly thank Drs. Naeem Sayed and Lea Wiesel for their support throughout my stay at JHI. I am grateful to Mr. K. V. Subramaniam for providing moral support during critical times of my thesis. I am very thankful to Ms. Sonoko Mitsui-Angwin at University

of Nottingham for her invaluable help with my thesis submission. I also thank my current post-doctoral advisor, Dr. Esten Mason, University of Arkansas, for his timely support during the process of final thesis submission.

Last, but not least, I am very thankful for funding from the University of Nottingham Tuition Fee Scholarship by the International Office and a University of Nottingham/SCRI studentship, which made my PhD programme possible. Any omission from this list is only an oversight and does not constitute a lack of gratitude.

DEDICATION

This thesis is affectionately dedicated to my family

DECLARATION

I hereby declare that the following thesis is based on the results of investigations conducted by myself, and that it is of my own composition. This thesis has not, in whole or in part, been previously presented for a higher degree or qualification. Work other than my own is clearly indicated in the text by reference to the relevant researchers or their publications.

Nithya Subramanian

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ARRREVIATIONS

%	Percent
α	Alpha
λ	Lambda
µg	Microgram
µl	Microlitre
ABC	ATP-binding cassette
ACA	Auto-inhibited Ca^{2+}-ATPase
AFLP	Amplified fragment length polymorphism
AKT	Arabidopsis K^+ transporter
Al	Aluminium
AMT	Ammonium transporter
ANNAT2	Annexin Arabidopsis 2
ANOVA	Analysis of variance
ANX	Annexin
APS	Ammonium persulphate
AtKC1	Shaker-like potassium channel
ATOX1 (ATX1)	Antioxidant 1
BAC	Bacterial artificial chromosome
BC	Backcross
bHLH	Basic helix-loop-helix transcription factor
BiP	Luminal binding protein
BLAST	Basic local alignment search tool
BLUP	Best linear unbiased predictors
BOR1	Boric acid/borate exporter protein
bp	Basepair
BSA	Bulked segregant analysis
bZIP	Basic region/leucine zipper motif
CaBP	Calcium-binding protein
CaM	Calmodulin
CaMK	Calcium/calmodulin-dependent protein kinase
CAX	Ca^{2+}/H^+ antiporter
CBL3	Calcineurin B-like protein 3
CBP	Calcium binding peptide
CCC	Cation chloride cotransporters
CCH	Cu chaperone
CCS	Cu chaperone for Cu/ZnSOD
CCX	Cation exchanger
CDF	Cation diffusion facilitator

CEC	Cation-exchange capacity
CGIAR	Consultative Group on International Agricultural Research
CHoR1	Calcium homeostasis regulator
CHX	Cation-H^+ exchangers
CIP	International Potato Center
CIPC	Isopropyl N-(3-chlorophenyl) carbamate
Cl	Chloride
CLC	Chloride channel transporters
cm	Centimetre
cM	Centimorgans
CN-B	Calcineurin B
CNGC	Cyclic nucleotide gated channel
CNX	Calnexin
COPT	High affinity Cu transporter
COX	Cu chaperone for Cytochrome c oxidase
CPA	Cation-proton antiporter
CPC	Commonwealth Potato Collection
CRR	Common root rot
CRS	Corky ringspot
CRT	Calreticulin
CRY1B	Cryptochrome 1B
Cs	Caesium
CSQ	Calsequestrin
Cu	Copper
CUC3	Cup-shaped cotyledon 3
cvs	Cultivars
CXIP4	CAX-interacting protein
DACC	Depolarization-activated Ca^{2+} channels
DAE	Days after crop emergence
DArT	Diversity arrays technology
DH	Doubled haploid
DM	Dry matter
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DRC	Dynamic reaction cell
DRI	Dietary reference intake
DW	Dry weight
ECA	Endoplasmic reticulum calcium transporting ATPase
EDS	Energy dispersive X-ray spectrometry
EDTA	Ethylenediaminetetraacetate
ENH1	Enhancer of SOS3-1

Fe	Iron
Fe^{2+}	Ferrous
Fe^{3+}	Ferric
FIT1	Fe-deficiency induced transcription factor 1
FPN	Ferroportin
FRD3	Ferric reductase defective 3
FRO	Ferric-chelate reductase
FW	Fresh weight
g	Gram
GLR	Glutamate receptor
GLuR5	Glutamate receptor subunit
GP179	RFLP marker loci on Potato chromosome V
GP21	RFLP marker loci on Potato chromosome V
GS	Glutamine synthetase
H	Hydrogen
H^2	Broad sense heritability
H_2O_2	Hydrogen peroxide
HACC	Hyperpolarization-activated Ca^{2+} channels
HAK9	High affinity H^+-coupled K symporters
HATS	High-affinity S transporters
HKT	High-affinity K^+ transporter
HMA	Heavy-metal ATPase
HNO_3	Nitric acid
I	Iodine
ICAP	Inductively coupled argon plasma
ICP-ES	Inductively coupled plasma-emission spectrophotometry
ICP-MS	Inductively coupled plasma-mass spectrometry
IHN	Internal heat necrosis
IM	Interval mapping
IREG3	Iron-regulated protein
IRT	Iron regulated transporter
ITP	Iron transport protein
JHI	James Hutton Institute
K	Potassium
K_2SO_4	Potassium sulphate
KAT2	Potassium channel in *Arabidopsis thaliana* 2
kb	Kilo basepair
KCl	Potassium chloride
KCO	Ca^{2+} activated outward rectifying K^+ channel

KEA	K^+ exchange antiporter
kir	K^+ inward rectifier
KIRC	Potassium inward regulated channel
KLT	Potassium channel
KORC	Ca^{2+} -permeable outward-rectifying K^+ channels
KUP1	K^+ uptake permease
LCT1	Low-affinity K+-permeable transporter in wheat
LD	Linkage disequilibrium
LEA	Late embryogenesis abundant protein
LGs	Linkage groups
LHA	Plasma membrane proton ATPase
LOD	Logarithm (base 10) of odds
M	Molarity
MAR1	Multiple antibiotic resistance 1
MATE	Multi-drug and toxin efflux
MFLP	Microsatellite-anchored fragment length polymorphism
MFS	Major facilitator superfamily
mg	Milligram
Mha	Million hectares
MHX	Magnesium/proton exchanger
MIP	Major intrinsic protein
ml	Millilitre
mm	Millimetre
mM	Millimoles
Mo	Molybdenum
mPub	Pubescent leaf blades
MRS2	Mitochondrial RNA splicing-2
MS	Mechanosensitive-Ca^{2+} channels
MSL5	Mechanosensitive channel of small conductance-like 5
MT	Metallothionein
MTO1	Methionine overaccumulation1
MTP	Metal tolerance protein
MΩ	Megaohm
NA	Nicotianamine
Na	Sodium
NAC	NAC transcription factor
NAM	No apical meristem
NAS	Nicotianamine synthase
NBS-LRR	Nucleotide-binding site leucine-rich repeat proteins
ng	Nanogram

NHX	Na^+/H^+ exchanger
Ni	Nickel
NIP	Nodulin-26 like intrinsic proteins
NIST	National Institute of Standards and Technology
No.	Number
NR	Nitrate reductase
NRAMP	Natural resistance-associated macrophage protein
NRT	Nitrate transporter
NTB	Neotuberosum
O	Oxygen
°C	Degree Celsius
OPT	Oligopeptide transporter
P	Phosphorus
PAA	P-type ATPase
PAGE	Polyacrylamide gel electrophoresis
PCA	Principal component analysis
PCM	Potato calmodulin
PCN	Potato cyst nematode
PCR	Polymerase chain reaction
PFA	Perfluoro alkoxy
PGSC	Potato Genome Sequencing Consortium
pH	Negative logarithm of the hydrogen ion concentration
PHA1	Plasma membrane proton ATPase 1
PHO1	Phosphate1
PHO2	Phosphate2
PHT2	Pi transporters
PIC1	Permease in chloroplasts 1
PIP	Plasma membrane intrinsic protein
PLRV	Potato leafroll virus
Ppiases	Pyrophosphatases
ppm	Parts per million
PSs	Phytosiderophores
PS	Potato standard
PT	Phosphate transporter
PTR	Peptide transporters
PV	Potato virus
p-value	Probability value
QTL	Quantitative trait loci
r	Correlation coefficient
RAN1	Responsive-to-antagonist1
REMAP	Retrotransposon-microsatellite amplified polymorphism

REML	Restricted maximum likelihood
RFLP	Restriction fragment length polymorphism
RH	Relative humidity
RIL	Recombinant inbred lines
ROS	Reactive oxygen species
SAM	S-Adenosyl methionine
SAT-1	Serine acetyl transferase 1
SCRI	Scottish Crop Research Institute
Se	Selenium
SE	Standard error
SED	Standard error of the difference of means
Si	Silicon
SIP3	SOS3-interacting proteins
SKOR	Stelar K^+ outward rectifier
SNP	Single nucleotide polymorphism
SO_4	Sulphate
SOD	Superoxide dismutase
SOS1	Salt overly sensitive1
SRAP	Sequence-related amplified polymorphism
SS	Superscaffolds
SSR	Simple sequence repeat
STA1	Starik1
SULTR	SO_4 transporters
SV	Slow vacuolar channel
Taq	*Thermus aquaticus*
TBE	Tris-borate/EDTA
TE	Tris-HCl/EDTA
TEMED	Tetramethylethylenediamine
TIP	Tonoplast intrinsic protein
TLS	Tomato leaf standard
TORK1	Potassium channel
TPC	Two-pore channels
TPK	Tandem pore K+ channel
TPT	Triose phosphate/Pi translocators
Tris-HCl	Tris (hydroxymethyl) aminomethane hydrochloride
UHD	Ultra-high density
UV	Ultraviolet
V	Volts
VCaB	Vacuole-associated annexin
VICC	Voltage-independent cation channels

VIT	Vacuolar iron transporter
VP-SEM	Variable pressure scanning electron microscope
WRKY	Transcription factor
YS1	Yellow stripe-1
YSL	Yellow stripe-like
ZIF1	Zinc-induced facilitator 1
ZIFL1	Zinc induced facilitator-like 1
ZIP	Zinc-regulated transporter (ZRT)- iron-regulated transporter (IRT)-like protein
Zn	Zinc

CHAPTER 1 GENERAL INTRODUCTION AND OBJECTIVES

1.1 MINERALS AND HUMAN HEALTH

1.1.1 Mineral elements required by humans

Food is essential for sustaining life and adequate intake of nutritious food is vital to achieve and maintain good health and well-being. Mineral nutrients are essential for proper functioning of the human body having both structural and functional roles. It has been found that at least 25 mineral elements are essential for the well-being of humans (Stein, 2010) and these can be acquired through a balanced diet comprising of plant and/or animal sources. However, the actual concentration of minerals in food crops may not always closely relate with their bioavailability for the human body. Bioavailability depends on the chemical form in which the mineral is present and on the nature of interactions with other dietary compounds in food. In particular, the bioavailability of minerals from plant based foods is limited by anti-nutritional compounds such as oxalates, polyphenols (tannins), and phytates, which typically affect the absorption of mineral elements by the gut tissues (White and Broadley, 2005, 2009). Nevertheless, plants appear to contain certain promoter substances such as organic and amino acids that stimulate the absorption of essential minerals in the gut (White and Broadley, 2005, 2009).

1.1.2 Mineral deficiency in humans

Mineral malnutrition is one of the major health challenges affecting humans worldwide (Copenhagen Consensus 2008). It is estimated that about 60, 30, 30 and 15% of the current world population is affected by iron (Fe), zinc (Zn), iodine (I) and selenium (Se) deficiency, respectively (White and Broadley, 2005). Additional mineral elements including copper (Cu), calcium (Ca), magnesium

(Mg) and Se are often also deficient in human diets (Broadley *et al.*, 2006; Broadley and White, 2010; White and Broadley, 2005, 2009).

Mineral malnutrition is not limited to developing countries but is common throughout the world. The occurrence of mineral deficiencies is linked to the consumption of produce grown on soils with low mineral phytoavailability and a high dependency on crops with inherently low tissue mineral concentrations, such as edible portions with low concentrations of mineral elements due to restricted phloem mobility (White and Broadley, 2009). In addition, intake of refined foods such as polished rice, milled wheat, pearled barley and decorticated sorghum (Zhao and Shewry, 2011), removal of skin from vegetables, and other food preparation methods also contribute to dietary mineral deficiency.

The general decline in concentration of certain mineral elements in food crops might be attributed to genetic and/or environmental factors, such as use of varieties with improved yield or mineral depletion within the soil (Davis, 2009; Fageria *et al.*, 2002; Grzebisz, 2011). The primary objective of plant breeding programmes over the last several decades has been focused on increasing yield potential and improving disease resistance of plants, generally without attention being paid to mineral traits in the modern cultivars. Increase of crop yield by agronomic practices, such as fertilization and irrigation, also tends to decrease the mineral concentrations in food crops (Davis, 2009). Other environmental factors include soil mineral status/availability, farming systems and intensive cropping systems (reviewed in Fageria *et al.*, 2002; Khoshgoftarmanesh *et al.*, 2010). Most farming systems are increasingly dependent on chemical fertilizers to increase crop yield and less plant residue is recycled back to the soil. Although the soil often receives macronutrients through fertilization, the micronutrient status can deplete due to continuous crop uptake and leaching also takes place from mineralised soils. As a result, micronutrient deficiency in agricultural soil is now widespread throughout the world (Fageria *et al.*, 2002), leading to reduced

levels of micronutrients in the edible parts of food crops. Furthermore, changes in agricultural production from diversified cropping system to monoculture crop systems have contributed to micronutrient deficiencies in humans by limiting food-crop diversity (Welch and Graham, 1999).

1.2 PLANT MINERAL NUTRITION

1.2.1 Mineral requirements of plants

Mineral elements are essential for the normal growth and development of plants. In addition to the three major elements, carbon (C), hydrogen (H) and oxygen (O), 14 other elements are required for the normal functioning of plants (Marschner, 1995; Mengel *et al.*, 2001; White and Brown 2010). Minerals are classified, based on the amount required by plants, as macro-nutrients (nitrogen (N), phosphorus (P), potassium (K), Ca, Mg and sulphur (S)) or micro-nutrients (Fe, manganese (Mn), boron (B), Zn, Cu, chlorine (Cl), molybdenum (Mo) and nickel (Ni)). A wealth of literature exists on the physiological functions of mineral macro- and micro-nutrients in plant systems (Marschner, 1995; Mengel *et al.*, 2001; Hansch and Mendel, 2009; Maathuis, 2009).

Plants acquire mineral elements primarily from the soil solution through their roots and they are delivered to the shoots through the xylem network along the transpiration stream (Karley and White, 2009; Marschner, 1995; Mengel *et al.*, 2001; White and Broadley, 2009; White and Brown, 2010). The subsequent redistribution of minerals within the plant, and delivery of minerals to non-transpiring or xylem-deficient tissues, occurs *via* the phloem network.

1.2.2 Mineral deficiency in plants

Most of the world's agricultural soils are deficient in important minerals including N (85% of the total cultivated land), P (73%), K (55%), Zn (49%), B

(31%), Mo (15%), Cu (14%), Mn (10%) and Fe (3%) (Sillanpää, 1982, 1990). Mineral deficiency typically affects plant growth and development, leading to reduced crop yield and quality. When an element is deficient, plants exhibit symptoms and the nature of the deficiency symptom depends on the specific function and mobility of the mineral element in the phloem. The mineral elements such as K, Na, Mg, N, P, S and Cl are considered as the most mobile minerals in phloem tissues, whereas Zn, Ni and Cu are regarded as having an intermediate phloem mobility, and Ca, Fe and Mn as less mobile elements in the phloem (Marschner, 1995). The phloem mobility of B, however, is found to be species dependent (Brown and Hu, 1996). Hence, in case of phloem-mobile minerals, deficiency symptoms appear first on older leaves, whereas for phloem-immobile minerals, the symptoms appear first on young leaves (Westermann, 2005). For example, Fe plays an important role in chlorophyll synthesis and photosynthesis and as such, Fe deficient plants exhibit leaf chlorosis. Fe is less mobile within the phloem and as a result, deficiency symptoms typically appear in the youngest leaves first as interveinal chlorosis. Details on specific function and deficiency symptoms of different mineral elements were summarized by Marschner (1995).

1.2.3 Physiological genetics of plant mineral nutrition

Minerals are inorganic elements that are not synthesized by plants, but must be obtained from the soil. As plants are sessile and anchored to the soil with their root system, they have developed complex mechanisms to adapt to the environment. In order to maintain adequate but non-toxic levels of minerals in tissues, plants have evolved a complex network of homeostatic mechanisms that control the uptake, distribution, accumulation and detoxification of minerals. The homeostatic network maintains the transport, chelation, trafficking and sequestration of the different metals (Clemens, 2001). As the metabolic and biochemical functions of mineral elements are closely associated with all aspects

of physiology, biochemistry and molecular biology of plants, their acquisition and distribution have been the subject of many studies over the past few decades (Marschner, 1995; Mengel *et al.*, 2001; Karley and White, 2009; Miller *et al.*, 2009; Miwa *et al.*, 2009; Puig and Peñarrubia, 2009; White and Broadley, 2009). The following sections contain a detailed overview of the physiological and molecular aspects of the uptake, transport, distribution and homeostasis for minerals of human dietary significance such as Fe, Zn, Ca, K, Cu and Mg. Review on minerals with more significance to plants such as N, P, S, B and Mn and other minerals (Na and Ni) were given in Appendix I. Most of the mineral homeostasis genes were identified and reported in the model species, *Arabidopsis thaliana* (At) and genes from other species were mentioned in the text.

1.2.3.1 Iron

Plants acquire Fe from the soil through two general mechanisms (Grotz and Guerinot, 2006; Marschner, 1995; White and Broadley 2009). Dicots and non-graminaceous monocot species use a reduction-based mechanism (Strategy I), whereas grasses and cereals use a chelation-based mechanism (Strategy II), to obtain Fe from the soil. Strategy I plants acidify the rhizosphere to lower the soil pH and increase ferric-iron (Fe^{3+}) concentrations in the soil. Acidification is accomplished by releasing protons into the rhizosphere through the activation of specific H^+-ATPase pumps. Fe^{3+} is subsequently reduced to ferrous-iron (Fe^{2+}) at the root surface by plasma-membrane bound ferric reductases, which belong to the ferric reductase oxidase (FRO) gene family. Fe^{2+} is then transported into root cells by iron regulated transporter1 (IRT1), a member of zinc-regulated transporter (ZRT)-IRT-like protein (ZIP) family (Vert *et al.*, 2002). Another class of transporters, the iron regulated gene 2/ Ferroportin 2 (AtIREG2/FPN2) group, is known to control the divalent metal efflux in root vacuoles (Schaaf *et al.*, 2006). Further, AtIREG2/FPN2 are found to be expressed particularly during Fe deficiency and serve to buffer Fe uptake by sequestering excess free Fe in the

vacuole (Morrissey *et al.*, 2009; Schaaf *et al.*, 2006). IREG2 is also involved in Fe-dependent Ni detoxification in the roots.

Strategy II plants depend on phytosiderophores (PSs) for Fe uptake. PSs, compounds of the mugineic acid family, are derived from the non-proteinogenic nicotianamine (NA), a condensation product of S-adenosyl methionine (SAM) synthesized by nicotianamine synthase (NAS). The PSs are secreted into the rhizosphere and typically form complexes w ith Fe^{3+}. The homologues of the maize yellow stripe-1 (YS1) transporters, which belong to the oligopeptide transporter (OPT) family, are responsible for Fe^{3+}-PS complex uptake by roots (Curie *et al.*, 2001, 2009). Rice appears to be an exception in this respect. Ishimaru *et al.* (2006) demonstrated that rice (a monocot) can take up Fe through IRT transporters as for strategy I plants.

Once taken up, Fe is chelated within the plant system to improve its mobility and to protect cells from harmful reactive oxygen species (ROS) produced by ferrous-iron catalyzed Fenton reactions (Cvitanich *et al.*, 2010). In particular, Fe is likely to be transported as a Fe^{3+}-citrate complex or as a Fe^{3+}-NA complex (Palmer and Guerinot, 2009). The pH of the xylem (5.5–6.0) favours the chelation of Fe to citrate rather than to NA (Hell and Stephan, 2003). Thus, it is most likely that Fe is transported through the xylem as a Fe^{3+}-citrate complex. A key factor of Fe xylem loading is ferric reductase defective 3 (AtFRD3), which is important in loading citrate into xylem and transporting Fe to shoot tissues (Durrett *et al.*, 2007). FRD3 belongs to the multi-drug and toxin efflux (MATE) family and is localized in the plasma membrane of cells in the pericycle and vascular cylinder (Green and Rogers, 2004). However, FRD3 appears to have a limited function during Fe deficiency, and the Iron regulated1/Ferroportin1 (IREG1/FPN1) is thought to load Fe into the vasculature under these circumstances (Morrissey *et al.*, 2009).

In the phloem, on the other hand, Fe^{2+} is thought to be transported as NA chelates. The role of NA in internal Fe transport was supported by a study conducted by Ling *et al.* (1996) in tomato. The study found that the NAS-defective tomato mutant *'chloronerva'* exhibited interveinal chlorosis, a typical symptom of Fe deficiency. In Arabidopsis, yellow stripe-like transporters (YSLs) and related OPTs are thought to be involved in moving Fe^{2+}-NA complex into and out of the phloem (reviewed by Curie *et al.*, 2009). In addition to NA, a Fe binding protein known as iron transport protein (ITP) is thought to be involved in phloem-mediated long distance transport by preferentially binding with Fe^{3+} (Kruger *et al.*, 2002). ITP belongs to the late embryogenesis abundant (LEA) family, and was first identified in castor bean (*Ricinus communis* L.). It has been suggested that NA has a shuttle function in phloem loading and unloading of Fe by chelating Fe^{2+} from ITP-bound Fe^{3+} complexes (Kruger *et al.*, 2002). In this process, an oxido-reductase enzyme should be involved in oxidising Fe from Fe^{2+}-NA to Fe^{3+} for binding ITP and reducing Fe^{3+} from ITP to Fe^{2+} for binding with NA.

Fe-chelates are subsequently transported to the various cell organelles through specific transporters. In particular, vacuoles are considered as an essential compartment for Fe sequestration and storage within plant cells. In Arabidopsis, Fe is transported into the vacuoles by vacuolar iron transporter 1 (VIT1), which has been shown to be essential for proper localization of Fe in the seed (Kim *et al.*, 2006). Upon Fe deficiency, Fe is released from vacuoles by members of the natural resistance-associated macrophage protein (AtNRAMP) family of divalent metal transporters (Lanquar *et al.*, 2005; Thomine *et al.*, 2003).

Most of the iron taken up by plants (>90%) is transported to the chloroplast, where it is required for electron transport, chlorophyll biosynthesis, Fe-S cluster assembly and for heme synthesis (Jeong and Guerinot, 2009; Kim and Guerinot, 2007). Fe transport to the chloroplasts is mediated by the permease in chloroplasts 1 (PIC1) protein in Arabidopsis (Duy *et al.*, 2007), which is localised in the chloroplast inner envelope and appears to be important for chloroplast

development. Furthermore, a chloroplast membrane-bound Fe^{3+} chelate reductase (FRO7) was found to reduce Fe, and thought to be important for Fe uptake into chloroplasts (Jeong *et al.*, 2008). Recently, AtMAR1/IREG3 (multiple antibiotic resistance1/iron-regulated protein3) was described as a plastid member of the FPN/IREG transporter family (Conte *et al.*, 2009). IREG3 has been proposed to function in the uptake of NA into chloroplasts (Conte *et al.*, 2009; Conte and Lloyd, 2010). Fe is also required in mitochondria for proper functioning of the respiratory electron transport chain and for the synthesis of Fe-S clusters (Palmer and Guerinot, 2009). STA1 (STARIK1)/AtATM3, an ABC (ATP-binding cassette) transporter in Arabidopsis, has been found to transport the Fe-S cluster assembly out of mitochondria (Chen *et al.*, 2007; Kispal *et al.*, 1999; Lill and Kispal, 2000).

Iron deficiency in plants is controlled through coordinated transcriptional activation of genes. Upon Fe deficiency in tomato (*Solanum lycopersicum*), the FER (LeFER) gene is found to be expressed at the root tip, and induce Fe mobilization responses in roots by regulating the expression of FRO and IRT1 proteins (Ling *et al.*, 2002). LeFER encodes a basic helix-loop-helix (bHLH) transcription factor (Ling *et al.*, 2002) and its orthologue in Arabidopsis is the Fe-deficiency induced transcription factor 1 (FIT1) (Colangelo and Guerinot, 2004; Jakoby *et al.*, 2004; Yuan *et al.*, 2005). Over-expression of LeFER and AtFIT1 enhanced the Fe-uptake responses, but only at low Fe supply, suggesting the existence of additional regulatory mechanisms (Jakoby *et al.*, 2004).

1.2.3.2 Zinc

Zn is taken up by plant roots primarily in the form of divalent cation (Zn^{2+}) or as complex organic ligands (Broadley *et al.*, 2007; Grotz and Guerinot, 2006), and the uptake is mediated by ZIP transporters (Broadley *et al.*, 2007; Colangelo and Guerinot, 2006; Palmgren *et al.*, 2008). In Strategy II plants (i.e. monocots), the YSL protein has been proposed to take up the Zn-phytosiderophore complex from soil (Suzuki *et al.*, 2006; von Wirén *et al.*, 1996). In the xylem, Zn may be

transported as Zn^{2+} or complexed with organic acids or NA (Broadley *et al.*, 2007; Palmgren *et al.*, 2008). Xylem loading of Zn is thought to be mediated by the heavy metal ATPases (AtHMA) localised in the plasma membrane of cells in the root and shoot vasculature (Hussain *et al.*, 2004). In addition to heavy metal transport, AtHMA1 can also transport Ca^{2+} (Moreno *et al.*, 2008). In the phloem, Zn^{2+} is thought to be transported by YSL proteins mostly in the form of NA chelates (Curie *et al.*, 2009).

In Arabidopsis, Zn is transported to the vacuoles by several transporters including members of the metal tolerance protein (MTP) family, also referred to as the cation diffusion facilitator (CDF) family (Arrivault *et al.*, 2006; Desbrosses-Fonrouge *et al.*, 2005; Gustin *et al.*, 2009; Kobae *et al.*, 2004), zinc-induced facilitator 1 (ZIF1) transporter (Haydon and Cobbett, 2007), and the Mg^{2+}/H^{+} (MHX) antiporter (Elbaz *et al.*, 2006; Shaul *et al.*, 1999). In addition, VIT was proposed to transport Zn into the protein storage vacuoles of the aleurone in barley grains (Tauris *et al.*, 2009). However, the transporters involved in Zn remobilization from vacuoles have not yet been determined. In the chloroplast, Cu and Zn or Fe are required as cofactors for the activity of superoxide dismutases (SODs) (Alscher *et al.*, 2002), but the transporters in chloroplast have not yet been determined. Zinc is transported to mitochondria most likely by ZIP family members, but no ZIP transporters have been assigned to this function so far (Palmer and Guerinot, 2009). More recently, members of the basic region/leucine zipper motif (bZIP) transcription factor gene family have been shown to regulate the adaptation to Zn deficient conditions in *A. thaliana* (Assunção*et al.*, 2010).

1.2.3.3 Calcium

Calcium is obtained from the soil in the form of Ca^{2+} by root cells and its transport from the soil to the root system is facilitated by the mass flow of water (Barber, 1995). Calcium moves across the root cortex either by diffusion or by displacement exchange in the free space (Bangerth, 1979; White, 1998). Calcium

reaches the xylem through the apoplast (regions of root where Casparian bands are absent) or *via* the symplast (where Casparian bands are present) (White, 2001). However, the relative contribution of these two pathways to the delivery of Ca to the xylem is not yet known.

A variety of Ca^{2+}-permeable cation channels have been found to mediate the influx of Ca into root cells (Demidchik and Maathuis, 2007; Hashimoto *et al.*, 2005; Kaplan *et al.*, 2007; Miedema *et al.*, 2008; Mortimer *et al.*, 2008; Roy *et al.*, 2008; Wheeler and Brownlee, 2008; White 2001; White *et al.*, 2002; White and Broadley, 2003, 2009). These include hyperpolarization-activated Ca^{2+} channels (HACC) formed by plant annexins, voltage-independent cation channels (VICC) formed by members of the cyclic nucleotide gated channel (CNGC) and/or glutamate receptor (GLR) proteins, depolarization-activated Ca^{2+} channels (DACC), Ca^{2+}-permeable outward-rectifying K^+ channels (KORC), mechanosensitive (MS) and second messenger-activated Ca^{2+} channels. As explicit perturbations in cytoplasmic Ca^{2+} concentrations initiate cellular responses to many developmental and environmental stimuli, the activity of these ion channels is tightly regulated in the plant system (White and Broadley, 2003, 2009).

Within the xylem, Ca is transported as Ca^{2+} or complexed with organic acids (White and Broadley, 2003). Depending on the plant species and phytoavailability of Ca in the environment, Ca is stored in leaf vacuoles as soluble (Ca^{2+} complexes with proteins and/or organic acids) or insoluble (as Ca-oxalate and Ca-phytate) forms (Franceschi and Nakata, 2005; White and Broadley, 2003). Ca is relatively immobile in the phloem, resulting in high Ca concentrations in highly transpiring parts such as leaves and low Ca concentrations in low transpiring organs such as fruits, seeds and tubers (White and Broadley, 2003; White *et al.*, 2009). Hence, it is likely that organs with low transpiration rates are more prone to Ca deficiencies, such as blossom end rot in tomatoes (Ho and

White, 2005) and brown centre, hollow heart, internal brown spot in potatoes (reviewed in Palta, 1996).

Within the cytosol, Ca^{2+} concentrations are maintained at very low levels (~100nM) by Ca^{2+} - ATPases, which are encoded by members of the P_{2A}-ATPase (ECA, endoplasmic reticulum-type Ca^{2+} - ATPase) or P_{2B}-ATPase (ACA, auto-inhibited Ca^{2+} - ATPase) gene families, and by Ca^{2+}/H^+ antiporters such as those encoded by Ca^{2+}/H^+ antiporter (CAX) genes that export Ca^{2+} to the apoplast, endoplasmic reticulum, plastids or vacuoles (George *et al.*, 2008; Hirschi, 2001; Mills *et al.*, 2008; Shigaki and Hirschi, 2006; White and Broadley, 2003). The influx of Ca^{2+} into the vacuoles by CAX genes is energized by the H^+ gradient generated by vacuolar ATPases and/or PPiases (pyrophosphatases) (Shigaki and Hirschi, 2006). On the other hand, the release of Ca^{2+} from vacuole is mediated by Ca^{2+}- permeable cation channels, which include homologues of AtTPC (two pore channel) and/or annexins (Mortimer *et al.*, 2008; Pottosin and Schonknecht 2007; Wheeler and Brownlee, 2008). In the cytosol and endoplasmic reticulum, Ca^{2+} is complexed by diverse proteins including calmodulin (CaM), calmodulin-related proteins, calcineurin-B-like proteins (CBLs), Ca^{2+}-dependent protein kinases (CDPKs) and annexins (ANX), and calreticulin (CRT), calsequestrin (CSQ), calnexin (CN) and lumenal binding proteins (BiP), respectively (White and Broadley, 2003). The vacuoles may also contain Ca^{2+}-binding proteins, such as the radish vacuolar calcium-binding protein (VCaB) (Yuasa and Maeshima, 2000)

1.2.3.4 Potassium

Potassium is taken up by plant roots as K^+ via low affinity ion channels and high affinity H^+-coupled symporters (Maathuis and Sanders, 1994; White and Karley, 2010). The H^+-coupled K^+ symporters are encoded by genes belonging to three ion transporter families: KT/HAK/KUP (K^+/H^+ symporters), HKT/TRK (high-affinity K^+ transporter, later shown to be a K^+/Na^+ symporter), and CHX (cation-H^+ exchangers) (Gierth and Maser, 2007; Zhao *et al.*, 2008; White and Karley, 2010). K^+ selective channels are also found in plasma membrane, which include the

voltage-gated shaker-type channels, such as AKT1 (Arabidopsis K^+ transporter), and members of the tandem pore K^+ (TPK/KCO; KCO-Ca^{2+} activated outward rectifying K^+ channel) channel family (Gambale and Uozumi, 2006; Lebaudy *et al.*, 2007). In addition non-specific K^+-permeable cation channels, encoded by the members of the CNGC and GLR gene families, are also present (Demidchik and Maathuis, 2007; White and Karley, 2010). A low-affinity cation transporter in wheat (LCT1, a K^+-permeable transporter) has been shown to transport both K^+ and Na^+ (Schachtman *et al.*, 1997).

Generally, the xylem and phloem loading of K^+ is mediated by the Shaker channels. A shaker-type K^+ efflux channel, stelar K^+ outward rectifier (SKOR), is shown to function in xylem loading of K^+ (Gaymard *et al.*, 1998), whereas, the phloem loading and unloading of K^+ is mediated by another shaker-type K channel, AtAKT2/AKT3 (Deeken *et al.*, 2002). K^+ is transported to the vacuoles of root cells by members of cation-proton antiporter (CPA) family, and calcium cation exchanger (CCX) family (Gierth and Maser, 2007; Morris *et al.*, 2008). CPA has two major sub-families, CPA1 and CPA2, which are further classified into several sub-sub-families such as Na^+/H^+ exchanger (NHX), cation/H^+ exchanger (CHX), and K^+ exchange antiporter (KEA). Members of the TPK/KCP (KCP, voltage-gated K channel) and Kir-like (K^+ inward rectifier, formerly KCO3) gene families encode channels that are involved in releasing K^+ from the vacuole (Lebaudy *et al.*, 2007).

1.2.3.5 Copper

The uptake of Cu as Cu^+ by roots is mediated by a conserved family of high-affinity Cu transporters (COPT) (Palmer and Guerinot, 2009; Pozo *et al.*, 2010; Puig *et al.*, 2007; Sancenón *et al.*, 2004). The most available form of Cu in soil is Cu^{2+} and it is possible that Cu^{2+} is reduced by FRO2 to Cu^+ (Puig *et al.*, 2007). In addition to COPT transporters, the ZIP family of transporters may also be involved in the uptake of Cu^{2+} (Wintz *et al.*, 2003). The expression of ZIP

transporters was found to be up-regulated in roots under Cu-deficient conditions (Wintz *et al.*, 2003).

Cu is chelated to NA and transported from root to shoot tissues (Curie *et al.*, 2009; von Wirén*et al.*, 1999). However, specific proteins involved in xylem loading of Cu have not yet been discovered, although heavy metal P_{1B}-type ATPases (HMA) are thought to be involved in Cu efflux from the cytoplasm. In Arabidopsis, eight HMAs have been found, and among them, AtHMA1-AtHMA4 transport divalent cations and AtHMA5-AtHMA8 transport monovalent Cu^+ (Grotz and Guerinot, 2006; Puig *et al.*, 2007). Cu is loaded into the phloem as chelates with NA or small proteins by YSL proteins (DiDonato *et al.*, 2004; Guo *et al.*, 2003, 2008a; Waters and Grusak, 2008a). In addition, members of OPT family (OPT3) may also transport Cu^{2+} in the phloem (Wintz *et al.*, 2003).

The transport of Cu into chloroplasts is mediated by heavy metal P_{1B}-transporting P-type ATPases such as PAA1 (P-type ATPase 1) (HMA6) and PAA2 (HMA8) and HMA1 (Abdel-Ghany *et al.*, 2005; Baxter *et al.*, 2003; Shikanai, *et al.*, 2003; Williams and Mills, 2005). PAA1 and HMA1 localize Cu to the inner chloroplast envelope, whereas, PAA2 transports Cu across the thylakoid membrane. The responsive-to-antagonist1 (RAN1), also known as HMA7, has been shown to deliver Cu^+ across post-Golgi membranes to produce functional ethylene receptors (Hirayama *et al.*, 1999). However, transporters involved in Cu transport into the mitochondria or vacuoles are not known.

Following transport into the cell, Cu is bound to metallothioneins (MTs) (Guo *et al.*, 2003, 2008a) and metallochaperones (Huffman and O'Halloran, 2001; O'Halloran and Culotta, 2000). Metallochaperones are soluble Cu binding proteins that mediate intracellular Cu delivery to particular cell compartments or proteins where it will be used. The cytosolic Cu chaperones in Arabidopsis include CCH (Cu chaperone), ATX1 (antioxidant 1) and CCS (Cu chaperone for Cu/ZnSOD). It has been found that cytosolic CCS, and the Cu chaperone for

cytochrome C oxidase (COX) deliver Cu to chloroplast and mitochondria respectively (Abdel-Ghany *et al.*, 2005; Grotz and Guerinot, 2006; Hall and Williams, 2003; Kramer *et al.*, 2007; Puig *et al.*, 2007).

1.2.3.6 Magnesium

Magnesium enters root cells as Mg^{2+} through members of the mitochondrial RNA splicing-2 (MRS2) family of transporters (AtMGT) and Mg^{2+}-permeable cation channels, but the former appears to dominate the Mg^{2+} influx across the plasma membrane (Deng *et al.*, 2006; Gardner, 2003; Shaul, 2002). MHX1 is likely to play a role in xylem loading of Mg (Shaul *et al.*, 1999), in the form of Mg^{2+} or as a complex with organic acids (Welch, 1995). The import and export of Mg^{2+} across the tonoplast is mediated by Mg^{2+}/H^{+} antiporters (AtMHX) (David-Assael *et al.*, 2006; Shaul *et al.*, 1999), and by Mg^{2+}-permeable cation channels, including the slow vacuolar (SV) channel, respectively (Pottosin and Schonknecht, 2007). The entry of Mg^{2+} into chloroplast is mediated by the MRS2-11 transporter (Drummond *et al.*, 2006), and about 15 – 20% of leaf Mg content is associated with chlorophyll formation (Wilkinson *et al.*, 1990).

1.3 ENHANCING MINERAL CONCENTRATIONS IN EDIBLE CROP PARTS FOR HUMAN NUTRITION

1.3.1 The need for crop biofortification

About 7,000 species of plants have been cultivated throughout the world for human consumption, but only 30 species constitute about 95% of the world's food supply (Cakmak *et al.*, 2002; FAO 1998, 2010). Among these crops, rice, wheat, maize, and potato provide more than 60% of the global plant derived food intake. The number of food crop species used for human consumption has been gradually declining over years with a parallel decrease in genetic diversity. The substantial loss in genetic diversity of modern cultivars could be attributed to the high selection pressure exerted by humans for high crop yield. In

particular, the genetic basis of food crops is further narrowed by the selection of superior plants from diverse landraces, and by repeated use of a restricted range of parental material for breeding. The germplasm collections, wild species and cultivated landrace crops, however, have a broad genetic background that could be utilized in plant breeding programmes for improving the nutritional qualities of staple food crops.

Staple crops are usually considered as an important source of carbohydrates and not minerals. As such, increasing the mineral status of major staple crops will improve human nutrition and health. Previous efforts to alleviate mineral deficiencies in humans mainly comprised of supplements, food fortification with minerals, and dietary diversification (Bouis and Welch, 2010; Maberly *et al.*, 1994; White *et al.*, 2009). However these approaches have not been effective because of the high cost of implementation coupled with the low coverage of people in developing countries. An alternative and perhaps more sustainable approach would be the enrichment of staple food crops, which could be achieved through the application of fertilizers (agronomic biofortification), and via genetic fortification through plant breeding to increase mineral concentrations and their bioavailability, or by genetic engineering (Bouis 1996; Cakmak 2008; Graham *et al.*, 2007; Pfeiffer and McClafferty, 2007; Rengel *et al.*, 1999; Theil *et al.*, 1997; White and Broadley, 2009).

1.3.2 Agronomic biofortification

The mineral status of the soil will ultimately influence mineral concentrations in food products. In mineral deficient soils, fertilizers are applied to soil and/or foliage to improve the plant health and to enhance mineral concentration in edible parts. However, addition of a particular mineral fertilizer may increase or decrease the concentration of other minerals in the edible plant parts due to the complex interactions among different minerals in the soil system, and also to the

effects of tissue mineral composition on the redistribution of elements within the plant (White *et al.*, 2009).

Although enhancing the mineral content of staple food crops through applications of fertilizers appears to be feasible, there are several confounding factors, including application methods, soil characteristics, mineral availability for plant uptake, and mineral mobility and accumulation sites within the plant (Zhu *et al.*, 2007). In addition, fertilizers are energy-demanding, limiting resources, and long term use of mineral enriched fertilizers may result in soil mineral toxicity. Even if minerals are efficiently taken up by plants, they may not necessarily be accumulated in edible parts such as fruits, tubers or seeds (Frossard *et al.*, 2000), as phloem transport and mobility of minerals also govern the level of mineral accumulation in edible plant parts. Phloem transport of minerals largely depends on the capacity of phloem loading and therefore application of fertilizers might be effective for xylem-fed minerals and less effective for phloem-fed minerals. Recently White *et al.* (2012) has reported the effect of foliar Zn-fertilizer application on tuber Zn concentration in the variety Maris Piper. The results showed that foliar application of Zn increased Zn concentration two-fold in tubers and a 40-fold in shoots compared to the control tuber and shoots. This indicates that mineral accumulation in edible plant parts are limited by their mobility in phloem. Therefore agronomic fortification of mineral nutrients is only applicable to specific crops and scenarios, and cannot be regarded as a general strategy to enhance the nutritional quality of edible plant parts (Hirschi, 2009). Genetic biofortification, on the other hand, seems to be a promising, sustainable approach to optimise mineral concentration in food crops and to help alleviate mineral deficiency in humans.

1.3.3 Genetic biofortification

Genetic biofortification of crops can be achieved by exploiting genetic variation in breeding by conventional and transgenic routes and particularly by utilizing

genetic variation in germplasm collections (Mayer *et al.*, 2008). The probability of success in developing high micronutrient rich food crops through genetic approaches depends on various factors including the existing genetic variation, trait heritability, gene action, associations among traits, and available diagnostic tools and screening techniques (Cakmak *et al.*, 2010).

Targeted breeding to enhance the mineral content of staple foods should consider the redistribution and remobilization mechanisms such as phloem loading and unloading, transporters, chelators and storage proteins. Mineral allocation to the seeds (grains) or tubers depends on the mobility of minerals and vascular anatomy (i.e. xylem and phloem). The loading of minerals might be different between species depending on the nature of the xylem and phloem network. In crops such as rice, the xylem is found to be continuous (Krishnan and Dayanandan, 2003; Zee, 1972), whereas in potato, wheat, and barley, the xylem is discontinuous (Artschwager, 1924; Kirby and Rymer, 1975; Zee and O'Brien, 1970). In rice, Zn can be loaded directly through xylem into the grains, whereas in wheat and barley Zn must be redistributed to phloem before entering the grain (Stomph *et al.*, 2009). Understanding the molecular mechanisms controlling mineral homeostasis in grains and tubers of staple food crops will be useful to devise suitable strategies for improving human mineral nutrition. In addition to the effect on the human diet, breeding for mineral-rich crop varieties may improve their resistance to diseases and environmental stresses and also enhance the crop yield when grown on mineral-deficient soils.

Considerable genotypic variation in the concentration of essential minerals has been found in the edible portion of food crops such as seeds or tubers (reviewed in White and Broadley, 2005, 2009). For example, the concentrations of Fe and Zn were found to vary 1.5 to 4 fold in cereal grains and 1.4 to 6.6 fold in legume seeds. This suggests that there is sufficient genetic potential within cultivated food crops that could be used in breeding programmes for manipulating the tissue concentrations of essential mineral elements. Additionally, concentrations

of mineral elements such as Fe and Zn were positively correlated with each other, and hence it is possible to enhance these mineral concentrations simultaneously through breeding (reviewed in White and Broadley, 2009).

Several plant breeding initiatives have set out to increase micronutrient concentration of staple crops to improve human nutrition. HarvestPlus, the Biofortification Challenge Program was established by the Consultative Group on International Agricultural Research (CGIAR) in 2003. HarvestPlus aims to increase Fe, Zn and vitamin A concentrations in cereal grains and tuber crops such as rice, wheat, pearl millet, beans, maize, cassava and sweet potato. Furthermore, HarvestPlus, in cooperation with the scientists at the International Potato Center (CIP), are focussing on enhancing Fe, Zn, vitamin C and polyphenols in potato tubers.

1.3.4 Potatoes as a candidate for mineral enhancement

Among the staple food crops, potatoes represents one of the best candidates for mineral enhancement because they are the third most important staple crop in the world, are highly nutritious, have high mineral bioavailability in humans, and have both large germplasm collections and genome sequences available. Unlike the major cereals, potato is not a globally traded commodity and its price is determined by local production costs. Hence it is a highly recommended food security crop and promoted as a better staple food for the world's poor (International Year of the Potato, 2008). Typically, production costs are relatively low, and potatoes are one of the most extensively consumed vegetable crops in the world.

1.4 ABOUT POTATOES

1.4.1 History of potatoes

Potatoes (*Solanum tuberosum* L.) were domesticated in the Andes of South America, where they have been grown for over 7000 years (Hawkes 1990; Spooner *et al.*, 2005). They were cultivated in Peru and Bolivia as a staple of the diet of the Inca and pre-Inca peoples, spreading to Chile in the South and Colombia and Venezuela in the North during pre-history. It was not until 1570s that potatoes were known to the New World, when Spanish explorers brought them to Europe as a botanical curiosity. From the 17th century onwards, potatoes made their way from Europe to other parts of the world (Pandey and Kaushik, 2003). The modern cultivated potato (*S. tuberosum*) belongs to the nightshade family (Solanaceae), which comprises 90 genera and about 2800 species of plants (Dodds, 1962). Potatoes are classified under the genus *Solanum*, which consists of about 2000 species and includes the domesticated crops such as tomato and eggplant. The cultivated potato is one of about 220 tuber-bearing species classified under the section Petota within the genus *Solanum* (Hawkes, 1990).

The cultivated species *S. tuberosum* includes four distinct sub-groups which have been given specific rank by some authors but are best formally regarded as Groups selected under domestication. *S. tuberosum* Groups Chaucha, Juzepczukii and Curtilobum are hybrid types which do not breed true and are generally absent from genebanks. The non-hybrid types comprise two diploid groups (Phureja and Stenotomum), and two tetraploid groups (Andigena from the Andes and Tuberosum from Chile) (Dodds, 1962). Among them, *S. tuberosum* Group Phureja was selected from *S. tuberosum* Group Stenotomum for the lack of tuber dormancy and faster tuber development so as to make it suitable for multiple cropping each year. The tetraploid *S. tuberosum* Group Andigena arose from Group Stenotomum through chromosome doubling and were superior to

diploids in terms of yield and some other traits. They were adapted to tuberise under the short day conditions of Central Andes and thus have become the dominant type grown by Andean farmers. From there, potato cultivation spread south into coastal Chile and the Andigena type is thought to have adapted to the long day conditions of Chile and hybridised with additional species along the way giving rise to the Chilean Tuberosum type.

Potatoes comprise one of the richest genetic resources among the domesticated plants available to plant breeders, in terms of the access to both landrace types and compatible wild relatives (Hawkes, 1994), which are important in developing improved crop varieties. Potatoes are characterized by 12 chromosomes (x=12) and consist of a series of ploidy levels ranging from diploids (2x=24) to hexaploids (6x=72) (Hawkes, 1994). Most of the cultivated potatoes are highly heterozygous autotetraploid outbreeders with a haploid genome size of ~850Mb. The tetraploids and hexaploids are mostly self-compatible allopolyploids and nearly all of the diploid species are self-incompatible outbreeders (Hawkes, 1990).

1.4.2 Recent advances in potato genetics

Significant progress has been made in sequencing the potato genome which is being carried out by a number of international research partners making up the Potato Genome Sequencing Consortium (PGSC, http://www.potatogenome.net). The PGSC made the *S. tuberosum* Group Phureja doubled monoploid (DM 1-3 516 R44) genome assembly (DM v3.2) available to the public in August 2010. The ongoing potato and tomato genome sequencing projects will be useful for the Solanaceae research community to help undertake fundamental and applied biological studies, including plant breeding (Bryan, 2010; The Potato Genome Sequencing Consortium, 2011).

1.4.3 World potato production and consumption

Potato is the world's most-important non-grain food commodity and fourth most important food crop after wheat, maize and rice. Potato is considered to be one of the world's most efficient crops in converting natural resources (land and water), labour and capital into a highly nutritious food (Horton, 1980). In 2009, global potato production comprised of 183 million hectares (Mha) and a total production of 329 million tonnes with China, Russia, India and USA being the top four producers (FAOSTAT, 2010).

Potato cultivation has witnessed a tremendous growth in the past few decades particularly in the developing world, due primarily to the crop's ability to adapt to a wide range of environments, its high yielding potential and nutritional qualities combined with its isolation from volatile world markets. Currently, developing countries represent more than a third of the global potato production. There has been a dramatic increase in potato production in developing countries, where the output grew from 32 million tonnes in 1961 to 177 million tonnes in 2009 (FAOSTAT, 2010). Moreover, potatoes represent about 30% of total food crop production in the developing countries (Millam, 2004) with a vast majority of the produce marked for domestic consumption.

As a result, potato consumption has been steadily increasing in the developing countries and according to the FAOSTAT 2005, the annual per capita consumption of potatoes was 14 and 26 kg, respectively, in Africa and Asia. Europe, on the other hand, represents the highest per capita potato consumption in the world (96 kg) (FAOSTAT, 2005). In the developed world, potato is also used as an animal feed and as a feedstock for the production of starch and alcohol (Horton and Sawyer, 1985).

1.4.4 Nutritional value of potatoes

Potatoes are rich sources of nutritionally important compounds. Potato tubers contain 75–80% water, 16–20% carbohydrates, 2.5–3.2% protein, 0.8–2% minerals, 0.6% fibre and 0.1–0.2% fat on a fresh weight (FW) basis (Bajaj, 1987). On a dry weight basis, potatoes contain about 10% protein, which is comparable to that of most staple cereals including rice and wheat (McCay *et al.*, 1987). Potato contains substantially more lysine that the cereal staples, making them a good supplement to cereals (Horton and Sawyer, 1985; Woolfe, 1987). Potatoes are known to be good source of antioxidants including polyphenols, carotenoids and tocopherols (Lachman and Hamouz, 2005). In addition, potatoes are important sources of vitamins (such as B_6, B_3 and C) and minerals particularly K, P, Ca, and Mg (Andre *et al.*, 2007). Cultivated potatoes also contain negligible amounts of β-carotene (pro-vitamin A) (Storey 2007). Vitamin C is the main vitamin in potatoes (Storey, 2007) and a single medium-sized potato supplies about half of the daily adult requirement of vitamin C, while other staple food crops such as rice and wheat lack this compound.

Potatoes contain about 4–6% minerals on a dry weight basis (Burton, 1989). A single medium-sized potato of 200 g FW provides about 26% of Dietary Reference Intake (DRI) of Cu, 17–18% of K, P and Fe, 5–13% of Zn, Mg and Mn, and 2.2% of Ca (White *et al.*, 2009). Interestingly, the bioavailability of minerals is potentially high in potatoes because of the presence of high concentrations of ascorbate, β-carotene, protein cysteine and other organic and amino acids that stimulate micronutrient absorption (White *et al.*, 2009). Moreover, low concentrations of anti-nutritional factors such as phytates (0.11–0.27% dry matter (DM); Frossard *et al.*, 2000; Phillippy *et al.*, 2004) and oxalates (0.03% DM; Bushway *et al.*, 1984) also improve the bioavailability of mineral nutrients in potatoes. For example, several vegetables contain higher levels of tissue Fe content than potatoes, but the bioavailability of Fe for humans is greater in

potatoes than in most other vegetables, making potatoes a nutritious choice (Fairweather-Tait, 1983).

Recently a study on bioavailability of Fe in cooked potato tubers in a set of nine pigmented Andean landraces was reported by Ariza-Nieto *et al.* (2007) using the in vitro digestion/Caco-2 cell model. The results showed that yellow-fleshed potatoes had greater bioavilability of intrinsic iron than the pink and purple-fleshed potatoes. Furthermore, all of the studied varieties showed a positive effect on absorption of extrinsic iron (ferrous sulfate and ferric chloride), suggesting that potatoes can promote the absorption of iron present in other components of the meal. Although the purple-fleshed potato had highest iron concentration, their bioavailability was very low. The yellow-fleshed potatoes are reported to have high concentrations of total carotenoids (Burgos *et al.*, 2008). The purple and red fleshed potatoes contain the main phenolics, anthocyanins and chlorogenic acid respectively (Al-Saikhan *et al.*, 1995, Friedman, 1997). Together these results imply that iron content and its promoters or inhibitors need to be considered separately in breeding programmes aiming to enhance the iron uptake in humans.

1.5 PHYSIOLOGY OF MINERAL ACCUMULATION IN POTATO TUBERS

1.5.1 Anatomy and morphology of the potato tuber

To understand the physiology of accumulation of minerals in potato tubers knowledge of their anatomical details is important. Detailed anatomical study on potato tubers has been previously done by Artschwager (1924), Reed (1910) and Reeve *et al.* (1969a, 1973) and reviewed by Cutter (1992). A brief overview is given below.

Potato t ubers arise from a modified underground stem developed from the swelling sub-apical region of underground lateral stolons. The stolons have a typical Solanaceae stem structure with a set of four or five bicollateral vascular

bundles. Reed (1910) noted an additional structure, namely an endodermis, surrounding the vascular tissue of the stolons. Oparka (1986) showed that this endodermis possesses a Casparian strip, which restricts the apoplastic flow of water and solutes outward from the vascular tissue. Under specific environmental conditions, such as short photoperiod, high light intensity, low nitrogen levels and low temperature, the stolons are induced to form tubers (Ewing and Struik, 1992). During tuber initiation, the stolon ceases to elongate, but the cells in the pith and cortex of the stolon enlarge and divide, resulting in swelling of the stolon tip (Artschwager, 1918, 1924; Reed, 1910). The vascular bundles lose their clear structure as the stolon tip swells, and the bundles disperse as cambial cells divide and create intercalary cortical cells (Reed, 1910). The tubers gradually develop from the sub-apical region. The tuber cells predominantly divide longitudinally until the tuber attains a diameter of about 0.8 cm. They then follow randomly oriented divisions, and subsequent cell enlargement in the perimedullary region (Xu *et al.*, 1998). During tissue enlargement, the endodermis is lost with its Casparian strip (Oparka 1986), and in this way, the xylem and phloem elements are dispersed throughout the tuber tissues. As the tuber develops, the apoplastic movement of solutes occurs throughout the parenchymatous tissues without any barrier.

Although the xylem tissue remains as a loose dispersed ring around the outer part of the cortex, the central cortical tissue is mostly covered by a dispersed network of internal phloem. The internal phloem from the stolon divides into many strands which then undergo anastomoses (Cutter, 1992). As the tubers mature, they have abundant internal and external phloem strands, occupying about 5% of total tuber volume. In contrast, the xylem tissues constitute only a very small proportion, which is usually restricted to the area between the internal and external phloem strands in a relatively peripheral location.

The surface layers of tubers are not static, but constantly change during tuber development. An epidermis exists for a short time on the youngest tuber and is

subsequently replaced by the periderm layer (Artschwager, 1924; Peterson and Barker, 1979; Reeve *et al.*, 1969a). The formation of periderm begins at the stem end of the young tuber and gradually extends over the entire tuber. A typical potato periderm consists of three types of cells: phellem (cork), phellogen (cork cambium), and phelloderm (parenchyma-like cells) (Artschwager, 1924; Reeve *et al.*, 1969a). The phellogen layer, which is the central layer, functions as a lateral meristem and undergoes periclinal divisions to produce outer phellem and inner phelloderm layers (Artschwager, 1924; Peterson and Barker, 1979). Moreover, the phellogen layer largely remains active throughout the growth of the tuber. The outer phellem cells, on the other hand, suberize and form a protective skin of the tuber during periderm maturation (Lulai, 2001; Reeve *et al.*, 1969a). The skin of young developing tubers is found to be enriched with proteins, and about two thirds of this protein is involved in plant-defence responses to biotic and abiotic stresses (Barel and Ginzberg, 2008).

1.5.2 Potato roots and mineral acquisition

The architecture of roots is important for effective and efficient acquisition of mineral elements from soil. The potato plant has a fibrous root system (Cutter, 1992), which is made up of four different types of roots, as described by Kratzke and Palta (1985). They are (1) basal roots (roots originating from the base of the main stem), (2) junction roots (roots arising at the junction between stolons and the main stem), (3) stolon roots (roots on the stolon nodes) and (4) tuber roots (roots on the forming tuber). Few studies have investigated the role of these roots in relation to mineral nutrition of tubers although some attention has been focused on Ca uptake and accumulation in tubers. The results suggest that stolon and tuber roots contribute to Ca uptake and accumulation in tubers, whereas, basal roots contribute to Ca accumulation in shoots (Busse and Palta, 2006; Kratzke and Palta, 1986). Significant variation in stolon and basal root length among potato genotypes have been reported by Wishart *et al.* (2009). In

addition to the uptake of Ca through stolon and tuber roots, studies have shown that tubers also receive Ca through direct transport across the periderm (Davies and Millard, 1985; Habib and Donnelly, 2002; Krauss and Marschner, 1971).

Most of the minerals present in tubers other than Ca appear to have been taken up originally by the main (basal) roots that deliver them first to the shoot *via* the xylem (Kärenlampi and White 2009). The low-transpiring tubers receive minerals mostly through redistribution from the above-ground tissues *via* the phloem network. Therefore, tuber mineral composition is not related simply to shoot mineral composition, but also depends on the extent of phloem loading and mobility of specific minerals.

1.5.3 Mineral composition of tubers during development

Kolbe and Stephan-Beckmann (1997a, b) have reported the changes in concentration of mineral elements in shoots and tubers of potato plants throughout their development. They suggest that the tubers start to accumulate minerals as early as 30 days after crop emergence (DAE), and minerals accumulate rapidly until about 90 DAE. The tuber attained a maximum concentration of minerals (N, P, K, Ca, Mg, Mn and Na) by about 120 DAE (Kolbe and Stephan-Beckmann 1997a, b). However, the maximum accumulation (concentration) of tuber N, P, Mg and Mn was found to occur between 60 and 75 DAE. For Ca, Na, and K, maximum accumulation occurs much earlier between 30 and 45 DAE, and 45 and 60 DAE respectively. The mineral concentrations of potato tubers generally decrease with dry matter accumulation during tuber bulking (Kolbe and Stephan-Beckmann, 1997b). Nitrogenous compounds and several other minerals (P, K, Zn, S and Cu) are typically remobilized from vegetative plant parts and translocated to the tubers during the course of haulm senescence. Thus, mature tubers at harvest contain the greatest proportion of several mineral nutrients. A fully mature potato tuber contains about 76–89% of total plant N, P and K, 84% of Cu, 55% of S, 50% of Zn, 42% of B, 20–40% of Mn,

Na and Mg, 37% of Fe, and 6–7% of Ca, (Harris, 1978; Heard, 2004; Kolbe and Stephan-Beckmann, 1997b) and rest of the minerals remains in the shoot. Further, the physiological gradients of mineral elements might be different between immature and mature potato tubers as the tubers accumulate different mineral elements at different times and rates during their development. Macklon and DeKock (1967) noted an even distribution of major cations and anions, citric acid, malic acid, and Fe in immature tubers, but a significant polarity for these nutrients in the mature tubers.

1.5.4. The potential to modify/enhance tuber mineral concentrations

Studies have shown that tuber mineral concentrations can be increased by application of mineral fertilizers such as N, P, K, Ca, Mg, Zn and Se (reviewed by White *et al.*, 2009; White *et al.*, 2012; Turakainen *et al.*, 2004). Addition of a particular mineral fertilizer may increase or decrease the concentration of other mineral(s) in the tubers due to the complex interactions among different minerals in the soil system and their consequences for uptake by plants, and also to the effects of tissue mineral composition on the redistribution of elements within the plant (White *et al.*, 2009).

Recent studies have shown a substantial genetic variation for mineral accumulation in potato tubers (Andre *et al.*, 2007 (Ca, Fe and Zn); Bamberg *et al.*, 1993 and 1998 (Ca); Brown 2008 and Brown *et al.*, 2010 and 2011 (Fe and Zn); Burgos *et al.*, 2007 (Fe and Zn); Casañas-Rivero *et al.*, 2003 (Ca, Cu, Fe, K, Mg and Zn); Ekin, 2011 (Ca, Cu, Fe, K, Mg and Zn); Haynes *et al.*, 2012 (Cu, Fe, Mn and Zn); Lefevre *et al.*, 2012 (Ca, Cu, Fe, K, Mg, Mn, Na and Zn); Luis *et al.*, 2011 (Ca, Cu, Fe, K, Mg and Zn); Öztürk *et al.*, 2011 (Cu, Fe and Zn); White *et al.*, 2009 (Ca, Cu, Fe, K, Mg and Zn) and 2011 (Zn)), suggesting that breeding for enhanced mineral levels can be achieved.

1.6 LINKAGE MAPPING AND QTL ANALYSIS IN POTATOES

Construction of genetic linkage maps is a prerequisite for linking specific genetic loci and eventually DNA sequences of genes to specific traits. A major use of linkage maps is to identify quantitative trait loci (QTL). Linkage mapping and identification of QTLs is important for dissecting and understanding the genetic control of agronomically important qualitative and quantitative traits (Salvi and Tuberosa, 2005). DNA-based markers are used to detect/visualise sequence variation at a specific genetic locus, developing the genetic maps, to identify QTL and subsequently for tagging genes. Various types of markers are now available for plant breeders and these include Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Diversity Arrays Technology (DArT) and Single Nucleotide Polymorphism (SNP). Using a combination of marker technology, genetic maps have been generated for potato since the late 1980s (Bonierbale *et al.*, 1988; Gebhardt *et al.*, 1989). Genetic mapping in potato has provided researchers with knowledge of genome regions that confer a number of important traits such as pest and disease resistance, tuber nutritional quality, and tuber shape and colour (Bryan and Hein, 2008).

Genetic mapping and QTL studies in potato have been generally conducted at the diploid level and several diploid maps have been generated (Gebhardt, 2007). However, linkage mapping and QTL analysis is more challenging in tetraploid potatoes than diploids due to the complexities of polysomic inheritance. Advanced analytical software tools such as the TetraploidMap for Windows (Hackett *et al.*, 2007) have been designed to develop linkage maps for autotetraploid species for each of the parents of an F_1 mapping population (full-sib offsprings). It handles both dominant and co-dominant markers in all possible configurations and takes into account null alleles in the analysis. The TetraploidMap software also includes algorithms for interval mapping of QTL. So far, six genetic linkage maps have been constructed in tetraploid potatoes using

this software (Bradshaw *et al.*, 2004, 2008; Kelley *et al.*, 2009; Khu *et al.*, 2008; McCord *et al.*, 2010, 2011; Sagredo *et al.*, 2006, 2009, 2011).

QTL analysis is considered as a powerful genetic approach for dissecting complex traits (Paran and Zamir, 2003), such as plant mineral accumulation. Most QTL studies reported in potatoes to date have focused on abiotic and biotic resistance, plant chlorosis and tuber quality traits (Anithakumari, 2011; Bradshaw *et al.*, 2004, 2008; Bonierbale *et al.*, 1988; Bryan *et al.*, 2004; Kelley *et al.*, 2009; Khu *et al.*, 2008; Kloosterman *et al.*, 2010; McCord *et al.*, 2010; Campbell, 2010; Gebhardt *et al.*, 1989, 1991; Sagredo *et al.*, 2006, 2009, 2011; Sliwka *et al.*, 2008; Simko *et al.*, 2008; Sorensen *et al.*, 2008; Werij *et al.*, 2007; van den Berg *et al.* 1996a, b). In potatoes, there has been no report on QTL(s) controlling mineral concentrations in tubers. Given the importance of mineral nutrition in staple foodstuffs, there is a need to identify genetic factors and relate gene to function for tuber mineral concentration.

Understanding of mineral homeostasis in plants came from the studies on the model plant *A. thaliana* and a number of QTL studies for mineral concentrations in shoots and seeds of *A. thaliana* has been reported (Bentsink *et al.*, 2003; Ghandilyan *et al.*, 2009; Harada and Leigh, 2006; Loudet *et al.*, 2003, 2007; Payne *et al.*, 2004; Vreugdenhil *et al.*, 2004; Waters and Grusak, 2008b; White, 2005). However, reports of QTL information in food crops is very limited to rice, wheat, maize, barley, brassica, common bean and soybean (Table 1.1 and references therein). Several QTLs impacting mineral concentrations in edible portions (grain and leaf) have been identified in these studies and several candidate genes underlying these QTLs have been suggested in many of these species.

Table 1.1: QTL studies reported for essential mineral concentrations in edible portions of crop species

Crop species	Tissue	Mineral elements	References
Barley (*Hordeum vulgare*)	Grain	Zn	Lonergan *et al.* 2009
Barley (*Hordeum vulgare*)	Grain	Zn	Sadeghzadeh *et al.* 2010
Bean (*Phaseolus vulgaris*)	Seed	Fe & Zn	Beebe *et al.* 2000
Bean (*Phaseolus vulgaris*)	Seed	Fe & Zn	Blair *et al.* 2009, 2010a
Bean (*Phaseolus vulgaris*)	Roots	Iron reductase activity	Blair *et al.* 2010b
Bean (*Phaseolus vulgaris*)	Seed	Fe, Zn & phytic acid	Cichy *et al.* 2009
Bean (*Phaseolus vulgaris*)	Seed	Zn	Gelin *et al.* 2007
Bean (*Phaseolus vulgaris*)	Seed	Ca, Fe & Zn	Guzman-Maldonado *et al.* 2003
Brassica oleracea	Leaf	Ca & Mg; Zn	Broadley *et al.* 2008 & 2010
Brassica oleracea	Leaf	K	White *et al.* 2010
Brassica rapa	Leaf	Ca & Mg	Broadley *et al.* 2009
Brassica rapa	Leaf	Ca, Cu, Fe, K, Mg & Zn	Wu *et al.* 2008
Maize (*Zea mays*)	Grain	Fe	Lung'aho *et al.* 2011
Maize (*Zea mays*)	Grain	Fe, Zn, Mg	Šimić *et al.* 2011
Oilseed Rape (*Brassica napus*)	Seed	Fe, Zn, Mn, Cu, Ca, Mg	Ding *et al.* 2010
Rice (*Oryza rufipogon*)	Grain	Ca, Cu, Fe, K, Mg & Zn	Garcia-Oliveira *et al.* 2009
Rice (*Oryza sativa*)	Grain	Fe	Gregorio *et al.* 2000
Rice (*Oryza sativa*)	Grain	Ca, Cu, Fe, Mn & Zn	Lu *et al.* 2008
Rice (*Oryza sativa*)	Grain	Ca, Cu, Fe, K & Mg	Norton *et al.* 2010

Rice (*Oryza sativa*)	Grain	Cu, Fe, Zn & phytate	Stangoulis *et al.* 2007
Rice (*Oryza sativa*)	Grain	Zn	Zhang *et al.* 2011
Soybean (*Glycine max*)	Seed	Ca	Zhang *et al.* 2009
Wheat (*Triticum aestivum*)	Grain	Ca, Cu, Fe, K, Mg & Zn	Peleg *et al.* 2009
Wheat (*Triticum aestivum*)	Grain	Zn	Shi *et al.* 2008
Wheat (*Triticum aestivum*)	Grain	Fe & Zn	Genc *et al.* 2009
Wheat (*Triticum monococcum*)	Grain	Cu, Fe & Zn	Ozkan *et al.* 2007
Wheat (*Triticum turgidum* ssp. *dicoccoides* (Körn) Thell.))	Grain	Fe & Zn	Distelfeld *et al.* 2007
Wheat (*Triticum boeoticum* x *Triticum monococcum*)	Grain	Fe & Zn	Tiwari *et al.*, 2009

The gene(s) underlying the QTLs can be identified either by positional cloning (Fridman *et al.*, 2000) or by a candidate gene approach (Pflieger *et al.*, 2001). Among these two, the candidate gene approach has been mostly used for isolating genes underlying specific plant traits based on their functional role or genomic location. Bulked segregant analysis (BSA), developed by Michelmore *et al.* (1991) is an efficient method to tag genes and find markers closely linked to a desired trait. BSA is performed by comparing two pooled DNA samples of extreme phenotypes for a target trait in a segregating population. Within each bulk, the individuals are ideally identical with regard to the gene of interest but arbitrary with regard to other genes. BSA is not only used for high resolution mapping to find simply inherited traits, but also for detecting markers linked to QTL (Liu, 1998).

A more recent mapping approach is association mapping which involves an understanding of the marker-trait associations among a diverse collection of individuals with different genetic backgrounds and related ancestry. Association mapping explores all the allelic variation resulting from multiple meiotic recombination events rather than just two parents of a mapping population, providing higher resolution than standard linkage mapping. Extensive knowledge of high throughput SNPs across the genome is required for genome-wide association mapping, which has the potential to find markers very close to or in candidate genes. Several association mapping studies have been undertaken in potatoes to identify markers associated with resistance to late blight (Gebhardt *et al.*, 2004, Malosetti *et al.*, 2007; Pajerowska-Mukhtar *et al.*, 2009), resistance to *Verticillium* (Simko *et al.*, 2004a, b), tuber quality traits (D'hoop *et al.*, 2008; Urbany *et al.*, 2011) and cold-induced sweetening (Baldwin *et al.*, 2011; Li *et al.*, 2005).

The availability of the full genome sequence of potato from PGSC along with the high throughput SNP genotyping will facilitate genetic dissection of complex traits such as mineral accumulation in tubers. The knowledge of the potato genome sequence will be an invaluable resource for the identification of candidate genes and their allelic variants contributing to the trait of interest.

1.7 THESIS OBJECTIVES AND EXPERIMENTAL APPROACH

The present study aims to understand the natural variation and genetic control of mineral accumulation in potatoes and to develop molecular markers to assist breeding programmes to enhance mineral concentration in tubers (genetic biofortification). To achieve this goal, the following four research objectives were defined:

1. Assess the spatial distribution of mineral elements within potato tubers following post-harvest storage
2. Explore variation in diverse potato germplasms (CPC accessions, Phureja-Tuberosum Core Collection and Neotuberosum population) for tuber mineral concentration
3. Improve marker density of an existing F_1 tetraploid mapping population (12601ab1 x Stirling) using DArT markers and seek QTLs for tuber mineral concentrations
4. Identify molecular markers linked to micronutrient (Fe, Zn and Ca) accumulation in tubers using BSA in F_1 tetraploid mapping population and Neotuberosum population

The starting point for this thesis was to explore the mineral distribution in potato tubers to gain a better understanding of the patterns of tuber mineral accumulation and sampling strategies. Following this, the mineral

concentration in diverse potato germplasm was surveyed to obtain a comprehensive picture of the extent of natural genetic variation for tuber mineral accumulation and its potential for improvement through breeding. Further, the genetic basis for tuber mineral concentration was studied using an existing F_1 tetraploid mapping population, 12601ab1 x Stirling. The mineral concentrations of field-grown potato tubers were analysed for patterns of segregation among the progenies. The existing SSR- and AFLP-based linkage maps of the mapping population were considerably improved by including genome-linked DArT markers. QTL analysis was then performed on the updated linkage map, and the genomic loci controlling tuber mineral accumulation were identified. Finally, the BSA approach was employed to identify the genome-linked molecular markers (such as SSRs and DArTs) associated with candidate genes and/or QTLs controlling tuber micronutrient (particularly Fe, Zn, and Ca) accumulation in the F_1 mapping population and also in the Neotuberosum population.

CHAPTER 2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

2.1.1 Mineral distribution studies

2.1.1.1 Distribution of minerals within Stirling tubers shortly after harvest (Experiment 1)

The potato cultivar 'Stirling' was used in this study. The tubers were planted at the Balruddery Farm (Dundee, UK, 56°28'38.95" N; 3°07'03.31" W) during April 2007. The crop was grown using standard agronomic practices and was harvested manually during September 2007. The tubers were then stored in a controlled environment, with an initial temperature of 12°C followed by a gradual decline until 4°C over a two-week period. Following the two-week storage, five tubers of uniform size and shape were selected and subsequently stored in an ambient store (13°C) for four weeks before they were subjected to mineral analysis.

2.1.1.2 Distribution of minerals within tubers of two genotypes following long-term cold storage (Experiment 2)

The potato cultivar 'Stirling' and the clone '12601ab1' were used in this study. They are the parents of the tetraploid mapping population used in this study for QTL mapping (see section 2.1.3 for more details). The tubers were planted at Balruddery Farm (Dundee, UK, 56°28'48" N; 3°8'13.2" W) during April 2009. The crop was grown using standard agronomic practices and was manually harvested during September 2009. The tubers were transferred to controlled environment stores, with an initial temperature of 12°C followed by a gradual decrease until 4°C over two weeks. The tubers were then maintained at 4°C for an extended period of six months. No sprout inhibitors were applied to

the tubers during the storage. Following the long-term cold storage, samples consisting of about 15 to 20 tubers from each genotype were obtained randomly among the tubers harvested from field plots. From each sample (i.e., each genotype), five un-sprouted tubers of uniform size and shape were selected for mineral analysis.

2.1.2 Diverse populations

The Commonwealth Potato Collection (CPC) is maintained by JHI in true seed form and is a major gene bank containing around 1500 accessions, of which about two-thirds are wild potato species and the rest one-third are cultivated types from S. America (Bradshaw and Ramsay, 2005; CPC, http://germinate.scri.ac.uk/germinate_cpc/app/). Selected accessions of wild potato species (Table 2.1) originating from different habitats were grown in a glasshouse for rejuvenation (15 to 20 plants per accession) prior to initiation of the project in 2007, and tuber samples were obtained from this material for mineral analysis. The Phureja-Tuberosum Core Collection (Core Collection) of potato genotypes comprises of 64 clones and was grown at Gourdie Farm during 2007 and 2008 (Appendix Table A2.5). Within the Core Collection, 36 clones belong to diploid *Solanum tuberosum* Group Phureja lines selected from CPC accessions for performance in UK field conditions, 25 clones were diploid and tetraploid European *S. tuberosum* Group Tuberosum and three were hybrids between Phureja and diploid Tuberosum clones (Table 2.2). Field plots were bulk harvested and tubers were stored at ambient temperature (8 to 12°C). In the 2008 field trials, two Tuberosum cultivars (2DH.36 (50) and 2DH.40 (3)), a Phureja clone ((TC.43 (45)) and a hybrid (99.FT 1(5)) were not planted, but were replaced by a Tuberosum cultivar, *Edzell blue*. The 60 genotypes grown in both years were used in principal component analysis (PCA) and correlation analysis (see Chapter 4).

The Neotuberosum (NTB) population is a diverse tetraploid population derived from Andean landrace potatoes by recurrent selection for early tuberisation under the long-day conditions of the UK summer (Bradshaw and Ramsay, 2005; Glendinning 1975a). The 450 clones of this population together with two control lines (the tetraploid Tuberosum Desiree and the diploid Phureja Mayan Gold) were grown in two replicate plots (four tubers per replication) at Gourdie Farm in 2005. Thirty replicates of the two control clones were randomly planted throughout the experimental field. Five representative tubers from each clone were selected, sampled, freeze-dried and milled prior to starting this project.

Table 2.1: Particulars of the different species from the Commonwealth potato collection (CPC) used in the study

Species	CPC number	Series	Country of origin
S. acaule	2109	Acaulia	Bolivia
S. acaule	2113	Acaulia	Bolivia
S. acaule	2456	Acaulia	Argentina
S. bulbocastanum	7638	Bulbocastana	Mexico
S. bulbocastanum ssp. *partitum*	7650	Bulbocastana	Unknown
S. capsicibaccatum	3554	Circaeifolia	Bolivia
S. violaceimarmoratum	7782	Conicibaccata	Bolivia
S. infundibuliforme	2477	Cuneolata	Argentina
S. brachycarpum	7031	Demissa	Mexico
S. brachycarpum	7027	Demissa	Mexico
S. demissum	1345	Demissa	Mexico
S. demissum	1126	Demissa	Mexico
S. demissum	7524	Demissa	Mexico
S. hougasii	7049	Demissa	Mexico
S. hougasii	7048	Demissa	Mexico
S. lopetalum	2922	Demissa	Mexico
S. fendleri	7214	Longipedicellata	Mexico
S. fendleri	2605	Longipedicellata	USA
S. fendleri	2601	Longipedicellata	USA
S. hjertingii	5697	Longipedicellata	Mexico
S. polytrichon	3987	Longipedicellata	Mexico
S. stoloniferum	2639	Longipedicellata	Mexico
S. megistacrolobum	3273	Megistacroloba	Bolivia
S. megistacrolobum	2482	Megistacroloba	Argentina
S. cardiophyllum	5908	Pinnatisecta	Mexico
S. trifidum	7124	Pinnatisecta	Mexico
S. canasense	3059	Tuberosa	Peru
S. canasense	3672	Tuberosa	Peru
S. gourlayi	7161	Tuberosa	Argentina
S. kurtzianum	6065	Tuberosa	Unknown
S. kurtzianum	3783	Tuberosa	Argentina
S. kurtzianum	5890	Tuberosa	Unknown
S. microdontum	3764	Tuberosa	Argentina
S. microdontum	3757	Tuberosa	Argentina
S. microdontum	3740	Tuberosa	Argentina
S. medians	7617	Tuberosa	Unknown
S. multidissectum	7180	Tuberosa	Peru
S. multidissectum	7699	Tuberosa	Peru
S. multidissectum	7171	Tuberosa	Peru
S. marinasense	6020	Tuberosa	Peru
S. marinasense	7739	Tuberosa	Peru
S. neocardenasii	7612	Tuberosa	Unknown
S. neorossii	7628	Tuberosa	Unknown
S. okadae	7775	Tuberosa	Unknown
S. demissum	4630	Tuberosa	Unknown
S. tuberosum Group Andigena	61	Tuberosa	Bolivia
S. tuberosum Group Andigena	573	Tuberosa	Peru
S. chacoense	3732	Yungasensa	Argentina
S. chacoense	3504	Yungasensa	Unknown

Table 2.2: Potato lines and cultivars comprised by the Phureja–Tuberosum Core Collection

Genotypes in the Phureja -Tuberosum Core Collection				
Group Phureja				
71.P.10	71.T.46	71.T.6	80.CP.23	81.S.66
842.P.75	851.T.8 [1]	DB 384 (4)	DB 520 (11)	DB.161 (10)
DB.168 (11)	DB.170 (35)	DB.175 (5)	DB.199 (10)	DB.207 (35)
DB.226 (70)	DB.244 (37)	DB.257 (28)	DB.270 (43)	DB.271 (39)
DB.299 (39)	DB.323 (3)	DB.333 (16)	DB.337 (37) [2]	DB.354 (901)
DB.358 (23)	DB.358 (24)	DB.358 (30)	DB.375 (1) [3]	DB.375 (2)
DB.377 (4)	DB.378 (1) [4]	DB.441 (2)	PHU.950 (412)	PHU.951 (901) [5]
TC.43 (45)[¤]				
Group Tuberosum				
12601ab 1	2DH40 (3) (2x) [¤]	Ailsa	Anya	Brodick
Cara	2DH36 (50) (2x) [¤]	Desiree	Edzell Blue^	Estima
Golden Millennium	Home Guard	Home Harvest	Hermes	Maris Piper
Montrose	Nadine	Pentland Dell	Pentland Squire	Record
Saxon	Scarborough	Stirling	Tay	Vales Everest
Wilja				
Hybrids [6.]				
99.FT 1 (5) [¤$] [2DH 40 (3) x DB337 (37)]				
HB.165 (1) [PDH182 x DB 226 (70)]				
HB.171 (13) [PDH 247 x DB 226 (70)]				

[1.] 851.T.8 ExMS 86 (13), [2.] Mayan Gold, [3.] Inca Dawn, [4.] Inca Sun, [5.] Mayan Twilight, [6.] Parents given in square brackets. [¤] Planted in 2007 only. ^Planted in 2008 only. [$] Identity was doubtful and not used in the analysis.

2.1.3 Tetraploid genetic mapping population

The 12601ab1 x Stirling population, also known as GenPop1, is an established tetraploid F_1 mapping population, consisting of 228 clones from a cross between the table cultivar Stirling, which carries resistance to late blight (*Phytophthora infestans*) obtained from *S. demissum,* and the clone 12601ab1 (SCRI advanced breeding clone), which confers resistance to the potato cyst nematode (*Globodera pallida*) obtained from *S. tuberosum* Group Andigena (Bradshaw *et al.*, 2004, 2008). In addition, Stirling possesses good agronomic traits such as high yield and good tuber appearance, and 12601ab1 has high

tuber dry matter content and good fry colour. In field trials, the parents were included thrice in each replication along with six control cultivars (Brodick, Estima, Maris Bard, Maris Piper, Montrose and Pentland Dell) and the trials were surrounded by a guard row. Three to five medium-sized, healthy tubers were selected from the field plots for mineral analysis.

2.2 GROWTH CONDITIONS

2.2.1 Glasshouse and field conditions

The seeds of selected CPC accessions were planted on 9 April 2007 in a standard peat-based potting compost in 15 cm depth pots and the plants were grown to maturity in the glasshouse. The compositions of the potting compost and the base fertilizer Sincrostart (William Sinclair, Lincoln, UK) are given in Appendix Tables A2.1 and A2.2.

The potato clones and cultivars used in this study were grown in field trials. The Neotuberosum population was grown in two replicate blocks, while the others had an addition of an α-design (Paterson *et al.*, 1988) with two complete replicate blocks, each subdivided into sub-blocks. Within each sub-block, tubers were planted in single-drill plots of four, five or eight tubers (Table A2.5) spaced about 35 to 40 cm apart within drills and about 75 to 90 cm between drills. Sixteen such plots make a section, with single guard plot at each end of a section. Each guard plot was of 2m in width from the end of the plot to allow for machine harvesting. Field-planting of the clones took place during April–May of the study years and the drills were covered on the same day of planting.

The replicated field trials were established in the Gourdie Farm during 2005, 2007 and 2008, and in the Balruddery Farm during 2009. Gourdie Farm is

located 3.7 km north-west of Dundee, at Liff and Balruddery Farm is located 6 km north-west of Dundee. Additional data given in the Appendix include field maps (Figures A2.1 and A2.2), soil survey summaries (Table A2.3) and soil mineral content of 10 ha units bulked from 20 samples across the field collected in a W-pattern (Table A2.4). The field study was conducted following standard agronomic practices.

To obtain optimum plant growth and maximize the quantity and quality of crop yield, recommended amounts of fertilizers were applied to the field based on soil test reports (Table A2.4). Soil P levels were slightly lower than the guideline level in two experimental sites, Reservoir Field and Dron Field during 2008 and 2009, respectively. Although lower levels of Mo and Na were found on both farms, these elements were of low importance in potato production. However, the levels of soil K, S and Ca were lower than the guideline level at all the trial sites. Soil Mg and Zn levels were at an adequate level at all sites except at Reservoir Field (2008) and at Dron Field (2009), respectively. Nevertheless, Cu and Fe were within the guideline levels at all the trial sites. The cation exchange capacity (CEC) was slightly lower in trial sites during 2007–2009.

Following standard field preparations for potato planting, fertilisers were incorporated into the soil during April before planting. Herbicide, aphicide and fungicide applications were standard for a ware crop in South-East Scotland (see Appendix Table A2.5 for a summary).

2.2.2 Meteorological data

Weather data was collected from the JHI weather station (East Loan, Invergowrie), about 2 to 5 miles from the trial sites at Gourdie and Balruddery (Appendix Table A2.6). JHI trial sites were planted during April–May and

active above-ground growth was completed during June–August. During the active growth period (June–August), 2005 had a rainfall total below the 30 year (1971–2000) mean; therefore, irrigation was applied to the crop. Rainfall during 2007–2009 was well above normal, particularly in 2007 with almost twice as that of the 30-year mean (Table A2.7). In 2007, much of the rainfall was early in the growing period, whereas in 2008 and 2009 much of the precipitation was received later in the growing period. Mean daily maximum temperatures for the active growing season were about 1°C above the 30-year mean in 2007 and 2009, about 4.3°C above in 2005 and about 1°C below in 2008. Each year, the field trials were harvested during the last week of September following haulm destruction, which typically begins during early September.

2.3 TUBER SAMPLING

2.3.1 Harvesting

Tubers from five glasshouse-grown CPC plants of each accession were harvested in January 2008. As they were relatively smaller than selected cultivated types, the whole tubers were used for mineral analysis. Five medium-sized healthy tubers, one each from the five plants, were selected and bulked for mineral analysis. The tubers were washed thoroughly using tap water, rinsed in deionised water, and briefly air-dried to remove any moisture on the tuber skin. The dried whole tubers were then chopped, freeze-dried, milled and then used for mineral analysis.

Plant emergence and maturity were scored for the Core Collection and mapping population, as described by Bradshaw *et al.* (2008). Plant emergence was scored on a scale of one (none) to nine (all plants in a plot well established) during the last week of May (May 24, 27 and 29, respectively in

2007, 2008 and 2009). Likewise, maturity scores were carried out on a scale of one (all plants in a plot turned brown (dead)) to nine (all plants still green) during the third week of August (Aug 20 in 2007, 2008 and 2009). Additionally, flower colour was recorded for all the clones in the mapping population during 2009 and 2010 using a 0 or 1 score, with 0 being white (e.g., Stirling) and 1 being blue (e.g., 12601ab1). The tubers from each plot were harvested about two weeks after foliage burn-down in September (Table A2.8). The harvested tubers from each plot (with four to five plants each) were bulked by replication and stored in net bags at ambient temperatures (8 to 12°C). Tuber yield from each plot was determined during October to November, using an Avery weighing balance connected to an Epson HX-20 portable computer. The dry matter (DM) content of the Core Collection tubers were estimated indirectly using the specific gravity method, as described by Bradshaw *et al.* (2008). For all other populations, DM content was determined based on the weight difference of tuber pieces prior to and after freeze-drying. Five (three in 2007 trials) medium sized healthy tubers were randomly selected and stored in brown bags at 4°C prior to sample preparation and mineral analysis. To reduce tuber to tuber mineral variation, five tubers per clone/replication were used for mineral analysis. All the populations used in this study were also maintained every year in JHI's high health seed site (Balruddery Farm, Dundee and Derachie Farm, Forfar) for seed stock maintenance.

2.3.2 Sample preparation

2.3.2.1 Mineral distribution studies (Experiments 1 and 2)

The position of tuber was determined first before chopping the tubers for Experiments 1 and 2. In order to understand the physiology of mineral

accumulation in potato tubers, the position of tubers were taken into account. Inspection of a number of different cultivars growing in the field and glasshouses at JHI confirmed that the previous orientation of harvested tubers in the field soil can be determined. Some, but not all, cultivars are slightly flattened dorso-ventrally, and most have two additional features which can be used to determine their orientation during growth in the soil. At the apical or bud end of the tuber, more buds are present on the upper (adaxial) surface than the lower surface. At the basal or stolon attachment end of the tuber (herein, the stem end) there is usually a pronounced bulge below the attachment point.

For Experiment 1, the tubers were washed, rinsed in deionised water and briefly air-dried. Five concentric skin (periderm and associated cortex) samples were carefully removed with a vegetable peeler to a depth of 0.3 to 0.4 mm from the apical to the stolon (stem) end of the tuber (Figure 2.1). The five skin samples were labelled from S01 to S05. The peeled potato tubers were then cut longitudinally into top (A), middle (B) and bottom (C) slices of equal thickness according to the orientation of the tuber in the soil. Each of the three slices was cut longitudinally into five strips and each of the five strips was further cut into five pieces of equal length (Figure 2.1B, C). After measuring fresh weights, each of the pieces was freeze-dried and weighed again to determine the DM content. Dried samples were then powdered using a clean glass rod and stored in a freezer at -20°C until mineral analysis.

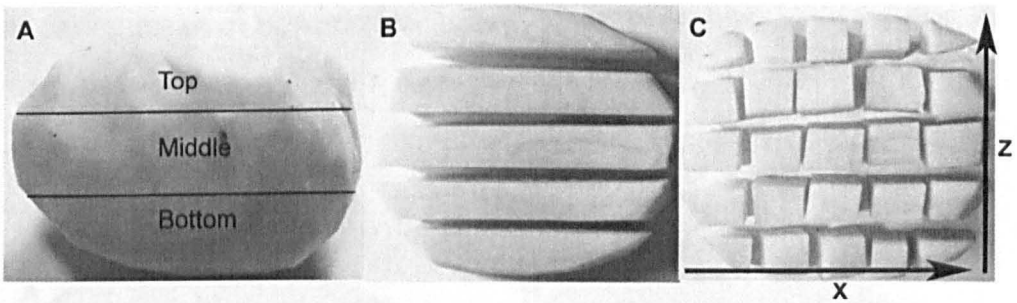

Figure 2.1: Tuber sampling for mineral analysis (Experiment 1). (A) Peeled tuber showing the bulge of abaxial side below the stolon attachment point with three longitudinal slices (top, middle and bottom). (B) The middle slice of the three longitudinal slices cut into five strips. (C) Each strip further divided into five pieces. X: Bud to stem end pieces of each strip; Z: Five strips of a slice.

Mineral concentrations of the bud end, centre and stem end of whole tubers were estimated by averaging the concentrations at bud end pieces (first and second piece of each strip in top, middle and bottom slices and S01 and S02 of skin), central slice (third piece of each strip in all three slices and S03) and stem end pieces (fourth and fifth piece of each strip in all three slices and S04 and S05).

For Experiment 2, tubers from the two genotypes, Stirling and 12601ab1, were washed, rinsed in deionised water and air-dried briefly. The tubers were first chopped into eight pieces (numbered 1 to 8) and the central transverse section of 1 cm was then chopped from the tuber eighths (numbered 1a to 8a) as shown in the Figure 2.2. In total, there were four slices: slice 1 (1, 1a, 2a and 2), slice 2 (4, 4a, 3a and 3), slice 3 (5, 5a, 6a and 6) and slice 4 (8, 8a, 7a and 7). After slicing, fresh weights of each of the pieces were determined. The samples were freeze-dried and weighed to determine the DM content. Dried samples were then powdered using a mortar and pestle and stored in a freezer at -20°C until used for mineral analysis. Mineral concentration of bud, centre and stem end of Stirling and 12601ab1 (whole) tubers were estimated

by taking mean of bud end pieces 1, 4, 5 and 8, central pieces 1a to 8a and stem end pieces 2, 3, 6 and 7, respectively.

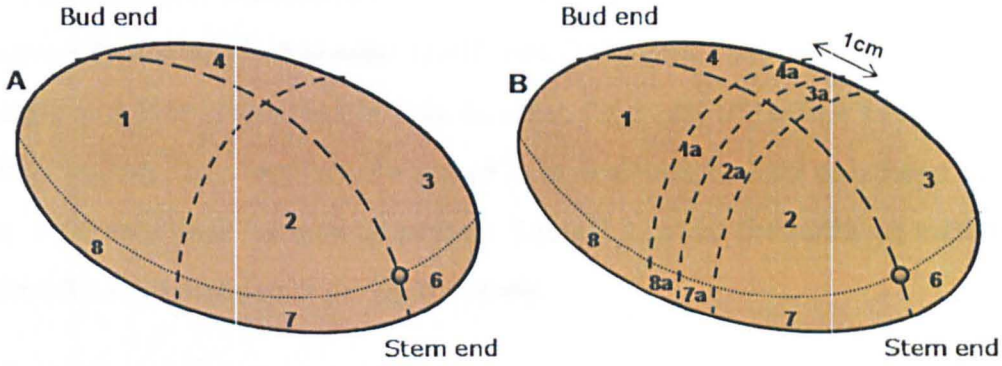

Figure 2.2: Sampling tuber sections for Experiment 2. (A) Tuber chopped into 8 pieces (1 to 8). (B) Transverse sections of 1cm chopped from tuber eighths (1a to 8a).

2.3.2.2 Diverse (Core Collection and NTB) and mapping populations

The selected potato tubers from each population used in this study were washed thoroughly using tap water, rinsed in deionised water and dried. The unpeeled tubers were cut longitudinally into two equal halves from bud to stem end and each half was then cut into four quarters by placing two cuts perpendicular to the flat cut surface giving rise to eighths. Representative tuber sub-sample, two opposite sections from each half tuber (1 and 3, and 6 and 8) (Figure 2.3, Table 2.3) was obtained for the mineral analysis. In 2008 and 2009, due to compromises with colleagues sharing the trial harvest, diagonally opposite segments (3 and 5) or the central transverse section of tuber (Figure 2.3B) were sampled for the mapping population. Therefore, for the mapping population, three different sampling methods were used in three different years (Table 2.3). Hence data from individual years were analysed separately and over-year means were also calculated (Chapter 5). The selected sections from each tuber were bulked for the five-tuber samples,

diced and subsequently frozen in liquid nitrogen and freeze-dried or freeze-dried directly without flash-freezing in liquid nitrogen. Freeze-drying was carried out in a Millitorr S3921 vacuum freeze-drying unit (Millitorr Engineering Ltd, Manchester, UK) for four days. The freeze-dried samples were milled in a coffee blender (2007 trial; DeLongHi, Italy) or in a Retsch mill (2008 and 2009 trials; Tecator Udy, Boulder, Colorado, US) with a 1 mm sieve. After milling, the samples were stored in re-sealable, air-tight polythene bags at -20°C until used for mineral analysis. Table 2.3 details the sampling method used for each population during the study.

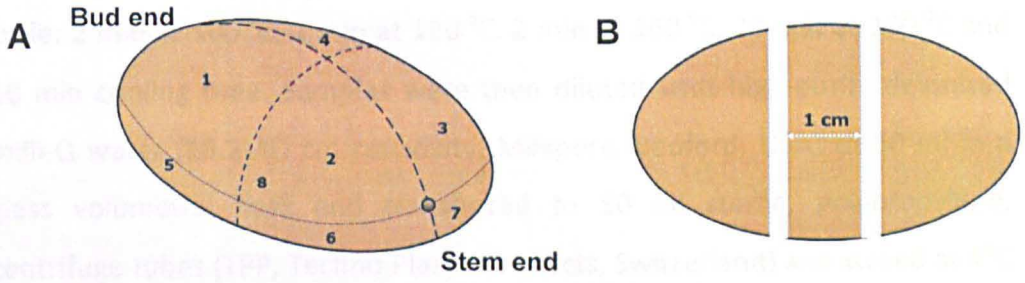

Figure 2.3: Representative sub-sampling of potato tubers for mineral analysis. A) sampling of tuber eighths. B) sampling of central transverse section.

Table 2.3: Tuber sub-sampling protocol for mineral analysis

Year	Protocol and population		
	Neotuberosum	Core Collection	Tetraploid mapping
2005	A [1.]		
2007		B [2.]	B
2008		B	A
2009			C [3.]

[1.] Diagonally opposite segments (3 and 5), [2.] Two opposite sections from each half of the tuber (1and 3, and 6 and 8), [3.] Central transverse section from each tuber.

2.4 ACID DIGESTION AND MINERAL ANALYSIS

The freeze-dried and milled tuber samples were acid-digested at JHI with concentrated nitric acid and hydrogen peroxide to dissolve the mineral elements for measurement. Samples of 0.1 g of the freeze-dried tuber material were first pre-digested with 3ml of concentrated nitric acid (HNO_3, 69%, Aristar grade, VWR) in a Teflon PFA (perfluoro alkoxy) microwave digestion tube, before adding 1 ml of hydrogen peroxide (H_2O_2, 30%, Aristar grade, VWR) to the digestion. The samples were then placed in batches of 40 in a carousel in a closed vessel 1600W microwave oven (MARS Xpress, CEM Corporation, Matthews, NC, USA) and digested at the following temperature cycle: 2 min at 100 °C, 1 min at 120 °C, 2 min at 160 °C, 20 min at 180 °C and 20 min cooling time. Samples were then diluted with high-purity deionised milli-Q water (18.2MΩ cm resistivity, Millipore, Bedford, USA) to 50 ml in a glass volumetric flask and transferred to 50 ml sterile, polypropylene, centrifuge tubes (TPP, Techno Plastic Products, Switzerland) and stored at 4°C prior to analysis. Each batch of digestion consisted of 2 digestion blanks, one or two in-house prepared potato reference material, and 37 or 36 samples. The extracts were analyzed at JHI using the inductively coupled plasma-mass spectrometry, ICP-MS (PerkinElmer ELAN DRC-e, Monza, Italy) equipped with a DRC (Dynamic Reaction Cell) for the mineral ions P, Cl, S, K, Mg, Ca, Mn, Zn, Fe, Cu, Cs and Ni. The extracts were directly used in ICP-MS without any further dilution.

The tuber samples from the populations used in the study were analysed at JHI, Warwick HRI (University of Warwick, UK) or both (Table 2.4). At Warwick HRI, 0.1 g (DW) of the freeze-dried tuber samples were digested for 1 hour with 1ml of H_2O_2 and 2ml of a H_2SO_4 (sulphuric acid/Se catalyst micro-Kjeldahl digestion method), as described by Broadley *et al.* (2010). The acid-digested

samples were then analysed for minerals such as N, P, K, Mg, Ca, B, Cu, Mn, Fe, Zn, S, and Na, using the inductively coupled plasma-emission spectrophotometry (ICP-ES, JY Ultima 2, Jobin Yvon Ltd, Stanmore, Middlesex, UK).

Table 2.4: Site of mineral analysis of different material used in this study

Year	Tuber material and site of analysis					
	NTB	CPC	Core Collection	Tetraploid mapping popn	Mineral distribution	
					Expt1	Expt2
2005	JHI					
2007		JHI	JHI/HRI	JHI/HRI	JHI	
2008			HRI	HRI		
2009				HRI		
2010						JHI

NTB-Neotuberosum population; CPC-Commonwealth Potato collection; popn-population; JHI-The James Hutton Institute, Dundee; HRI-Horticultural Research Institute, Warwick; Expt-Experiment.

Mineral analysis at JHI was performed using the ICP-MS (standard mode) with mathematical corrections for polyatomic interferences. The rejectionparameters RPa and RPq were fixed to 0.0 and 0.25 during the analysis. The oxide and doubly charged ions were fixed at <3%, hence no correction was necessary for their interference. The complete parameter values used for the ICP-MS were provided in Appendix Table A2.9. We used matrix-matched in-house prepared potato tuber samples (Stirling and 12601ab1) from the 2007 harvest as an internal standard reference to correct for non-spectral interferences and instrumental drifts.

Instrument optimization was achieved prior to sample analysis, using a multi-element daily performance check solution (SmartTune solution, ELAN DRC plus II) of 10 elements, selected across the desired mass range at a known concentration (10 µg/l barium and 1 µg/l of beryllium, cerium, cobalt, indium, iron, lead, magnesium, thorium and uranium).

The ICP-MS had been just commissioned at the start of the project, therefore, a NIST-SRM 1573a (a tomato leaf standard (TLS) reference material from the National Institute of Standards and Technology, Gaithersburg, MD, USA), was used with each run. Two potato standards (PS) (Stirling (PS1) and 12601ab1 (PS2)) were run along with the NIST standards at the beginning and end of the run. Furthermore, measures were taken to ensure the accuracy of ICP runs by performing palindromic arrays of test samples with standards at the beginning and end of the run and by including the tuber standards at multiple intervals. Each analytical run included at least one batch of acid-digested potato standards and chemical standards.

Each analytical run was carried out in the following order: a calibration blank, external calibration standards, milli-Q water, 1% HNO_3, digestion blanks, NIST-TLS and/or PS1 and PS2 and then the test samples with additional standards at the end of the run. Based on the standard calibration plots, the software (ELAN® ICP-DRC-MS software, PerkinElmerSCIEX, Massachusetts, USA) automatically calculates the elemental concentration in samples. The calibration curves for multi-element anion and cation standards in ICP-MS showed a high R^2 value (0.999) for both types of ions. The autosampler can accommodate up to 149 samples per run. The drift for mineral concentrations was minimal in within-run standards, and a good repeatability was observed between runs (Appendix Figures A2.3 and A2.4 for three standards at different points in eight runs, and Figure A2.5 for drift in test samples). The progressive change (drift) in mineral concentrations with sample number within a run was confirmed by running palindromic arrays (Figure A2.6). Therefore, two corrections were applied to the data to correct for inter-run differences and for drift during runs, assuming a regular progression throughout the run.

2.5 MOLECULAR METHODS

2.5.1 Genomic DNA extraction

Genomic DNA was extracted from 100mg of fresh leaf material from young, fully expanded leaves, using the DNeasy Plant DNA Extraction kit (Qiagen, Cat. no. 69106) and stored at -20°C until further analysis.

The quality and quantity of the DNA samples were tested using the electrophoresis procedure on a 1% agarose gel, with SYBR safe (approx. 5 µl/50 ml of gel) (Invitrogen™, Life Technologies Ltd., UK) and ran in 1X Tris/borate/EDTA (TBE) buffer along with known quantities of uncut λ DNA and a standard size 1Kb marker ladder (Promega). The DNA samples were run at 100V for about 25 minutes and the bands were visualized on an UV transilluminator. The band images were printed using the CCD photo documentation system (UVI DOC-008-XD).

2.5.2 Amplified Fragment Length Polymorphism procedure

AFLP assays were performed following the protocol of Vos *et al.* (1995). Genomic DNA (0.50 µg) was fully digested for 1 hour at 37°C using a reaction mix containing 2.5 units of *Eco*RI, a 6-bp cutting enzyme (10 units/µl, Pharmacia), 2.5 units of *Mse*I, 4-bp cutting enzyme (4 units/µl, New England Biolabs) and 4µl of 5x restriction–ligation buffer (comprised of 100µl of 10x One-Phor-All Buffer (Pharmacia), 5µl of bovine serum albumin (BSA), 5µl of 1M dithiothreitol (DTT) and 90µl of sterile deionised water) in a final reaction volume of 20 µl. The One-Phor-All universal buffer was compatible with all enzymes used in the study.

Restricted DNA was then ligated to enzyme *Eco*RI and *Mse*I adapters, which were made by annealing forward and reverse adapter strands (3000 pmol of each strand). Sterile distilled water was added to give a 5 pmol/μl solution of *Eco*RI adapter and a 50 pmol/μl solution of *Mse*I adapter. The adapters were ligated to digested DNA in a 5 μl ligation mixture containing 2.5 pmol of *Eco*RI adapter, 25 pmol of *Mse*I adapter, 0.5 μl of 10mM ATP, 1 μl of 5x restriction-ligation buffer and 0.5 unit of T_4 DNA ligase (10 units/μl, Pharmacia). The template was incubated for a further 3 hours at 37°C using a PE 9700 thermal cycler (Perkin Elmer, USA).

Sequence of the *Eco*RI adapter:

Forward - 5'- CTCGTAGACTGCGTACC
Reverse - 3' - CTGACGCATGGTTAA

Sequence of the *Mse*I adapter:

Forward - 5'- GACGATGAGTCCTGAG
Reverse - 3'- TACTCAGGACTCAT

This results in a primary template.

The adapter-bound DNA was 'pre-amplified' using non-selective primers that are strictly complementary to their respective adapters. This generates a large amount of secondary template DNA for use in the subsequent selective amplification using radioactively labelled primers. The sequences of the non-selective primers are:

*Eco*RI primer (EOO- core primer): 5' – GACTGCGTACCAATTC - 3'

*Mse*I primer (MOO- core primer): 5' – GATGAGTCCTGAGTAA - 3'

Five μl of the primary template (restriction-ligation product) was added to a pre-amplification mixture containing 30 ng EOO primer, 30 ng MOO primer, 2

µl of 2 mM dNTPs (Roche), 2 µl of 10x PCR buffer (Roche), 0.1 µl of Taq DNA polymerase (5 U/µl) and 10.3 µl of distilled water to give a final reaction volume of 20 µl. The reaction was performed in a PE 9700 thermal cycler (Perkin Elmer, USA) using the following programme: 24 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 56°C and 60 sec extension at 72°C. To verify whether the restriction and ligation procedures had been successful, a sample of the amplification product was subjected to electrophoresis on a 1% agarose gel with SYBR safe in 1x TBE; a smear ranging from 100 to 500 bp was expected. This template was then diluted in sterile deionised water depending on smear intensity (generally 25-fold dilution) to obtain a working concentration of 0.25 µg/µl (secondary template). The working sample was stored at -20°C.

Selective amplification was performed by amplifying diluted template using primers with specific base extensions: a 3-base extension on the E (*Eco*RI) primers (E+3) and a 3-base extension on the M (*Mse*I) primers (M+3). AFLP marker analyses for the tetraploid mapping population have been previously done (Pande, 2002), and one of the *Eco*RI and *Mse*I combinations, EAACMCAG (E32 M49), was used in this study to verify the identity of the existing clones in the mapping population (Appendix III).

For detecting fragments after the selective amplification, the forward primer (E32 primer) was end-labelled with radioactive phosphorus (^{33}P). The labelling was carried out using 5 ng of E primer and 1.0 µCi $\gamma(^{33}P)$-ATP in 1x T4 kinase buffer using 0.1 units of T4 polynucleotide kinase (10 units/µl, New England Biolabs), making a final volume of 0.5 µl. The mixture was incubated at 37°C for 30 min prior to amplification.

The selective amplification was performed using 5ng of $\gamma(^{33}P)$ ATP end-labelled E32 primer and 30ng of unlabelled M49 primer added to 5 µl of secondary template. PCR components and their volumes were similar to that of the pre-amplification. The reactions were performed using the following touch-down profile: 12 cycles of 30 sec at 94°C, 30 sec at 65°C and 60 sec at 72°C. At the end of each cycle, the annealing temperature was decreased by 0.7°C. These initial cycles were followed by 24 cycles of 30 sec at 94°C, 30 sec at 56°C and 60 sec at 72°C.

Following final PCR, an equal volume of loading dye consisting of 98% deionised formamide, 0.5mM of EDTA (pH 8.0), 25 mg of bromophenol blue and 25 mg of xylene cyanol was added to the samples and subsequently denatured at 95°C for five minutes and 'snap cooled' on ice. About 4 µl aliquot of each sample was loaded on a 5% polyacrylamide gel (polyacrylamide composition: 1x TBE and 7.5M urea; gel composition: 100 ml 5% polyacrylamide, 250 µl 10% ammonium persulphate and 100 µl TEMED). The size of each marker was identified by comparing with the known bands of the Sequamark 500bp-ladder (Promega, Madison, WI). Electrophoresis was carried out using a BioRad electrophoresis system (Richmond, VA, USA) buffered with 1x TBE for 3 hours at 90 watts. The gels were then transferred on to Whatman filter paper and dried using a gel drier. Dried gels were exposed to X-ray (Biomax MS, Kodak film) film for 1 to 5 days at room temperature before developing the images. The autoradiograms were scored manually for the presence or absence of alleles.

AFLP markers were designated by the first letter of the forward primer used (P, in the case of *Pst*I and E for *Eco*RI*)* and the letters of the selective nucleotide extension at the 3' end of the primer followed by the first letter of the reverse primer (M for *Mse*I) and the letters of the selective nucleotide

extension followed by an underscore and the molecular size of the amplified product (e.g., EAACMCAG_200.0). The approximate size of each marker was estimated by comparing with the Sequamark 500 bp ladder (*fmol* sequencing system, Promega) by linear interpolation.

2.5.3 Simple Sequence Repeats

The forward primer was radio-labelled by mixing 0.1 µl of forward primer (10 µM) with 0.04 µM of (γ^{33}P) ATP (10 mCi/ml), 1X T4 polynucleotide kinase buffer and 0.1 unit of T4 polynucleotide kinase (New England Biolabs), at a final reaction volume of 0.5 µl per assay. The reaction mixture was incubated at 37°C for 30 min followed by heating to 65°C for 10 minutes to inactivate the kinase.

PCR was performed using 50 ng of DNA, 0.2 mM of dNTPs (Roche), 0.1 µl of Taq DNA polymerase (5 U/µl, Roche), 0.1 µl of unlabelled reverse primer (10 µM) and 0.5 µl of labelled forward primer, in a total volume of 20 µl. The reactions were conducted in the PE 9700 thermal cycler using the following procedure: 3 min at 94°C followed by 35 cycles of 15 sec at 94°C, 30 sec at the specific annealing temperature for each primer, 30 sec at 72°C and then 5 min at 72°C.

Following PCR, an equal volume of loading dye (prepared as described earlier) was added to the reaction products and the samples were denatured at 95°C and 'snap cooled' on ice prior to loading in the gel. Amplified products were separated by gel electrophoresis and detected by autoradiography as described above for AFLP analysis. SSR and AFLP marker scores were cross-checked to ensure the reliability of scoring and to minimize scoring errors.

2.5.4 Diversity Arrays Technology

Diversity Arrays Technology (DArT) is a DNA hybridisation-based method of generating molecular markers using restriction enzymes that allows whole genome scanning using a microarray platform (Jaccoud *et al.*, 2001). The whole genome fingerprints generated by DArT were scored based on the presence or absence of hybridisation to individual array elements. This allows for simultaneous detection of variation at numerous genomic loci, without the need for sequence information.

DArT detects primarily dominant markers, mostly resulting from single nucleotide polymorphisms (and InDel) at restriction sites at hundreds to thousands of arbitrary genomic loci (Wenzl *et al.*, 2004). DArT markers are developed from a representation generated from a pool of DNA from several genotypes (accessions, cultivars or breeding lines) representing a good range of variability within the target species (Jaccoud *et al.*, 2001; Kilian *et al.*, 2005). The genotyping technology involves the digestion of the genomic DNA with methylation-sensitive restriction enzymes, thereby reducing genome complexity and enriching for low copy sequences for marker development. Following digestion, the fragments are ligated with enzyme-specific adapters, amplified and cloned. The cloned fragments are then spotted on to glass slides to produce the DArT array. For genotyping, genomic representations were generated for all the individuals from a mapping or a diverse population, and are hybridized to the microarray panel together with a reference DNA fragment (labelled with a different dye (cy5) than the target DNA sample (cy3)). The reference DNA is a polylinker sequence of vector used to prepare library common to all spotted fragments.The polymorphisms in the restriction site between individuals are detected through differences in hybridization

signal. The relative hybridization intensities (cy3/cy5) are then converted into scores as '0' (absent) or '1' (present) using DArTsoft software.

DNA from leaf samples of the tetraploid mapping population, 12601ab1 x Stirling, was extracted as described in section 2.5.1 and was sent to Diversity Arrays Technology, Pty Limited, Australia, for genotyping. The pooled DNA samples (bulks) from Neotuberosum population for tuber yield, Fe, Zn and Ca concentrations were also sent for DArT marker analysis.

2.6 STATISTICAL ANALYSIS

Data from different experiments of this project were analysed using the Genstat 13 version 13.2 (VSN International Ltd., Hemel Hempstead, UK).

2.6.1 Statistical analysis for the mineral distribution studies

For Experiments 1 and 2, the data was analyzed using the analysis of variance (ANOVA) procedure, with tuber as a blocking factor. Correlation analysis was performed on average mineral concentrations using the FCORRELATION procedure in GenStat and graphs were constructed using the spreadsheet program MS Excel 2007.

2.6.2 Statistical analysis for the diverseand mapping populations

Data obtained from the diverse (Core Collection and NTB) and mapping populations (Chapters 4 and 5) were analyzed using the restricted maximum likelihood (REML) method, with (year/ replication/section/plot) as a random factor and clone as a fixed factor. A Wald test statistic (W) was calculated so that significant variation sources could be identified using a chi-squared function based on the appropriate degrees of freedom. The data were

subjected to initial exploratory analysis using box and whisker plots (not shown). The outlier values which were consistently extreme in all the two or three years were retained, whereas others with more than 3 times the interquartile range were excluded from further analysis. Broad sense heritability (H^2) was estimated using variance components calculated from the mixed models procedure using the REML method according to Nyquist (1991),

$$H^2 = \sigma^2_c / ((\sigma^2_c + (\sigma^2_{ce}/e) + (\sigma^2/re))$$

where σ^2_c, σ^2_{ce}, and σ^2 are the variance components for clones, clone x environment interaction and plot to plot variation of residuals, respectively; e represents the number of environments (which is number of years in this study) and r is the number of replications. For NTB population, the field experiment was conducted in a single year (2005) with two replications and the heritability was calculated using the function

$$H^2 = \sigma^2_c / ((\sigma^2_c + (\sigma^2/r)).$$

The variance components were calculated with a random term [(Year/Replication/Section/Plot) + (Year x Clone)] and no defined fixed factors to allocate sources of variation in the measured traits. Clone means over year were calculated based on the best linear unbiased predictors (BLUP) of clone effects from REML analysis across the three year replicate data sets balanced by year. Principal component analysis (PCA, based on correlation matrix) and correlation analyses were used to determine the associations among the different variables (plant and tuber traits). Multivariate (PCA) analysis was used to reduce data dimensionality and to serve as a tool for understanding relations between mineral elements and potato clones. The purpose of PCA ordination was to identify traits that account for the most variation observed

in the population, and to identify whether there was a biological relationship among such traits. The contributions of each variable to the first two principal components were shown in the biplot. An obtuse angle between two mineral elements in a biplot represents a negative correlation between them, an acute angle represents a positive correlation, and a right angle represents no correlation.

2.7 CONSTRUCTION OF LINKAGE MAPS AND QTL ANALYSIS

Linkage analysis and QTL mapping was performed using the TetraploidMap software package for Windows, as described by Hackett *et al.* (2007) and Bradshaw *et al.* (2004, 2008). This software is designed to infer parental genotype of an autotetraploid species from the marker of the parents and those of a segregating progeny. This software accommodates dominant (such as AFLP, DArT markers in simplex, duplex, or double-simplex configuration) and co-dominant (such as SSRs, SNPs) markers. TetraploidMap works with Windows XP and can handle a maximum of 800 markers per project and a maximum of 50 markers per linkage group.

In an autotetraploid species like *S. tuberosum*, a dominant marker can have one (AAAB, simplex), two (AABB, duplex), three (ABBB, triplex), and four (BBBB, quadruplex) copies. With the random mating of each one of the four homologous chromosomes in meiosis (without considering double reduction), the expected ratios in the gametes of a dominant marker are 1:1 (simplex) and 5:1 (duplex) (Hackett *et al.*, 1998). Triplex and quadraplex markers did not show segregation and are not used for mapping.

2.7.1 Selection of molecular markers for map construction

The following types of markers were selected for map construction: simplex dominant markers (segregation ratio of 1:1) with a p-value < 0.001, duplex dominant markers (segregation ratio of 5:1) with a p-value < 0.01 and all co-dominant SSR markers. The marker segregation ratios and chi-squared (X^2) values were analysed using the software. Once the parental genotypes were determined at each locus, markers were assembled into linkage groups using the cluster analysis. Following initial clustering of markers, the final linkage group composition and marker order were determined.

2.7.2 Marker ordering

TetraploidMap has various options for marker ordering, a two-point linkage analysis, an initial ordering (based on seriation algorithm), a ripple search and a simulated annealing ordering. The two-point and initial marker ordering results can be distant from the optimal results, therefore, in most cases ripple ordering is used due to its rapidity and accuracy. Marker ordering by the simulated annealing method can provide more accurate ordering results than ripple ordering, but the computation takes a long time especially if there are more than 20 markers in a group. Homologous chromosomes in a linkage group can be identified by simplex markers linked in repulsion, duplex markers and SSRs. Any marker that was a poor fit in the ordering with low LOD (logarithm (base 10) of odds) values was moved to a different group or omitted from analysis. Both ripple ordering and simulated annealing methods can handle only up to 50 markers. Hence, in case of linkage groups (LGs) with more than 50 markers (Table 7.2) the markers occupying the same locus were removed from the analysis. The phase information of markers within linkage

groups was determined manually and each linkage group was reconstructed into four homologous chromosomes (Figure 7.2).

2.7.3 Chromosomal identity

Chromosomal identification of LGs was carried out by the presence of mapped SSRs, co-migrating AFLP markers in the SH x RH UHD (ultra-high-density) reference population (see Bradshaw *et al.*, 2008) and by alignment of DArT markers to the potato genome sequence/chromosomes. DArT markers that have been mapped previously in other diploid populations (01H15 (Campbell, 2010) and PGSC reference map) were also used for aligning or identifying chromosomes. The location on the potato genome is available for most DArT markers, allowing the easy alignment of parental genetic maps to physical maps.

2.7.4 Alignment of the parental genetic maps

The linkage maps of the parents were bridged by the use of double-simplex (3:1) markers present in both parents and also by the use of multiallelic SSRs. The alignment of linkage groups from parental maps was achieved for all linkage groups, using the linkage of double-simplex (3:1) markers to simplex (1:1) markers, as described in Bradshaw *et al.* (2008). Further, different DArT markers from the same superscaffold that were mapped in both parents were also used for aligning the parental genetic maps.

2.7.5 QTL analysis using interval mapping

QTLs were analysed for the measured phenotypic traits (described in Chapter 5), including plant emergence, maturity, flower colour, tuber yield, dry matter

and 13 mineral elements (N, Ca, K, Mg, P, S, B, Cu, Fe, Mn, Ni, Zn and Na). Following map construction, QTL analysis for these traits was performed using interval mapping method of TetraploidMap package. All the measured traits were analysed separately for each year (2007, 2008 and 2009) and for over-year means. During 2007, mineral analyses were carried out at JHI and HRI using two different analytical techniques (see Section 2.4), therefore, QTL analysis was performed separately for each dataset. The full model fits six means corresponding to the six possible QTL genotypes (Q12, Q13, Q14, Q23, Q24 and Q34), reflecting the combinations transmitted to offspring. The simple models can also be analysed and compared to the full model by a likelihood-ratio test. Ten simpler models (four simplex genotypes at chromosomes 1–4 and six dominant duplex genotypes) that reflect a dominant effect of the QTL are also analysed and compared. Permutation tests of 100 iterations were performed to determine the significance for either the full or the set of reduced models. When a simpler model was not significantly different from the full model and passed the permutation tests, then the simpler model was presented. In cases where a simpler model didn't pass the permutation test, but the full model did, then the full model was reported.

The QTL(s) were named using the trait abbreviation suffixed with the year, for example, Fe_08. QTLs with LOD scores ≥ 2.5 were considered for the analysis, including the ones declared non-significant by interval mapping analysis. In some cases, secondary QTLs were also found. The interval mapping analysis assumes only one QTL per chromosome and gives only the effects of the most significant QTL, ignoring the effects of other QTL lying elsewhere in the genome. Hence, the location and LOD score for all secondary QTLs were reported here. The characteristics of QTLs detected were listed in Appendix Table A8.1 and the QTLs were presented in Figure 8.1. The linkage maps and

QTLs were reconstructed using the MapChart software package version 2.2 (Voorrips, 2002).

For LGs with less than four homologous chromosomes (12601ab1 IXa), QTL analysis was not performed and a single-point analysis using ANOVA was carried out to test the association of markers in these LGs with the measured traits. Only markers with associated p-values <0.001 were reported here (Appendix Table A8.2).

CHAPTER 3 DISTRIBUTION OF MINERAL ELEMENTS WITHIN POTATO TUBER FOLLOWING POST-HARVEST STORAGE

3.1 INTRODUCTION

Potatoes are the most important non-grain food crop in the world, with a production rate of 329 million tonnes in 2009 (FAOSTAT 2010), ranking fourth in terms of global food production. Potatoes contribute to human health by providing calories and nutrients. They also deliver a significant proportion of the minerals in the human diet (Kärenlampi and White, 2009). However, the distribution of mineral elements, as well as their concentrations, can vary within the potato tuber. The concentration of some minerals has been found to be greater in the skin than the flesh of the tuber (McGuire and Kelman, 1984; Trehan and Sharma, 1996; Wszelaki *et al.*, 2005). In addition, a substantial number of studies have shown variation in tuber mineral concentration between the stem end and the bud end of the potato tuber (Bretzloff, 1971; Bretzloff and McMenamin, 1971; DeKock *et al.*, 1979; Ereifej *et al.*, 1998; Heisler *et al.*, 1963; Hughes and Swain, 1962; Johnston *et al,*. 1968; LeRiche *et al.*, 2006, 2009; Macklon and DeKock, 1967; Wurster and Smith, 1963, 1965) (Table 3.1).

Different sampling methods have been used by researchers to investigate the distribution of minerals within potato tubers (Table 3.1). Although these studies have attempted to establish the distribution of minerals in potato tubers, a full understanding of the three-dimensional distribution of nutritionally significant minerals within the potato tuber would be more informative. Knowledge of the three-dimensional distribution of minerals will guide the understanding of the processes and patterns of tuber mineral accumulation and thereby inform strategies to enhance the mineral status

through agronomy and/or breeding. It will also permit the exploration of the effect of alternative sampling strategies.

The pattern of mineral distribution in tubers could vary between different stages, including tuber development, maturity, post-harvest storage and transition from dormancy to sprouting. As a seasonal crop, potatoes are usually stored at low temperatures for prolonged periods in order to ensure year-round supply. Therefore, it is vital to understand the changes in tuber mineral concentration during storage. Previous studies that investigated the effect of storage on distribution of tuber nutrients have mainly focused on carbohydrates, nitrogen compounds, proteins, pH, polyphenols, polyphenol oxidase, glycoalkaloids, minerals such as Ca, Mg, K and P, and ascorbic acid (Baijal and van Vliet, 1966; Kazunori *et al.*, 2008; Ortiz-Medina and Donelly, 2003; Pett, 1936; Shekhar and Iritani, 1978; Weaver *et al.*, 1978 a, b, c; Zgórska and Frydecka-Mazurczyk, 2003). However, little is known about the changes in the distribution of other nutritionally important mineral elements during storage. Furthermore, the extent of changes in mineral concentrations during post-harvest storage could vary between genotypes. In this chapter, the following specific aspects were investigated: a) differences in mineral concentrations between the skin and the flesh region of tuber, and b) pattern of dry matter (DM) and mineral distribution within the tuber flesh as influenced by i) genotype, and ii) storage conditions.

The study consisted of two different experiments (Chapter 2, Section 2.1.1 and 2.3.2). In Experiment 1, the potato cultivar Stirling was used to investigate the mineral partitioning between skin and tuber flesh and the pattern of distribution for DM and mineral content within tuber flesh. Experiment 2 investigated genotypic differences in DM and mineral distributions in Stirling and 12601ab1. Data for tuber DM and mineral

Table 3.1: Previous studies and current work on mineral variation within potato tuber flesh

Reference	Cultivar/clone	Post-harvest storage	Sampling details	Minerals analyzed	FW/DW
Arteca *et al.* (1980)	Russet Burbank	Not available	Longitudinal slice into stem end, centre and bud end	Ca, Cl & K	DW
Bretzloff (1971)	Red La Soda, Wauseon, Lenape & clone B725-61	Not available	Transversal slice into inner pith, outer pith and cortex	Mg & Ca	FW
Bretzloff and McMenamin (1971)	Kennebec	Harvested tubers treated with sprout inhibitor, CIPC[€] and held at 6°C for eight months	Six different tuber sampling- longitudinal slices, saggital^ slices, cross-section, paired opposite sectors, concentric zones & subsamples of blends	Mg, Ca & K	FW
Davies and Millard (1985)	Maris Piper	Not available	Seven tissue types in a transverse section of tuber, from periderm to pith	Ca, N, Mg, P & K	FW
DeKock *et al.* (1979)	Majestic	Not available	Peeled core into 15 pieces from stem to bud end	Ca, K, Mg, P, Fe, Cu & Mn	FW
Ereifej *et al.* (1998)	10 cvs[&]	Stored at 8°C for 2 months and reconditioned at 21°C & 65% RH for 2 weeks	Peeled tuber into bud end, stem end vascular ring and central core	Ca, Na, Mg, P, Cu, Fe, Mn, Zn & K	DW
Glynne and Jackson (1919)	King Edward	Stored at a dark cool room or stored as a clamp (conditions not mentioned)	Whole tuber into skin, outer cortex, inner cortex, outer medulla and inner medulla	N	FW & DW
Hughes and Swain (1962)	Ulster Torch, Majestic & King Edward	Not available	Central longitudinal cores into 10 sections with removal of vascular tissues at both ends	Fe & inorganic P	DW
Johnston *et al.* (1968)	Russet Burbank	Not available	Longitudinal central slice divided into 5 concentric sections. Each section into four segments from stem to bud end	K, Mg, Ca, Na, Fe, Mn, Zn, Cu, N, P & Cl	FW
Klein *et al.* (1982)	Katahdin, Kennebec & clone NY 61	Control: stored at 5°C for 6 months. Sprouting: stored at 5°C	Control: Whole tuber into cortex and pith. Sprouted tubers: sprouts, cortex and pith	N, K, P, Ca, Mg, Mn, Fe, Cu, B & Zn	DW

		for 6 months followed by storage at 21°C for one month in light and dark conditions			
LeRiche *et al.* (2006 & 2009)	Shepody & Russet Burbank	Stored at 15°C for 14 days at 95% RH and gradually decreased over a month period to 9°C	Central pith strip from one half of tuber divided into 8 equal segments from stem to bud end (VP-SEM/EDS); 3 tuber subsamples-stem end, centre and bud end (ICAP)	Mg, Ca & P (2006); 14 minerals* (2009)	DW
Macklon and DeKock (1967)	7 varieties	Not available	Central longitudinal cores divided into 16 equal cylindrical sections	K, P, S, Fe, Ca, Mg & Cl	FW
Shekhar and Iritani (1978)	Russet Burbank	3 storage methods: (i) 5.5 and (ii) 15.5°C for 8 weeks & (iii) stored at 5.5°C for 5 weeks followed by storage at 15.5°C for 3 weeks	Peeled tuber into basal and apical portions	Ca, Mg, P (total and inorganic) & K	DW
Westermann *et al.* (1994)	Russet Burbank	Stored at 15°C and within 45 days after harvest, tubers were reconditioned to 25°C & used for analysis	Removed 1.25 cm tip from apical and basal end of tuber and 0.6cm slice adjacent to the tip from both ends used for analysis	Cl, S, P, Zn, Cu, Mn, Fe, K, Ca, Mg & N	DW
Wurster and Smith (1963)	Katahdin	Not available	Longitudinal and cross-sections (radio-iron, Fe^{59})	Fe	DW
Present study, Expt¥ 1	Stirling	Stored at 4°C (95% RH) for 2 weeks followed by storage at 13°C (95% RH) for 4 weeks	Peel: 5 concentric skin samples from bud to stem end. Flesh: Peeled tuber into top, central and bottom slices. Each slice into 5 strips and each strip into 5 pieces.	P, Ca, Mg, K, S, Fe, Cu, Zn, Mn & Cl	FW & DW
Present study, Expt¥ 2	Stirling & clone 12601ab1	Stored at 4°C (95% RH) for 6 months	Whole tuber into 4 slices. Each slice into 4 sections: bud end, 2 pieces of 0.5cm centre section and stem end.	P, Ca, Mg, K, S, Fe, Cu,Zn, Mn & Cs	FW & DW

FW: fresh weight; DW: dry weight; ^Saggital:dividing tubers into right and left sections; €CIPC: isopropyl N-(3-chlorophenyl) carbamate; &cultivars; RH: relative humidity; *14 elements: P, Ca, Mg, K, S, Fe, Cu, Na, Zn, B, Mn, Al (aluminium), Si (silicon) and Cl (chloride); ICAP: inductively coupled argon plasma; VP-SEM/EDS: variable pressure scanning electron microscope (VP-SEM) equipped with energy dispersive X-ray spectrometry (EDS); ¥Expt: Experiment; Cs: Caesium.

distribution of Stirling from Experiments 1 and 2 were used to explore the effect of storage conditions on DM and mineral distribution, because this cultivar was analysed after different storage conditions in Experiments 1 and 2.

3.2 RESULTS

3.2.1 Partitioning of minerals between the skin and flesh regions of tuber

Mineral concentrations of tuber skin and flesh regions were determined using the dataset from Experiment 1. The concentration of mineral elements was generally higher in the skin compared to the flesh region on a fresh weight (FW) basis, with the exception of P, S and Cl, which were comparable in both skin and tuber flesh tissues (Appendix Table A3.1). However, due to the much greater mass of the tuber flesh, the total mineral amounts were greater for all minerals except Fe in the flesh as a whole compared to the skin region. For instance, the tuber peel region contributed about 17%, 34%, and 55% of the total tuber Zn, Ca and Fe, respectively.

3.2.2 Pattern of DM and mineral distributions within the tuber

3.2.2.1 General patterns of distribution

This section summarizes the general patterns observed in DM and mineral distribution within the tuber flesh over the entire study (Experiments 1 and 2). The distributions of DM and mineral elements were investigated by sampling 75 portions of the tuber flesh (Figure 2.1; Chapter 2). The DM and mineral distribution differed significantly between the top, middle, and the bottom slices of tubers orientated according to their position in the soil (Appendix Table A3.2). Tuber DM was lower in the middle of the tuber compared to the peripheral region. In particular, the central portion of the middle slice had a much lower DM content (around 10%) compared to the periphery of the tuber (up to 24%). In the top and the bottom slices, an

increasing gradient of DM was observed from the bud end to the stem end (Figure 3.1). The tuber flesh contained higher DM content (20%) than the tuber peel (14%) (data not shown).

The tuber mineral concentrations were expressed on a FW basis (Figures 3.1 and 3.2) as expressing them on a dry weight basis gives patterns dominated by DM profiles, and is less relevant to consumers. Although tubers from a single cultivar were used in this study, mineral concentrations differed considerably from tuber to tuber within the cultivar, ranging from a 1.2-fold difference for P to a 2.05-fold difference for Mg between tubers with lowest and highest concentrations. Further, the ranking of tubers differed for each mineral, and no single tuber exhibited lowest concentration for all minerals. Nevertheless, the general patterns of distribution for various minerals did not differ among the tubers, and therefore, the mean values for all five tubers are presented.

Among the minerals investigated, Ca, P, and Cu followed the same pattern of distribution, with higher concentrations in the peripheral cortex tissues compared to the medulla tissues in the centre of the tuber (Figure 3.1). However, the decreasing gradient towards the centre of the tuber was much stronger for Ca than for P and Cu. K showed a gradual increase from the stem end to the bud end, while Cl and Fe exhibited an opposite trend, with high concentrations towards the stem end (Figures 3.1 and 3.2). Fe was particularly high near the point of stolon attachment. Similarly, the concentrations of Mg, Mn, S and Zn were generally high at the stem end (Figure 3.2); however each of these elements showed a unique pattern of distribution. For example, Zn showed a dorso-ventral gradient at the stem end of the tuber with the level rising towards the upper side of the tuber, whereas Mn, although higher near the stolon attachment point, was distinctly lower at the stem end of the tuber away from the attachment point.

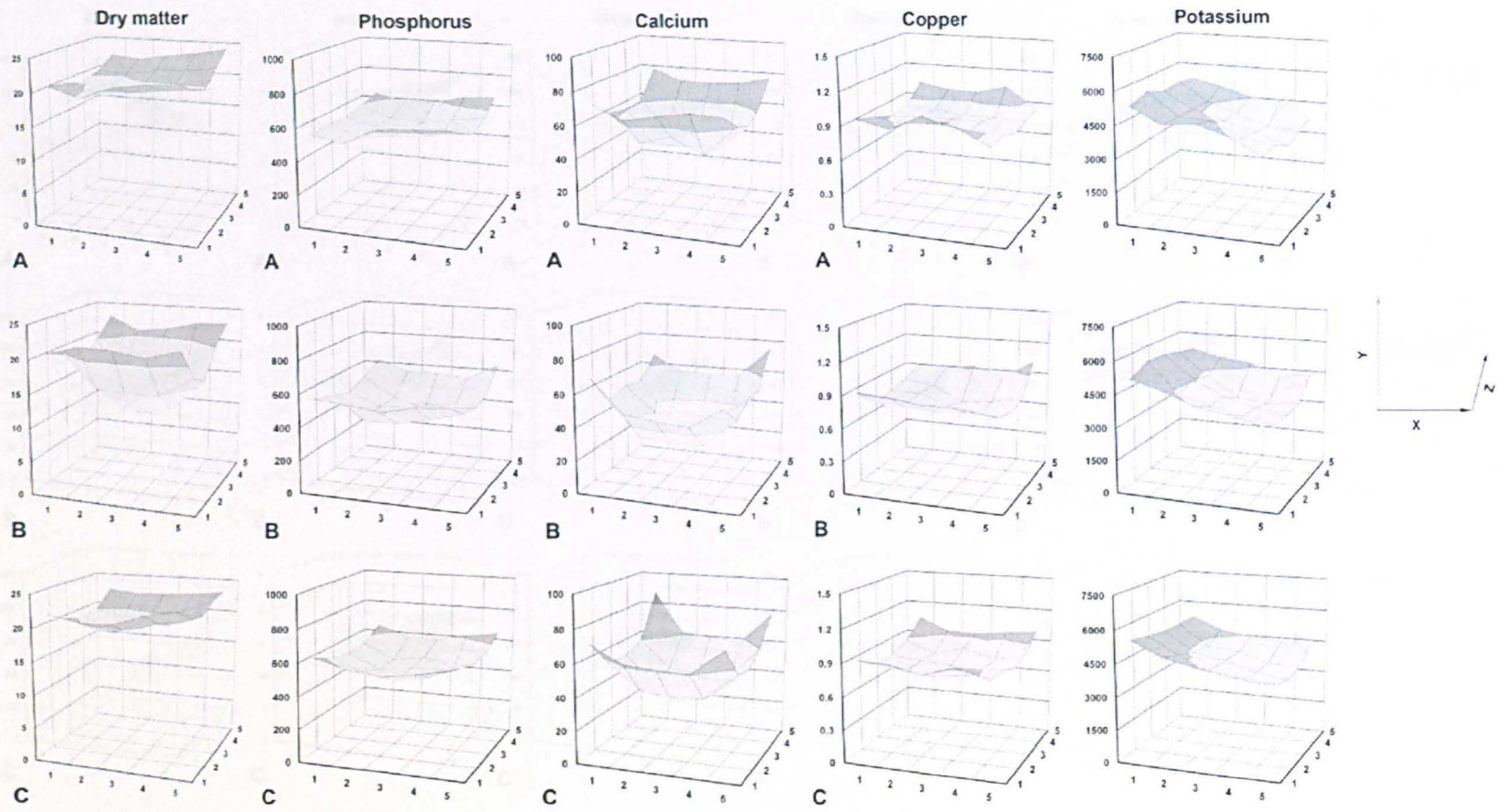

Figure 3.1: Dry matter (% DM) and mineral (µg g^{-1}, FW basis) distribution compared among the three longitudinal slices of potato. (A) top, (B) middle and (C) bottom slices. X-axis: Bud to stem end pieces of each strip; Y-axis: DM or mineral concentration; Z-axis: Five strips of a slice.

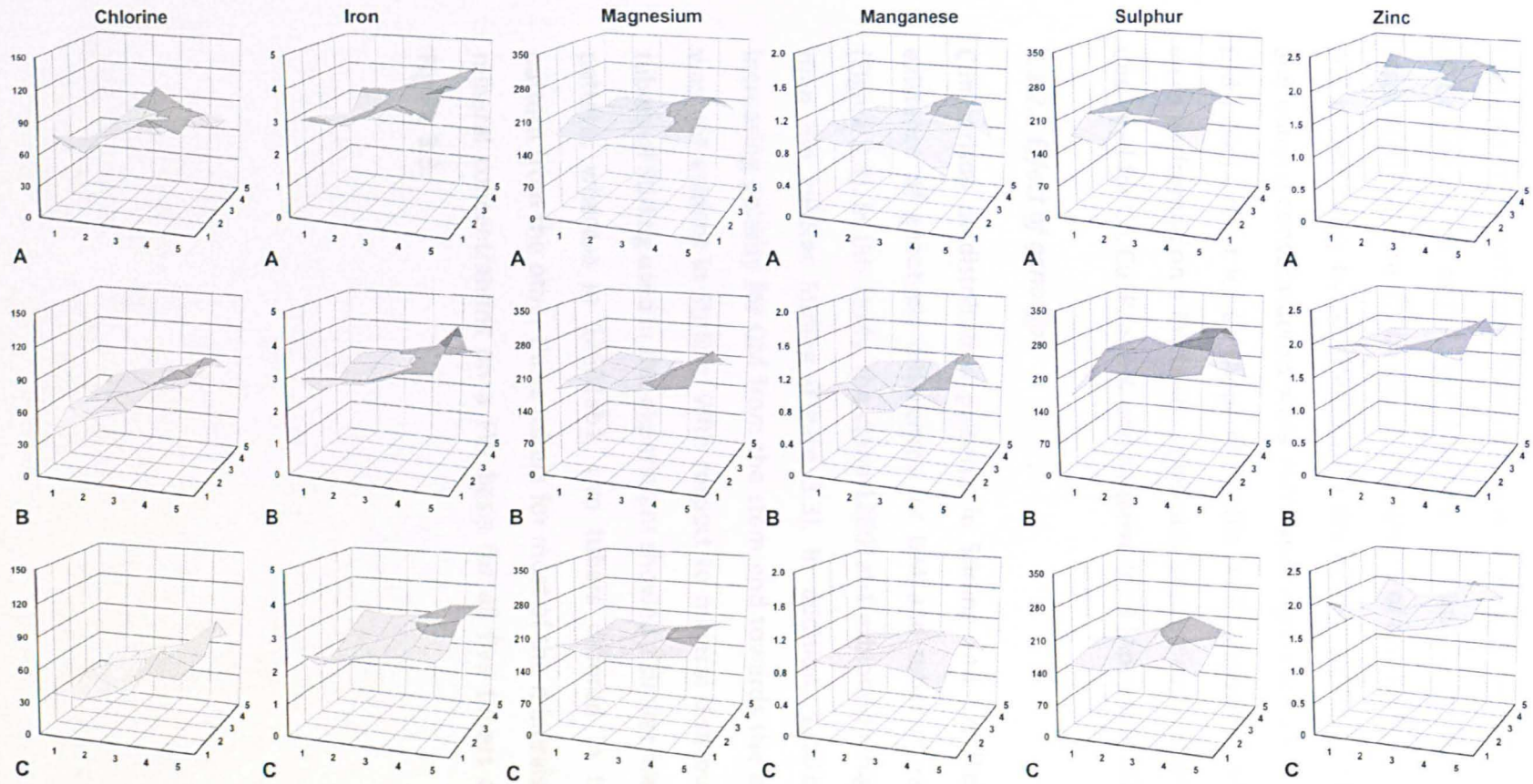

Figure 3.2: Mineral distribution in flesh tissue of potato tubers compared among the three longitudinal slices (µg g^{-1}, FW basis). (A) top, (B) middle, and (C) bottom. X-axis: Bud to stem end pieces of a strip; Y-axis: DM or mineral concentration; Z-axis: Five strips of a slice.

The different mineral elements were also found to be correlated significantly with each other when correlations were expressed on a DM basis (Appendix Table A3.3). In particular, high correlations were observed between the pairs of Ca and P, Cu and P, and Mg and Mn, and such high correlation was associated with the similarities in the pattern of distribution in the tuber flesh, and driven partly by covariance with dry matter content. Tuber DM content and mineral concentrations were correlated significantly but negatively (on a DM basis) except for Ca (Appendix Table A3.3). However, when correlations were performed on a FW basis, significant positive correlations with DM were observed for Ca, Cu, P, and K, and negative for S (Appendix Table A3.3).

3.2.2.2 Effect of genotype

Comparison of distribution patterns in Stirling and 12601ab1 suggests the existence of genotypic differences for DM and tuber mineral distributions (Figure 3.3). In this study, the clone 12601ab1 exhibited a higher DM content than the cultivar Stirling (Figure 3.3). In addition, 12601ab1 showed an increasing polarity for DM from the stem end towards the bud end, but this was not evident in Stirling. With respect to mineral distribution, all the five tubers of Stirling used in this experiment showed a similar mineral distribution pattern, whereas in 12601ab1, two tubers differed in their distribution pattern from the other three tubers for most of the minerals. Mean values of mineral concentrations on a FW basis for all five tubers are presented in Figure 3.3.

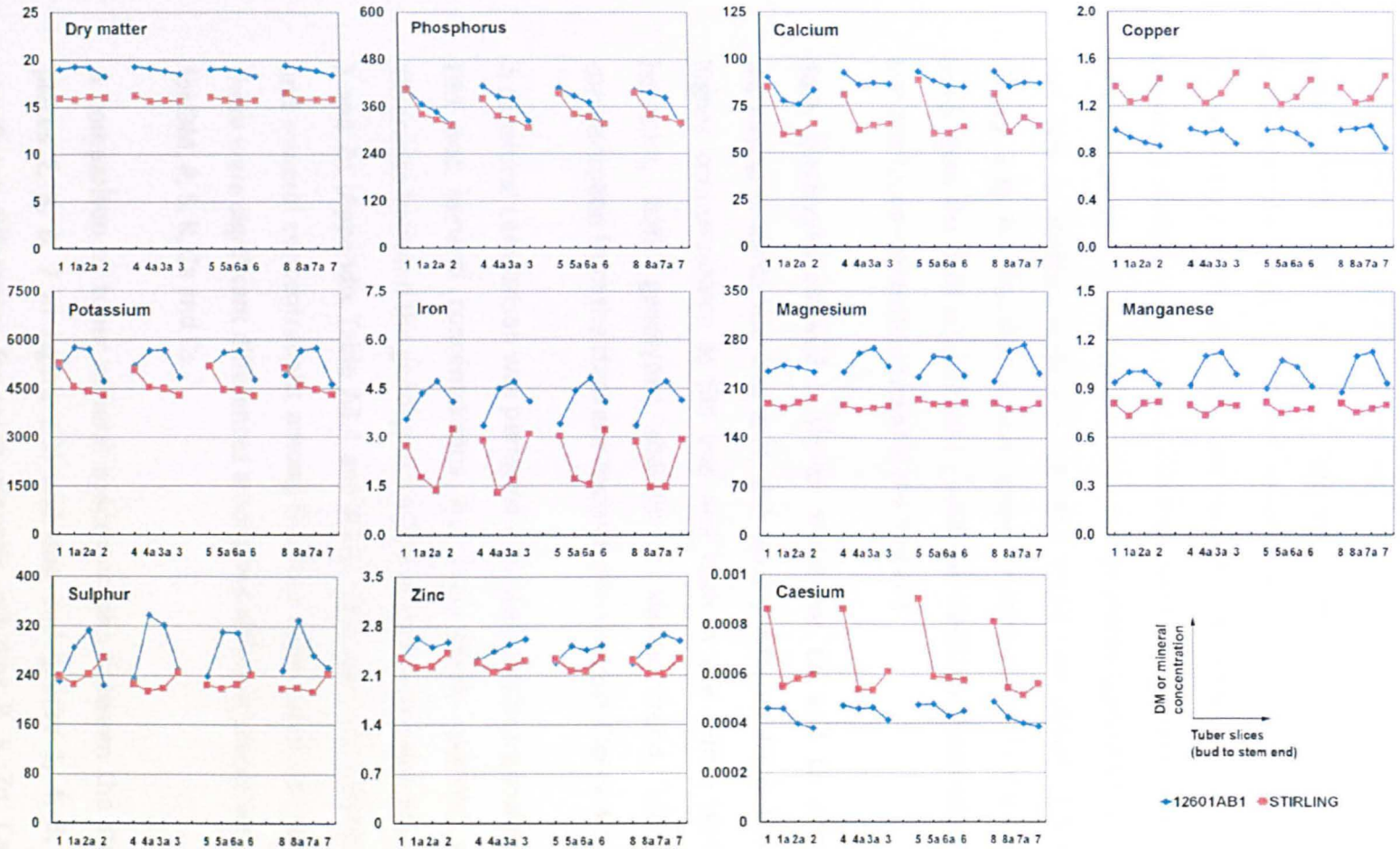

Figure 3.3: Pattern of dry matter (%) and mineral (µg g^{-1} FW) distribution in the potato genotypes Stirling and 12601ab1, from the bud end (left) to the stem end (right) of the tuber.

For Mg and Mn, 12601ab1 showed an increase from the bud end towards the centre and then a decrease near the stem end, whereas in Stirling, the distribution of Mg and Mn was almost uniform across this transect. For S and K, 12601ab1 had higher concentrations in the centre than the bud and stem ends, whereas Stirling exhibited high concentrations of S at the bud end and K at the stem end (Figure 3.3). With respect to Zn, 12601ab1 showed increasing concentrations from the bud end towards the stem end, whereas Stirling showed a minimum concentration in the centre of the tuber. Similar to Zn, the concentration of Fe was low in the central pieces compared to the stem or bud end in Stirling, while the opposite trend was observed for 12601ab1 (Figure 3.3). For Cu, Stirling had lower concentrations in the centre of the tuber than the bud or stem ends, whereas 12601ab1 showed an increasing trend in Cu concentration towards the bud end.

Both genotypes showed a similar trend for Ca and Cs concentrations, increasing towards the bud end, but Stirling differed from 12601ab1 with higher concentrations at the bud end than in the centre portion. For P, however, both genotypes showed a similar trend, with increasing concentration from the stem end towards the bud end (Figure 3.3).

A statistical comparison was performed between the two genotypes for tuber DM and mineral concentrations and the results revealed a significant difference between the genotypes for DM and for most minerals, except for P, S and Zn (Appendix Table A3.4 and A3.5). However, the differences for DM and mineral concentrations among the four slices were not significant, but there were significant differences among the different pieces within each slice for DM, P, S, K, Ca and Cs.

A comparison of tuber mineral concentrations between the stem end (i.e. pieces 2, 3, 6, 7 in Figure 2.2) and bud end (pieces 1, 4, 5, 8) revealed significant differences for most minerals including P, K, Zn, Ca, Fe and Cs (Appendix Table A3.4; Figure 3.4). In addition, there was also a significant genotype by piece interaction for S, K, Mn, Fe, Cu and Cs and genotype by

stem vs bud end interaction for minerals including K, Zn, Ca. Significant differences were also found among the stem end, centre and bud end portions of whole tuber for both genotypes for tuber DM and mineral concentrations, except for Mg and Mn (Appendix Table A3.6; Figure 3.4).

A correlation analysis showed high correlation among the mineral elements that exhibited similar distribution pattern within the tuber (Appendix Table A3.7). For example in the Stirling genotype, P and K had a similar pattern of distribution and a high correlation between them (r= 0.99) (Appendix Table A3.7). Generally, tuber mineral concentrations did not correlate well with tuber DM content, with an exception in Stirling, where K and P showed a significant positive correlation with DM content.

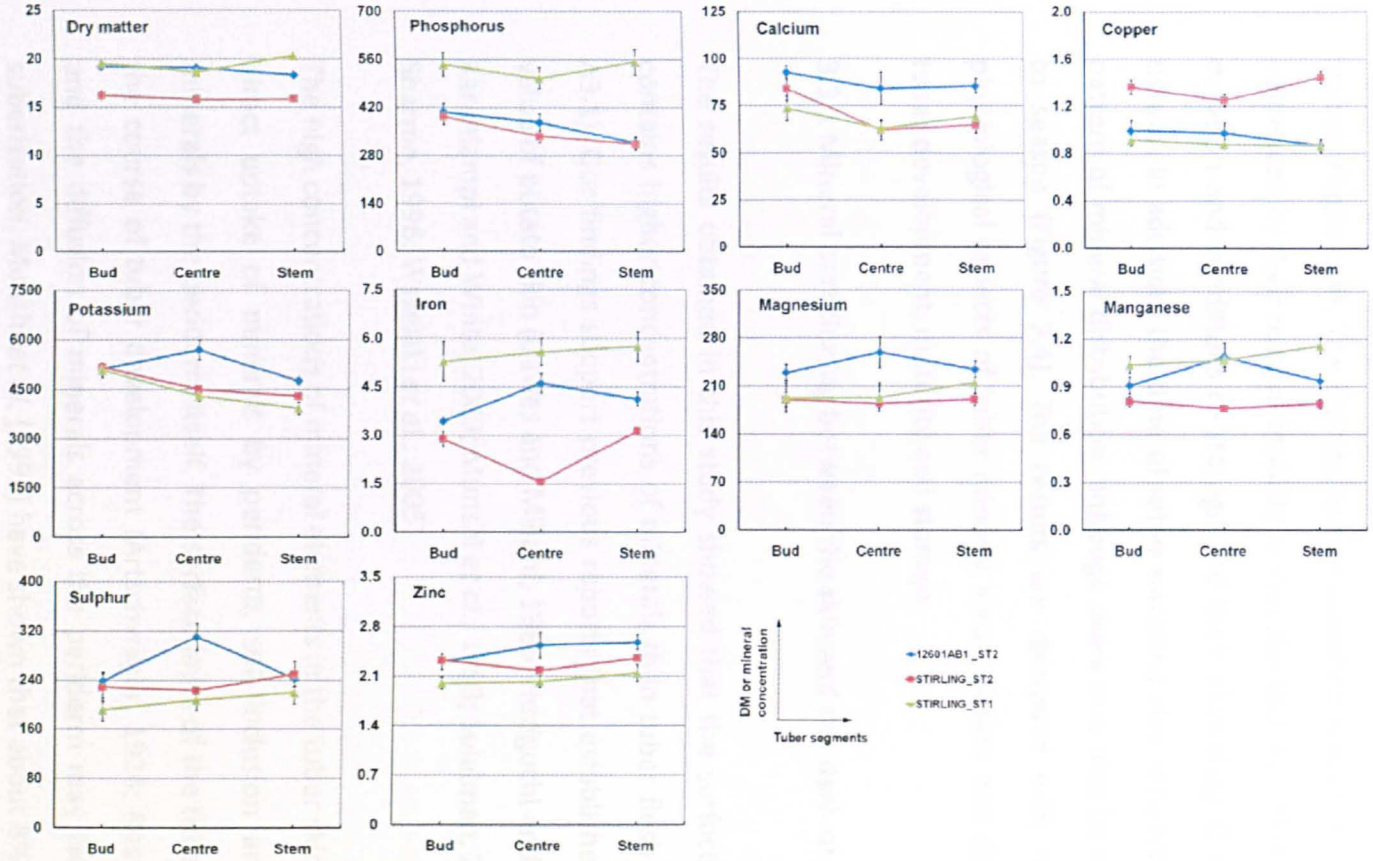

Figure 3.4: Pattern of dry matter and mineral (µg g^{-1} FW) distribution among the bud end, centre and the stem end segments of the tuber compared between Stirling and 12601ab1.

ST1: Tubers from the 2007 harvest, which were stored at 4°C for 2 weeks followed by 13°C for 4 weeks, before being used for mineral analysis; ST2: Tubers from the 2009 harvest, which were stored at 4°C for 6 months. Error bars represent ± standard error, n=5.

3.3 DISCUSSION

As potato tubers are highly dynamic, the distribution of nutrients within the tuber may continue to change at different stages such as tuber maturity and sprouting (Macklon and DeKock, 1967) and during post-harvest storage (this study) (Figure 3.4). The distribution of specific minerals in a tuber might be influenced by their relative mobility in vascular tissues, phloem unloading, the duration and conditions of storage and their physiological function in tuber tissues. In addition, the time of tuber sampling also appears to influence the pattern of mineral distribution, although there may also be a component due to season (Figure 3.4). The results are discussed with reference to the physiological aspects of tuber mineral accumulation and distribution during tuber development, maturity and storage.

3.3.1 Mineral partitioning between the skin and the flesh of potato tubers

The results obtained in this study showed that the surface layer of potato contains higher concentrations of minerals than tuber flesh (Appendix Table A3.1). Our findings support previous reports that established the nutritional value of potato skin (Davies and Millard, 1985; Horiguchi and Nishihara, 1981; Kärenlampi and White, 2009; Munshi *et al.*, 1993; Sulaiman, 2005; Trehan and Sharma, 1996; Wszelaki *et al.*, 2005).

The high concentrations of mineral elements in the tuber skin might be due to direct uptake of minerals by periderm, soil inclusion and/or binding of minerals by the periderm itself. The surface layer of the tuber changes during the course of tuber development (Artschwager, 1924; Reeve *et al.*, 1969a) and the diffusion of minerals across the periderm may likely occur before suberization. Munshi *et al.* (1993) have shown that about 8% of Ca, 5% of Mg, 95% of Al, 88% of Fe, and 4% of Mn in the potato peel could be accounted for soil entrapment by periderm during its growth. Hence, the mineral concentration of potato skin appears to be affected by edaphic conditions in which it is grown. The quantity of soil entrapment by periderm, however, is

typically influenced by the thickness of the periderm (Munshi *et al.*, 1993), which is known to be affected by genotype, tuber developmental stage and by prevailing environmental conditions (Artschwager, 1924; Reeve, 1974; Tyner *et al.*, 1997).

Although the peel contained higher concentrations of most minerals than the flesh on a FW basis, given the relatively small mass, the overall contribution of tuber skin to the total mineral content of the tuber was low (Appendix Table A3.1). An exception was Fe, where the total content was higher in the tuber skin. This is perhaps because Fe might be tightly bound to the proteins in the periderm. In addition, the concentration of soluble proteins is higher in the periderm than in the cortex or the pith tissues of the tuber (Ortiz-Medina and Donelly, 2003, 2009). Nevertheless, our findings confirm the need for including tuber skin in our diet to obtain the maximum benefit from tuber minerals, as the skin is often peeled off before food preparation or is not readily consumed.

However, potato plants grown on contaminated soils or on sludge-amended soils will accumulate high concentration of undesirable mineral elements such nickel, arsenic, cobalt, lead, cadmium and aluminium in tuber peels (Davies and Crews, 1983; Muñoz*et al.*, 2002; Munshi *et al.*, 1993; Queirolo *et al.*, 2000; Reid *et al.*, 2003; Stegen *et al.*, 2002). In this case, although peeling of tubers reduces the amount of essential mineral elements, it will reduce the intake of toxic elements in humans.

3.3.2 DM and mineral distributions in tubers as influenced by genotype and post-harvest storage conditions

A considerable variation in the distribution of DM and different mineral elements was found among potato tubers (Figure 3.4). This is possibly due to the differences in redistribution of nutrients within tubers, and the extent of redistribution could be influenced by genotype and storage conditions. In Experiment 2, which compared the two genotypes (Stirling and 12601ab1) for

differences in DM and mineral distribution, the tubers were planted and harvested at the same time, and were maintained under the same storage conditions, allowing for effective comparison of genotypes. Although, 12601ab1 and Stirling have similar plant emergence and maturity scores (main crop maturity), they differed in their sprouting tendency, with 12601ab1 sprouting less than Stirling (Bradshaw *et al.*, 2008). However, sprout growth is negligible for potatoes stored at 4°C (Burton, 1989) and no visible signs of sprouting were observed in the tubers used in this study. Hence, the marked difference observed between the genotypes for mineral redistribution might be due to cold-induced sweetening or preparation of tubers for sprouting. As starch constitutes a major part of tuber DM content (Burton, 1989), the differences in the distribution pattern of minerals between Stirling and 12601ab1 tubers upon cold storage (Figure 3.3) might be influenced by changes in tuber starch content.

It has long been known that storage of potatoes at low temperatures induces breakdown of starch with subsequent accumulation of reducing sugars (Burton *et al.*, 1992). However, the sugar content of potatoes during storage varies with cultivar (Dale and Mackay, 1994). Tubers of Stirling and 12601ab1 after storage at 4°C for three or four months were found to have high and low concentrations of reducing sugars respectively (Pande, 2002; Shepherd *et al.*, 2010). Thus, the two genotypes, Stirling and 12601ab1 behave differently at low-temperature storage with respect to reducing sugars (Pande, 2002; Shepherd *et al.*, 2010) and distribution of mineral elements (this study).

The cultivar Stirling was used in Experiments 1 and 2; the results on the distribution of DM and mineral concentration (Figure 3.4) should be interpreted carefully because comparisons were made between the tubers harvested in two different years (i.e. 2007 and 2009) and stored at different conditions (4°C followed by 13°C for 6 weeks ('storage condition 1') and 4°C for six months ('storage condition 2')). Figure 3.4 shows that, in general, the short- and long-term storage Stirling tubers have differences in their

distribution of minerals. In each storage condition the differences between the bud end, centre, and the stem end for DM and mineral concentrations were mostly significant except for Cu in condition 1 and for Mg in storage condition 2 (Appendix Tables A3.8 and A3.6). In addition to the influence of different storage temperatures on enzymes and associated kinetic processes, it is also possible that the differences in environmental conditions between those two years could also have contributed to the differences in DM and mineral distribution to some extent. The results from this study and previous studies (refer to Table 3.1 for references), suggests that storage conditions of tuber might influence the distribution pattern of minerals. Nevertheless, results of this study contribute to the existing knowledge and provide baseline information for future investigations. The influence of genotype and storage conditions on the distribution of DM and individual mineral elements are discussed in the following sections. The mineral elements that followed a similar distribution pattern in the tuber flesh are discussed together.

3.3.2.1 Dry matter

The DM content of tuber flesh was higher than the tuber peel as reported by Glynne and Jackson (1919), Johnston *et al.* (1968) and Ortiz-Medina and Donelly (2009). A general gradient for DM content within the tuber was observed in this study, from the outer cortical layer to the central pith, and from stem end to bud end (Figure 3.1). The distribution of DM content within tubers observed in this study corroborates previous studies (Baijal and Van Vliet, 1966; Cole, 1975; Dinesh and Ezekiel, 2004; Glynne and Jackson, 1919; Houghland, 1930; Johnston *et al.*, 1968; Karlsson and Eliasson, 2003; Pritchard and Scanlon, 1997; Subedi and Walsh, 2009; Zgórska and Frydecka-Mazurczyk, 2003). The predominant constituent of DM content in potato is starch (65–75%) (Burton, 1989). The distribution of starch within a tuber follows that of the DM (Woolfe, 1987), increasing from the skin towards the vascular ring and then decreasing towards the centre. Tsuchiya *et al.* (1993) suggested that density of starch grains within tuber is related to the distribution of vascular bundles in the tuber. The authors also reported that

parenchyma cells around phloem in cortex and perimedulla had higher density of starch grains than those in central medulla.

The distribution of DM content from stem to bud end of tubers might be related to cell size. Chen and Liao (1993) has reported a positive correlation (r = 0.82) between tuber cell size and DM content. The stem end of tuber is histologically more mature than the bud end (Artschwager, 1924). In addition, Reeve *et al.* (1971, 1973) showed that in mature tubers a decreasing gradient in the cell size of storage parenchyma was found from the stem end to the bud end. Furthermore, Karlsson and Eliasson (2003) have reported that storage parenchyma cells at stem end of tubers had higher DM content than at bud end. This perhaps explains the decreasing gradient in DM distribution towards the bud end observed in this study (Figure 3.1). Among the genotypes investigated, Stirling had lower DM content than 12601ab1 (Figure 3.3; Appendix Table A3.5), as reported by Bradshaw *et al.*, (2008). These two genotypes are of same maturity class (Bradshaw *et al.*, 2008) and in this study they were grown and stored in the same conditions (refer Chapter 2, Section 2.1.1). Hence the differences observed for DM content between Stirling and 12601ab1 tubers (Figure 3.3) could have been due to the genetic differences for DM production and partitioning to tubers.

3.3.2.2 Magnesium, sulphur, zinc, iron and manganese

Tuber Mg concentration decreased from the stem end to the bud end in Stirling tubers stored at 13°C (Experiment 1) in this study (Figure 3.2). These results support the findings of DeKock *et al.* (1979), Johnston *et al.* (1968), LeRiche *et al.* (2009) and Shekhar and Iritani (1978). However, Bretzloff and McMenamin (1971) reported a uniform distribution of tuber Mg when stored at a much lower temperature (6°C) for eight months. In this study, a uniform distribution of Mg was observed in Stirling tubers stored at 4°C for six months, but not in 12601ab1 stored under the same conditions (Experiment 2; Figure 3.3). Bretzloff and McMenamin (1971) in their experiment applied the sprout inhibitor CIPC to the tubers, and in our study no sprout inhibitor

was used. Nevertheless, the uniform distribution of Mg in tubers might be due to the effect of storage conditions on Mg mobilization and redistribution within the tuber. Further, it appears that there may be a genotype by storage interaction, but we did not test this explicitly as our study was not designed to explore this.

In Experiment 1, the distributions of Fe, Mn and Zn in Stirling tubers share similarities with those of Mg and S, suggesting shared features of accumulation and distribution in these minerals (Figure 3.2). All of these elements were high in the central slice of the tuber flesh and had higher concentrations near the stolon attachment point (stem end). Gradients in Fe concentration declining towards the apical (bud) end of the tuber (observed in Experiment 1), is in agreement with previous studies (DeKock *et al.*, 1979; Heisler *et al.*,1963; Macklon and DeKock, 1967; Reeve *et al.*, 1969b; Westermann *et al.*, 1994; Wurster and Smith, 1963, 1965). The protein concentration within tuber was found to increase from bud to stem end (van Loon and Muller, 1984). This suggests that Fe, Mn and Zn could be bound to proteins, such that the stem-end had higher concentrations of these mineral elements than the bud-end (Figure 3.2). In addition, the percentage of Fe associated with protein was found to be higher at the stem end than the bud end of tubers (Heisler *et al.*, 1963). The dorso-ventral polarity seen in the distribution of some minerals could be a result of the asymmetric distribution of the vascular tissue at the stem end of the tuber and the short distance migrated by these minerals following exit from the vascular tissue. It is also possible that the similarity in the pattern of S and heavy metal deposition (Fe, Zn, Mn) in tubers is related to the role of S-containing proteins and peptides such as the metallothioneins and phytochelatins, in binding metal ions (White and Broadley, 2009).

The chemical form in which the mineral is present in the edible tissues may also influence its pattern of distribution. In plants, Fe, Mn, Zn, and Cu occur in various chemical forms such as inorganic ions, inorganic metal oxides, organic

acid salts, and organic complexes (Broadley *et al.*, 2007; White and Broadley, 2009). In potatoes, Horiguchi and Nishihara (1981) have shown that the major proportion of these elements was found in the soluble low molecular fraction, which comprises free ions and complexes of amino acids and organic acids. The similarities in the chemical forms among these elements would have in turn contributed to the similarities in the distribution pattern observed in this study.

Further, the proportion of the mineral in the protein vs starch fraction of the tuber flesh may also govern the pattern of distribution. Horiguchi and Nishihara (1981) found higher concentrations of Fe, Mn, Zn and Cu in the protein fraction compared to the starch fraction, leading to a comparable distribution pattern among them. Levitt and Todd (1952) reported that 25% of total Fe, Cu, and Zn in potatoes were associated with the protein fraction in the form of metalloprotein complexes.

When comparisons were made among the genotypes stored in the same conditions (Experiment 2), the distribution of minerals such as Mg and Mn, and Fe and Zn followed a similar distribution pattern within each of the genotypes (Figure 3.3), indicating the similarities in the mobilization and redistribution pattern between these pair of minerals (Mg and Mn; Fe and Zn), and the occurrence of genotypic differences for mineral distribution patterns. Although S showed a distribution pattern similar to Fe, Mg and Mn in 12601ab1, in that high concentrations were found in the centre sections compared to the bud or stem end (Figure 3.3). This strengthens the possibility of a causal association between these minerals in tuber tissue.

3.3.2.3 Phosphorus, calcium and copper

Within the Stirling tubers in Experiment 1 (stored at condition 1), P followed a distribution pattern very similar to DM distribution, with high concentrations around the periphery and decreasing towards the centre (Figure 3.1), but no significant difference between the bud and the stem end (Appendix Table

A3.8). These results are in accordance with Shekhar and Iritani (1978). Samotus (1965) suggested that most of the phosphate delivered to the tuber is metabolised into a range of compounds, while the remainder is bound to phytate and starch, whereas Quick and Li (1976) have shown that most of the tuber P is present within starch (37.6% of total tuber P), phytic acid (26.8%), and inorganic phosphate (23.8%). The P content of potato starch can vary between cultivars and is influenced by environmental conditions (Hasse and Plate, 1996; Noda *et al.*, 2004a, b; Quick and Li, 1980; Yusuph *et al.*, 2003), and also known to increase with increasing plant maturity (Samotus and Schwimmer, 1962).

As most of the DM is made up of starch, and P is one of the important non-carbohydrate components present in starch (Schoch, 1942), the distribution patterns of P and DM were similar (Figure 3.1). A general decrease in P concentration was found from the outer to the inner parts of the tuber, reinforcing the likelihood that most of the tuber phosphate is associated with starch content. Furthermore, the starch concentration was found to decrease from the periphery of the tuber towards the pith (Houghland, 1930; Whittenberger and Nutting, 1950). However, such a pattern was not prominent with respect to phytic acid distribution within potato tubers, as Phillippy *et al.* (2004) has reported a non-significant increase of phytic acid concentration from the tuber periphery towards the centre.

In contrast to the storage condition 1, potato tubers stored under condition 2 (i.e. 4°C for six months) showed a higher concentration of P at the bud end than the stem end (Figure 3.4), a pattern previously reported in other similar studies (Johnston *et al.*, 1968; LeRiche *et al.*, 2009, inductively coupled argon plasma (ICAP) mineral analysis; Macklon and DeKock, 1967; Reeve *et al.*, 1969b; Shekhar and Iritani, 1978; Wager, 1963; Westermann *et al.*, 1994). The effect of post-harvest storage on different forms of P in potato tubers was studied by Samotus and Schwimmer (1962). They found that tubers stored at 25°C for five or six weeks exhibited a decrease in the levels of non-starch,

TCA-insoluble P (P from proteins, nucleic acids, and phospholipids), and an increase in inorganic P. However, in the same study, the tubers stored at a much lower temperature (0°C) for six or seven weeks showed a considerable decrease in starch and phytic acid P concentrations and an increase in the inorganic as well as the non-starch, TCA-insoluble P. Furthermore, Shekhar and Iritani (1978) reported that tubers stored at 5.5°C for eight weeks had high concentrations of inorganic and total P in the stem and bud end respectively. A similar decrease in the P concentration in the starch after post-harvest storage of potatoes was also reported by Golachowski (1985), Mica (1976) and Sabiniano *et al.* (1995). This possibly explains the reason for low DM content at the stem end and high total P at the bud end of tubers observed in this study (Figure 3.3 and 3.4).

As Ca typically moves with water in the xylem, transpiration is the main driving force for Ca transport in plants (Busse and Palta, 2006; Karley and White, 2009; White and Broadley, 2003). As such, the potato tuber, which is a low-transpiring organ, accumulates less Ca than the leaves of the plant (Kärenlampi and White, 2009; Ozgen *et al.*, 2006; Palta, 1996). Within the tuber (subjected to storage condition 1), the Ca concentrations were higher in the surface layers than the flesh and, within the flesh, it declined from the periphery towards the central pith (Appendix Table A3.1; Figure 3.1). This distribution pattern is consistent with earlier work (Bretzloff, 1971; Bretzloff and McMenamin, 1971; Johnston *et al.*, 1968; Park *et al.*, 2005). However, no significant difference in Ca concentration between the stem end and the bud end of tubers was observed (Appendix Table A3.8), as was previously reported by Johnston *et al.* (1968). High Ca concentrations were observed at the stem end by Arteca *et al.* (1980), Ereifej *et al.* (1998; 7 cultivars), LeRiche *et al.* (2009), and Westermann *et al.* (1994) and at the bud end by Ereifej *et al.* (1998; 3 cultivars) and Shekhar and Iritani (1978). On the other hand, the tubers subjected to the storage condition 2 in this study (i.e. 4°C for six months) exhibited high Ca concentrations at the bud end (Figure 3.3), which was contrary to the observations of Bretzloff and McMenamin (1971) and

Shekhar and Iritani (1978) who showed high concentrations of Ca at the stem end after storage at 5.5°C and 6°C respectively. DeKock *et al.* (1979) reported a positive correlation between soil pH and the Ca concentration in the stem end of tubers. Therefore, it appears that the distribution of Ca concentration within the tuber is a complex phenomenon, influenced by genotype, production environment (soil pH, soil Ca concentrations), and the post-harvest storage conditions (temperature, length of storage).

With respect to Cu, an effect of genotype on the distribution pattern was evident (Figure 3.3). In Stirling tubers (stored under conditions 1 and 2), a uniform distribution of Cu between the stem and the bud end of the tuber was found (Appendix Table 3.6 and 3.8), which corroborates the observations of Johnston *et al.* (1968). In 12601ab1, on the other hand, the tubers had a high concentration of Cu in the bud end. In a similar study, DeKock *et al.* (1979) and Le Riche *et al.* (2009) reported high concentrations of Cu at the stem end of tubers in their study material. These findings suggest that there is genetic variability for Cu distribution in potato tubers.

3.3.2.4 Potassium, caesium and chlorine

A genotypic effect was observed in tuber K distribution (Figure 3.3). In Stirling (Experiments 1 and 2), K concentration decreased from the bud end to the stem end (Figure 3.4), and this is in accordance with Arteca *et al.* (1980), Johnston *et al.* (1968), Macklon and DeKock (1967), Reeve *et al.* (1969b), Shekhar and Iritani (1978) and Westermann *et al.* (1994). However, 12601ab1 exhibited a different distribution pattern for K, with higher concentrations in the centre segment than in the bud or stem ends. Further, the distribution pattern of K in 12601ab1 was similar to that of S, Mg and Mn (Figure 3.4).

Nitsos and Evans (1969) observed that K is required for the activity of starch synthase; however, only about 1.8 % of the total K in tuber dry matter is needed for starch synthesis in potatoes (Forster and Beringer, 1983; Lindhauer and De Fekete, 1990). The equal decline towards the stem end

across the three slices observed in Stirling tubers (Experiment 1; Figure 3.1) suggests that K concentrations are not directly related to starch accumulation. Within the tuber, starch concentration was found to decrease from stem end towards the bud end (van Loon and Muller, 1984). This suggests that the distribution of starch and K were opposite to each other. As the bud end of tuber is histologically younger than stem end (Artschwager, 1924), starch synthesis might be actively occurring at bud end of tubers. However, an optimal osmotic environment within the cell was found to be essential for starch synthesis (Oparka and Wright, 1988), and hence the bud end of tubers might require more K to maintain cellular osmoticum for starch synthesis.

The distribution pattern of Cs was similar to that of K and Ca in Stirling tubers (Figure 3.3), indicating that these cations may share common uptake and transport mechanisms. Cs has no established role in plant nutrition, but can be toxic to plants (Hampton *et al.*, 2004). Cs is chemically similar to K and interferes with the uptake and biochemistry of K (White and Broadley, 2000; Hampton *et al.* 2004).

Chlorine has a number of essential biochemical functions in plants (White and Broadley, 2001). In particular, it regulates the activities of several cytoplasmic enzymes, provides a major osmoticum in the vacuole, and acts as a counter-ion for cation transport (Westermann, 2005; White and Broadley, 2001). In this study, Cl concentration in Stirling tubers (Experiment 1) decreased from the stem end to the bud end (Figure 3.2), which is in agreement with Arteca *et al.* (1980) and Johnston *et al.* (1968). It is possible that the pattern of Cl distribution within tubers is affected by the type of fertilizers applied to the soil. Westermann *et al.* (1994) found an equal concentration of Cl in the tuber ends with KCl application but a relatively higher concentration of Cl in the bud end than in the stem end with K_2SO_4 application.

3.4 CONCLUSIONS

3.4.1 Implications for understanding mineral accumulation in tubers

The variation in nutrient concentration and distribution within a tuber is associated with the developmental anatomy of the tuber and different tuber tissue zones (Reeve *et al.*, 1969a, 1970). A well-marked distribution pattern of anionic and cationic minerals within the potato tubers was observed in this study following both short- and long-term cold storage. Within the flesh tissue of short-term stored Stirling tubers (Experiment 1), the minerals showed distinct distributions, and most of them were consistent with phloem unloading in tubers. This information is important for understanding the mechanisms responsible for mineral accumulation in tubers and will provide insight to more focussed molecular studies later in this thesis (Chapter 6 and 7). In Experiment 2, in which two genotypes were stored under the same conditions, there were significant differences in the distributions of several minerals between genotypes, suggesting that mineral mobilization and redistribution in tubers is influenced by genotype. The difference in distribution of minerals observed in the tubers stored under 4°C (i.e. condition 2) might be linked to cold-induced sweetening of potatoes and/or preparation of tuber for sprouting, which are known to be associated with hydrolysis of starch and subsequent accumulation of reducing sugars. In addition, Stirling tubers harvested in two different years and stored under different conditions (Experiment 1 and 2) gave an indication that the distribution of minerals can differ between storage conditions.

3.4.2 Implications for human nutrition

The concentrations of all mineral elements studied, with the exception of P and S, were greater in potato peels than in tuber flesh. Fe was particularly concentrated in the peels of the tubers, such that 55% of all tuber Fe was found there. Thus, peeling tubers will remove considerable amounts of

minerals, and therefore, tuber peels should be included in food preparations to improve mineral intakes of humans. Potatoes need to be cooked before consumption, due to the indigestibility of ungelatinized potato starch (Burton, 1989) and hence, loss of minerals could occur as a result of different methods of preparation and cooking (Woolfe, 1987). Studies have shown that including potato peels during cooking will minimize the loss of minerals from the tuber flesh (reviewed by Woolfe, 1987).

3.4.3 Implications for tuber sampling protocol for mineral analysis

In this study, variations in the distribution of minerals within the tuber were observed and were found to be influenced by genotype and storage conditions. Since the distribution of minerals within the tuber is not homogeneous, protocols for sampling tuber tissues for mineral analyses could influence estimates of total tuber mineral concentrations. Moreover different sampling methods have been used by different researchers to analyze mineral concentrations in tubers (Table 4.9 and references therein). Using the data from Experiment 2, an attempt was made to verify the impact of different tuber sub-sampling protocols on estimates of tuber mineral concentrations (Appendix Figure A3.1, Appendix II). The results (based on DW basis) suggest that mineral estimates are influenced by genotype, distribution patterns of mineral within the tuber and the method of sub-sampling (Appendix II). However the data used for the analysis was from potato tubers stored at 4°C for six months, and the results could be different if the analysis was performed on tubers stored for a short-term. Additional experiments are required to test this hypothesis.

CHAPTER 4 TUBER MINERAL CONCENTRATIONS IN DIVERSE POTATO GERMPLASM

4.1 INTRODUCTION

Mineral elements are essential for the normal functioning of human body and mineral malnutrition is considered to be one of the most serious challenges facing the ever-increasing global population (WHO/FAO 2004). The dietary availability of minerals in staple food crops depends on the concentration of minerals in edible tissues and their bioavailability for absorption in human body. The mineral nutritional status of food crops can be enhanced through genetic (breeding for efficient cultivars) and/or agronomic means (application of mineral fertilisers) (White and Broadley, 2009). Modern potato varieties available in Europe derive, to a large extent, from a small number of varieties are introduced from their ancestral home in South America. The available diversity in landrace types of potato and wild relatives offers prospects for significant improvements for many traits (Bradshaw *et al.* 2006). Therefore, it is valuable to explore the genetic resources of the wild relatives of potatoes and other diverse populations as sources for the genetic enhancement of tuber minerals, because they harbour a vast allelic richness for useful traits including tuber mineral concentration. Identifying the valuable alleles found in the wild and cultivated diversity populations will allow researchers to understand the physiological and genetic basis of mineral accumulation in potato tubers and thereby facilitate the introduction of such useful alleles into the modern cultivars.

The domesticated groups of *S. tuberosum* including Group Andigena, Phureja, and Tuberosum are likely to contain greater genetic diversity than existing modern cultivars and as such they are expected to have a greater diversity for mineral traits. In this study, a comprehensive genetic diversity analysis for tuber mineral concentrations was carried out in large germplasm collections, including CPC accessions (representing the eco-geographical distribution of wild potatoes), the Core Collection (comprising Phureja clones and

Tuberosum cultivars) and the Neotuberosum (NTB) population (diverse tetraploid Andean potatoes derived from Group Andigena) (see section 2.1.2). The overall objective of this study was to determine the genetic variability for tuber mineral concentrations among diverse potato germplasm collections and to seek patterns amongst the variation detected. The glasshouse/field conditions and the method of tuber sampling for mineral analysis were given in 2.2.1 and 2.3 (Chapter 2).

4.2 RESULTS

4.2.1 Mineral variation in CPC accessions

The CPC accessions grown in the glasshouse showed a wide variation for tuber dry matter (DM) and mineral concentrations (Table 4.1). A detailed summary of the mineral composition of 44 wild accessions and 5 cultivated taxa is presented in Appendix Table A4.1. Among the germplasm investigated, there was a greater range in the concentration of minerals of nutritional significance (Ca, Fe and Zn; 6.7, 3.6, and 4.5-fold respectively) than several of the minerals important for primary plant metabolism (e.g. K, P and S all below

Table 4.1: Range of values for tuber dry matter (DM) and mineral concentrations observed in CPC accessions

Trait[1]		Mean	Range	Ratio[2]
DM	%	28.9	17.3–48.4	2.8
Ca	mg g^{-1}	0.2	0.1–0.7	6.7
K	mg g^{-1}	19.6	15.0–26.9	1.8
Mg	mg g^{-1}	1.4	0.8–2.2	2.5
P	mg g^{-1}	3.6	2.4–5.2	2.1
S	mg g^{-1}	1.6	1.0–2.8	2.9
Cu	µg g^{-1}	5.6	2.6–10.8	4.0
Fe	µg g^{-1}	22.0	12.2–43.6[3]	3.6
Mn	µg g^{-1}	7.1	3.9–11.7	3.0
Zn	µg g^{-1}	13.6	5.9–26.9	4.5

[1] Mineral concentrations presented on a DW basis; [2] Maximum/minimum trait value; [3] Excluding one outlier (*S. bulbocastanum* CPC 7650) 3.8-fold higher than the next highest value.

3-fold, Table 4.1). Differences between taxonomic groups were not tested for statistical significance because some of the series and species used in this study contained only a few accessions.

To explore the patterns of variation amongst the 49 different accessions, a PCA analysis was carried out opting for the correlation matrix method using all the 10 variables (DM and 9 mineral elements). The first two principal components (PC1 and PC2) accounted for 60.9% (46.0% and 14.9% respectively for PC1 and PC2) of the total variability found among the 49 accessions (Figure 4.1). The contributions of each variable (tuber DM and mineral concentrations) to the first two PC scores are shown in the biplot in Figure 4.2A.

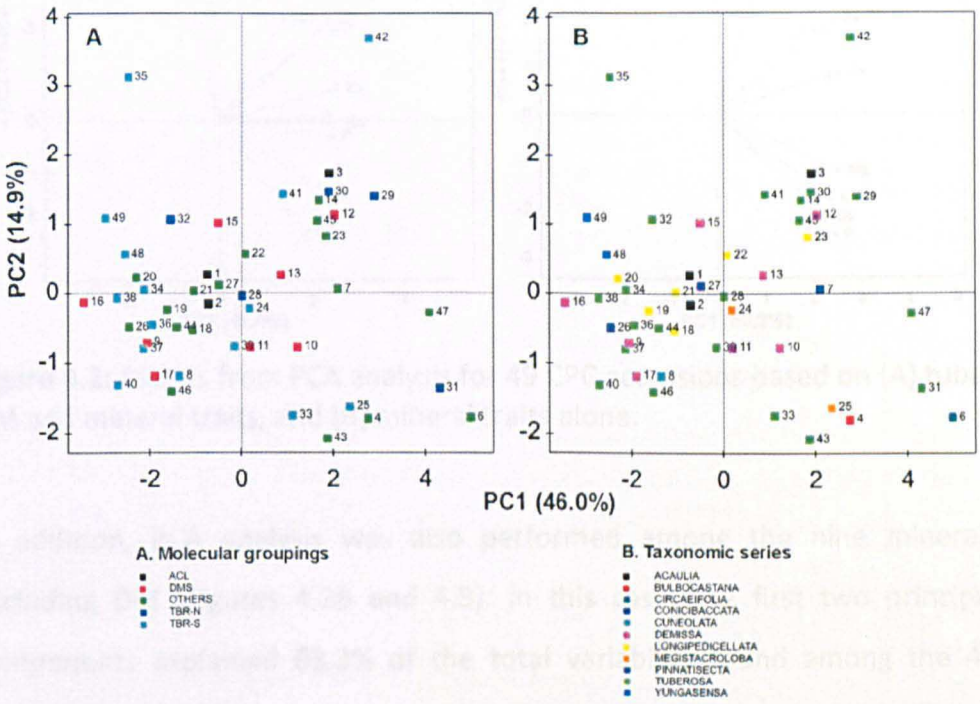

Figure 4.1: PCA plots for 49 CPC accessions based on tuber DM and mineral concentrations. The accessions are colour coded based on (A) molecular groupings, and (B) taxonomic series, as per Hawkes (1990). Abbreviations: TBR-S, *Solanum* series *Tuberosa* Southern Group (Argentina and Bolivia); TBR-N, *Solanum* series *Tuberosa* Northern Group (Peru); DMS, Mexican hexaploids in series *Demissa*; ACL, series *Acaulia*. Numbers 43-47 are cultivated species and rest are wild species (refer Appendix Table A4.1 for more details).

The PC1 was positively associated with mineral elements (Mn, Cu, Fe, Zn, S, Mg, P, K and Ca), while DM showed a negative association with this axis. The PC2, on the other hand, was positively associated with DM, Cu, Fe and Mn and negatively with Ca and K. The Spearman's correlation analysis conducted on these accessions also established the negative relationships between DM, and Ca and K (Appendix Table A4.2). The accessions that fall within 15% of low and high extreme values for tuber Ca, K and DM are given in Appendix Table A4.3. In the PCA plot, the accessions were colour coded according to molecular grouping and taxonomic series (Figures 4.1 A and B).

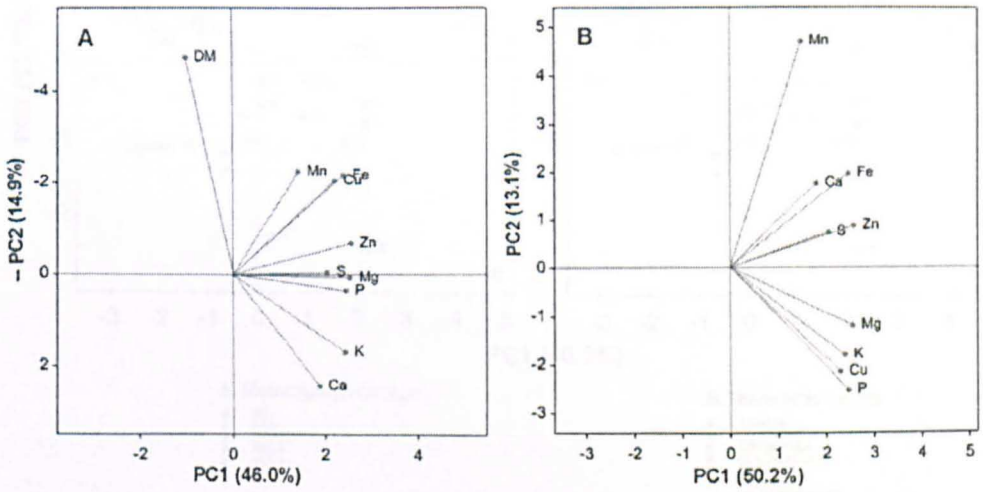

Figure 4.2: Biplots from PCA analysis for 49 CPC accessions based on (A) tuber DM and mineral traits, and (B) mineral traits alone.

In addition, PCA analysis was also performed among the nine minerals excluding DM (Figures 4.2B and 4.3). In this case, the first two principal components explained 63.3% of the total variability found among the 49 accessions, with PC1 alone accounting for 50.2% of the variability (Figure 4.2B). The loadings plot indicated that all minerals were positively associated with PC1, whereas the PC2 was positively associated Mn, Ca, Fe, S and Zn, and negatively with Mg, K, Cu and P. Comparing the loadings plot with and without DM, the relative positions of most minerals is similar with the exception of Ca and Cu which exchange positions. Correlations between variables (Table A4.2) show a negative link between Ca and DM, which may

explain the shift in the loadings plot position of Ca. Weak separations of groups of accessions can be seen in the PCA plots for analyses with DM (Figure 4.1) and particularly without DM (Figure 4.3) as a variable. There was a tendency for the northern and southern series Tuberosa species to separate into two groups, and the Acaulia and Demissa groups also form their own space shared with a broad spread of other accessions (Figure 4.3).

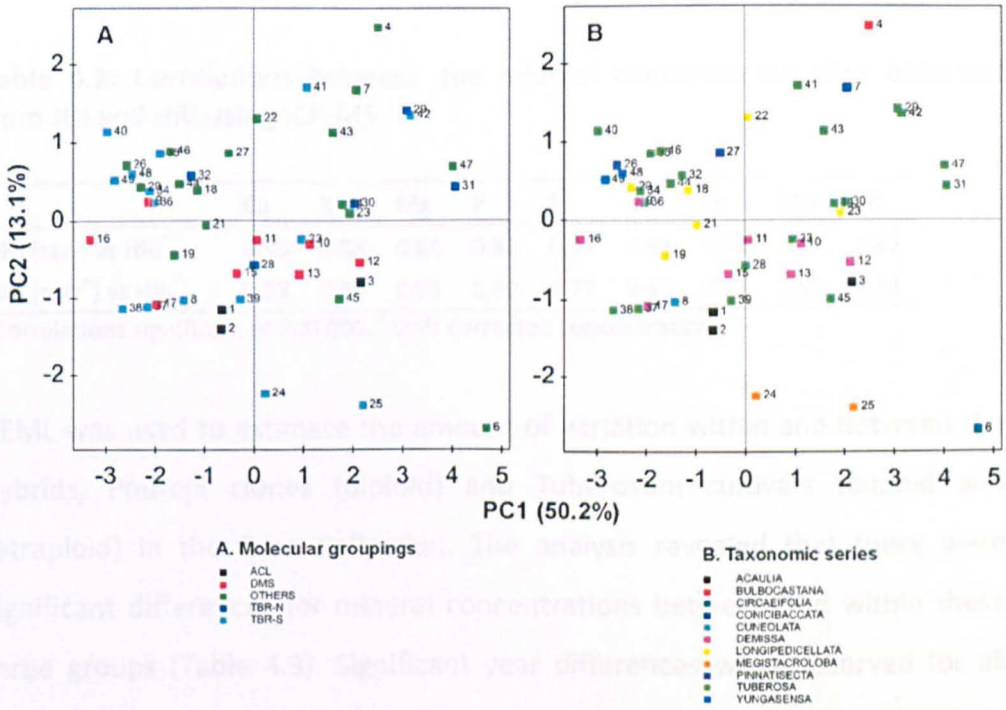

Figure 4.3: PCA plots for 49 CPC accessions based on tuber mineral concentrations alone. The accessions are colour coded based on (A) molecular groupings and (B) taxonomic series, as per Hawkes (1990). Abbreviations as in Figure 4.1.

4.2.2 Mineral variation in the Core Collection

4.2.2.1 Genetic diversity for tuber mineral concentrations

The genetic diversity for mineral concentrations in cultivated potatoes was studied using the Core Collection comprising of two hybrids, 36 Phureja lines and 26 Tuberosum cultivars across two years (2007, 2008). In 2007, tuber mineral concentrations were analysed separately at JHI and HRI and the

results from these two analyses showed a similar trend for mineral composition except for S (Table 4.2). Nevertheless, the drift-corrected JHI data (Table 4.2) improved the correlation for S and other minerals. In 2008 mineral analysis was carried out only at HRI, and hence the 2007 data obtained from HRI were chosen for further analysis. In addition to Ca, K, Mg, P, S, Cu, Fe, Mn and Zn, minerals such as B, N and Na were measured at HRI and therefore for consistency, data measured at HRI were taken for further analysis.

Table 4.2: Correlations between the mineral concentration data obtained from JHI and HRI using ICP-MS

	Ca	K	Mg	P	S	Cu	Fe	Mn	Zn
JHI (raw) vs HRI^	0.95	0.83	0.85	0.81	0.33	0.83	0.72	0.91	0.88
JHI (corr[R]) vs HRI^	0.93	0.87	0.91	0.89	0.77	0.93	0.79	0.95	0.91

^ Correlations significant at P≤0.001. [R] Drift corrected concentrations.

REML was used to estimate the amount of variation within and between the hybrids, Phureja clones (diploid) and Tuberosum cultivars (diploid and tetraploid) in the Core Collection. The analysis revealed that there were significant differences for mineral concentrations between and within these three groups (Table 4.3). Significant year differences were observed for all variables excluding DM, K, P, S, B and Cu in Tuberosum cultivars and K, S, B, Cu, Mn and Zn in Phureja clones. Further, there was a significant year interaction in Tuberosum cultivars for plant maturity, yield, DM, S, and Zn and in Phureja lines for tuber yield and Na concentration.

The frequency distributions for mean mineral concentrations showed that diploid Phureja clones and hybrids tended to have low yields, low Cu and Mg concentrations and high concentrations of Ca, Na and Zn when compared to high yielding tetraploid Tuberosum (Figure 4.4). Significant correlations were found between 2007 and 2008 mineral concentrations in Phureja and Tuberosum genotypes, with the exception of Fe in Phureja and Tuberosum and P and Cu in Tuberosum cultivars (Figure 4.5). The individual mineral

Table 4.3: REML analysis of the plant maturity, tuber yield, DM and mineral traits for two hybrids, 36 Phureja lines and 26 Tuberosum cultivars grown in replicated field trials during 2007 and 2008

Source of variation	Mat[+]	Yield	DM	N	Ca	K	Mg	P	S	B	Cu	Fe	Mn	Na	Zn
Genotype (H, P, T)	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Year	***	**	*	***	***	ns	*	ns	ns	*	ns	**	ns	*	**
Genotype* Year	**	***	***	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	**	ns
Between P_T_H	***	***	***	***	***	***	****	***	ns	***	***	ns	***	***	***
Between P_T_H.Year	ns	ns	***	*	*	ns	ns	ns	**	ns	***	ns	ns	*	***
Within P_T_H	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Within P_T_H. Year	**	***	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns
SED (Between P_T_H)	0.722	1.679	1.412	0.116	0.005	0.119	0.007	0.017	0.009	0.302	0.464	4.429	0.563	3.242	1.154
SED (Within P_T_H)	0.513	2.376	1.003	0.165	0.007	0.175	0.009	0.025	0.013	0.431	0.659	6.342	0.796	4.611	1.640
Within T	***	***	***	***	***	***	***	**	***	***	***	***	***	***	***
Year	**	***	ns	***	***	ns	*	ns	ns	ns	ns	**	*	*	**
T* Year	***	*	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	*
Within P	***	***	***	***	***	***	***	***	***	***	***	*	***	***	***
Year	***	*	***	***	***	ns	*	*	ns	ns	ns	**	ns	***	ns
P*Year	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns

[+]Plant maturity; H-Hybrid; P-Phureja; T-Tuberosum; Significance of the effects is given in three levels: * P≤0.05; ** P≤0.01; ***P≤0.001; ns-non-significant. SED-standard errors of differences of mean.

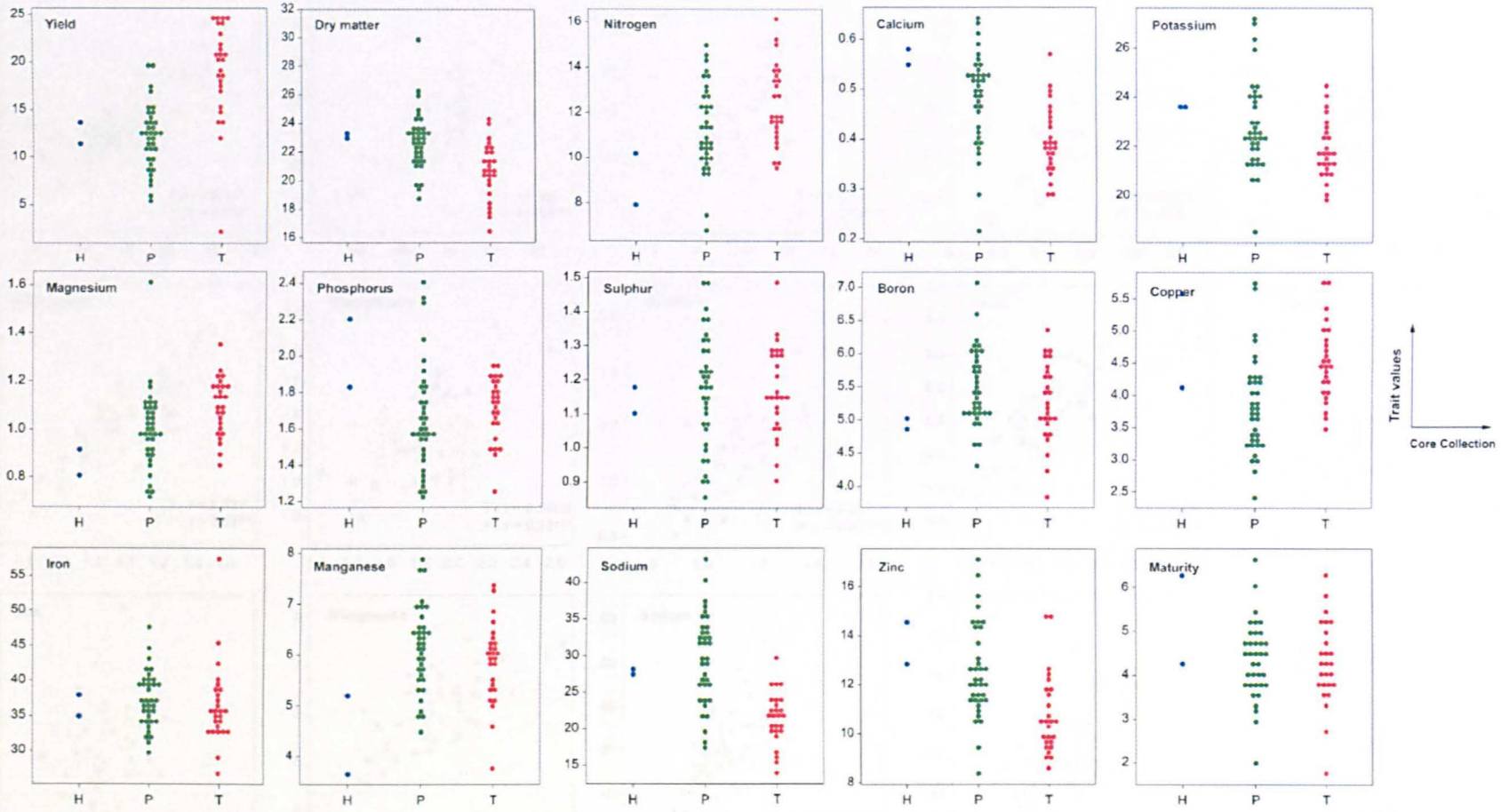

Figure 4.4: Frequency distributions for mean tuber yield, DM and mineral concentrations (DW basis) and plant maturity of two hybrids (H), 36 Phureja (P) lines and 26 Tuberousm (T) cultivars grown in replicated field trials during 2007 and 2008. Yield in kg, FW plot-1, FW; Dry matter in %; Concentrations of N, Ca, K, Mg, P and S in mg g^{-1} DW; B, Cu, Fe, Mn, Na and Zn in µg g^{-1} DW.

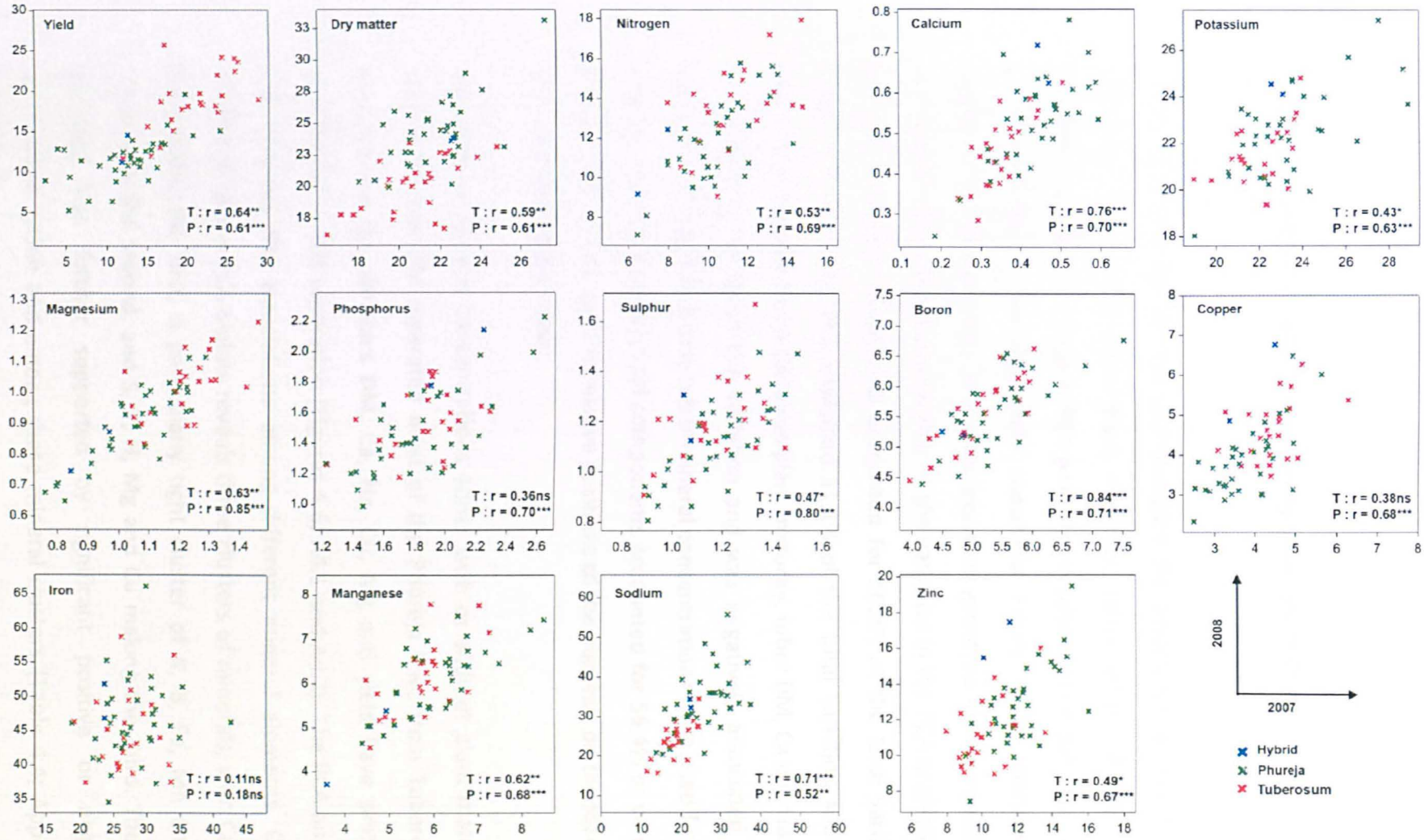

Figure 4.5: Correlations [r] between mean tuber yield, DM and mineral concentrations of Core Collection (2 Hybrids, 35 Phureja & 23 Tuberosum genotypes) in the 2007 (X axis) vs the 2008 (Y axis) field data. Yield in kg plot^{-1}, FW; Dry matter in %; Concentrations of N, Ca, K, Mg, P and S in mg g^{-1} DW; B, Cu, Fe, Mn, Na and Zn in µg g^{-1} DW. Significance of the effects is given in three levels: * P≤0.05; ** P≤0.01; ***P≤0.001; ns-non-significant.

concentrations for hybrids, Phureja clones and Tuberosum cultivars are given in Appendix Tables A4.4 to A4.6, respectively for 2007, 2008 and combined data.

4.2.2.2 Associations among plant maturity, tuber yield, DM and mineral concentrations

PCA analysis was carried out to investigate the association among the 60 genotypes for the variables plant maturity, tuber yield, DM and the concentrations of 12 minerals. PCA analysis revealed similar patterns when applied for each year separately (Appendix Figure A4.1), therefore a combined PCA analysis was conducted based on genotype means across two years (Figure 4.6). When considering all the variables in the PCA analysis, the first two principal components accounted for 50.3% of the total variation (Figure 4.6 A and B). PC1 explained 31.1% of the total variation and was negatively associated with variables plant maturity, tuber DM, Ca and Na. PC2 contributed to 19.2% of the variation and was negatively associated with yield, Cu and Mg. When only tuber mineral concentrations were used for PCA analysis, the first two principal components accounted for 56.4% of variance (Figure 4.6 C and D), and the relative positions of the minerals on the loadings plot were almost the same.

PCA plots of mineral concentrations either with or without plant maturity, tuber yield and DM separated most of the Phureja lines from Tuberosum cultivars and the variables DM, Ca, Na, Cu, Mg and yield have primarily contributed to this separation (Figures 4.6; A4.1 and A4.2). The PCA analysis also revealed the associations among different mineral elements. Closer inspection of the PCA biplots reveals three clusters of minerals, with Ca and Na forming the first, a particularly tight cluster of K, B, Fe, Mn and Zn comprising the second, and S, P, N, Mg and Cu making the third. The PCA grouping was further supported by significant positive or negative correlations within and among these mineral clusters (Table 4.4; Appendix Table A4.7).

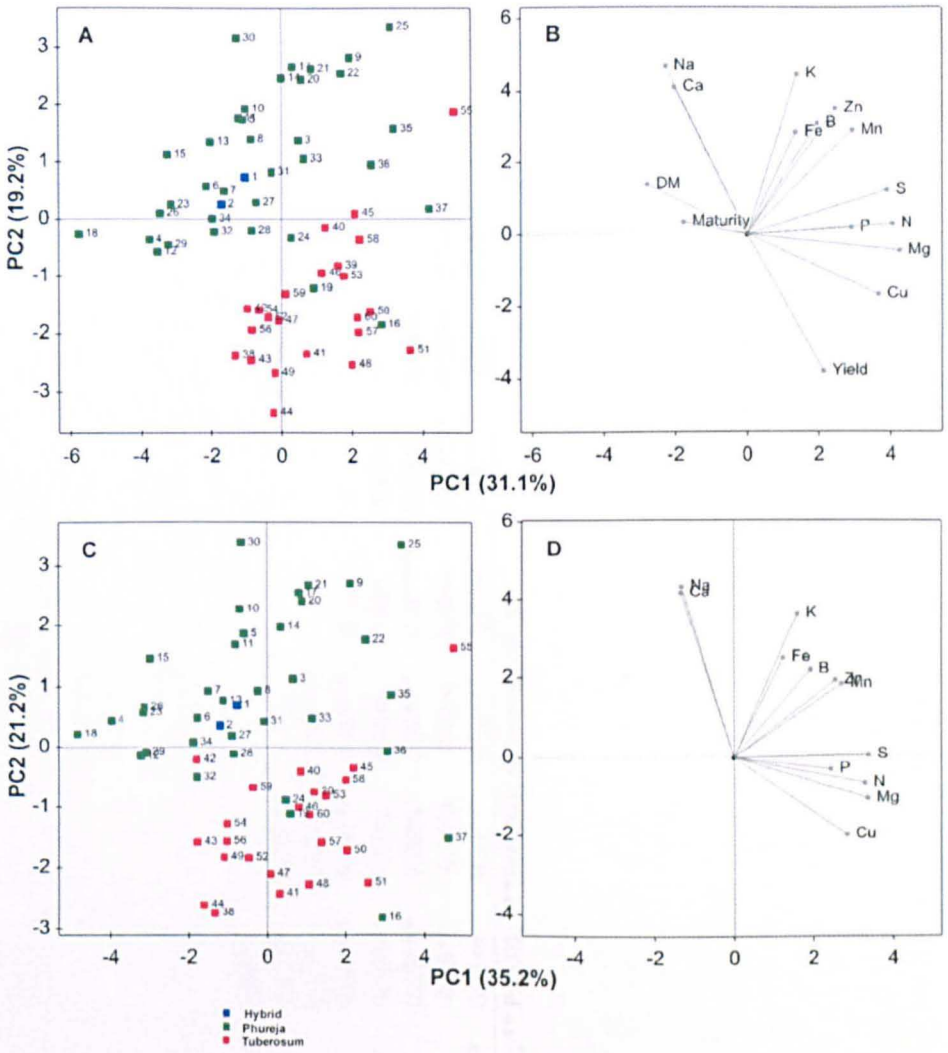

Figure 4.6: PCA plots and biplots of tuber mineral traits estimated on 60 genotypes of the Core Collection over two years field experiment with (A and B) and without (C and D) plant maturity, tuber yield and DM showing genotypes (A and C) and the loadings of the variables (B and D). Numbers 1 and 2 are Hybrids, 3–37 are Phureja clones and 38–60 are Tuberosum cultivars (see Appendix Table A4.6 for more details).

When PCA analyses were performed separately for Phureja and Tuberosum genotypes, the results were different to that obtained using the whole dataset (Figure 4.7; A4.3). A stronger negative relationship was found between tuber DM and mineral concentrations (Figure 4.7), which was also supported by correlation analysis (Table 4.5; Appendix Table A4.8 and A4.9), and the relationship of mineral concentrations to yield became more complex. This could be explained by the large difference in yield between Phureja and Tuberosum genotypes, a factor which is removed by within-

Table 4.4: Correlation coefficients (r) among plant maturity, tuber yield, DM and mineral concentrations in the Core Collection of 60 potato genotypes tested over two years (2007 & 2008)

	Mat[+]	Yield	DM	N	Ca	K	Mg	P	S	B	Cu	Fe	Mn	Na
Yield	-0.07ns	-												
DM	0.21ns	-0.51***	-											
N	-0.40**	0.18ns	-0.37**	-										
Ca	0.20ns	-0.34**	0.12ns	-0.32*	-									
K	0.19ns	-0.20ns	-0.21ns	0.07ns	0.36**	-								
Mg	-0.31*	0.43***	-0.39**	0.78***	-0.36*	0.14ns	-							
P	0.03ns	0.27*	-0.29*	0.20ns	-0.16ns	0.42***	0.30*	-						
S	-0.25*	0.16ns	-0.22ns	0.65***	-0.24ns	0.26*	0.63***	0.35**	-					
B	-0.07ns	-0.11ns	-0.14ns	0.31*	0.13ns	0.33**	0.27*	0.06ns	0.38**	-				
Cu	0.04ns	0.45***	-0.45***	0.40**	-0.40**	0.13ns	0.49***	0.71***	0.42***	0.16ns	-			
Fe	-0.09ns	-0.06ns	-0.29*	0.20ns	0.28*	0.36**	0.10ns	0.13ns	0.08ns	0.30*	0.17ns	-		
Mn	-0.30*	0.05ns	-0.08ns	0.57***	-0.04ns	0.33*	0.59***	0.08ns	0.56***	0.46***	0.05ns	0.24ns	-	
Na	0.16ns	-0.47***	0.40**	-0.28*	0.64***	0.37**	-0.35**	-0.25ns	-0.20ns	0.10ns	-0.57***	0.10ns	0.12ns	-
Zn	-0.19ns	-0.28*	0.03ns	0.35**	0.03ns	0.45***	0.25ns	0.51**	0.50***	0.22ns	0.36**	0.26*	0.40**	0.15*

[+]Plant maturity; Significance of the effects is given in three levels: * P≤0.05; ** P≤0.01; ***P≤0.001; ns-non-significant.

Table 4.5: Spearman's correlation coefficients among traits in Core Collection Phureja and Tuberosum genotypes over two years

	Mat[+]	Yield	DM	N	Ca	K	Mg	P	S	B	Cu	Fe	Mn	Na
Yield	0.11ns	-												
	-0.24ns	-												
DM	0.09ns	0.09ns	-											
	0.22ns	-0.74***	-											
N	-0.31*	0.001ns	-0.12ns	-										
	-0.39*	-0.26ns	-0.13ns	-										
Ca	0.26*	0.13ns	-0.22*	-0.18ns	-									
	0.06ns	-0.19ns	-0.14ns	0.08ns	-									
K	0.07ns	0.02ns	-0.49***	0.30*	0.40***	-								
	0.43**	0.25ns	-0.25ns	-0.01ns	0.07ns	-								
Mg	-0.28*	0.36**	-0.19ns	0.73***	-0.07ns	0.46***	-							
	-0.35*	-0.22ns	0.04ns	0.68***	-0.06ns	0.22ns	-							
P	-0.27ns	0.30*	-0.33**	0.39**	-0.19ns	0.40**	0.47***	-						
	0.17ns	0.31*	-0.26ns	-0.19ns	0.18ns	0.47**	0.26ns	-						
S	-0.38**	0.26*	-0.23*	0.80***	-0.13ns	0.36**	0.87***	0.42***	-					
	-0.03ns	0.03ns	-0.15ns	0.53***	-0.18ns	0.30*	0.49**	0.17ns	-					
B	-0.17ns	-0.20ns	-0.09ns	0.52***	0.07ns	0.47***	0.38**	-0.05ns	0.38**	-				
	0.14ns	0.10ns	-0.31*	0.29*	0.24ns	0.44*	0.34*	0.50***	0.49**	-				
Cu	-0.11ns	0.15ns	-0.17ns	0.51***	-0.33**	0.25*	0.53***	0.73***	0.45***	0.11ns	-			
	0.11ns	0.39*	-0.50**	0.11ns	0.17ns	0.56***	0.33*	0.69***	0.48**	0.73***	-			
Fe	-0.04ns	0.02ns	-0.39**	0.22*	0.06ns	0.36**	0.15ns	0.24*	0.07ns	0.30*	0.34**	-		
	-0.03ns	0.20ns	-0.37*	0.07ns	0.45**	0.20ns	0.12ns	0.26ns	0.01ns	0.22ns	0.39*	-		
Mn	-0.23*	0.32*	-0.12ns	0.54***	-0.004ns	0.40**	0.69***	0.36*	0.68***	0.44***	0.26*	0.21ns	-	
	-0.14ns	-0.22ns	0.12ns	0.55***	-0.08ns	0.06ns	0.53***	-0.01ns	0.33*	0.52***	0.22ns	0.13ns	-	
Na	0.22*	0.18ns	-0.002ns	-0.26*	0.49***	0.29*	-0.17ns	-0.18ns	-0.26*	0.02ns	-0.47***	-0.09ns	-0.01ns	-
	-0.03ns	-0.11ns	0.07ns	0.43**	0.12ns	0.45**	0.29*	-0.11ns	0.16ns	0.10ns	0.07ns	0.20ns	0.36*	-
Zn	-0.39**	0.10ns	-0.22*	0.58***	-0.30*	0.30*	0.66***	0.68***	0.65***	0.06ns	0.68***	0.15ns	0.50***	-0.33**
	-0.37*	0.03ns	-0.21ns	0.76***	0.06ns	0.12ns	0.75***	0.18ns	0.70***	0.54***	0.43**	0.19ns	0.55***	0.23ns

[+]Plant maturity; The upper and lower numbers refer to the Phureja and Tuberosum respectively; * P≤0.05; ** P≤0.01; ***P≤0.001;ns-non-significant.

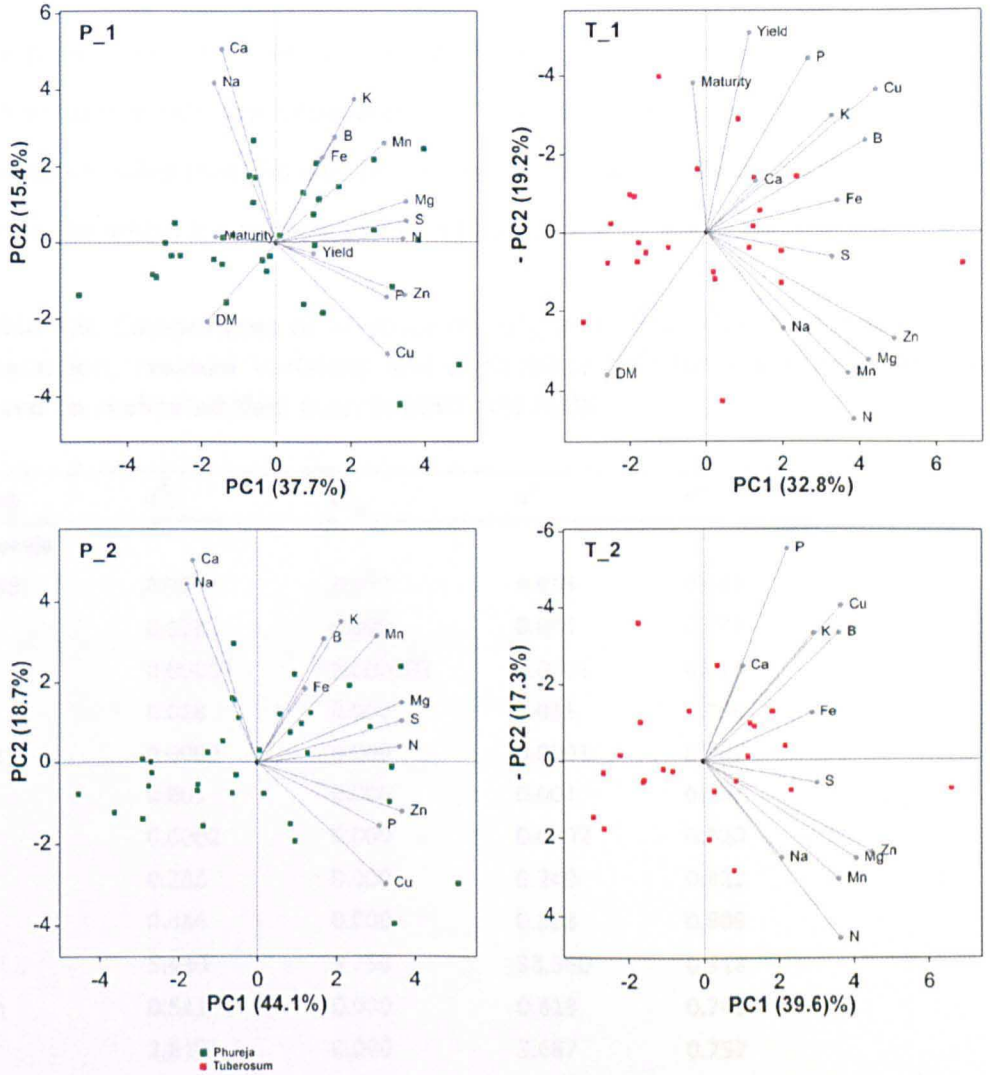

Figure 4.7: PCA biplots for tuber mineral traits measured on 35 Phureja (P) clones and 23 Tuberosum (T) cultivars of the Core Collection over two years with (1) and without (2) plant maturity, tuber yield and DM.

group analysis. The biplots also revealed that late maturity of plants had positive and negative associations with different minerals (Figure 4.7). In Phureja lines late maturity showed positive associations with tuber Ca and Na and negative associations with other minerals. In Tuberosum cultivars, late maturity was negatively associated with N, Mg, Mn, S, Na and Zn and positively associated with K, P, Cu, Ca and B.

4.2.2.3 Heritabilities

The broad-sense heritability of mineral traits was determined for Phureja and Tuberosum genotypes separately for the two study years (Table 4.6). The results revealed that most of the minerals had high heritability (>50%) except for Fe, for which it was only 15.7% in Phureja and 31.8% in Tuberosum.

Table 4.6: Components of variance σ^2_c, σ^2_{cy} and σ^2 for clones, clones x years interaction, residual variation and heritability (H^2) for the Core Collection based on replicated field trials in 2007 and 2008

Trait	σ^2_c	σ^2_{cy}	σ^2	H^2
Phureja				
Yield	8.157	4.977	4.616	0.693
N	0.029	0.000	0.035	0.777
Ca	0.00007	0.000003	0.0001	0.813
K	0.028	0.000	0.035	0.756
Mg	0.0002	0.000	0.0001	0.897
P	0.001	0.000	0.001	0.801
S	0.0002	0.000	0.0002	0.820
B	0.288	0.000	0.249	0.822
Cu	0.446	0.000	0.398	0.809
Fe	5.430	3.750	38.560	0.318
Mn	0.543	0.000	0.818	0.740
Zn	2.819	0.000	3.687	0.757
Na	24.290	9.580	27.520	0.676
Tuberosum				
Yield	22.042	3.320	6.726	0.864
N	0.017	0.008	0.021	0.651
Ca	0.0001	0.00001	0.00002	0.862
K	0.008	0.002	0.015	0.630
Mg	0.0001	0.00002	0.0001	0.796
P	0.0002	0.0001	0.0004	0.523
S	0.0001	0.0001	0.0001	0.640
B	0.305	0.011	0.082	0.911
Cu	0.186	0.105	0.407	0.540
Fe	2.690	13.100	28.800	0.157
Mn	0.448	0.002	0.437	0.799
Zn	1.708	0.660	1.287	0.710
Na	13.250	0.000	12.690	0.816

4.2.3 Mineral variation in the NTB population

4.2.3.1 Genetic diversity for tuber minerals

The genetic diversity for tuber mineral concentrations was also investigated in a tetraploid NTB population grown in replicated field trials during 2005. Frequency distributions for mean tuber yield, DM and mineral concentrations are presented in Figure 4.8 and the values are given in Appendix Table A4.10. REML analysis for tuber yield, DM and minerals showed significant differences between the NTB clones and the two control lines DB337 (37) belonging to Group Phureja and Desiree (Des) belonging to Group Tuberosum, for all the variables except for Cu concentration (Appendix Table A4.11). In addition, significant differences were also detected among clones within the NTB group for all the measured variables. The heritability estimates revealed high values for all minerals except for Fe (H=33.6%) (Table 4.7).

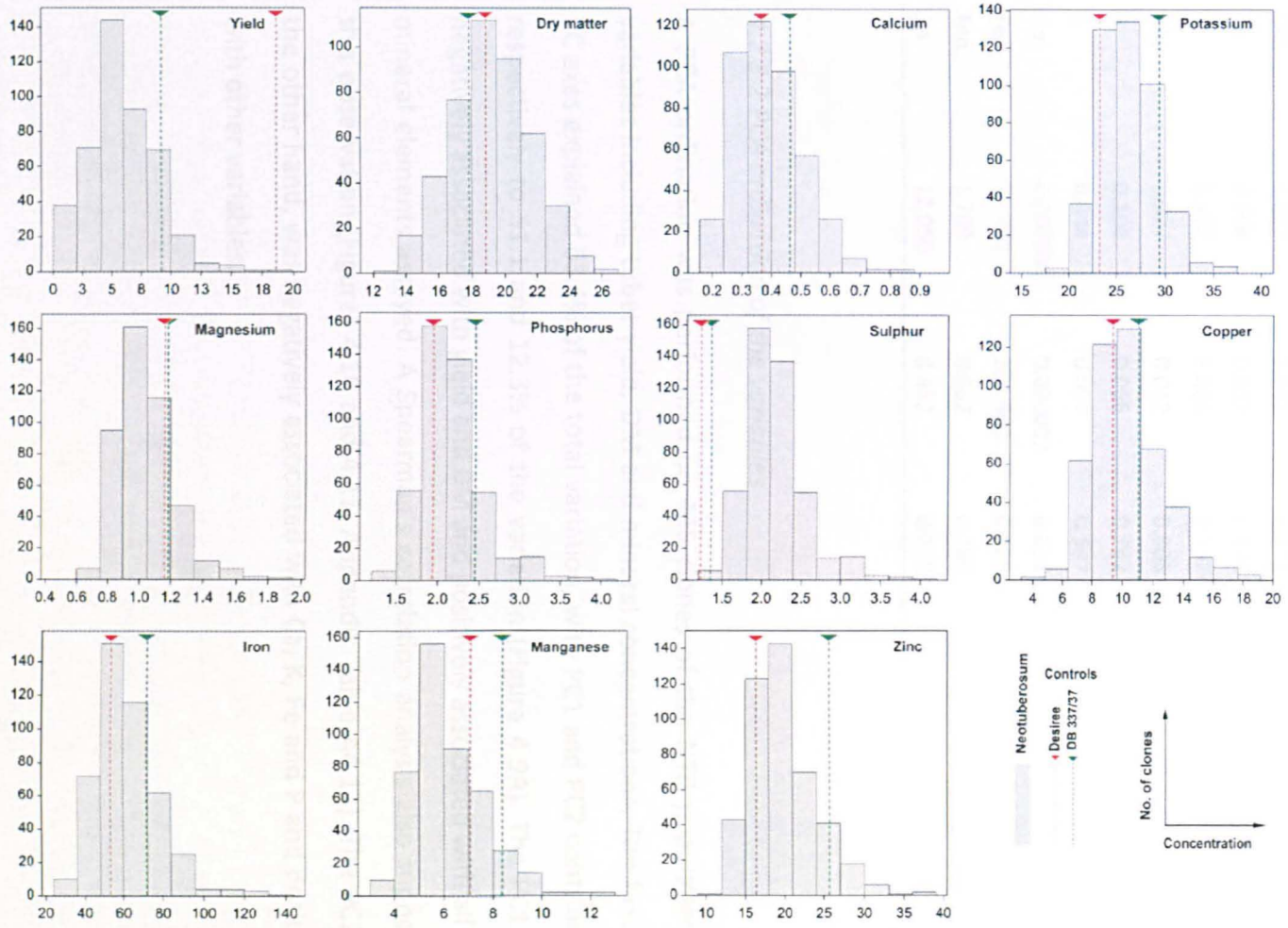

Figure 4.8: Frequency distributions for tuber yield, dry matter and mineral concentrations (DW basis) of Neotuberosum clones with controls Desiree (Tuberosum, tetraploid) and DB 337/37 (Phureja, Mayan Gold, diploid) grown in replicated field trials during 2005. Yield in kg, FW plot^{-1}; Dry matter in %; Concentrations of Ca, K, Mg, P and S in mg g^{-1} DW; Cu, Fe, Mn and Zn in µg g^{-1} DW.

Table 4.7: Components of variance σ^2_c and σ^2 for clones and residual variation and heritability (H^2) in the NTB population based on replicated field trial in 2005

Trait	σ^2_c	σ^2	H^2
Yield	7.073	1.886	0.889
DM	4.237	2.602	0.773
Ca	0.008	0.007	0.694
K	6.257	3.886	0.757
Mg	0.024	0.012	0.806
P	0.108	0.066	0.762
S	0.044	0.057	0.597
Cu	0.000004	0.000003	0.695
Fe	77.700	294.300	0.336
Mn	1.709	0.962	0.780
Zn	12.056	8.452	0.738

4.2.3.2 PCA ordination of the variables

A PCA ordination was performed on 448 clones of the NTB population for variables including tuber yield, DM and mineral concentrations. The first two PC axes explained 63.4% of the total variation, with PC1 and PC2 contributing respectively to 51.1 and 12.3% of the variation (Figure 4.9A). The PC1 was negatively associated with yield and DM and positively associated with all nine mineral elements assayed. A Spearman's correlation analysis also supported this observation (Figures 4.10 and 4.11; Appendix Table A4.12). The PC2, on the other hand, was negatively associated with Ca, K, Fe and P and positively with other variables.

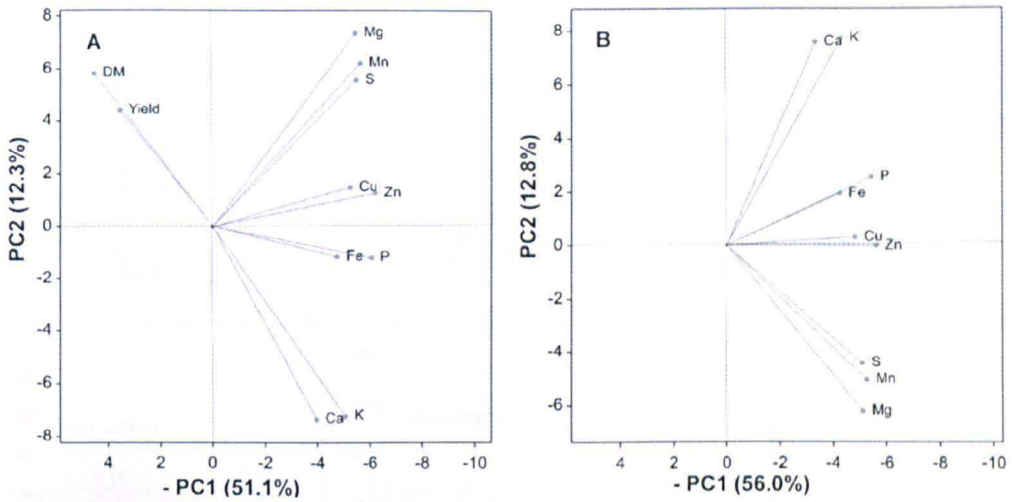

Figure 4.9: PCA biplots for tuber mineral concentrations estimated on 448 Neotuberosum clones with (A) and without (B) tuber yield and DM content.

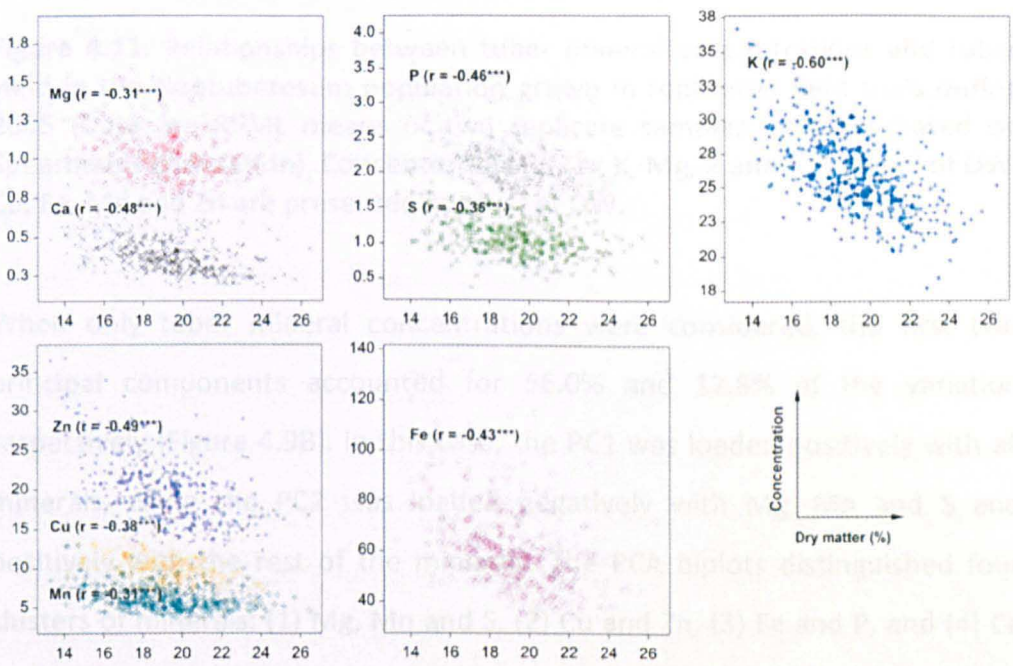

Figure 4.10: Relationships between tuber mineral concentrations and tuber dry matter content in the Neotuberosum population. (Data are REML means of two replicate samples; r values based on Spearman's correlation). Concentrations of Ca, K, Mg, P and S in mg g^{-1} of DW; Cu, Fe, Mn and Zn are presented in µg g^{-1} of DW.

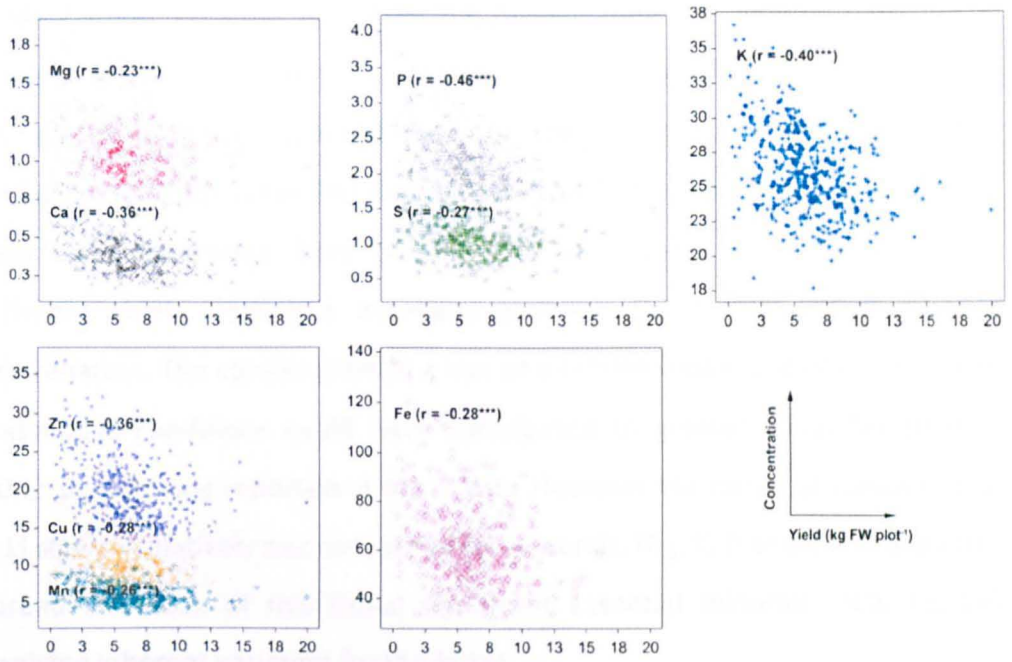

Figure 4.11: Relationships between tuber mineral concentrations and tuber yield in the Neotuberosum population grown in replicated field trials during 2005 (Data are REML means of two replicate samples; r values based on Spearman's correlation). Concentrations of Ca, K, Mg, P and S in mg g^{-1} of DW; Cu, Fe, Mn and Zn are presented in µg g^{-1} of DW.

When only tuber mineral concentrations were considered, the first two principal components accounted for 56.0% and 12.8% of the variation respectively (Figure 4.9B). In this case, the PC1 was loaded positively with all minerals, while the PC2 was loaded negatively with Mg, Mn and S and positively with the rest of the minerals. The PCA biplots distinguished four clusters of minerals: (1) Mg, Mn and S, (2) Cu and Zn, (3) Fe and P, and (4) Ca and K (Figures 4.9 A and B).

4.3 DISCUSSION

4.3.1 Genetic variation for tuber mineral concentrations

4.3.1.1 CPC accessions

The CPC accessions used in this study represented different taxonomic series and diverse geographical origins within South and Central America (Table 2.1),

making them valuable resources for investigating the genetic variation in mineral accumulation in wild potatoes adapted to different environments. The glasshouse study demonstrated considerable variability among the wild CPC accessions for tuber DM and mineral concentrations (Table 4.1). Mineral data was determined from one bulk of five tubers harvested from five different plants from each accession grown in the annual CPC glasshouse rejuvenation. The combination of a lack of a randomised replicated design and glasshouse conditions could have contributed to greater variability than in other populations reported in this thesis. However the range of values (Table 4.1) show a relatively narrow spread for minerals, Mg, K, P andS, and a greater spread for those of nutritional significant essential minerals (Ca, Fe, Zn) implying inherent variation for the latter.

PCA plots of the accessions for tuber DM and mineral traits (Figure 4.1) showed much overlap of accessions based on molecular groups and taxonomic series. However when PCA analysis was performed using only mineral elements (Figure 4.3), although there was some overlap among accessions based on molecular group, some groups of accessions did form separate groups. The Mexican (DMS) and ACL accessions formed separate groups within the plot area. Also, the two main molecular groups within series Tuberosa *sensu* Hawkes (1990), the Peruvian species (TBR-N) and the Bolivian and Argentinian species (TBR-S) also occupied different areas in the biplot of PC1 and PC2. The level of diversity observed in the CPC collection indicates the existence of differences in tuber mineral accumulation between different potato species.

Significant variation was observed in the CPC germplasm collection particularly for tuber DM, Ca and K accumulation in tubers and the data corroborate those of McCann *et al.* (2010) and Bamberg *et al.* (1993, 2008). In this study, tuber DM content varied from 17–48% (Table 4.1), which is in agreement with the range (18–35% DM) reported by McCann *et al.,* (2010) in wild *Solanum* species. Research by Bamberg *et al.* (1993) suggests that there

is great genetic variation within *Solanum* germplasm for the ability to accumulate Ca in tubers. They screened wild *Solanum* species in control levels (80 ppm (parts per million) of Ca in solution) and high Ca (800 ppm Ca) levels in 21 *Solanum* species (three accessions per species) in glasshouse. With an ample supply of Ca (80 ppm) to potato plants, Bamberg *et al.* (1993) found that the tuber Ca concentrations ranged from 0.16 – 0.74 mg g^{-1} DW similar to the range reported here (0.10 – 0.67 mg g^{-1} DW, Table 4.1). Among the *Solanum* species investigated in this study, *S. bulbocastanum* (CPC 7638) and *S. chacoense* (CPC 3504) exhibited the highest and the lowest tuber Ca concentrations, respectively (Appendix Table A4.3). When species in common between this study and that of Bamberg *et al.* (1993) were considered, the ranking of genotypes were similar, such that *S. chacoense* and *S. kurtzianum* had low tuber Ca concentrations and *S. gourlayi* and *S. stenotomum* had high tuber Ca concentrations (Appendix Table A4.3; Bamberg *et al.*, 1993).

With regard to K accumulation, *S. capsicibaccatum* (CPC 3554) and *S. chacoense* (CPC 3504) exhibited high and low tuber K concentrations respectively (Appendix Table A4.3). A 1.8-fold range for tuber K concentrations was found in this study (Table 4.1). With an ample supply of K (90 ppm solution) to potato plants, Bamberg *et al.* (2008) reported that *S. acaule, S. chacoense, S. okadae* and *S. pinnatisectum* had high tuber K concentrations.

In general, accessions with low tuber DM content showed high concentrations of Ca and K and *vice versa* (Appen dix Table A4.3). About 65–75% of DM content in tubers is found to be starch (Burton, 1989). The concentrations of minerals (including Ca and K) in tubers were found to decrease following DM (starch) accumulation during tuber bulking (Kolbe and Stephan-Beckmann, 1997b). Within the tuber, Ca is concentrated around the periphery and decreases towards the centre of tubers (Subramanian *et al.*, 2011). As Ca is relatively immobile in the phloem (Marschner, 1995, Westermann, 2005), its concentration tends to decrease with increasing DM accumulation. On the

other hand, K is mobile in the phloem and it occupies a different location in tuber from that of Ca (Subramanian *et al.*, 2011). Within the cells K is required for many physiological functions, including osmoregulation, enzyme activation and membrane transport processes (Marschner, 1995; White and Karley, 2010). Nitsos and Evans (1969) first observed that starch synthesizing enzymes have a specific requirement for K and about 1.8% K in tuber DM is needed for high starch concentrations in potatoes (Forster and Beringer, 1983; Lindhauer and De Fekete, 1990). However, tuber K concentrations above 2% DM were found to reduce starch content (Marschner and Krauss, 1980), which might be explained in terms of an osmotic optimum for starch synthesis (Oparka and Wright, 1988). Furthermore, Perrenoud (1993) reported that K fertilization increases tuber yield by promoting large-sized tubers through an increased water accumulation and a decline in DM content of tubers.

The association of S and Zn as evident from PCA plots could be attributed to the phloem transport of Zn by non-proteinogenic nicotianamine and/or binding of Zn to S-containing proteins and peptides (Broadley *et al.*, 2007; White and Broadley, 2009). On the other hand, the association of Fe and Ca might be due partly to the high entrapment of these minerals by periderm of the potato tuber. This is supported by the fact that potato surface layers had 55% and 34% of total tuber Fe and Ca concentrations (Subramanian *et al.*, 2011).

4.3.1.2 Core Collection

The distinction in clustering patterns of minerals in PCA biplots between Phureja and Tuberosum groups (Figure 4.6) indicates differences in the mechanisms and patterns of mineral uptake and accumulation in tubers. Additionally, high heritability values for the measured minerals (Table 4.6), with the exception of Fe indicate the strong genetic control of mineral accumulation in tubers. The Phureja clones in JHI's Core Collection were found

to contain a range of useful traits including, but not limited to, high levels of tuber carotenoid content, improved flavour, reduced cooking time and resistance to *Erwinia* disease (De Maine *et al.*, 2000). Similarly, this study has established the existence of variability for tuber mineral concentration in the Core Collection including elevated levels of the nutritionally significant minerals, Ca, Fe and Zn (Figure 4.4).

The overall differences between the Phureja and Tuberousm groups for tuber mineral concentrations might be linked, in part, to differences in root traits. Roots play an important role in acquiring and transporting water and minerals in plants and hence the architecture of roots greatly influences water and mineral acquisition. Studies conducted at the JHI on the root traits of 28 potato genotypes have revealed the presence of more stolon and longer basal roots in Phureja clones and the hybrid HB171 (13) when compared to Tuberosum cultivars (Wishart *et al.,* in preparation). In potatoes, the functional roots present on stolon and tubers have been shown to supply water and inorganic nutrients to tubers, while basal roots appear to transport water and mineral elements to aerial parts of the plant (Busse and Palta, 2006; Kratzke and Palta, 1985). Moreover, studies have also established that Ca in tubers is primarily taken up through the periderm, stolon and tuber roots and little transport occurs from basal roots (Busse and Palta, 2006; Habib and Donnelly, 2002; Kratzke and Palta, 1986). This may explain the occurrence of higher tuber Ca levels in the Phureja clones than Tuberosum cultivars. The strong association between Ca and Na in Phureja clones as evident from the PCA plots (Figure 4.7) suggest that these minerals might share an uptake pathway directly to the tuber from the stolon roots.

In addition to root characteristics, the size of the potato tuber may also be an important factor governing the mineral concentrations in tuber. In general, Phureja clones had smaller tuber size than Tuberosum cultivars (Nithya Subramanian, personal observation). High concentrations of minerals in Phureja clones compared to Tuberosum suggest that a negative association

may exist between tuber size and concentration of most minerals. Recently, White *et al.* (2011) reported a significant decrease in tuber Zn concentrations with increasing tuber size in *S. tuberosum* cultivars. Low Ca concentration in large tubers can possibly be ascribed to the low surface area to volume ratio, low mineral accumulation potential in cultivated species that typically have large tubers (Bamberg et al., 1993), and/or due to the dilution effects caused by high dry matter accumulation in tubers.

The results obtained in this study are in agreement with previous studies that demonstrated an inverse relationship between tuber yield and the accumulation of some minerals in different potato genotypes (reviewed in White *et al.*, 2009). As an exception, among the 12 minerals studied over the two years in this study, Cu showed a consistent positive relationship with tuber yield in *S. tuberosum* cultivars (Appendix Table A4.9). This result supports the findings of White *et al.* (2009). The negative correlation found between tuber DM and mineral concentrations might be due to the dilution effect caused by higher DM (starch) accumulation in tubers, as the rate of mineral deposition was reduced with accumulation of starch in tubers (Kolbe and Stephan-Beckmann, 1997b).

In addition to the above mentioned factors, the maturity and senescence of plants might influence mineral concentrations in tuber. In this study significant negative correlations were found between the late maturity of plants and tuber N, Mg, S, Mn and Zn concentrations (Table 4.5). Leaf senescence is characterized by degradation of macromolecules and chlorophyll, followed by remobilization and translocation of mobile nutrients from senescing leaves to other plant parts (including tubers) via the phloem (Marschner, 1995). Hence the phloem-mobile minerals can be efficiently mobilized to tubers during senescence. Early potato cultivars have been reported to have a higher N content in the tubers than late cultivars (Burton, 1989). This suggests that early plant maturity and senescence followed by effective remobilization of minerals to tubers could lead to enhanced

accumulation in tubers. In this study, potato haulms were killed four weeks prior to harvest as part of normal practice. As haulm destruction was done at the same time on different plant maturity classes; late maturing genotypes could have less mineral remobilization and low concentration in tubers.

Overall, the variation for tuber mineral concentrations observed between Phureja clones and Tuberosum cultivars might be attributable to inherent genetic variability for mineral uptake, partitioning and accumulation, and differences in rooting and maturing characteristics. Some of this might be driven by detailed molecular physiology such as transporters, whereas some may be due to the interactions with the route of transport, means of deposition and life cycle timing.

4.3.1.3 NTB population

The NTB population showed greater variability for tuber yield, DM and mineral concentrations than the Core Collection (Figures 4.8 and 4.4). NTB clones had not been selected for field performance other than tubering ability and had low yield compared to the control lines, Phureja and Tuberosum (Figure 4.8). In addition, tuber yield and DM were positively associated with each other in the NTB population (Figure 4.9), and this correlation was absent in the Phureja lines and Tuberosum cultivars in the Core Collection (Figure 4.7). Nevertheless, tuber mineral concentrations showed a significant negative relationship with tuber yield and DM as observed in other populations used in this study (Figure 4.10, 4.11). As such, the NTB clones accumulated greater concentrations of mineral elements in tuber tissues than Phureja and Tuberosum genotypes (Table 4.8). In particular, NTB clones showed three- to five-fold variation for Ca, Fe and Zn (Table 4.8). This could be linked to the lower tuber yield, efficient uptake, partitioning and accumulation of minerals in tubers. Moreover, a previous investigation of potato root traits has revealed that the NTB clones had longer stolon roots than Phureja genotypes (Wishart *et al.*, in preparation). Therefore, the very diverse mineral

profile witnessed in the NTB population (Figure 4.8) could be related to the existence of high level of polymorphism within this population. In addition, mineral concentrations of potato tubers reflect the mineral composition of the soil in which the plants were grown (Anderson *et al.*, 1999). In this study the NTB was grown in fields with high soil mineral concentrations and cation-exchange capacity (CEC) (Appendix Table A2.4) favouring enhanced mineral cation uptake by plants.

The NTB population was primarily derived from recurrent mass-selection of short-day adapted Andigena potatoes for high tuber yield under long-day conditions of the UK (Bradshaw and Ramsay, 2005; Glendinning 1975a), leading to the capturing of the high level of genetic polymorphism available in the parental Andigena stock. It was shown to be a highly diverse set of potato germplasm with resistance to diseases such as blight, wart, and potato viruses X and Y (Glendinning, 1975b). In addition, the present study showed a wider variation in the NTB population for tuber yield, DM and mineral concentrations than among the existing clones/cultivars in the Core Collection (Table 4.8). Given the levels of diversity, the NTB population could be considered as a valuable gene pool that could be exploited as a source of desirable alleles for marker assisted breeding for mineral traits.

Table 4.8: Mean and extremes obtained for various traits in the Core Collection and Neotuberosum population

Trait	Range											
	Gp. Tuberosum (n=23)	Mean	S.E.	Ratio^	Gp. Phureja (n= 35)	Mean	S.E.	Ratio^	NTB (n=450)	Mean	S.E.	Ratio^
Yield	2.1–25.1	19.8	0.8	12.0	5.3–19.7	12.1	0.6	3.7	0.44[&]–19.9	5.9	0.1	45.3
DM	16.4–24.2	20.1	0.3	1.5	18.6–30.2	20.9	0.2	1.6	13.3–26.4	19.1	0.1	2.0
N	9.6–16.3	12.4	0.4	1.7	6.8–14.9	11.2	0.3	2.2	-	-	-	-
K	19.7–24.4	21.7	0.2	1.2	18.5–27.4	22.8	0.3	1.5	17.7–36.7	26.1	0.1	2.1
Mg	0.8–1.3	1.1	0.02	1.6	0.7–1.6	1.0	0.02	2.3	0.66–1.8	1.0	0.01	2.7
P	1.2–1.9	1.7	0.04	1.6	1.2–2.4	1.6	0.05	2.0	1.3–4.1	2.2	0.01	3.2
S	0.9–1.5	1.2	0.03	1.7	0.9–1.5	1.2	0.03	1.7	0.4–2.0	1.1	0.01	5.0
B	3.8–6.3	5.3	0.1	1.7	4.3–7.1	5.5	0.1	1.7	-	-	-	-
Ca	288.3–565.6	403.6	14.7	2.0	215.1–652.2	483.0	15.8	3.0	168.8–852.4	390.0	5.3	5.0
Cu	3.5–5.8	4.5	0.1	1.7	2.4–5.8	3.8	0.1	2.4	3.4–19.1	10.2	0.1	5.6
Fe	26.7–58.1	36.0	0.8	2.2	29.4–48.0	37.3	0.7	1.6	32.2–136.6	61.6	0.8	4.2
Mn	3.8–7.4	5.9	0.1	1.9	4.5–8.0	6.1	0.1	1.8	3.6–12.9	6.2	0.1	3.6
Na	13.9–29.5	21.5	0.8	2.1	17.4–43.9	29.7	1.0	2.5	-	-	-	-
Zn	8.5–14.7	10.7	0.3	1.7	8.4–17.3	12.4	0.3	2.1	11.9–36.7	19.7	0.2	3.1

n - number of clones or cultivars; S.E. Standard Error; ^Ratio between lowest and highest tuber traits; [&] excluding three very low yielding genotypes (NTB363, 440 & 207) having one replicate value; Yield in kg. FW plot^{-1}; DM in %; Concentrations of N, K, Mg, P and S in mg g^{-1} DW; B, Ca, Cu, Fe, Mn, Na and Zn in µg g^{-1} DW.

4.3.2 Prospects for mineral enhancements in potatoes

Overall, the present study revealed a wide variability for tuber mineral concentrations across the wild and cultivated germplasms. This could be ascribed primarily to genetic factors (including root characteristics, Wishart *et al.*, in preparation) and also to environmental factors (eg. soil pH, CEC and soil mineral composition). The range of values observed in this study (Table 4.8) is mostly comparable with previous reports on tuber mineral concentration in *Solanum* species (Table 4.9). The differences in mineral concentration of tubers in the different studies (Table 4.9) could be attributed to genotype and environmental factors and also to the tuber sampling method, as suggested by Andre *et al.* (2007).

The extensive genetic variation observed in the potato germplasm indicates the possibility of enhancing tuber mineral levels by breeding. Although mineral homeostasis has been extensively studied in the model plant species *Arabidopsis thaliana*, it is largely undocumented in potatoes. *Arabidopsis thaliana* does not possess tubers and therefore, detailed research is needed to elucidate the mechanisms of mineral accumulation in tubers. These efforts will be hastened by the potato genome sequence that has become available recently (Visser *et al.*, 2009; Th e Potato Genome Sequencing Consortium, 2011) and by the new genomic tools (Bryan and Hein, 2008). Utilization of these resources, in combination with natural variation in potato mineral traits, will allow identification of the underlying genetic factors affecting tuber mineral concentrations. Furthermore, natural variants of genes controlling tuber mineral concentrations in germplasm collections can be exploited by association mapping studies. The desired alleles can then be introduced into commercial varieties for enhancing mineral accumulation in tubers along with other useful traits. In addition, the positive correlations among mineral elements suggest the common mechanisms controlling their uptake, transport and accumulation in tuber tissues. This suggests the possibility of simultaneous selection for enhanced levels of tuber minerals.

Table 4.9: Variation in tuber mineral concentrations among *Solanum* genotypes in diverse field trials[§] (DW basis)

Element	Genotypes	TSM	n	Conc" (range)	Reference
N	*S. tuberosum* varieties	A	26	8.1–14.7	White *et al.* (2009)
K	*S. tuberosum* cultivars[¥]	B	8	4.9–6.9	Casañas-Rivero *et al.* (2003)
K	*S. tuberosum* cultivars	B	8	19.5–23.4	Ekin (2011)
K	*S. tuberosum* cultivars	C	10	11.4–16.9	Ereifej *et al.* (1998)
K	Andean cultivars	B	21	25.0–39.0	Lefevre *et al.* (2012)
K	*S. tuberosum* varieties[^]	D	9	18.7–30.3	Luis *et al.* (2011)
K	*S. tuberosum* varieties	A	26	18.2–24.2	White *et al.* (2009)
Mg	*S. tuberosum* cultivars[¥]	B	8	0.2–0.3	Casañas-Rivero *et al.* (2003)
Mg	*S. tuberosum* cultivars	B	8	1.35–1.67	Ekin (2011)
Mg	Andean cultivars	B	21	0.7–1.3	Lefevre *et al.* (2012)
Mg	*S. tuberosum* cultivars	C	10	1.1–1.6	Ereifej *et al.* (1998)
Mg	*S. tuberosum* varieties[^]	D	9	0.8–1.9	Luis *et al.* (2011)
Mg	*S. tuberosum* varieties	A	26	0.9–1.2	White *et al.* (2009)
P	*S. tuberosum* cultivars	B	8	2.23–2.80	Ekin (2011)
P	*S. tuberosum* cultivars	C	10	0.9–3.0	Ereifej *et al.* (1998)
P	*S. tuberosum* varieties	A	26	0.9–1.6	White *et al.* (2009)
S	*S. tuberosum* varieties	A	26	0.9–1.4	White *et al.* (2009)
Ca	Andean landraces[♦]	B	74	271.1–1092.9	Andre *et al.* (2007)
Ca	*S. tuberosum* cultivars[¥]	B	8	47.6–87.3	Casañas-Rivero *et al.* (2003)
Ca	*S. tuberosum* cultivars	B	8	940.0–1280.0	Ekin (2011)
Ca	*S. tuberosum* cultivars	C	10	514.0–1436.0	Ereifej *et al.* (1998)
Ca	Andean cultivars	B	21	200.0–780.00	Lefevre *et al.* (2012)
Ca	*S. tuberosum* varieties[^]	D	9	150.0–530.0	Luis *et al.* (2011)
Ca	*S. tuberosum* varieties	A	26	269.5–668.1	White *et al.* (2009)
Cu	*S. tuberosum* cultivars[¥]	B	8	0.5–2.1	Casañas-Rivero *et al.* (2003)
Cu	*S. tuberosum* cultivars	B	8	6.59–9.81	Ekin (2011)
Cu	*S. tuberosum* cultivars	C	10	7.0–26.0	Ereifej *et al.* (1998)
Cu	Potato clones (Atlantic & 17 tetraploid clones)	E	18	8.7–11.6	Haynes *et al.* (2012)
Cu	Andean cultivars	B	21	3.0–6.0	Lefevre *et al.* (2012)
Cu	*S. tuberosum* varieties[^]	D	9	5.5–13.0	Luis *et al.* (2011)
Cu	*S. tuberosum* cultivars	B	16	3.1–5.4	Öztürk *et al.* (2011)
Cu	*S. tuberosum* varieties	A	26	2.3–4.9	White *et al.* (2009)
Fe	Andean landraces[♦]	B	74	29.8–154.9	Andre *et al.* (2007)
Fe	*S. tuberosum* breeding clones/varieties	B	38	18.0–65.0	Brown (2008)
Fe	*S. tuberosum* breeding clones/varieties	F	33	16.1–62.6	Brown *et al.* (2010)
Fe	Germplasm accessions*	G	37;	13.6 – 36.7;	Burgos *et al.* (2007)

	& cultivars		12	9.4–25.2	
Fe	*S. tuberosum* cultivars[¥]	B	8	7.2–11.2	Casañas-Rivero *et al.* (2003)
Fe	*S. tuberosum* clones/varieties[λ]	H	315; 280	11.2–27.0; 12.3–30.8	CIP & HarvestPlus (2011)
Fe	*S. tuberosum* cultivars	B	8	67.2–97.2	Ekin (2011)
Fe	*S. tuberosum* cultivars	C	10	60.0–162.0	Ereifej *et al.* (1998)
Fe	Potato clones (Atlantic & 17 tetraploid clones)	E	18	41.5–53.0	Haynes *et al.* (2012)
Fe	Andean cultivars	B	21	31.0–48.0	Lefevre *et al.* (2012)
Fe	*S. tuberosum* varieties[^]	D	9	15.5–63.0	Luis *et al.* (2011)
Fe	*S. tuberosum* cultivars	B	16	48.9–72.6	Öztürk *et al.* (2011)
Fe	*S. tuberosum* varieties	A	26	32.3–91.9	White *et al.* (2009)
Mn	*S. tuberosum* cultivars[¥]	B	8	1.2–2.1	Casañas-Rivero *et al.* (2003)
Mn	*S. tuberosum* cultivars	B	8	7.77–9.94	Ekin (2011)
Mn	*S. tuberosum* cultivars	C	10	6.0–11.0	Ereifej *et al.* (1998)
Mn	Potato clones (Atlantic & 17 tetraploid clones)	E	18	8.3–12.9	Haynes *et al.* (2012)
Mn	Andean cultivars	B	21	4.5–11.5	Lefevre *et al.* (2012)
Mn	*S. tuberosum* varieties[^]	D	9	5.0–10.0	Luis *et al.* (2011)
Mn	*S. tuberosum* cultivars	B	16	6.9–13.1	Öztürk *et al.* (2011)
Mn	*S. tuberosum* varieties	A	26	5.1–7.4	White *et al.* (2009)
Na	*S. tuberosum* cultivars[¥]	B	8	17.1–66.6	Casañas-Rivero *et al.* (2003)
Na	*S. tuberosum* cultivars	C	10	851.0–2217.0	Ereifej *et al.* (1998)
Na	Andean cultivars	B	21	85.0–210.0	Lefevre *et al.* (2012)
Na	*S. tuberosum* varieties[^]	D	9	323.5–1175.0	Luis *et al.* (2011)
Zn	Andean landraces[+]	B	74	12.6–28.8	Andre *et al.* (2007)
Zn	*S. tuberosum* breeding clones/varieties	B	38	12.5 – 20 .0	Brown (2008)
Zn	*S. tuberosum* breeding clones/varieties	F	36	12.3–16.7	Brown *et al.* (2011)
Zn	Germplasm accessions[*] & cultivars	G	37; 12	8.3 – 20.2; 9.5–14.8	Burgos *et al.* (2007)
Zn	*S. tuberosum* clones/varieties[ξ]	H	315; 286	6.3–32.5; 8.9–25.1	CIP & HarvestPlus (2011)
Zn	*S. tuberosum* cultivars[¥]	B	8	2.2–5.1	Casañas-Rivero *et al.* (2003)
Zn	*S. tuberosum* cultivars	B	8	14.1–15.94	Ekin (2011)
Zn	*S. tuberosum* cultivars	C	10	15.0–27.0	Ereifej *et al.* (1998)
Zn	Potato clones (Atlantic & 17 tetraploid clones)	E	18	17.6–25.9	Haynes *et al.* (2012)
Zn	Andean cultivars[π]	B	21	20.0–52.0	Lefevre *et al.* (2012)
Zn	*S. tuberosum* varieties[^]	D	9	16.5–26.0	Luis *et al.* (2011)
Zn	*S. tuberosum* cultivars	B	16	13.8–18.9	Öztürk *et al.* (2011)
Zn	*S. tuberosum* varieties	A	26	7.2–17.2	White *et al.* (2009)
Zn	*S. tuberosum* varieties	A	23	9.2–13.0	White *et al.* (2012)

[§] Studies involving genotypes equal to or greater than eight are reported here. Each trial comprised n genotypes grown under identical conditions; "Concentrations of N, K, Mg, P and S in mg g^{-1} DW; Ca, Cu, Fe, Mn, Na and Zn in µg g^{-1} DW; TSM-Tuber sampling method. A-Two opposite sections from each half of a tuber with skin; B-Whole tubers; C-Peeled tuber; D-Peeled pulp tissues; E-Pith tissue; F-Transverse section of unpeeled tubers; G-Two opposite longitudinal sections of peeled tubers & H-Peeled tubers, information on tuber sampling method is not available. [¥]Subspecies - Andigena & Tuberosum and Solanum. x Chaucha; ^ Calculated to DW basis assuming 20% tuber DM content; [*]Landraces at CIP core collection of 8 taxonomic groups (Ajanhuiri group, Andigenum group, Chaucha group, Chilotanum group, Curtilobum group, Juzepczukii group, Phureja group, Stenotonum group); ^18 wild species & 3 cultivated species; *5 taxonomic series (Phureja, Andigena, Tuberosum, Goniocalyx, Stenotomum, Chaucha); [λ]Andigena, Goniocalyx, Phureja, Stenotomum; [£]Chaucha, Andigena, Goniocalyx, Phureja, Stenotomum; [π]The study was conducted under control and drought conditions and the values reported here were the controls.

CHAPTER 5 VARIATION IN TUBER MINERAL COMPOSITION IN A TETRAPLOID MAPPING POPULATION

5.1 INTRODUCTION

Genetic variability for tuber mineral concentrations is a prime requirement for a successful breeding programme aiming at developing mineral-rich potato varieties. Extensive genetic variation for tuber mineral concentrations was discovered in potato germplasms (Chapter 4), paving the way for improving this trait through plant breeding efforts. A thorough understanding of the genetic control of tuber mineral accumulation as well as its relationship with other plant traits would greatly benefit plant breeding programmes that aim at enhancing mineral concentrations in tubers. Yet, this information is still lacking in the published literature and there have been no reports of QTL analysis for mineral accumulation in potatoes or in other members of the Solanaceae family.

In this study, an attempt was made to understand the genetic basis of tuber mineral variation using an F_1 tetraploid mapping population, 12601ab1 x Stirling (also known as GenPop1), comprising of 190 progenies. The mapping population was grown in replicated field trials over a three-year period (2007–2009), and tuber mineral concentrations were measured by ICP-MS. In this chapter, statistical analyses are presented on plant developmental traits, such as plant emergence and maturity, and tuber traits, such as tuber yield, DM and mineral concentrations. These data were subsequently used to identify QTL(s) that affect the phenotypic expression of the measured traits (Chapter 8). The phenotypic measurements of the two parents and the 190 clones of the mapping population are discussed in this chapter.

The mapping population originally consisted of 228 clones (Bradshaw *et al.*, 2008) and has been maintained in the field for more than 10 years. Hence, the identity of the clones was first verified by comparing the molecular marker scores and phenotypic expressions for each clone (Appendix III). This

way, the original identities of many clones were confirmed and those with uncertain identity were removed from further study. In total, 38 clones were removed, leaving the 190 clones for use in the study presented here. More details on the verification of the clones' identity are given in Appendix III.

5.2 RESULTS

5.2.1 Phenotypic variation among the clones

Phenotypic variation was studied among the 190 clones confirmed to be true to type in the 12601ab1 x Stirling mapping population (Appendix III). In 2007, tuber mineral concentrations were analysed separately in JHI and HRI using different mineral analysis procedures (Chapter 2; section 2.4.1). The results revealed similar trends between the two methods with high correlation coefficients (Table 5.1); however, statistical analysis showed significant differences in mineral concentrations between these two analyses (results not shown). Therefore, the data from each procedure were analysed separately and subjected to QTL analysis individually (Chapter 8).

Statistical (REML) analysis for plant emergence, maturity, tuber yield, DM, and mineral concentrations showed significant variations among the clones studied (Table 5.2). This was true for the measurements made at JHI during 2007, except for Ni (not shown). The analyses also revealed significant year differences for the measured variables except for P, Cu, Fe, Mn and Zn (Table 5.2). Further, a significant genotype by year interaction was noted for all the variables except for Fe (Table 5.2). Therefore, the phenotypic data from the clones were analyzed and presented separately for each year (Table 5.3), and the mean values for each clone and for each variable were summarized in Appendix Tables A5.1 to A5.5. The data were normally distributed for all the measured variables (Appendix Figure A5.1).

Table 5.1: Correlations between the mineral concentrations of mapping population analysed at JHI and HRI during 2007

	Ca	K	Mg	P	S	Cu	Fe	Mn	Zn
JHI vs HRI[€]	0.89	0.87	0.91	0.86	0.74	0.75	0.77	0.92	0.87

[€] Correlations significant at P≤0.001.

Table 5.2: REML analysis of the plant emergence, maturity, tuber yield, DM and mineral traits in 12601ab1 x Stirling tetraploid mapping population grown in replicated field trials during 2007 – 2009[≠]

Source of variation	Emer[Ø]	Mat[+]	Yield	DM	N	Ca	K	Mg	P	S	B	Cu	Fe	Mn	Na	Zn
Year	***	***	***	*	***	***	*	***	ns	*	**	ns	ns	ns	**	ns
Clone	***	***	***	***	***	***	***	***	***	***	**	***	***	***	***	***
Clone* Year	***	*	***	**	*	***	***	***	***	***	***	***	ns	*	***	***

[≠] Based on HRI mineral values; [Ø]Plant emergence; [+]Plant maturity; Significance of the effects is given in three levels: * P≤0.05; ** P≤0.01; ***P≤0.001; ns-non-significant.

Table 5.3: Summary data for plant emergence, maturity, tuber yield, DM and mineral traits for parents and F_1 progeny

Trait		2007			2008			2009		
		12601ab1 mean	Stirling mean	Progeny range	12601ab1 mean	Stirling mean	Progeny range	12601ab1 mean	Stirling mean	Progeny range
Plant development traits										
Emer[Ø]		2.67	1.83	0.95–7.46	-	-	-	4.99	3.67	0.95–7.55
Mat[+]		4.66	4.15	2.49–7.55	-	-	-	5.21	5.21	2.51–8.58
Tuber traits										
Yield_	kg	12.5	22.1	3.69–23.0	11.0	17.0	3.25–19.4	11.4	14.0	2.14–18.7
DM*	%	-	-	-	22.8	16.9	13.2–22.7	23.0	17.0	14.6–24.3
N	mg g^{-1}	10.78	9.56	6.29–18.1	9.88	8.25	6.01–18.0	11.2	13.2	8.98–20.9
Ca	mg g^{-1}	0.38 *(0.34)*	0.41 *(0.36)*	0.23–0.71 *(0.22–0.64)*	0.43	0.46	0.25–0.77	0.37	0.34	0.20–0.65
K	mg g^{-1}	21.4 *(21.1)*	23.5 *(23.1)*	20.0–29.4 *(19.1–29.8)*	22.6	22.1	19.0–29.6	22.0	23.8	18.7–30.9
Mg	mg g^{-1}	1.24 *(1.25)*	1.10 *(1.15)*	0.76–1.58 *(0.78–1.66)*	0.95	0.82	0.64–1.25	1.05	1.04	0.86–1.40
P	mg g^{-1}	1.71 *(1.73)*	1.75 *(1.75)*	1.01–2.50 *(0.99–2.34)*	1.74	1.89	1.21–2.40	1.48	1.85	1.08–2.61
S	mg g^{-1}	1.18 *(0.99)*	1.38 *(1.05)*	0.81–1.89 *(0.63–1.74)*	1.02	1.29	0.77–1.74	0.96	1.30	0.85–1.78
B	µg g^{-1}	4.25	6.33	3.75–7.16	4.07	5.50	3.46–6.62	4.89	6.13	4.16–6.98
Cu	µg g^{-1}	4.25 *(4.37)*	5.57 *(5.73)*	2.94–7.41 *(2.51–8.39)*	5.01	6.24	3.70–8.12	4.51	7.22	2.79–8.19
Fe	µg g^{-1}	28.2 *(21.5)*	36.5 *(25.1)*	24.5–50.0 *(19.2–38.2)*	33.6	39.4	30.2–56.3	29.7	38.6	25.0–54.1
Mn	µg g^{-1}	4.96 *(5.02)*	5.33 *(5.26)*	3.52–7.61 *(3.46–7.65)*	4.61	4.84	3.51–6.90	4.62	5.15	3.58–7.79
Ni	µg g^{-1}	- *(0.29)*	- *(0.22)*	- *(0.05–0.46)*	-	-	-	-	-	-
Zn	µg g^{-1}	9.88 *(10.6)*	11.0 *(11.9)*	6.63–17.2 *(7.76–19.5)*	9.98	10.7	7.92–18.5	9.29	12.9	7.83–15.9
Na	µg g^{-1}	16.3	10.8	8.13–28.4	22.5	16.9	11.9–39.1	22.6	18.4	11.8–38.9

[Ø]Plant emergence; [+]Plant maturity; Mineral concentration on DW basis; Values in brackets are mineral values measured at SCRI during 2007.

The broad-sense heritability estimates were generally high between 75 and 94% (except for Fe, 56%) of the variation in clone means over years and replicates due to genetic differences between clones (Table 5.4).

Table 5.4: Components of variance σ^2_c, σ^2_{cy} and σ^2 for clones, clones x years interaction and residual variation and heritability (H^2)

Trait	σ^2_c	σ^2_{cy}	σ^2	H^2
Plant development traits				
Emergence	2.04240	0.29740	0.80000	0.8581
Maturity	0.98240	0.05940	0.45800	0.8732
Tuber traits				
Yield	10.51800	0.99500	2.40900	0.9351
DM	1.80000	0.21200	1.67000	0.7832
N	0.02335	0.00151	0.03030	0.8074
Ca	0.00006	0.00000	0.00004	0.8783
K	0.02451	0.00247	0.02430	0.8322
Mg	0.00009	0.00002	0.00008	0.8111
P	0.00035	0.00005	0.00040	0.8029
S	0.00020	0.00005	0.00017	0.8137
B	0.22180	0.03000	0.17200	0.8433
Cu	0.41200	0.04610	0.52700	0.7910
Fe	6.72000	-1.14000	31.27000	0.5627
Mn	0.26370	0.01120	0.31600	0.8207
Zn	1.40700	0.39900	2.11200	0.7463
Na	12.99000	3.39000	11.57000	0.8013

In addition, significant correlations between years were found for all the variables among the three study years (2007, 2008 and 2009) (Table 5.5). The two parental lines, Stirling and 12601ab1, had similar scores for plant emergence, maturity, tuber N, Ca, K, Mg, P, S, Mn and Ni concentrations, but differed for tuber yield, DM, B, Cu, Fe, Zn and Na concentrations, with Stirling having higher tuber yield, B, Cu, Fe, and Zn, and lower DM and Na than 12601ab1 (Table 5.3).

Table 5.5: Correlation coefficients (r) between years for plant emergence, maturity, tuber yield, DM and mineral concentrations (mean values) of 190 clones in the 12601ab1 x Stirling mapping population[€]

Source of variation	2007 vs 2008	2007 vs 2009	2008 vs 2009
Emer[Ø]	-	0.76	-
Mat[+]	-	0.79	-
Yield	0.85	0.79	0.81
DM	-	-	0.62
N	0.65	0.47	0.5
Ca	0.69	0.72	0.66
K	0.65	0.64	0.59
Mg	0.77	0.48	0.54
P	0.62	0.48	0.59
S	0.73	0.49	0.55
B	0.71	0.55	0.61
Cu	0.61	0.46	0.58
Fe	0.35	0.24	0.32
Mn	0.64	0.58	0.61
Na	0.56	0.63	0.6
Zn	0.63	0.34	0.43

[€]Allcorrelations significant at P≤0.001; [Ø]Plant emergence; [+]Plant maturity.

5.2.2 Relationships among plant emergence, maturity, tuber yield, DM, and mineral concentrations

PCA analysis was performed on all the phenotypic traits measured on the 190 clones of the mapping population. The analysis revealed similar patterns when analysed for each year separately (Appendix Figure A5.2), and therefore a generalised PCA analysis was performed on the data pooled across the three years (Figure 5.1). When considering all the measured variables, the first two principal components accounted for 56.0% of the total variation (Figure 5.1A), with PC1 and PC2 individually explaining 32.5 and 23.5% of the total variation respectively. The PC1 was negatively associated with plant emergence, maturity, tuber yield and DM, and positively with all the 12 mineral elements. The PC2, on the other hand, was negatively associated with tuber yield, DM, Mg, S, Mn, N and B, and positively with the rest of the variables.

The negative associations between the mineral elements and tuber yield or DM revealed by the biplots (Figure 5.1A) were further confirmed by the correlation analysis (Table 5.6). The biplots also revealed that plant maturity was negatively associated with tuber yield, DM, Mg, S, Mn, N, B and Zn and positively with plant emergence, tuber Na, Ca, K, P and Fe. In addition, plant emergence showed positive associations with yield and DM, and negative associations with the different mineral elements (Figure 5.1A).

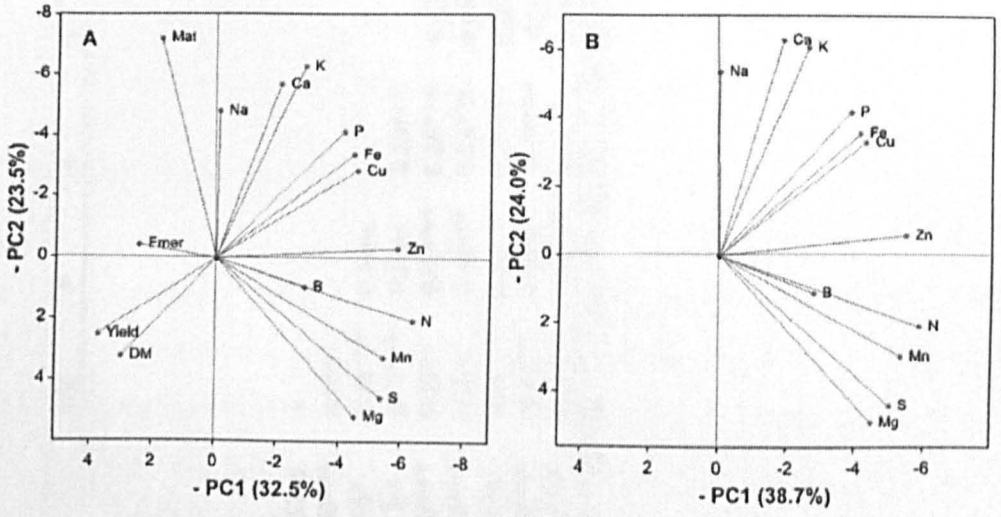

Figure 5.1: PCA biplots for phenotypic variables estimated on 190 clones of the mapping population over three years: (A) all measured traits; (B) mineral traits only.

When the PCA analysis was performed on mineral traits only (Figure 5.1B), the PC1 explained 38.7% of the total variance, while the PC2 explained 24.0% of the variance. The biplots for tuber mineral concentrations either with or without plant emergence, maturity, tuber yield and DM (Figure 5.1A and B) revealed four distinct clusters of minerals: (1) Na, Ca and K, (2) P, Fe and Cu, (3) Zn and (4) B, N, Mn, S and Mg (Figure 5.1; Appendix Figure A5.2). This pattern of PCA grouping was further supported by the presence of significant positive or negative correlations within and among these mineral clusters, as revealed by F correlation coefficients (Table 5.6). Correlation analyses were also performed among all the variables separately for each year (Appendix Table A5.6).

Table 5.6: F correlation coefficients (r) among plant emergence, maturity, tuber yield, DM and mineral concentrations (HRI measurements) in progenies of 12601ab1 x Stirling mapping population tested over three years (2007–2009)

	Emer[Ø]	Mat[+]	Yield	DM	N	Ca	K	Mg	P	S	B	Cu	Fe	Mn	Na
Mat[+]	0.09ns	-													
Yield	**0.49*** **	-0.13ns	-												
DM	0.02ns	-0.25***	0.10ns	-											
N	**-0.32*** **	-0.41***	**-0.43*** **	**-0.36*** **	-										
Ca	-0.08ns	**0.42*** **	**-0.35*** **	**-0.20** **	0.08ns	-									
K	0.04ns	**0.58*** **	**-0.32*** **	**-0.56*** **	**0.17***	**0.45*** **	-								
Mg	-0.13ns	**-0.69*** **	-0.03ns	**-0.06ns**	**0.70*** **	**-0.20** **	-0.12ns	-							
P	-0.10ns	**0.28*** **	**-0.38*** **	**-0.39*** **	**0.34*** **	0.31***	**0.66*** **	0.09ns	-						
S	**-0.33*** **	**-0.66*** **	**-0.28*** **	**-0.09ns**	**0.81*** **	-0.13ns	-0.15*	**0.78*** **	0.11ns	-					
B	-0.08ns	-0.14*	0.01ns	**-0.30*** **	**0.35*** **	0.04ns	0.13ns	**0.32*** **	0.12ns	**0.29*** **	-				
Cu	-0.16*	0.08ns	**-0.39*** **	**-0.23** **	**0.38*** **	**0.37*** **	**0.47*** **	**0.19** **	**0.69*** **	**0.26*** **	0.13ns	-			
Fe	-0.15*	0.17*	**-0.36*** **	**-0.41*** **	**0.43*** **	**0.44*** **	**0.52*** **	0.05ns	**0.44*** **	**0.28*** **	**0.22** **	**0.37*** **	-		
Mn	-0.13ns	**-0.50*** **	**-0.15***	-0.13ns	**0.75*** **	0.03ns	0.06ns	**0.78*** **	**0.26*** **	**0.71*** **	**0.41*** **	**0.30*** **	**0.35*** **	-	
Na	0.02ns	**0.36*** **	-0.12ns	**-0.16***	-0.09ns	**0.66*** **	**0.32*** **	**-0.21** **	0.07ns	-0.24***	-0.03ns	0.07ns	**0.19** **	-0.16*	-
Zn	-0.22**	-0.18*	**-0.48*** **	**-0.28*** **	**0.71*** **	**0.31*** **	**0.27*** **	**0.46*** **	**0.41*** **	**0.61*** **	0.12ns	**0.58*** **	**0.52*** **	**0.53*** **	0.03ns

[Ø]Plant emergence; [+]Plant maturity; Significance of the effects is given in three levels: * P≤0.05; ** P≤0.01; ***P≤0.001; ns-non-significant.; Bolded values are significant correlations over two or three years (refer Appendix Table A5.6).

5.3 DISCUSSION

The primary aim of this work was to analyse the tuber mineral concentrations in an existing F_1 tetraploid mapping population (12601ab1 x Stirling). This mapping population was developed originally for studying disease resistance and tuber quality traits (Bradshaw *et al.* 1995, 2004). It was unsurprising, therefore, that the results from this study showed that the variation between parents was small for most minerals except for B, Cu, Fe, Zn and Na (Table 5.3 and 5.7). However, the progenies showed a great variation for mineral concentrations, indicating the complex underlying mechanisms involved in mineral homeostasis in tubers.

5.3.1 Trait distributions and heritabilities

All the traits exhibited a near normal distribution (Appendix Figure 5.1), which enabled the execution of QTL analysis (Chapter 7) on the original trait scores without the need for data transformation. The heritability scores for all traits were generally high (Table 5.4), indicating that this mapping population is ideal for selection as well as identification of the QTLs associated with mineral traits in potatoes. A 1.5 to 3.0 fold variation in the concentration of tuber minerals has been found in the mapping population (Table 5.7). Furthermore, the population means were similar to that of the two parents, but the release of genetic variation can be seen in the large population ranges (Table 5.7), indicating the presence of transgressive segregation, suggesting that these traits may be under complex genetic control.

Table 5.7: Parents and F1 population mean and extremes for various traits in the 12601ab1 x Stirling mapping population measured over three years (2007–2009)*

Trait		12601ab1 mean	Stirling mean	Population mean	Population extremes	Ratio^
Plant development traits						
Emer[Ø]		3.83	2.75	3.67	0.98–7.03	7.2
Mat[+]		4.94	4.68	5.11	2.57–7.81	3.0
Tuber traits						
Yield	kg	11.61	17.71	13.02	3.21–19.80	6.2
DM	%	22.91	16.95	18.87	15.02–22.88	1.5
N	$mg\ g^{-1}$	10.62	10.33	11.39	7.96–19.98	2.3
Ca	$mg\ g^{-1}$	0.39	0.40	0.43	0.24–0.71	2.9
K	$mg\ g^{-1}$	21.98	23.13	23.64	19.66–29.42	1.5
Mg	$mg\ g^{-1}$	1.08	0.99	1.03	0.80–1.37	1.7
P	$mg\ g^{-1}$	1.64	1.83	1.77	1.23–2.32	1.9
S	$mg\ g^{-1}$	1.05	1.32	1.19	0.88–1.76	2.0
B	$\mu g\ g^{-1}$	4.41	5.99	5.20	3.82–6.74	1.8
Cu	$\mu g\ g^{-1}$	4.59	6.34	5.30	3.62–8.11	2.2
Fe	$\mu g\ g^{-1}$	30.51	38.15	38.21	30.44–49.10	1.6
Mn	$\mu g\ g^{-1}$	4.73	5.11	5.03	3.74–7.42	2.0
Na	$\mu g\ g^{-1}$	20.47	15.37	20.21	11.76–35.45	3.0
Zn	$\mu g\ g^{-1}$	9.72	11.52	11.05	8.54–15.97	1.9

*Mineral concentrations based on HRI measurements (DW basis); [Ø]Plant emergence; [+]Plant maturity; ^Ratio between lowest and highest plant and tuber traits.

5.3.2 Trait correlations

PCA and correlation analyses performed on 190 clones of the mapping population revealed positive associations among the different mineral elements (Figure 5.1; Table 5.6). This perhaps points to the existence of common mechanisms controlling the uptake and metabolism of minerals occurring within each cluster. The analysis also revealed that early maturity of plants is associated with high concentrations of tuber Mg, S, Mn, N, B and Zn and low concentrations of tuber Ca, K and Na (Figure 5.1; Table 5.6). Thus, selecting cultivars from specific maturity groups may be a useful strategy for selecting for associated mineral elements. The significant negative associations found between tuber DM content and mineral concentrations might be due to the dilution effects caused by starch accumulation. A highly

significant positive correlation (r = 0.49) between early plant emergence and tuber yield was detected (Table 5.6), in agreement with Bradshaw *et al.* (2008). As such, selection for high tuber yields may target the genotypes that exhibit rapid emergence after planting. However, significant negative correlation was found between tuber yield and mineral concentrations (Table 5.6). Therefore, while breeding for enhanced mineral concentrations in tubers, special attention should be given to avoid negative effects on yield. Furthermore, PCA and correlation analyses (Figure 5.1B; Table 5.6) revealed a similar pattern of associations among the different mineral elements as observed in the NTB population (Figure 4.9B; Appendix Table A4.12). This suggests that this mapping population captures a good representation of the variation available in the diverse set.

In summary, the results of the trait analyses suggest the presence of transgressive segregation for most traits and also point to the existence of complex genetic mechanisms controlling these traits. Moreover, the associations among the mineral traits could be linked to the physiological relationships among these traits. These relationships could be a result of physical linkage of genes on the same chromosome, pleiotropic effect of a gene affecting the traits or common pathways shared among minerals. However, the exact mechanisms for such associations are largely unknown. These knowledge gaps can in fact be filled by identifying the genetic loci affecting these traits on a linkage map (explored in Chapter 7). The results from this study will greatly assist the selection for useful mineral traits through plant breeding programmes in potatoes.

CHAPTER 6 BULKED SEGREGANT ANALYSIS TO IDENTIFY MOLECULAR MARKERS LINKED TO CALCIUM, IRON AND ZINC ACCUMULATION IN POTATO TUBERS

6.1 INTRODUCTION

Bulked segregant analysis (BSA) is a widely used efficient method to identify molecular markers in specific regions of a genome linked to a trait of interest (Michelmore *et al.*, 1991). This method involves genotyping two pools (bulks) of DNA, each pool containing samples from many individuals with a similar phenotype, and with the individual's contribution to one pool having a different phenotype to the individiuals contributing to the other pool for a specific trait from the tails of the distribution in a population. The differential presence of a marker allele in a pool indicates close genetic linkage to allele(s) controlling the phenotype initially used for pool construction. BSA has been very useful for tagging genes with major phenotypic effects and it has been successfully used in a number of crop species for various traits of interest, including disease resistance (Michelmore *et al.*, 1991), abiotic stress (Quarrie *et al.*, 1999) and tolerance to mineral toxicity (Patterson *et al.*, 2007).

In potatoes, the BSA approach has been used in both diploid and tetraploid populations to identify DNA markers linked to potato cyst nematode (PCN) resistance (Bryan *et al.*, 2004; Bakker *et al.*, 2004; Draaistra, 2006; Jacobs *et al.*, 1996), resistance to potato virus X (PVX) (Bendahmane *et al.*, 1997; De Jong *et al.*, 1997; Heldak *et al.*, 2009), PVY (Flis *et al.*, 2005; Heldak *et al.*, 2007; Hosaka *et al.*, 2001; Song, 2004), PVM (Marczewski *et al.*, 2006), *Phytophthora infestans* (van der Lee *et al.*, 2001; Li *et al.*, 1998, Wickramasinghe *et al.*, 2009), PVS (Marczewski *et al.*, 1998), anther culture response (Boluarte-Medina and Veilleux, 2002), tuber quality traits (Kloosterman *et al.*, 2010) and tuberisation (Fernandez-del-Carmen *et al.*, 2007). A few studies have used BSA analysis to identify DNA markers linked to the accumulation of minerals such as cadmium (Jegadeesan *et al.*, 2010; Penner *et al.*, 1995) and Zn (Sadeghzadeh *et al.*, 2010) in edible parts of staple crops.

Molecular markers that are closely associated with mineral accumulation in potato tubers have great potential in marker assisted selection for enhanced tuber mineral concentrations.

The objectives of the present study were to (1) identify the SSR markers that are closely linked to gene(s) controlling high mineral (particularly Ca, Fe and Zn) accumulation in potato tubers in a F_1 tetraploid mapping population (GenPop1), and (2) identify genome-linked DArT markers in the diverse tetraploid Andean NTB population that are linked to Ca, Fe and Zn concentrations, and tuber yield. The first of these objectives was adopted prior to the availability of high throughput marker systems in potato, and was intended to identify additional markers which would then be explored in the full mapping population. The second was an attempt to locate genome locations of genes using association mapping, which had become possible as a result of the availability of a DArT marker panel. A summary of polymorphic SSRs and DArT markers found in the mapping and NTB populations is given in this Chapter and these results will be discussed in Chapter 7 with particular reference to yield and mineral QTLs identified in the mapping population.

6.2 BULKED SEGREGANT ANALYSIS

6.2.1 Preparation of DNA bulks for the mapping population

For the 12601ab1 x Stirling mapping population, four DNA bulks (two bulks each from individuals with low or high concentration of a particular mineral element) were constructed for tuber Fe, Zn and Ca concentrations and were screened together with the parental lines using SSR markers. Ten non-duplicated clones, chosen using the spatially-adjusted means of 190 clones of the tetraploid mapping population were selected in each bulk. Data pertaining to tuber mineral concentrations from three study years (2007–2009) were used to select clones to be pooled for BSA (Appendix Tables 5.3-5.5, Chapter 5). The clones within the mapping population showed 3.0, 1.6, and 2.0, fold variation for tuber Fe, Zn and Ca

concentrations, respectively (Table 5.6, see Chapter 5). Firstly, the clones were ranked by trait means (low–high) for each year, and subsequently, clones falling in the lower or upper 15 to 20% were identified for each year. The clones were then selected based on their average ranking during the three years for low and high mineral values and also based on the consistency of values obtained in different years. The first 10 clones falling in the low and high extremes for mineral traits were labelled as L1 and H1, and the next 10 clones as L2 and H2, respectively (Table 6.1). Therefore, there were 12 bulks in total for three mineral elements (two bulks each for low and high Fe, Zn and Ca), and were labelled as low Ca (Ca-L1, Ca-L2), low Fe (Fe-L1, Fe-L2), low Zn (Zn-L1, Zn-L2), high Ca (Ca-H1, Ca-H2), high Fe (Fe-H1, Fe-H2) and high Zn (Zn-H1, Zn-H2). The mean and range of mineral concentrations of the parents and the clones are given in Table 6.1, and the mean and range values of tuber yield for clones that were used in constructing mineral bulks are presented in Table 6.2. Generally, tubers with a low yield showed high concentrations of Ca, Fe and Zn, and tubers with high yield displayed low mineral concentrations (Table 6.1 and 6.2).

Genomic DNA was extracted from individual clones from leaf tissues using a Qiagen DNeasy Plant Mini Kit (as described in section 2.5.1) in conjunction with a QIAcube apparatus (from Qiagen) for all samples. The DNA was quantified by electrophoretic comparison with a λ-DNA standard and by fluorometric measurement using the Picogreen quantitation method (Invitrogen, Paisley, UK). The DNA of each individual was then diluted with sterile distilled water to obtain a final concentration of 20 ng μl^{-1}. Equal volumes of the diluted DNA were combined to prepare bulks.

6.2.2 Preparation of DNA bulks for the NTB population

BSA using DArT technology as described by Wenzl *et al.* (2007) was employed to identify putative genomic regions and markers associated with tuber Ca, Fe and Zn concentrations and tuber yield in the NTB population. To minimize false positive and false negative results from BSA, 30 clones per bulk were used. Two bulks (from

clones with low or high concentrations of a specific mineral elements), each comprising of 30 clones were selected using means from the spatially explicit REML analysis of field trial data. The clones in the NTB population showed a 45.3, 5.0, 4.2 and 3.1 fold variation for tuber yield, Ca, Fe and Zn concentrations, respectively (Table 4.9, see Chapter 4). Ten bulks were prepared in total, with eight bulks for low and high concentrations of the four tuber traits (Fe, Zn, Ca and yield) and two random bulks. Random bulks were prepared from two randomly assorted DNA pools. Individuals in each trait bulk were selected based on phenotypic data from field trials conducted in 2005 (Appendix Table A4.10, Chapter 4) and also on the availability of DNA material for the clone. DNA samples were kindly provided by Gaynor McKenzie (Research assistant, JHI).

Table 6.1: Mean and range of tuber Ca, Fe and Zn concentrations (DW basis) for potato clones used for constructing the bulks in 12601ab1 x Stirling population clones^

Trait	Parents		High bulk						Low bulk					
	12601ab1	Stirling	H1			H2			L1			L2		
	Mean		Mean	S.E	Range	Mean	S.E	Range	Mean	S.E	Range	Mean	S.E	Range
Ca ($mg\ g^{-1}$)	0.39	0.40	0.60	0.014	0.55–0.71	0.54	0.005	0.52–0.57	0.28	0.007	0.24–0.31	0.32	0.004	0.30–0.33
Fe ($\mu g\ g^{-1}$)	30.51	38.15	46.55	0.95	45.90–49.10	43.49	0.46	40.47–45.87	32.36	0.50	30.44–33.82	33.41	0.20	32.31–34.30
Zn ($\mu g\ g^{-1}$)	9.72	11.52	14.33	0.26	13.16–15.97	13.09	0.20	12.31–14.05	8.96	0.07	8.54–9.31	9.30	0.10	8.7–9.64

^Ten clones per bulk. Mean concentrationsfrom 2007, 2008 and 2009 trials; L1-low trait bulked DNA; L2-second low trait bulked DNA; H1-high trait bulked DNA; H2-second high trait bulked DNA.

Table 6.2: Mean and range of tuber yields for potato clones used for constructing Ca, Fe and Zn bulks

Yield ($kg\ plot^{-1}$)														
Parents		Mineral bulks	High bulk						Low bulk					
12601ab1	Stirling		H1			H2			L1			L2		
Mean			Mean	S.E	Range	Mean	S.E	Range	Mean	S.E	Range	Mean	S.E	Range
11.61	17.71													
		Ca	11.07	1.16	5.11–15.95	12.00	1.24	4.83–16.05	15.16	0.59	12.51–17.69	14.43	0.81	8.75–17.76
		Fe	8.75	0.91	3.21–12.14	12.37	1.16	6.48–17.14	13.06	0.90	6.45–16.72	14.30	0.84	10.00–17.69
		Zn	7.42	0.94	3.21–11.98	12.41	0.91	6.98–16.29	15.49	0.55	13.01–18.59	14.58	0.69	10.00–17.69

The DNA concentration was measured with a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA) and was diluted to a final concentration of 20 ng μl^{-1}. Equal volumes of the diluted DNA were combined to prepare the bulks. Two replicates of each bulk were sent to Diversity Arrays Technology, Pty Limited, Australia to perform the DArT-BSA assay as described by Wenzl *et al.* (2007). The mean concentration and range of Fe, Zn, Ca and yield for different bulks (30 clones/bulk) in the NTB population are given in Table 6.3.

Table 6.3: Mean and range of tuber yield, Ca, Fe and Zn concentrations of potato clones used for constructing the bulks in the Neotuberosum population (30 clones/bulk)

		High bulk			Low bulk		
	Trait	Mean	S.E	Range	Mean	S.E	Range
Yield	(kg $plot^{-1}$)	11.92	0.40	9.65–19.91	1.25	0.09	0.44–2.17
Ca	(mg g^{-1})	0.63	0.01	0.55–0.83	0.22	0.005	0.17–0.27
Fe	(µg g^{-1})	91.24	3.12	70.32–130.4	41.23	0.68	32.42–51.04
Zn	(µg g^{-1})	28.93	0.55	24.76–36.73	13.91	0.14	11.90–15.14

About 7400 DArT markers were tested for polymorphism between the low and high DNA bulks for tuber yield, Ca, Fe and Zn concentrations in the NTB population (Table 6.7). DArT data were analyzed by examining the hybridisation intensity of low and high trait bulks to individual DArT markers. Markers with the largest difference in hybridisation intensity between the bulks were considered to have high probability of association with mineral traits. Each of the DNA bulks was independently analyzed up to five times and the data were subjected to a t-test. Markers associated with a p-value less than or equal to 0.0001 were considered significant and polymorphic between the low and high traits.

6.2.3 Bioinformatic analysis for putative candidate genes influencing Fe, Zn and Ca accumulation

A literature survey was performed to identify genes with a documented or putative function in mineral (particularly Fe, Zn and Ca) homeostasis and accumulation in plants. Efforts were also taken to include more markers for chromosome 5, which was identified during this study as a region of interest (Appendix Table A6.1). Potential candidate genes (genes with known or predicted function) reported in Arabidopsis and in *Solanaceae* that could influence tuber Fe, Zn and Ca concentrations were identified and their sequences were downloaded from GenBank (Appendix Table A6.1). Further, these were used as query sequences to search against the potato genome scaffolds (version 3) released by the Potato Genome Sequencing Consortium (PGSC). The sequences obtained from GenBank were searched using a local version of the BLAST similarity program, BLAST 2.2.18 (Altschul *et al.*, 1997). The scaffolds with low e-values (high sequence similarity, Appendix Table A6.1) were chosen for further analysis.

6.2.4 Identification of SSRs linked to candidate genes

SSRs in the selected genome scaffolds were identified using the Phobos® software, version 3.3.10 (Mayer 2009). Phobos is a highly accurate and fast search tool for finding microsatellites. Two to three primer pairs were designed targeting the SSRs within, or in close proximity to, the candidate gene hit regions in the scaffolds using the Primer 3® (multiple primer design) software. Primers were chosen with a length of 20–25 bases, an optimal annealing temperature of 55°C, GC content of 50% and an amplification product length ranging between 150 and 250 bp.

The primer sequences identified using the Phobos® software were verified for uniqueness within the scaffold, and other primers were chosen in those

regions if any sequence repeats were found. In cases where primers within the specified product size (150–250 bp) were not found, then the primers with product size ranging from 90–350 bp were identified and used. For each candidate gene, two to three flanking primers were designed, resulting in a total of 317 primer pairs. The resulting primer pairs with relevant scaffold and superscaffold information, primer sequences, and predicted product size are presented in Appendix Table A6.2. All primers were synthesised by Sigma-Aldrich Company Ltd (Poole, Dorset, UK). SSR analysis was performed as described in Section 2.5.3. The 317 SSR primer pairs were first used for screening the parents of the mapping population and the pairs that yielded polymorphic bands were then chosen for screening the mineral bulks. The individual primer pairs were named according to the respective scaffold number. For example, the SSR primer 589-2 indicates that it is the second primer designed from scaffold 589. The results of the SSR analysis are presented in Table 6.5 and Appendix Table A6.3.

6.3 RESULTS

6.3.1 BSA using SSRs in GenPop1 mapping population

Of the 317 SSR primers tested for polymorphism between the parents (Stirling and 12601ab1) of the mapping population, 187 (59%) produced polymorphic bands (Table 6.4; Appendix Table A6.2). Out of 187 parental polymorphic primers, 143 (76%) primers specific for mineral traits were screened on bulked DNA from low and high mineral (Ca, Fe and Zn) accumulating segregants for polymorphism. Markers showing polymorphism between the bulks are considered putatively linked to the target trait/candidate gene. Out of the 143 primer pairs tested, 37 (26%) primer pairs produced polymorphic bands between low and high mineral bulks (Table 6.4). A summary of the polymorphic SSRs found via BSA is given in Table 6.5. Bulks were scored as identical (0), as different as the two parents (1), or showing a clear difference

which did not match the difference between the parents (*) (Table 6.5). These polymorphic markers were distributed throughout the potato genome, except chromosomes IX and X (Table 6.5). Additionally, clones (used for preparing bulks), SSR markers and superscaffolds (identified after performing BSA) were shared among different mineral bulks (Table 6.6).

Table 6.4: Particulars of BSA in 12601ab1 x Stirling population using SSRs

Screening of SSR primers	No. of primers
Screening of parents	
Total SSRs screened	317
Successful polymorphic primers between parents	187
Monomorphic SSRs	78
Primers that produced non-specific product	19
Faint (low intensity) bands	10
PCR failure	9
No products	9
Amplification in only one of the parents	5
Screening of bulks (BSA)	
Successful polymorphic primers between parents	187
Total primers used for BSA	143
Polymorphic SSRs between bulks	37

Table 6.5: Results of bulked segregant analysis (BSA) using SSRs for tuber Fe, Zn and Ca concentrations in the 12601ab1 x Stirling mapping population

CHR^	Scaffold no.	Superscaff no.	Potential candidate genes	No. of SSR primers for BSA		Bulks[£]											
				Screened	Polymorphic	Ca-L1	Ca-L2	Ca-H1	Ca-H2	Fe-L1	Fe-L2	Fe-H1	Fe-H2	Zn-L1	Zn-L2	Zn-H1	Zn-H2
I	386	68	FRD3	3	2	0	0	0	1	0	0	0	0	0	0*	0	0
I	455	10	CaM, ECA2, CNGC, FRO	11	2	0	0	0	0	0	0	0	0	0*	0	0	1
I,III	1716	136	OPT4	2	1	0	0	0	0	0	0	0	0	0*	0	0	0
I	1743	412	CaM	1	1	0	0	0	0	0	0	0	0	0	0*	0	0
I	2734	92	ZIF1 & ZIFL1	2	2	0	0	0	0	0	0	0	0	0*	0	0	2
I,II	589	60	CRT, NAS, AtIREG3	6	4	0	0	1	0	0*	0	0	0	2	0	0	2
II	2386	12	LeNRAMP3 & HMA	1	1	0	1	0	0	0	0	0	1	0	0	1	0
II	874	490	ACA2	2	1	0	0	0	0	0*	0	0	0	0	0	0	0
II	903	569	LeOPT1	3	2	1*	0	0	0	1	1*	0	0	0	0	0	0
III	1131	39	YSL 7 & 8	2	1	0	0	0*	0	0	0	0	0	0	0	0	0
III	1571	126	BiP, YSL	3	1	0*	0	0	0	0	0	0	0	0	0	0	0
IV	187	189	VIT	2	1	0	0	0	0	0	0	0	0	0	0	0	0*
IV	249	185	Annexin	2	1	0	0	1	0	0	0*	0	0	0	0	0	0
IV	1089	249	ACA4	2	1	0	0	0	0	0	0	0	1	0	0	0	0
IV	1237	573	ACA	2	1	0	0	0	0	1	0	0	0	1	0	0	1
IV	1367	32	GLR 3.3, CUC3	3	1	1	0	0	0	0	0	0	0	0	0	0	0
V	154	202	ZIP & ferritin	6	3	0	0	1*	0	0	0*	0	0	0	0	0	0

VII	1893	666	CAX 4&5	1	1	0	0	0	0	0	1	0	0	0	0	0	0
VII	2050	300	NtMTP 1A &1B	3	1	0	0	0	0	0	0	0	0	0	0	0	1
VIII	64	342	ZIP4	1	1	0	0*	0	0	0	0	0	0	0	0	0	0
VIII	1525	48	BiP, YSL, ATOPT	3	1	0	0	1	0	0	0	0	0	0	0	0	0
VIII	7454	274	CBL1	3	2	0	0	1*	0	0	0	0	0*	0	0	0	0
XI	301	133	LeNRAMP1 & ATAF	3	2	1	0	0	0	0*	0	0	0	0	0	0	0
XII, III	827	376	Vacuolar H^+-ATPase , CAX6	3	1	0	0	0	0	0	0	0	0	0	0*	0	0
--	534	315	BiP, CBP4	5	2	0*	0	0	0	0	0	0	0	0*	0	0	0

^Chromosome number based on alignment of superscaffolds to pseudo-chromosomes; [£]L1-low trait bulked DNA, L2-second low trait bulked DNA, H1-high trait bulked DNA and H2-second high trait bulked DNA; 0-no difference between bulks for single traits, 1 or 2-one or two markers with difference between bulks as clear as in parents, and *clear bulk differences based on the intensity of bands between bulks. 0*- Out of two polymorphic markers, one is '0' with no difference between bulks for a trait, and the other is * with clear bulk differences based on the intensity of bands between bulks; 1*- Out of two polymorphic markers, one is '1' with clear difference between bulks as clear as in parents, and the other is * with clear bulk differences based on the intensity of bands between bulks; 2-two polymorphic SSRs from the same scaffold. For abbreviation of candidate genes see Appendix Table A6.1.

Table 6.6: Summary of shared input into bulks (number of shared clones in bulks, shown outside brackets) and shared markers differentiating bulks found during the study (numbers of markers; numbers of superscaffolds, SS, within brackets) in the mapping population

Mineral bulks	Shared clones (markers; SS)				
Low bulk					
	L1/L1	**L2/L2**	**L1/L2**	**L/L**	
Ca/Fe	1 (1; 1)	0 (0)	4 (2; 1)	5 (3; 2)	
Ca/Zn	1 (1)	2 (0)	3 (0)	6 (0)	
Fe/Zn	1 (3; 2)	3 (0)	3 (0)	7 (3; 2)	
Ca/Fe/Zn	0 (0)	0 (0)	3 (0)	3 (0)	
Ca/Ca	-	-	-	0 (2; 2)	
Fe/Fe	-	-	-	0 (1; 1)	
Zn/Zn	-	-	-	0 (0)	
High bulk					
	H1/H1	**H2/H2**	**H1/H2**	**H/H**	
Ca/Fe	2 (0)	0 (0)	4 (0)	6 (0)	
Ca/Zn	0 (0)	1 (0)	2 (1; 1)	3 (1; 1)	
Fe/Zn	5 (0)	2 (0)	1 (1; 1)	8 (1; 1)	
Ca/Fe/Zn	0 (0)	0 (0)	1 (0)	1 (0)	
Ca/Ca	-	-	-	0 (1; 1)	
Fe/Fe	-	-	-	0 (0)	
Zn/Zn	-	-	-	0 (1; 1)	
Low/high bulk					
	L1/H1	**L1/H2**	**L2/H1**	**L2/H2**	**L/H or H/L**
Ca/Fe	0 (0)	0 (0)	1 (2; 2)	0 (1; 1)	1 (3; 3)
Ca/Zn	1 (0)	2 (0)	0 (0)	0 (0)	3 (0)
Fe/Zn	0 (0)	0 (1; 1)	0 (0)	0 (0)	0 (1; 1)
Ca/Fe/Zn					0 (1; 1) L1/H2/H1
Ca/Ca	-	-	-	-	0 (0)
Fe/Fe	-	-	-	-	0 (0)
Zn/Zn	-	-	-	-	0 (2; 2)

Figures 6.1 and 6.2 provide examples of the potential marker loci (detected by BSA) that are linked to low or high mineral concentrations in bulks and their segregation patterns. Some of the SSR primer pairs produced band intensities that were less intense than the differences between the parents (e.g., SSR 589-2 and 589-4, Figure 6.1), and such bands were scored as *.

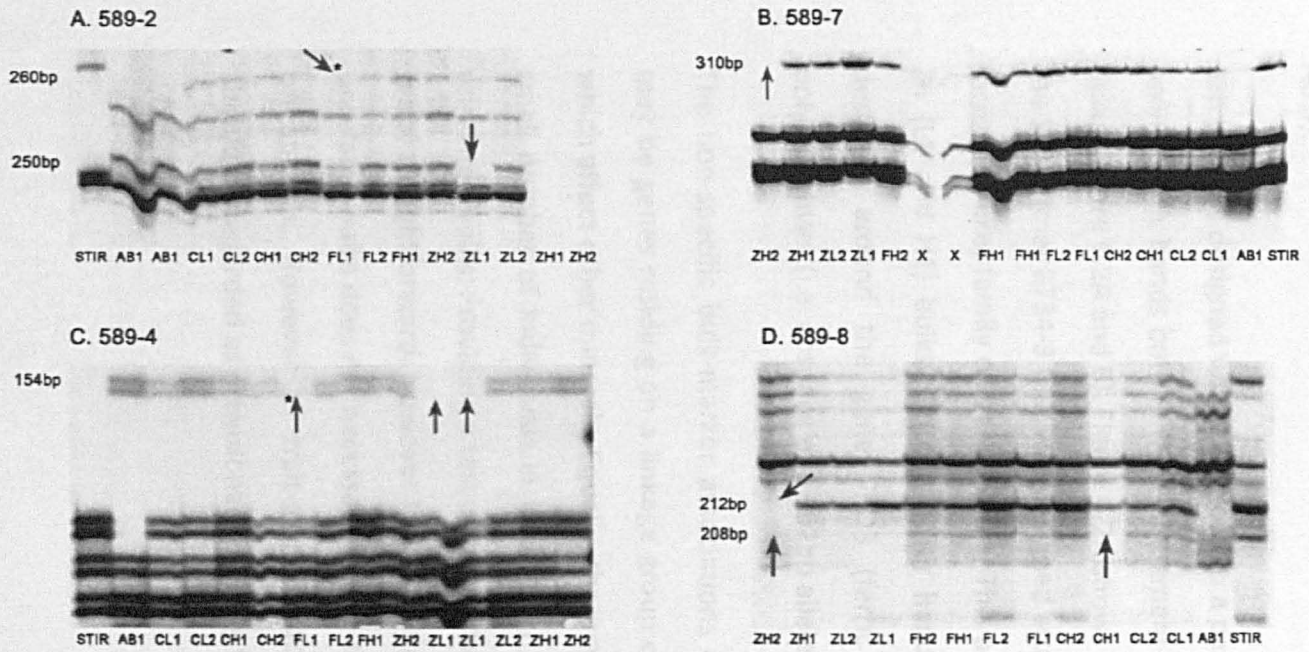

Figure 6.1: PCR amplification of SSR primer pairs (589-2, 4, 7 & 8) with parents and mineral bulks of 12601ab1 x Stirling mapping population using bulk segregant analysis. Polymorphic bands are indicated with arrows (absence of alleles), with respective product size at the left.
*indicates the bands that exhibited low intensity compared to other bulks. A, B, C, D displays 14 lanes of bulked DNA and parents. Each mineral bulk consists of 10 clones. Lane codes: Stir-Stirling (high Fe and Zn accumulating parent), AB1-12601ab1 (low Fe and Zn accumulating parent), both parents have similar concentrations of Ca; other lanes with different combinations of elements and trait levels (C-Calcium, F-Iron, Z-Zinc, L1-low trait bulked DNA, L2-second low trait bulked DNA, H1-high trait bulked DNA and H2-second high trait bulked DNA). See Appendix Table A6.2 for more details on SSRs used in this experiment.

Light and dark intensity of bands may indicate alleles with decreased and increased frequencies, respectively. Marker scores for each of the polymorphic SSR primers and the linkage phase of marker loci that were inferred from the parental bands are presented in Appendix Table 6.3. Among the parents, Stirling typically had higher tuber Fe and Zn concentrations than 12601ab1 (Table 6.1), thus, the linkage phase of the SSR marker locus in Fe and Zn bulks were assumed from the parental scores (Appendix Table A6.3). However, for tuber Ca, both parents had similar concentrations (Table 6.1), and hence the phase information of markers is not indicated (Appendix Table A6.3).

SSR primers designed within or close to a particular candidate gene produced polymorphic bands both in mineral element-specific bulks and in non-specific bulks (Figure 6.2A and B). Figure 6.2A shows the banding pattern produced by the SSR primer 2734-3 that was designed around ZIF1 (and ZIFL1, zinc-induced facilitator-like) family of transporters. This primer showed difference only for Zn (L1 and H1) bulks. On the other hand, SSR primer 386-3, which was designed around the gene FRD3 (ferric reductase defective3) showed polymorphism (i.e. absence of a 162bp allele) for Ca-H2 bulk (Figure 6.2B).

The non-specific bulk-marker associations can have multiple causes. There may be genes residing on a linkage group close to a specific candidate gene which affect other minerals. Equally, there may be stochastic effects from the small number of individuals in each bulk, or from the sharing of clones in unrelated bulks. Providing a test of statistical reliability in these circumstances is not straightforward. However Table 6.6 shows that sharing several clones in unrelated bulks does not necessarily cause shared markers or shared scaffolds on analysis. However all trait-gene associations suggested by this study should be regarded as provisional requiring further verification.

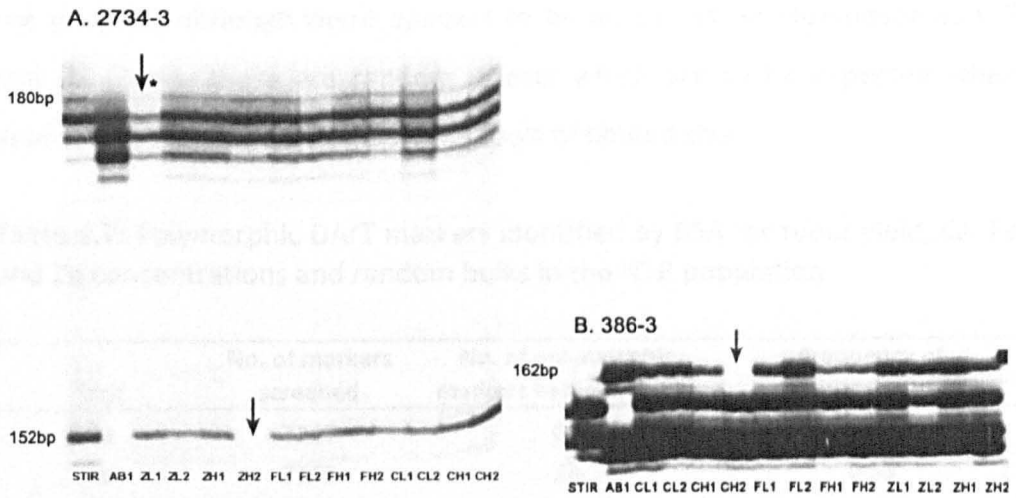

Figure 6.2: PCR amplification of SSR primer pairs (2734-3 and 386-3) with parents and mineral bulks of 12601ab1 x Stirling mapping population using bulk segregant analysis. Polymorphic bands are indicated by arrows (absence of alleles), with the respective product size at the left.

Each mineral bulk consists of 10 clones. Lane codes: Stir-Stirling (high Fe and Zn accumulating parent), AB1-12601ab1 (low Fe and Zn accumulating parent), both parents have similar concentrations of Ca; other lanes with different combinations of elements and trait levels (C-Calcium, F-Iron, Z-Zinc, L1-low trait bulked DNA, L2-second low trait bulked DNA, H1-high trait bulked DNA and H2-second high trait bulked DNA). See Appendix Table A6.2 for more details on SSRs used in this experiment.

6.3.2 Genome-wide screening using DArT-BSA in NTB population

Out of about 7400 DArT markers used for screening, 77 (tuber yield), 69 (Ca), 28 (Fe), 64 (Zn) and 29 (random bulks) markers were identified as polymorphic between low and high bulks for respective traits (Table 6.7; Appendix Table 6.4). The 238 DArT markers identified as polymorphic for tuber yield or mineral concentration were found to be distributed across all 12 chromosomes of the potato genome (Table 6.8; Appendix Table A6.4). Genes influencing mineral concentrations appear to be spread around the genome, with chromosomes I, II, III and IV all appearing to contain such genes. There is some overlap of mineral-associated markers with locations having an influence on yield but other locations, for example chromosome XI, have many DArT markers apparently associated with yield and few with minerals. Apparent linkages with random bulks are fairly evenly spread across

the genome, although there appears to be an excess on chromosomes I, II and III. Clearly there are random effects which are to be expected when associating very large data sets with pools of limited size.

Table 6.7: Polymorphic DArT markers identified by BSA for tuber yield, Ca, Fe and Zn concentrations and random bulks in the NTB population

Trait	No. of markers screened	No. of polymorphic markers between bulks	Frequency of polymorphisms (%)
Ca	7517	69	0.92
Fe	7452	28	0.38
Zn	7434	64	0.86
Yield	7463	77	1.03
Random	7413	29	0.39
Total	-	**267**	**3.58**

Table 6.8: Alignment of DArT markers identified by DArT-BSA in the NTB population with different chromosomes of the potato genome

CHR	No. of DArT markers				
	Ca	Fe	Zn	Yield	Random bulk
I	7	5	8	5	5
II	11*	4*	10*	12*	5
III	8*	5	5*	5	5*
IV	12*	0	5*	2	1
V	2	0	6*	2	1
VI	3	0	2*	8*	0
VII	4	2	0	4	0
VIII	1	3	5	2	1
IX	3	1	5	4*	0
X	2	1	4	2	0
XI	4*	1	3	14	0
XII	3	3	3	6	0
Unknown	9	3	8	2	11
Total	**69**	**28**	**64**	**77**	**29**

*Indicates superscaffolds that were shared between two chromosomes (see Appendix Table A6.4 for more details).

There were clones, DArT markers and superscaffolds shared among tuber yield, mineral and random bulks, and a summary is presented in Table 6.9. Further details on DArT markers and superscaffolds shared among different

trait bulks are provided in Appendix Table A6.5 and A6.6, respectively. Some of the superscaffolds were found more than once in different bulks with a repeat/frequency range of 2–6 (Appendix Table A6.7).

6.4 DISCUSSION

6.4.1 BSA using SSRs in the mapping population

The BSA method (Michelmore *et al.*, 1991) in combination with a candidate gene approach using SSRs enabled targeted identification of markers linked to loci influencing various traits, while minimising experimental work. Among the SSR primers used, 37 showed polymorphism in one or more bulks (Table 6.5) and markers linked in coupling/repulsion with the trait were inferred from both the parents. Some key examples of polymorphic SSRs identified using BSA are discussed here and the rest will be discussed in Chapter 8 with particular reference to the QTLs identified for tuber mineral concentrations.

The SSR primer 2734-3 was designed around the ZIF1 gene family of transporters (Figure 6.2A; Appendix Table A6.2), and showed a difference only for Zn (L1 and H1) bulks. AtZIF1 has been reported to be involved in transport of Zn into the vacuole (Haydon and Cobbett, 2007). The absence of a 152 bp allele in the Zn-H2 bulk is in repulsion with high concentrations of Zn (Appendix Table A6.3).

The SSR primer 386-3, which was designed around the gene FRD3 (ferric reductase defective3) showed polymorphism (absence of a 162 bp allele) for the Ca-H2 bulk (Figure 6.2B). This allele was also absent in Stirling (Figure 6.2B). However, the parents of the population, 12601ab1 and Stirling, have similar tuber Ca concentrations, and hence the linkage phase of this marker allele cannot be inferred. FRD3 is thought to transport citrate into the root xylem, which is required for efficient translocation of iron to the shoot (Durrett *et al.*, 2007; Green and Rogers, 2004; Yokosho *et al.*, 2009).

Table 6.9: Summary of shared clones, DArT markers and superscaffolds (SS) among the different bulk samples in the NTB population

Trait	Shared clones among bulks				Shared markers; SS among bulks
	L/L	L/H	H/L	H/H	
Ca/Fe	10	0	0	9	1 ;5
Ca/Zn	1	0	1	5	6 ;5
Fe/Zn	6	0	0	9	3 ;4
Ca/Yield	0	2	11	0	4 ;7
Fe/Yield	0	6	11	0	0 ;2
Zn/Yield	2	3	11	0	6 ;6
Zn & Fe/Yield	0	1	6	0	1 ;1
Zn & Ca/Yield	1	0	5	0	0 ;1
Fe & Ca/Yield	0	1	4	0	0 ;1
Ca/Random	-	-	-	-	0 ;2
Fe/ Random	-	-	-	-	2 ;2
Zn/Random	-	-	-	-	2 ;4
Yield/Random	-	-	-	-	1 ;2
Ca/Random1	1	-	-	2	-
Fe/Random1	1	-	-	1	-
Zn/Random1	2	-	-	1	-
Yield/Random1	0	-	-	1	-
Ca/Random2	0	-	-	0	-
Fe/Random2	1	-	-	0	-
Zn/Random2	0	-	-	1	-
Yield/Random2	1	-	-	4	-
Ca/Fe/Zn	1	0	0	3	0 ;0
Ca & Fe & Zn/Yield	0	0	3	0	0 ;0
Zn & Yield/Random1	0	1	0	0	-
Ca/Zn/Yield	-	-	-	-	0 ;1
Fe & Yield/Random	-	-	-	-	0 ;2
Ca & Yield/Random	-	-	-	-	0 ;1
Fe & Yield/Random	-	-	-	-	0 ;1
Ca & Fe & Yield/Random	-	-	-	-	0 ;1
Ca & Fe & Zn & Yield/Random	-	-	-	-	0 ;0
Ca & Fe & Zn & Yield/Random1	0	0	0	0	-
Ca & Fe & Zn & Yield/Random2	0	0	0	0	-

L-low trait bulk; H-high trait bulk; Each bulk contains 30 clones.

It is possible that the polymorphism found in the Ca-H2 bulk (Figure 6.2B) is closely associated with allelic variation in genes affecting Ca accumulation near to FRD3 or it might be occurring by chance and have no functional significance. When superscaffold 68 (to which FRD3 gene is assigned) was searched in the potato genome sequence data from the PGSC database, no Ca-related gene had been reported in the superscaffold as of July 2011. Nevertheless, about six out of 20 clones (2 clones in the H1 bulk of Ca and Fe and 4 clones in Ca-H1/Fe-H2 bulk, Table 6.5) were found to be shared between high Ca and Fe bulks. Whether or not the observations might be due partially to the sharing of clones among these bulks is unknown. However, it is also likely that further Ca-related genes will be present in the genome but not yet annotated.

The SSR primers designed around the calreticulin (CRT), nicotianamine synthase (NAS) and Fe-regulated protein 3 (IREG3) genes in scaffold no. 589 (superscaffold 60, Table 6.5), produced polymorphic bands in Ca-H1, Fe-L1 and Zn-L1 and Zn-H2 bulks (Figure 6.1; Table 6.5; Appendix Table A6.3). Calreticulin is a key Ca^{2+}-binding protein in the endoplasmic reticulum and plays an important role in many cellular processes, including Ca^{2+} storage and release, protein synthesis and molecular chaperone activity (White and Broadley, 2003). NAS is the key enzyme in the synthesis of nicotianamine (NA) and NA is an ubiquitous metal chelator which plays an important role in the homeostasis and transport of metals including Fe and Zn (Douchkov *et al.*, 2005; Ling *et al.*, 1999). IREG3 is thought to function in the uptake of the Fe-NA into chloroplasts (Conte *et al.*, 2009; Conte and Lloyd, 2010).

For the SSR primer 589-2, the absence of a 250 bp allele in the Zn-L1 bulk and the low intensity 260 bp allele in the Fe-L1 bulk are associated with opposite effects in the parental tuber Zn and Fe concentrations (Figure 6.1A and C; Appendix Table A6.3). Likewise, for the SSR primer 589-4, absence of a 154 bp allele in Zn-L1 bulk is associated with low concentrations of tuber Zn and the

low intensity allele (154 bp) in Fe-L1 bulk is associated with high parental tuber Fe concentration (Figure 6.1A and C). When clones shared among low (L1) Fe and Zn bulks were checked, only one clone was in common (Table 6.6). Taken together, the identified polymorphic allele for mineral traits suggests the close linkage of these SSR marker loci to an allele of the NAS gene which gives transgressive segregation for tuber Fe and Zn concentrations.

The absence of a 310 bp allele for the SSR primer 589-7 is in opposition to the parental high concentrations of tuber Zn (Figure 6.1C). Similarly, a 208 bp product was absent for the primer 589-8 in Ca-H1 and Zn-H2 bulk (Figure 6.1D). Only two clones were found to be shared between these bulks (Table 6.6), suggesting that the marker loci are closely linked to CRT and NAS genes.

The results obtained with the SSR primers 589-2, 4, 7 and 8 suggest that the CRT, NAS and IREG/FPN genes are possibly occurring in the genome in clusters. Using the PGSC website (www.potatogenome.net), superscaffold 60 was searched to find the location of CRT, NAS and IREG3 genes in the potato genome, and this showed that these genes are indeed occurring in a cluster in the genome. CRT, NAS and IREG/FPN are found between 1222–1226 kbp, 1304–1306 kbp and 1401–1409 kbp respectively in the superscaffold (Figure 6.3).

The results from this study suggest that BSA could be effective in a tetraploid cross to develop markers closely linked to a trait of interest. Polymorphic SSRs identified using BSA was found to be distributed throughout the potato genome except for chromosomes VI, IX and X (Table 6.5). Some SSR markers were unique to one predicted gene-mineral combination (eg. SSR primer 2734-3 and Zn), and some were apparently linked to a gene controlling a non-specific mineral element (eg. SSR primer 386-3 and Ca).

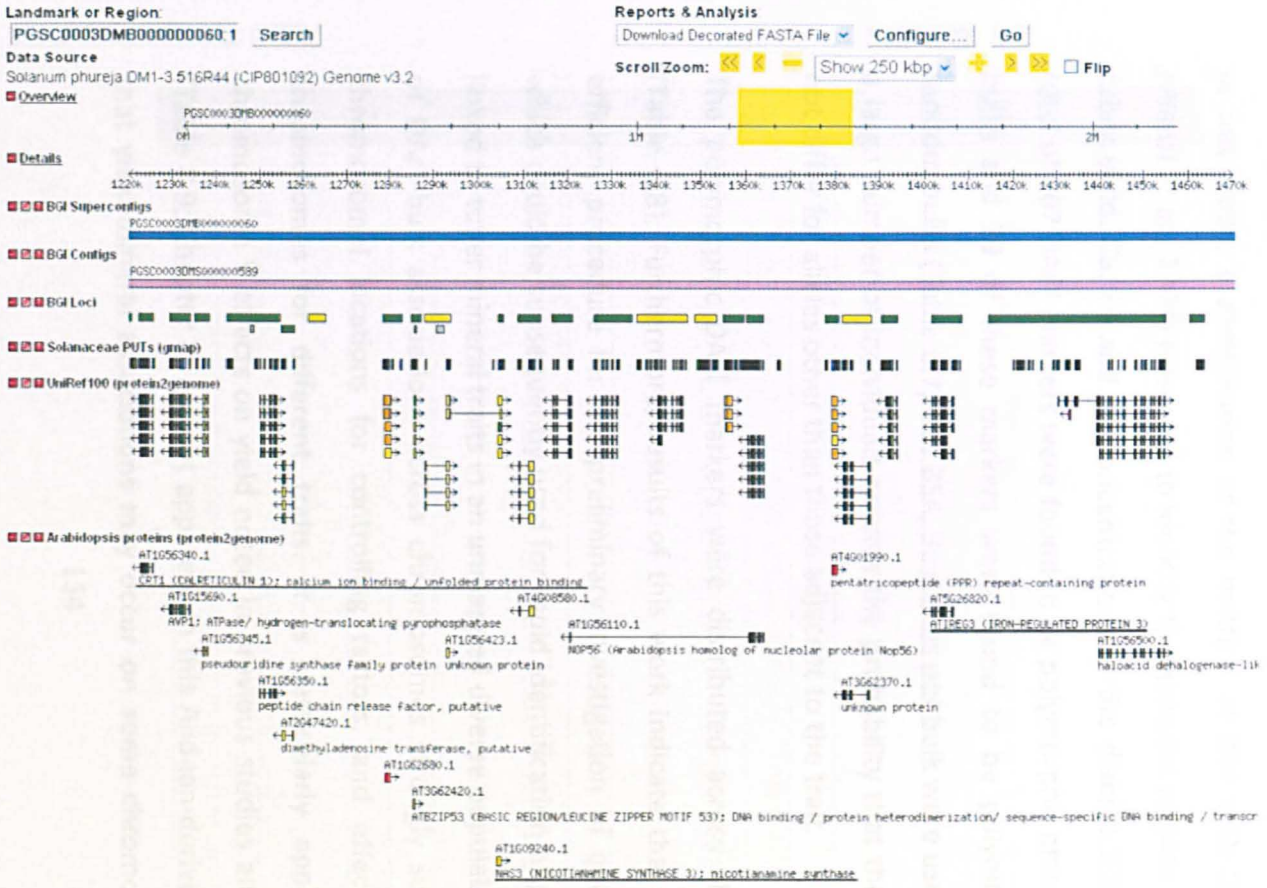

Figure 6.3: Arrangement of gene clusters CRT, NAS and IREG3 (underlined) on scaffold 589 (PGSC0003DMS000000589) aligned to superscaffold 60 (PGSC0003DMB000000060), and superscaffold 60 is mapped on chromosomes 1 and 2 (PGSC database mined during June 2011).

The SSR markers showing polymorphism between the parents, and between low and high mineral bulks of the GenPop1 mapping population might be linked to QTL regulating the mineral traits. If so, they are likely to co-segregate with the QTL. Due to time constraints, it was not possible to screen and map these polymorphic SSRs in the whole population. However, attempts were made to align the identified SSR markers to the GenPop1 genetic maps (using the information of superscaffold to chromosome alignment) and check for the presence of QTLs in these aligned genomic regions (see Chapter 8).

6.4.2 BSA using DArT markers in NTB population

In this study, a combination of the method of BSA with DArT technology (Wenzl *et al.*, 2007) was used to identify the genomic regions associated with tuber yield, Ca, Fe and Zn concentrations in the diverse NTB population. A total of 267 DArT markers were found to be polymorphic among the different bulks and 29 of these markers were found to be polymorphic between random bulks (Table 6.7). For BSA, 30 clones per bulk were used, as pooling of a large number of individuals assures the probability that the two pools will not differ for alleles other than those adjacent to the trait.

The polymorphic DArT markers were distributed across all chromosomes (Table 6.8). Furthermore, results of this work indicate that DArT-BSA is an efficient procedure for the preliminary investigation of quantitative traits, which could be subsequently used for rapid identification of genomic regions linked to tuber mineral traits in an unmapped diverse population. The pattern of DArT-bulk associations across chromosomes strongly suggests multiple chromosomal locations for controlling factors, and effects on different chromosomes for different traits. It is particularly apparent that the chromosome V effects on yield noted in previous studies and current study (Table 8.9; Chapter 8) are not apparent in this Andean-derived material, and that yield-mineral associations may occur on some chromosomes and not

others. Due to time constraints, the DArT markers identified in this study were not tested in individual clones in each trait bulk. However, as most of the DArT markers were aligned on the potato genome, efforts were made to align the polymorphic DArT markers identified for tuber yield and mineral bulks to the GenPop1 genetic map and check whether any QTL is present in these aligned genomic regions (see Chapter 8).

6.4.3 Markers shared between different trait bulks

The polymorphic markers shared between different bulks in the GenPop1 and NTB populations could be due to (1) sharing of clones between these bulks, (2) close physical location of genes affecting the accumulation of different minerals (eg. CRT, NAS and IREG3 gene clusters, Figure 6.3), (3) minerals having common metal chelators or transporters (Fe and Zn), or (4) by chance.

The sharing of DArT markers between bulks for yield and mineral (Ca, Fe and Zn) trait could be due to sharing of clones between the bulks (Table 6.9) or due to the significant negative associations existing between tuber yield and mineral concentrations (Figure 4.11, Chapter 4). Only a few clones were shared between random and different trait bulks (Table 6.9), suggesting that the DArT markers shared in common between them will be occurring by chance.

6.4.4 Use of random bulks in BSA

Along with the pooled samples for tuber yield, Ca, Fe and Zn, two random bulks were also used in the NTB population (section 6.2.2). The random bulks constructed from two randomly assorted DNA pools were used to evaluate the impact of stochastic effects associated with bulking (Wenzl *et al.*, 2007). 29 DArT markers were different between the two random bulks (Table 6.7)

and this might be due to the low bulk size leading to introduction of random fluctuations in allele frequencies in the pooling process.

The frequency of the potential markers for tuber yield, Fe, Zn and Ca concentrations was tested using the random bulks. Out of the 29 polymorphic random markers zero, one and two markers were shared with Ca, yield, and Fe and Zn bulks respectively (Table 6.9 and Appendix Table A6.5). In addition only a few clones were shared between random and different trait bulks (Table 6.9). Hence the very low frequency of markers shared between random bulk and the trait bulks suggest that the identified markers in the trait bulks might be closely associated with the trait and are not occuring by chance. Thus use of random bulks in BSA proves to be a useful control.

6.4.5 BSA in other food crops for yield and mineral traits

A few studies have implemented the BSA approach for identifying markers linked to the accumulation of minerals such as cadmium and Zn in edible parts of staple crops (Table 6.10 and references therein). BSA has also been successfully used to identify markers linked to grain/fruit yield under normal and abiotic stress conditions in rice, maize and tomatoes (Table 6.10 and references therein).

In previous studies, DArT-BSA has been used to identify markers linked to leaf rust and powdery mildew in wheat and common root rot resistance, leaf rust, aluminium tolerance and pubescent leaf blades in barley (Table 6.11 and references therein). All the reported studies except that of Quarrie *et al.* (1999) (Table 6.10 and 6.11) have been performed on F_1-F_8, DH, BC or RIL populations. To date, Quarrie *et al.* (1999) is the only reported study that has applied BSA in diverse (composite) populations. In their study, BSA using RFLPs was applied in two composite populations of maize to investigate changes in allele frequency between the base population and the

corresponding population obtained following several cycles of recurrent selection for grain yield under drought stress.

The polymorphic SSR and DArT markers identified in this study have shown that chromosomal loci/genes controlling mineral accumulation are distributed throughout the genome (Table 6.5 & 6.8), indicating the complexity of this trait and the involvement of multiple genetic loci. Furthermore, molecular markers that are closely linked with the traits of interest in a diverse population (such as NTB in this study) are very informative, as the diverse population had undergone more meiotic recombination, which decreases linkage disequilibrium (LD) to short chromosome intervals. The use of diversity populations also ensures that many alleles are being sampled unlike the situation in a bi-parental mapping population. On the other hand, the bi-parental mapping population, which has a limited number of recombination events, is likely to exhibit greater LD and so can be used to detect linkage more readily. The LD declines as greater numbers of markers can be accessed as technology improves. However NTB population need to be corrected for population structure and this is not possible with BSA alone.

6.4.6 Limitations of BSA

BSA can be used for a quantitative trait controlled by a few major genes (Michelmore *et al.*, 1991). In BSA, individuals with the highest and lowest phenotypic values for a target trait were pooled and the two bulks were tested with molecular markers to identify the trait linked marker(s). In case of QTLs with a large effect, markers linked to the gene can be obtained using BSA (Wang and Paterson, 1994). However, QTLs with small effects on the phenotype and explaining a large portion of the genetic variation in a trait probably escapes detection of markers using BSA.

Table 6.10: Present and other reported studies using the BSA approach to identify molecular markers linked to mineral concentrations and yield in edible parts of food crops

Trait	Crop	Plant part	Clones/bulk (H/L)	Population size & type	Marker	Reference
Minerals						
Cadmium (Cd)	Oat	Grain	9/9	150, F2	RAPD, REMAP, SRAP	Tanhuanpaa *et al.* (2007)
Cd	Soybean	Grain	10/10	138, F2:3, 166, RIL (F6:8)	SSR	Jegadeesan *et al.* (2010)
Cd	Wheat	Grain	8/6	70, F8	RAPD	Penner *et al.* (1995)
Zn	Barley	Grain	5/5*	150, DH	MFLP	Sadeghzadeh *et al.* (2010)
Ca, Fe & Zn	Potato	Tuber	10/10	190, F1	SSR	Present study (2011)
Ca, Fe & Zn	Potato	Tuber	30/30	450, diverse popn (NTB)	DArT-BSA	Present study (2011)
Yield						
Under drought	Maize	Grain	50/0	-, Two Composite popns	RFLP	Quarrie *et al.* (1999)
Under normal condn	Rice	Grain	10/10	89, DH lines	RAPD	Shashidhar *et al.* (2005)
Under lowland drought stress	Rice	Grain	20/20	490, BC1F4:5	SSR	Venuprasad *et al.* (2009)
Under heat stress	Tomato	Fruit	7-8/7-8	47, F7 RILs	RAPD	Lin *et al.* (2006)
Under normal condn	Potato	Tuber	30/30	450, diverse popn (NTB)	DArT-BSA	Present study (2011)

H/L- high and low trait bulk; *DNA not bulked, clones analysed separately; RAPD- random amplified polymorphic DNA; REMAP- retrotransposon-microsatellite amplified polymorphism; SRAP- sequence-related amplified polymorphism; MFLP- microsatellite-anchored fragment length polymorphism; Popn- population; RIL-recombinant inbred lines; DH-doubled haploid; BC-backcross.

Table 6.11: Present and reported studies using the DArT-BSA procedure in food crops to identify markers linked to traits of interest

Trait	Crop	Gen/bulk	Popn size & type	Reference
Al tolerance	Barley	20	F1, DH popn	Wenzl *et al.*, 2007
CRR	Barley	8	92, RIL	Lehmensiek *et al.*, 2010
Leaf rust	Wheat	29	94, F2 popn	Czembor *et al.*, 2008
Leaf rust	Barley	20	146 F3 lines	Golegaonkar *et al.*, 2009
mPub	Barley	20 & 40	F1, DH popn	Wenzl *et al.*, 2007
Powdery mildew	Wheat	25	139, F2:3 lines	Maxwell *et al.*, 2010
Tuber yield, Ca, Fe & Zn conc.	Potato	30	450, diverse popn (NTB)	Present study (2011)

CRR- Common root rot; Al-Aluminium; mPub-Pubescent leaf blades; Gen-genotypes; Popn-Population; Conc-concentration.

A common problem in BSA is the risk of detecting false positives from pooled DNA samples. In this study 10 clones per bulk for the mapping population and 30 clones per bulk for the NTB population were used to minimize detecting false positive markers. Futhermore, the false positives can be screened out by genotyping the individuals that were used to make up the bulks, resulting in detection of markers that are tightly linked to the target trait. Following this it is necessary to perform QTL mapping across the entire mapping population to verify BSA results. However, marker-linked QTLs in one mapping population should be verified in an independent, unrelated mapping population or germplasms to validate the linkage of markers. The validated markers can be then used for marker-assisted selection.

In summary, using a BSA approach, 37 polymorphic SSRs in a F_1 mapping population and 161 polymorphic DArT markers in the diverse NTB population were identified which might be linked to genes that influence tuber Ca, Fe and Zn concentrations. Furthermore, 77 DArT markers were found to be associated with tuber yield. The identified SSR and DArT markers have been distributed on all 12 chromosomes of the potato genome, suggesting that tuber yield and mineral traits are controlled by multiple genes. This is the first published study to identify genomic regions associated with tuber yield and

mineral concentrations in potatoes. The results of this work clearly demonstrate that BSA technique coupled with candidate gene approach using SSRs and genome-wide scan using DArT markers provide a good platform for rapid identification of genetic loci involved in mineral homeostasis in plants.

CHAPTER 7 CONSTRUCTION OF LINKAGE MAPS IN A TETRAPLOID MAPPING POPULATION, 12601ab1 X STIRLING (GENPOP1)

7.1 INTRODUCTION

Genetic linkage maps are very useful tools for studying the inheritance of both qualitative and quantitative traits. In potatoes, most genetic mapping studies have been conducted at the diploid level, mainly focussing on traits such as biotic and abiotic stress tolerance, agronomic traits and quality characteristics. However, the cultivated potatoes are autotetraploids (2n=4x=48), displaying tetrasomic inheritance (random pairing of four homologous chromosomes during meiosis, Bradshaw *et al.*, 2008). Hence the progress of linkage mapping in tetraploid potatoes has been considerably slower than in diploid potatoes. With the availability of advanced statistical package for tetraploids (TetraploidMap, Hackett *et al.*, 2007), linkage mapping is possible now in tetraploid potatoes. TetraploidMap can handle both dominant and co-dominant markers and includes algorithms for interval mapping of QTL. To date, six linkage maps have been constructed in tetraploid potatoes using this software (Bradshaw *et al.*, 2004, 2008; Kelley *et al.*, 2009; Khu *et al.*, 2008; McCord *et al.*, 2010, 2011; Sagredo *et al.*, 2006, 2009, 2011).

Mapping in a species with tetrasomic inheritance requires more markers to cover the genome than in a diploid organism. Recently, the Diversity Arrays Technology (DArT) platform, which is a DNA-hybridisation-based method of generating molecular markers across a genome, has been shown to be a quick and cost-effective genotyping method (Jaccoud *et al.*, 2001). Additionally, the DArT markers are now anchored on to the potato genome (The Potato Genome Sequence Consortium, 2011), enabling easy alignment of linkage maps with the potato genome and rapid identification of the candidate genes and quantitative trait loci (QTLs). The objective of this research was to add

genome-linked DArT markers to the existing (SSR and AFLP-based) linkage maps of Stirling and 12601ab1, the parents of F_1 tetraploid potato population.

7.2 RESULTS

7.2.1 Evaluation of DArT markers

In this study, the existing genetic map of the 12601ab1 x Stirling population (Bradshaw *et al.*, 2008) was updated with genome-linked DArT markers. The segregation of DArT markers in potatoes was assessed on 190 progeny genotypes from the cross of 12601ab1 x Stirling. Among 2785 polymorphic (from a total of 3998) DArT markers, 464 were 12601ab1-specific, 428 were Stirling-specific and 380 were present in both parents (Table 7.1). In the absence of segregation distortion, the expected segregation ratios for the tested mapping population were 1:1 for single-dose markers, 5:1 for double-dose markers present only in one parent and 3:1 and 11:1 for both bi-parental single-and double-dose markers, respectively. The majority of polymorphic DArT markers showed a 1:1 segregation ratio (269 in 12601ab1 and 249 in Stirling) (Table 7.1).

7.2.2 Construction of the GenPop1 genetic map

Genetic linkage analysis and map construction was carried out separately for Stirling and 12601ab1, using the TetraploidMap software (Hackett *et al.*, 2007). Initially, linkage maps were constructed separately with SSR and AFLP markers, and DArT markers (not shown). Linkage mapping with DArT markers revealed the presence of some DArT markers with similar scores. In total, about 100 DArT markers were found with the same scores across the segregating population and were subsequently removed for the integrated map analysis. Following the marker selection criteria described in section 7.2.1, the final map was constructed using all the three types of markers

(SSRs, AFLPs and DArT) for Stirling and 12601ab1. Due to the input limitation of the TetraploidMap software, only 800 markers (segregating in 1:1 and 5:1 ratios) were used for map construction.

Table 7.1: Details of polymorphic DArT markers in the 12601ab1 x Stirling mapping population

Marker type	No. of markers		
Total DArT markers	3998		
Monomorphic & questionable markers	2716		
Polymorphic markers	1282 (464+428+380)		
	12601ab1	**Stirling**	**12601ab1 & Stirling**
Polymorphic markers	464	428	380
Uni-parental single-dose markers (Aaaa x aaaa, 1:1)	269	249	
Uni-parental double-dose markers (AAaa x aaaa, 5:1)	195	179	
Bi-parental single-dose markers (Aaaa x Aaaa, 3:1)			238
Bi-parental, single/double-dose markers (Aaaa x AAaa, 11:1)			142

The final linkage maps of the parents Stirling and 12601ab1 varied in the map length and number and density of markers (Table 7.2; Figure 7.1). The 449 markers of the Stirling parent were assigned to 12 linkage groups and the linkage map spanned over a total genetic distance of 1451 cM with an average density of 3.2 cM per marker (Table 7.2). Likewise, the 546 markers of the 12601ab1 parent were assigned to 12 linkage groups covering a genetic distance of 1644 cM with an average density of 3.0 cM per marker (Table 7.2). The total number of mapped loci per linkage map ranged from 7 to 50 in Stirling and from 9 to 50 in 12601ab1.

Table 7.2: Summary of chromosomal alignment, length of linkage groups (LG) and number of markers in genetic maps of the parents Stirling and 12601ab1 constructed using 190 progenies of the cross

CHR	Stirling				12601ab1			
	No. of markers	LG >50 markers	Length (cM)	Mark den*	No. of markers	LG >50 markers	Length (cM)	Mark den*
Ia	18		82.0	4.6	49		82.0	4.6
Ib	50	81	125.4	2.5	45		125.4	2.5
II	49	77	119.7	2.4	50	67	119.7	2.4
III	32		120.3	3.8	46		120.3	3.8
IV	29		131.9	4.5	50	67	131.9	4.5
V	40		117.5	2.9	50	89	117.5	2.9
VI	50	63	106.3	2.1	36		106.3	2.1
VII	7		49.9	7.1	30		49.9	7.1
VIII	16		125.6	7.9	50	59	125.6	7.9
IX	27		119.7	4.4	-		-	-
IXa^	-		-	-	9		87.6	9.7
IXb^	-		-	-	25		110.5	4.4
X	31		118.7	3.8	16		104.7	6.5
XI	50	72	105.9	2.1	50	56	155.4	3.1
XII	50		128.4	2.6	40		94.0	2.4
Total	**449**		**1451.3**	**3.2**	**546**		**1644.1**	**3.0**

cM-centiMorgan; *Marker density (markers/cM); LG-linkage group; ^Chromosome IX in 12601ab1 has two linkage groups, IXa and IXb.

Because the maximum number of markers handled by the 'ripple' option in TetraploidMap is 50, only 50 markers were ordered in each LG (Table 7.2). For chromosme I, there were in total about 99 markers in Stirling and 94 in 12601ab1 (Table 7.2), and they were separated into two clusters with 81 and 18 markers in Stirling and 49 and 45 markers in 12601ab1, respectively. The two clusters within each parental group were ordered without any difficulty and were named Ia and Ib (Table 7.2; Figure 7.1) based on their alignment to the pseudo-chromosome (not shown). Similarly, for LG IX in 12601ab1, there were two LGs, namely IXa and IXb (Table 7.2 and Figure 7.1). When markers from both IXa and IXb were merged and ordered, the two clusters (group) of

markers failed to show any significant association between them, but the markers within the two clusters were ordered without any difficulties.

The linkage maps in Figure 7.1 are presented as overall maps, with data from four homologs combined into a single group, as described by Bradshaw *et al.* (2008). An example is given for 12601ab1 LG XII in Figure 7.2. All linkage groups contained all the four homologs, except for 12601ab1 LG IXa, which contained only three homologs (not shown).

7.2.3 Chromosomal identity

Chromosomal identifications of LGs were determined by the presence of mapped SSR and DArT markers and by alignment of DArT markers to the potato genome sequence. Eight SSR markers (STM5127, STM3016, STM3160, STM5140, STM3179, STM5148, STM0037 and STM5109) were mapped in this population, which allowed the tagging of chromosomes I, IV, V and XI in one or both parents (Figure 7.1). Following that, the position of the DArT loci mapped in this study was compared with the already available linkage maps of diploid potato (Campbell, 2010; The Potato Genome Sequence Consortium, 2011). A total of 48 and 50 mapped DArT markers were common between the 12601ab1 and Stirling linkage maps and the diploid potato maps (01H15 and PGSC reference maps), respectively (Table 7.3). Figure 7.3 shows the alignment of DArT markers on the genetic maps of Stirling and 12601ab1 LG XII with the diploid map 01H15 (Campbell, 2010). Based on the alignment with diploid maps, the tetraploid genetic maps were reversed vertically (denoted as R) as given in Figure 7.3.

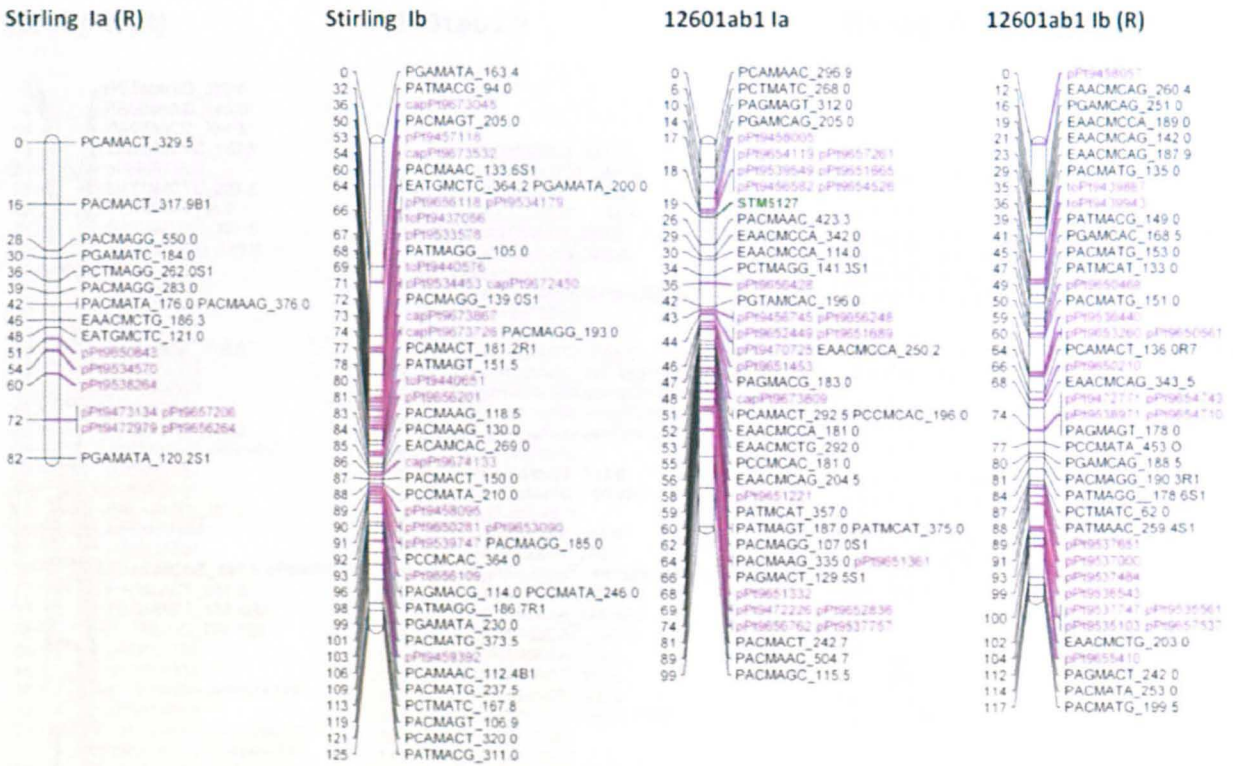

Figure 7.1 (Continued on next page): An integrated AFLP, SSR and DArT genetic linkage map for Stirling and 12601ab1. The vertical bars in the centre represent the chromosomes; the codes on the right indicate AFLP (black), DArT (pink) or SSR (green) marker loci, with corresponding map locations in the accumulative genetic distance (cM) on the left. The map fragments labelled R in parentheses are the vertical reversal of linkage maps based on an alignment with the physical map. Maps were constructed using MapChart 2.2 (Voorips, 2002).

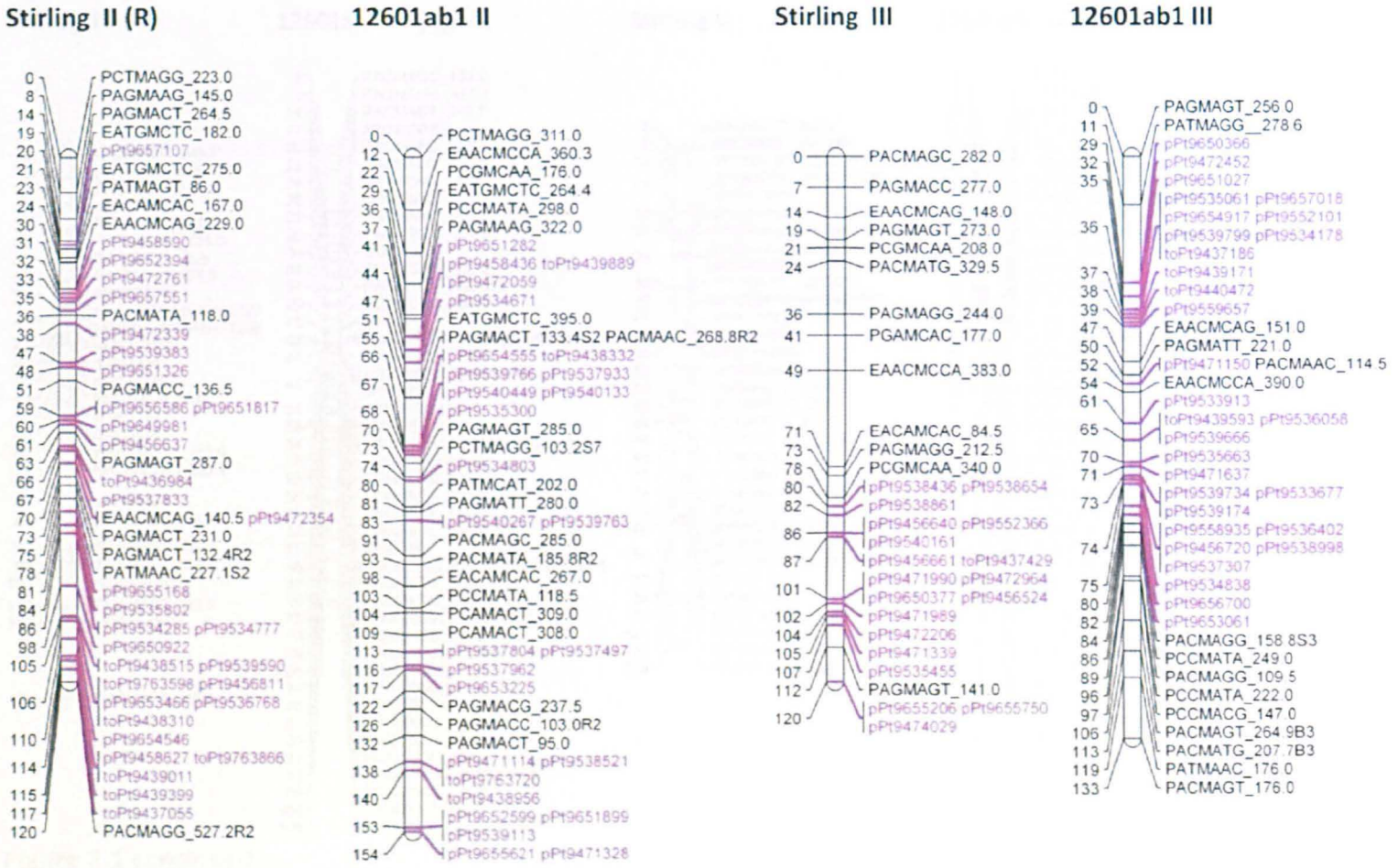

Figure 7.1 continued...

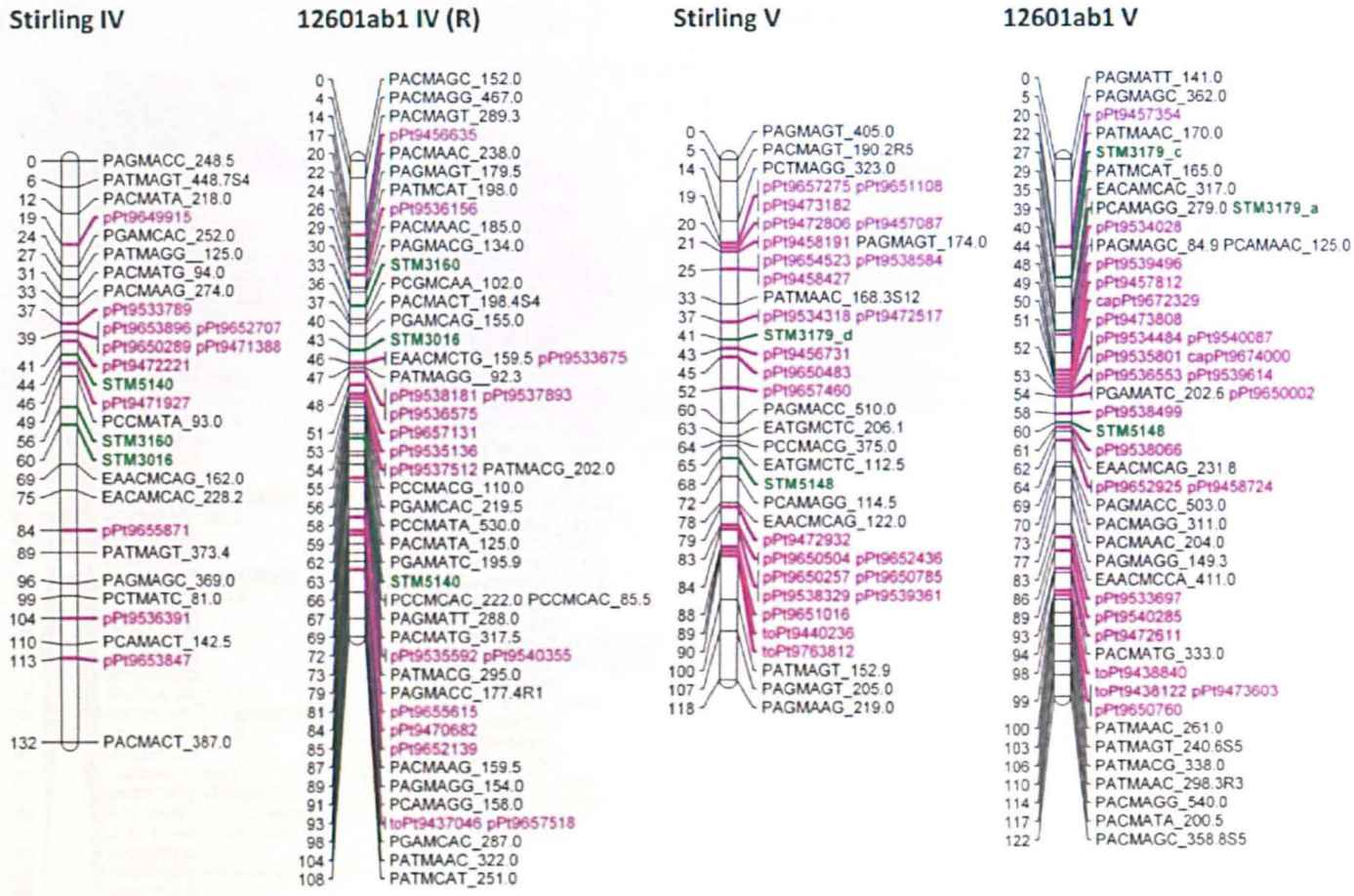

Figure 7.1 continued...

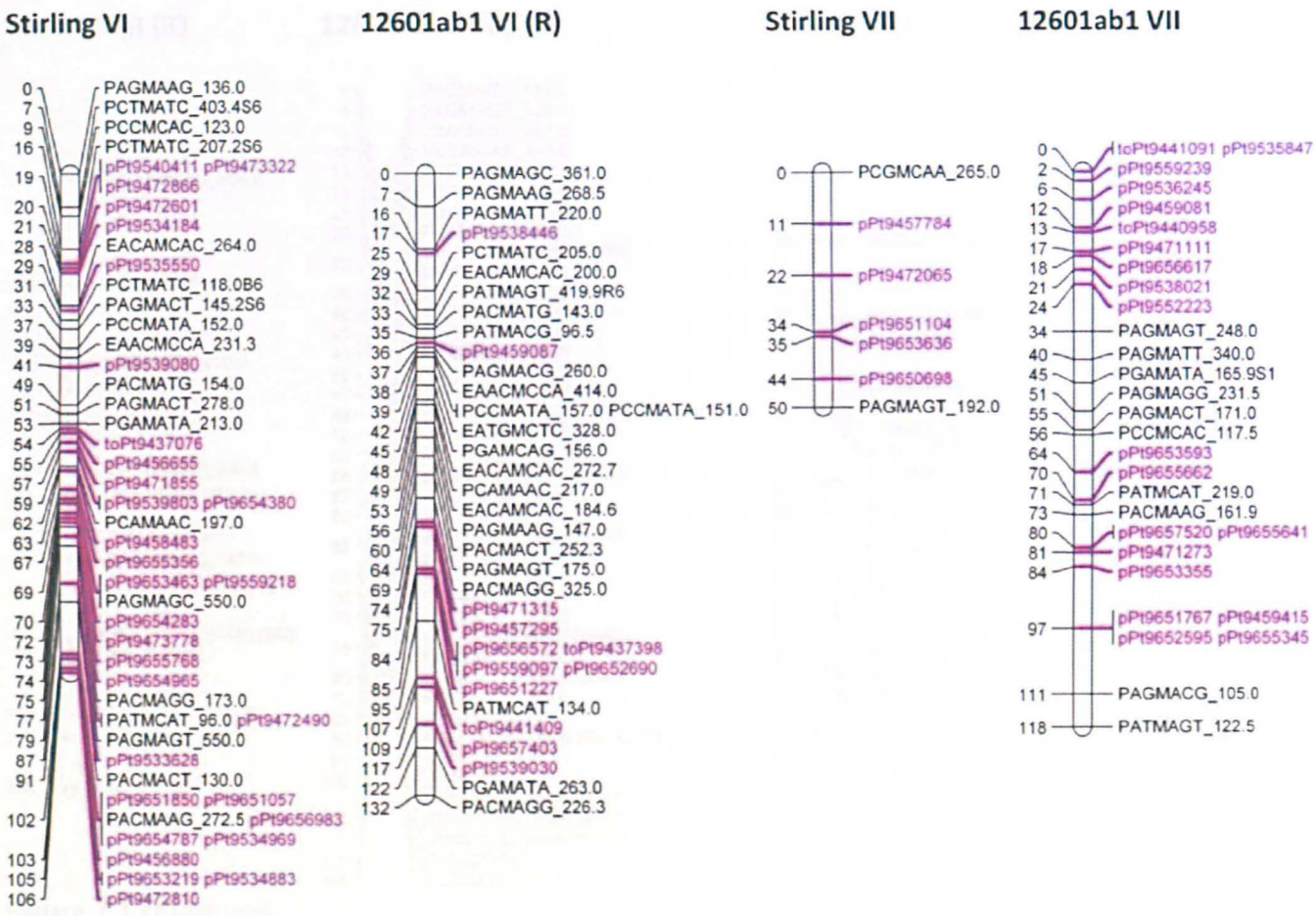

Figure 7.1 continued...

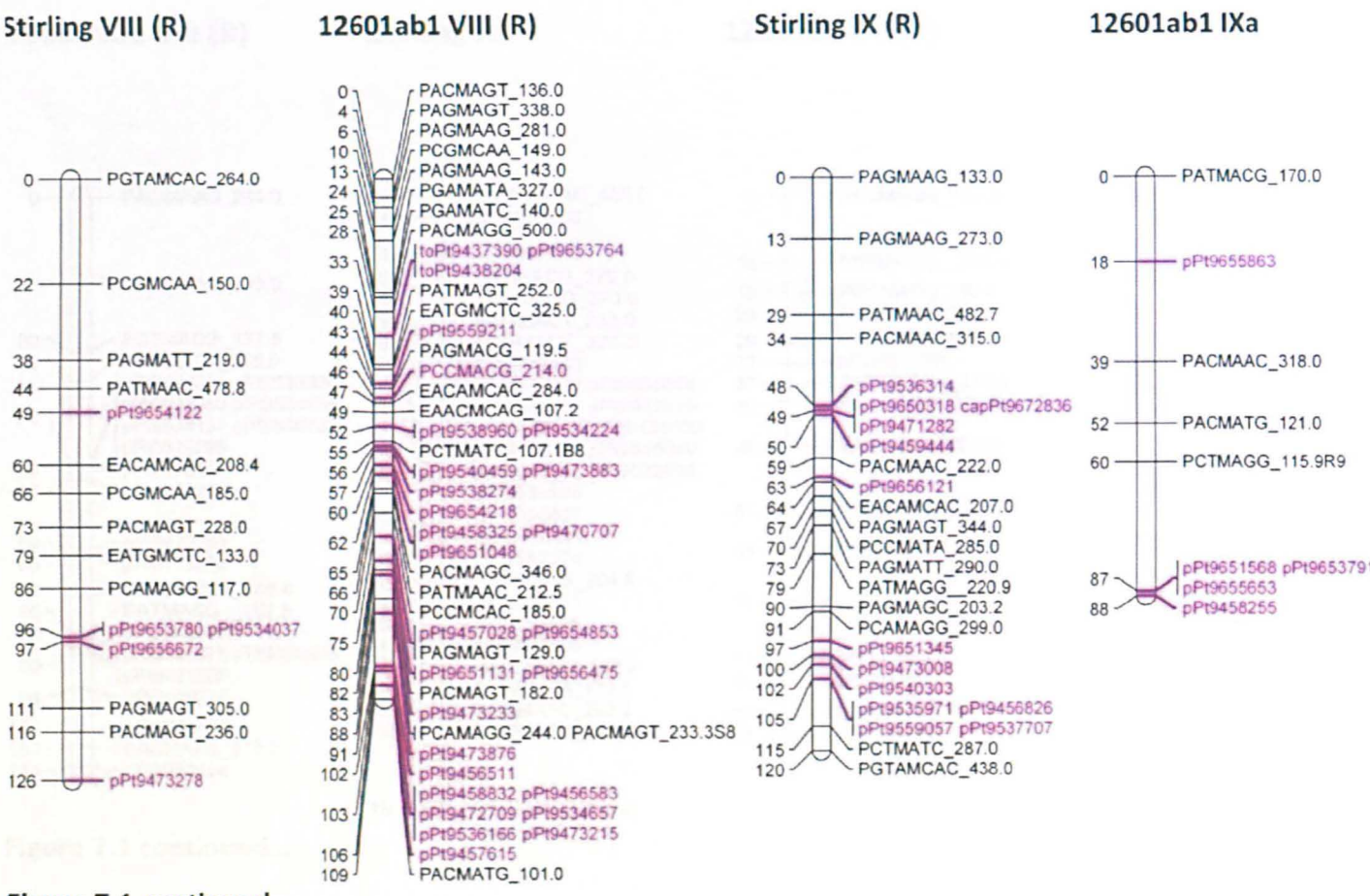

Figure 7.1 continued...

12601ab1 IXb (R) **Stirling X** **12601ab1 X (R)**

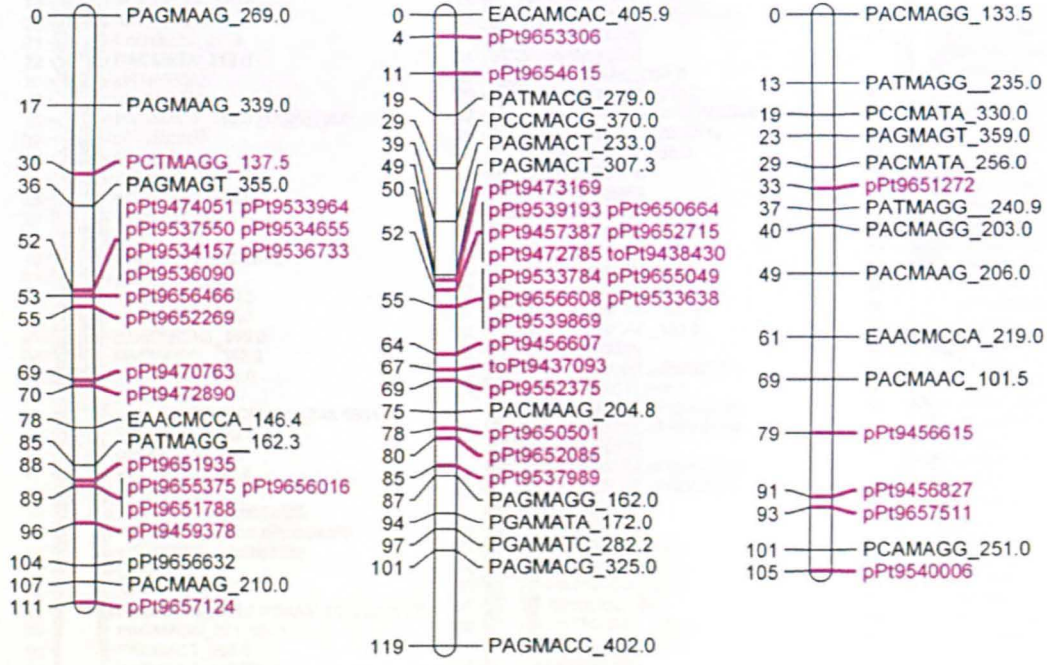

Figure 7.1 continued...

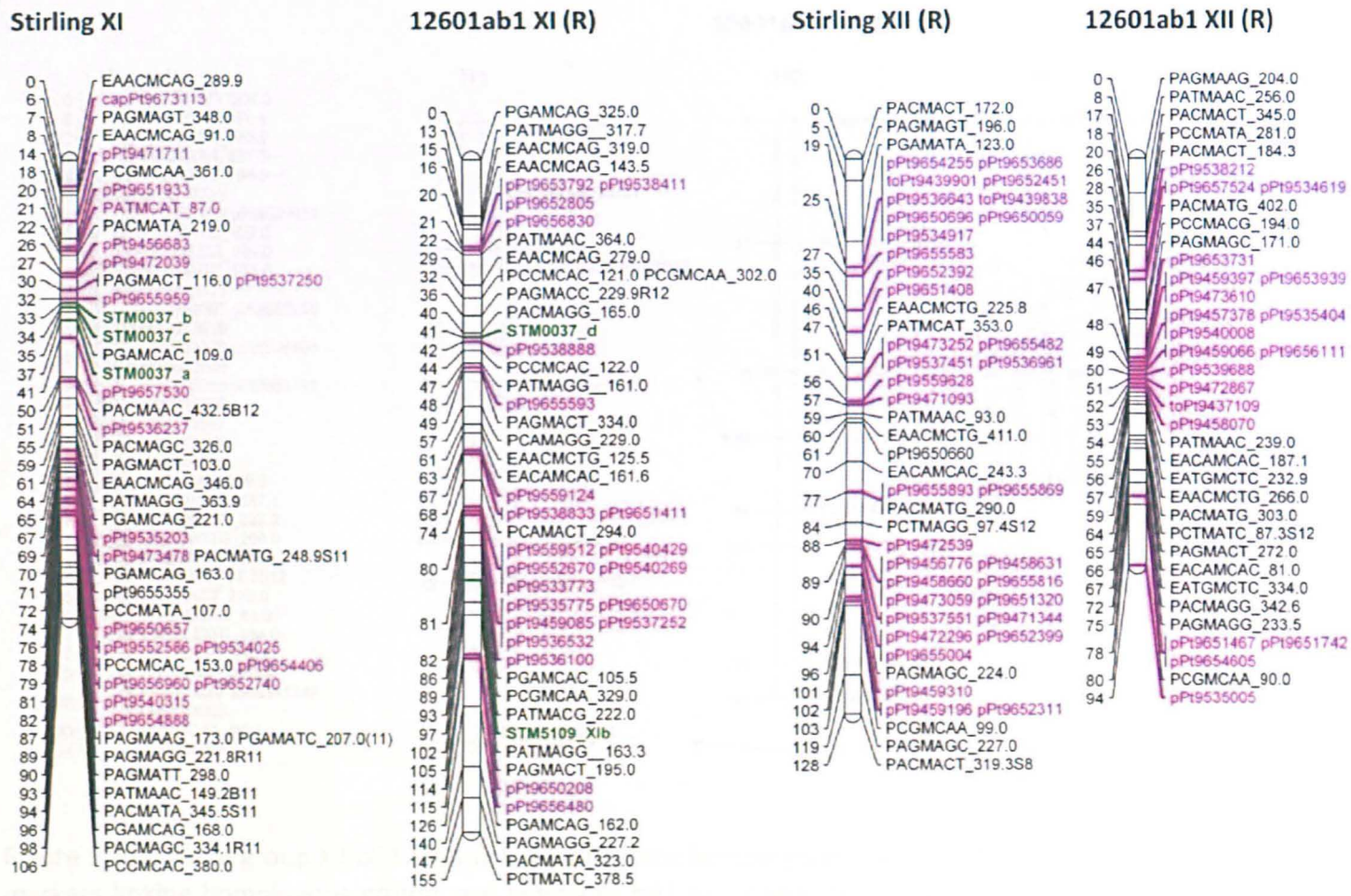
Stirling XI
12601ab1 XI (R)
Stirling XII (R)
12601ab1 XII (R)
0 EAACMCAG_289.9
6 capPt9673113
7 PAGMAGT_348.0
8 EAACMCAG_91.0
14 pPt9471711
18 PCGMCAA_361.0
20 pPt9651933
21 PATMCAT_87.0
22 PACMATA_219.0
26 pPt9456683
27 pPt9472039
30 PAGMACT_116.0 pPt9537250
32 pPt9655959
33 STM0037_b
34 STM0037_c
35 PGAMCAC_109.0
37 STM0037_a
41 pPt9657530
50 PACMAAC_432.5B12
51 pPt9536237
55 PACMAGC_326.0
59 PAGMACT_103.0
61 EAACMCAG_346.0
64 PATMAGG_363.9
65 PGAMCAG_221.0
67 pPt9535203
69 pPt9473478 PACMATG_248.9S11
70 PGAMCAG_163.0
71 pPt9655355
72 PCCMATA_107.0
74 pPt9650657
76 pPt9552566 pPt9534025
78 PCCMCAC_153.0 pPt9654406
79 pPt9656960 pPt9652740
81 pPt9540315
82 pPt9654888
87 PAGMAAG_173.0 PGAMATC_207.0(11)
89 PAGMAGG_221.8R11
90 PAGMATT_298.0
93 PATMAAC_149.2B11
94 PACMATA_345.5S11
96 PGAMCAG_168.0
98 PACMAGC_334.1R11
106 PCCMCAC_380.0
0 PGAMCAG_325.0
13 PATMAGG_317.7
15 EAACMCAG_319.0
16 EAACMCAG_143.5
20 pPt9653792 pPt9538411
pPt9652805
21 pPt9656830
22 PATMAAC_364.0
29 EAACMCAG_279.0
32 PCCMCAC_121.0 PCGMCAA_302.0
36 PAGMACC_229.9R12
40 PACMAGG_165.0
41 STM0037_d
42 pPt9538888
44 PCCMCAC_122.0
47 PATMAGG_161.0
48 pPt9655593
49 PAGMACT_334.0
57 PCAMAGG_229.0
61 EAACMCTG_125.5
63 EACAMCAC_161.6
67 pPt9559124
68 pPt9538833 pPt9651411
74 PCAMACT_294.0
80 pPt9559512 pPt9540429
pPt9552670 pPt9540269
pPt9533773
81 pPt9535775 pPt9650670
pPt9459085 pPt9537252
pPt9536532
82 pPt9536100
86 PGAMCAC_105.5
89 PCGMCAA_329.0
93 PATMACG_222.0
97 STM5109_Xlb
102 PATMAGG_163.3
105 PAGMACT_195.0
114 pPt9650208
115 pPt9656480
126 PGAMCAG_162.0
140 PAGMAGG_227.2
147 PACMATA_323.0
155 PCTMATC_378.5
0 PACMACT_172.0
5 PAGMAGT_196.0
19 PGAMATA_123.0
pPt9654255 pPt9653686
toPt9439901 pPt9652451
25 pPt9536643 toPt9439838
pPt9650696 pPt9650059
pPt9534917
27 pPt9655583
35 pPt9652392
40 pPt9651408
46 EAACMCTG_225.8
47 PATMCAT_353.0
51 pPt9473252 pPt9655482
pPt9537451 pPt9536961
56 pPt9559628
57 pPt9471093
59 PATMAAC_93.0
60 EAACMCTG_411.0
61 pPt9650660
70 EACAMCAC_243.3
77 pPt9655893 pPt9655869
PACMATG_290.0
84 PCTMAGG_97.4S12
88 pPt9472539
89 pPt9456776 pPt9458631
pPt9458660 pPt9655816
pPt9473059 pPt9651320
90 pPt9537551 pPt9471344
94 pPt9472296 pPt9652399
pPt9655004
96 PAGMAGC_224.0
101 pPt9459310
102 pPt9459196 pPt9652311
103 PCGMCAA_99.0
119 PAGMAGC_227.0
128 PACMACT_319.3S8
0 PAGMAAG_204.0
8 PATMAAC_256.0
17 PACMACT_345.0
18 PCCMATA_281.0
20 PACMACT_184.3
26 pPt9538212
28 pPt9657524 pPt9534619
35 PACMATG_402.0
37 PCCMACG_194.0
44 PAGMAGC_171.0
46 pPt9653731
47 pPt9459397 pPt9653939
pPt9473610
48 pPt9457378 pPt9535404
pPt9540008
49 pPt9459066 pPt9656111
50 pPt9539688
51 pPt9472867
52 toPt9437109
53 pPt9458070
54 PATMAAC_239.0
55 EACAMCAC_187.1
56 EATGMCTC_232.9
57 EAACMCTG_266.0
59 PACMATG_303.0
64 PCTMATC_87.3S12
65 PAGMACT_272.0
66 EACAMCAC_81.0
67 EATGMCTC_334.0
72 PACMAGG_342.6
75 PAGMAGG_233.5
78 pPt9651467 pPt9651742
pPt9654605
80 PCGMCAA_90.0
94 pPt9535005

Figure 7.1

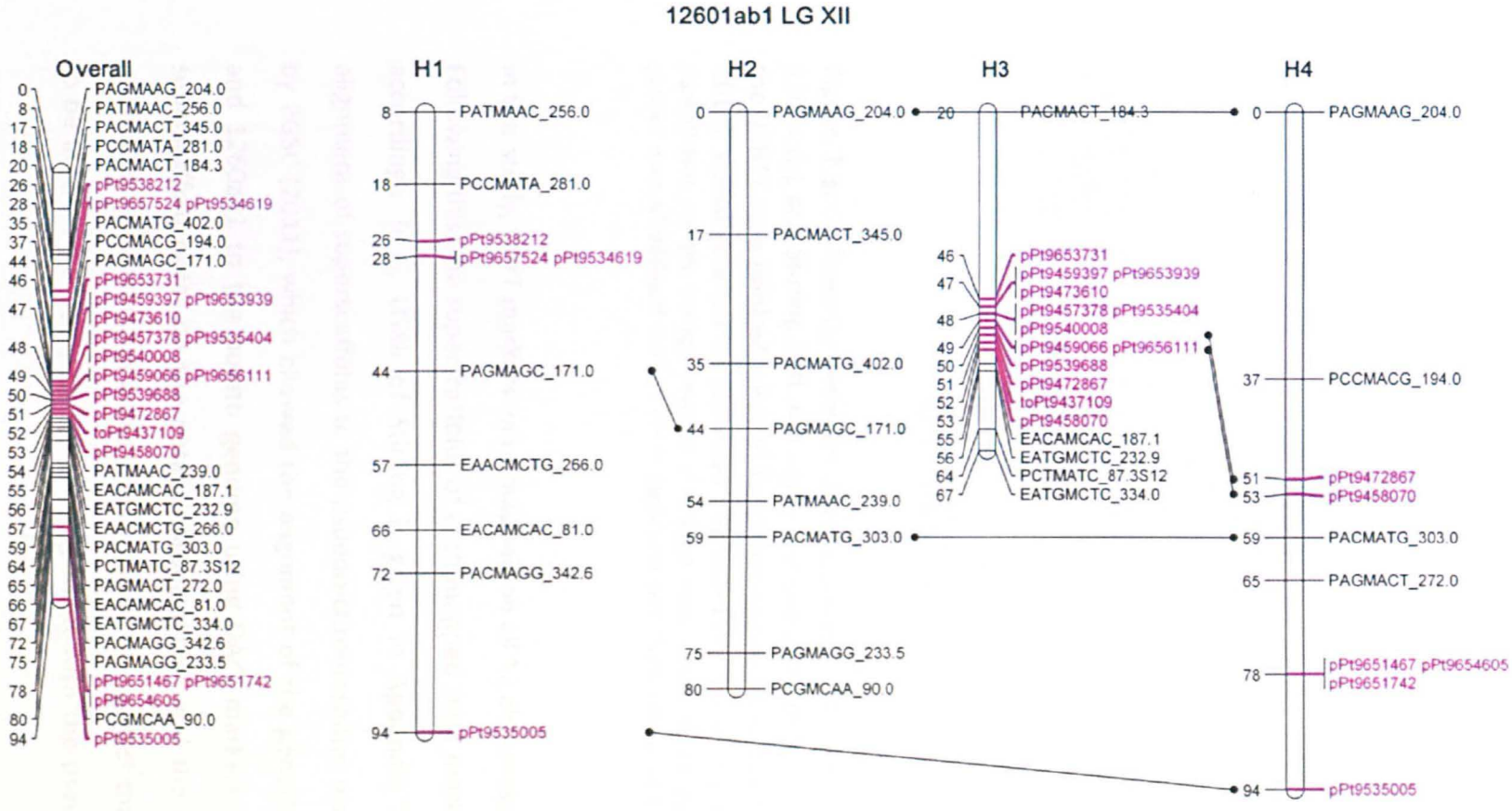

Figure 7.2: Linkage group XII of 12601ab1 showing four homologous chromosomes (H1 to H4) combined (overall) and separately. Duplex (5:1) markers linking homologous groups are indicated by lines. Markers on LGs in pink are DArT markers and those in black are AFLP markers.

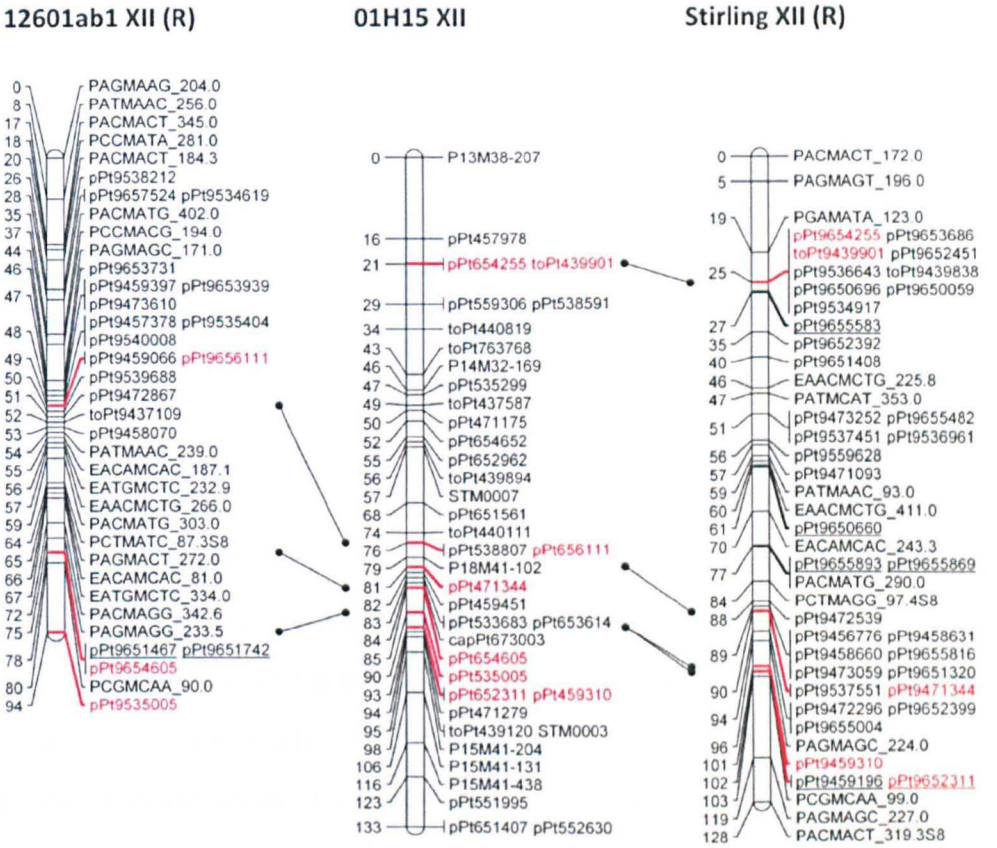

Figure 7.3: Co-linearity between DArT markers on the genetic linkage map of 12601ab1 and Stirling (left and right) and the same markers (red coloured) on the 01H15 map (diploid, middle) for chromosome 12 (adapted from Campbell, 2010). Homologous loci are connected with solid lines. The maps corresponding to Rs in parentheses are the vertical reversal of linkage maps based on an alignment with diploid genetic map. Underlined markers are in alignment with PGSC reference map (not shown).

In this study, DArT markers were mapped on all 12 chromosomes (Figure 7.1). Following this, the superscaffolds of each mapped DArT marker were assigned accordingly (e.g., LGXII of Stirling is given in Appendix Table A7.1). The alignment of superscaffolds to the psuedo-chromosomes was made available by PGSC (2011), which allowed the alignment of the genetic maps of Stirling and 1260ab1 to the potato genome using DArT markers (Figure 7.4). The superscaffold order on the potato chromosomes and the position of DArT markers on some linkage maps in this population suggest that the maps have to be inverted vertically to achieve alignment with the pseudo-chromosome

(not shown). Linkage maps that were reversed were denoted by R in parentheses (Figures 7.1, 7.3 and 7.4). Figure 7.4 shows the alignment of mapped DArT markers in Striling and 12601ab1 LG XII to pseudo-chromosome XII.

7.2.4 Alignment of the parental maps

Once the LGs were established for each parent, SSRs, double-simplex markers (present in both parents) and DArT markers from same superscaffold that were mapped in both parents were used for aligning the parental maps (Figure 7.5). Significant coupling linkages between simplex (1:1) and double-simplex (3:1) markers were identified as means of associating LGs from the two parents. An example is shown in Figure 7.5 for LG XII. Simplex markers linked to double-simplex markers are shown in bold underlined text and arrows indicate linkage to a common double-simplex marker. A permutation analysis was performed using the TetraploidMap software for testing the significance of linkage of a simplex to double-simplex marker as described by Bradshaw *et al.* (2008). The 3:1 markers reported here showed a chi-squared statistic of >17 from a test of independent segregation with one degree of freedom. This corresponds to a significance level of 0.00004 in an individual test.

Table 7.3: Summary of number of mapped DArT markers in common between tetraploid maps of Stirling and 12601ab1 and diploid potato maps, the 01H15 map and the PGSC reference map

	Number of DArTs										
CHR	Stirling^				12601ab1^				Stirling & 12601ab1*		
	01H15	Ref map	01H15 & Ref map	Total	01H15	Ref map	01H15 & Ref map	Total	01H15	Ref map	01H15 & Ref map
I	1	5	0	6	1	6	1	6	3	7	1
II	2	6	0	8	2	7	2	7	5	12	2
III	1	0	0	1	1	0	0	1	5	2	0
IV	0	0	0	0	3	2	1	4	2	0	0
V	1	2	0	3	2	5	1	6	1	8	1
VI	0	10	0	10	0	0	0	0	5	0	0
VII	1	2	1	2	1	2	0	3	3	0	0
VIII	0	0	0	0	0	9	0	9	1	2	0
IX	0	1	0	1	1	2	0	3	2	1	0
X	2	0	0	2	0	0	0	0	4	1	0
XI	5	3	1	7	3	1	0	4	4	2	0
XII	5	6	1	10	3	2	0	5	1	5	1
Total	18	35	3	50	17	36	5	48	36	40	5

^Mapped markers segregating in 1:1 and 5:1 ratio; *Markers segregating in 3:1 and 11:1 ratio present in both parents and were not used for mapping in tetraploid mapping population.

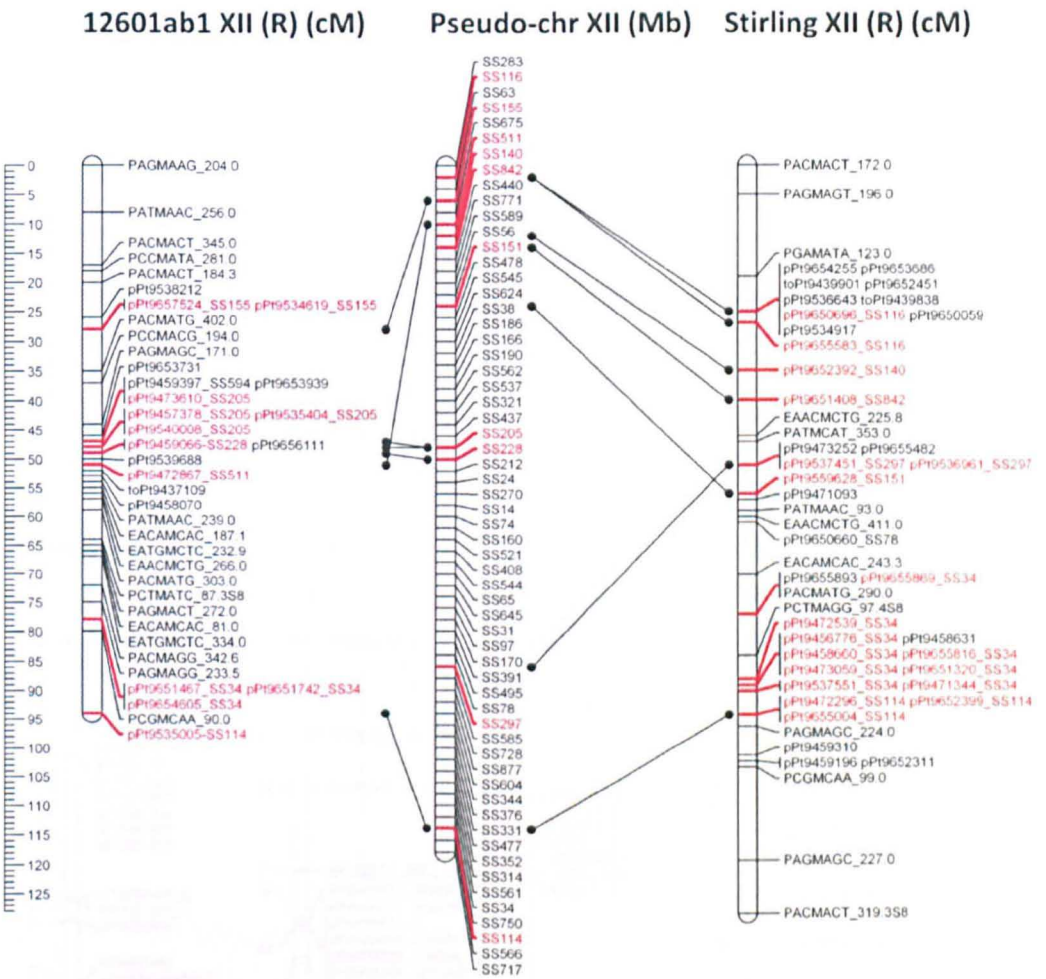

Figure 7.4: Comparison of 12601ab1 (left) and Stirling (right) linkage maps XII with the psuedo-chromosome (chr) XII of potato genome sequence. Map distances are given in centiMorgan (cM) and pseudo-chromosome positions are given in nucleotide, Mb. DArT markers with superscaffold alignment/hits are given with the suffix SS no. (eg., SS155). Lines between linkage maps and pseudo-chromosome indicate the alignment of DArT markers (superscaffolds) in genetic maps to pseudo-chromosome.

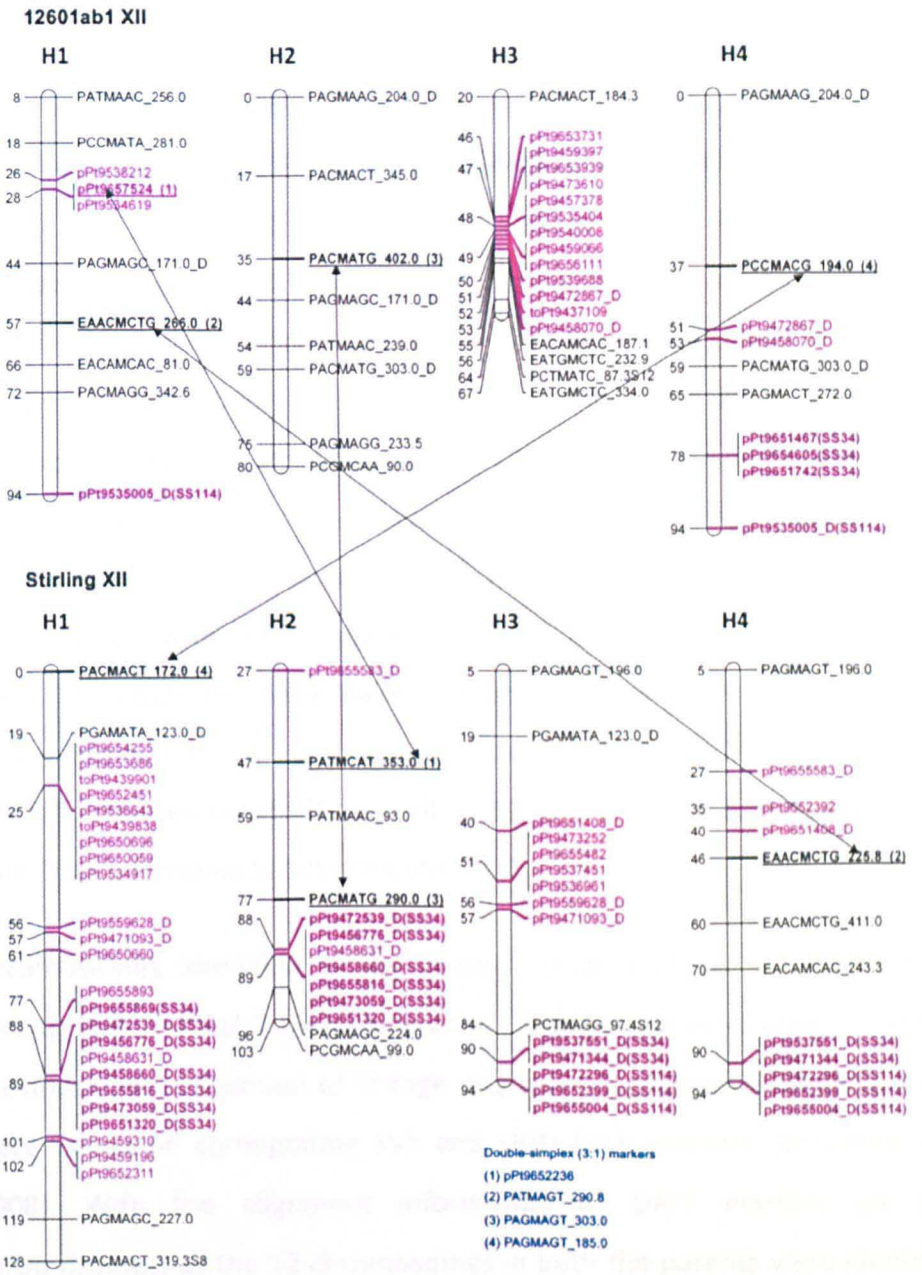

Figure 7.5: Alignment of the two parental (Stirling and 12601ab1) maps using the double-simplex (3:1) markers segregating in both parents. Linkage Group XII showing the four homologous chromosomes (H1 to H4) in 12601ab1 and Stirling. Markers on LGs in pink are DArT markers and those in black are AFLP markers. Duplex markers are indicated by suffix 'D' and simplex markers linked to double-simplex markers in coupling are shown in bold underlined text. The double-simplex DArT and AFLP markers (blue colour) bridging the two parental maps were given at the bottom of the figure. Arrows show that two simplex markers are linked to the same double-simplex marker, and hence they align the two parents. DArT markers from the same superscaffold (SS) that were mapped in both parents also align the parental maps.

7.3 DISCUSSION

The development of dense genetic linkage maps in tetraploid potatoes is the first step for understanding the genetic control (QTLs) of agronomic, tuber quality and nutritional traits. In the present study, linkage analysis was conducted in 12601ab1 x Stirling, a F1 tetraploid mapping population with a size of 190 clones. Genome-linked DArT markers were added to the existing linkage maps of Stirling and 12601ab1 (Bradshaw *et al.*, 2008). The published genetic map was constructed using 38 AFLP primer combinations and 23 SSRs (Bradshaw *et al.*, 2008). The addition of DArT markers improved the previous linkage maps from this population (Bradshaw *et al.*, 2008) by saturating the linkage maps with DArT markers (Figure 7.1). To date, DArT-based linkage maps in potatoes have been reported in wild species such as *S. bulbocastanum* and *S. commersonii* (Gao *et al.*, 2009; Iorizzo *et al.*, 2009), diploid cultivated potato (Campbell, 2010) and a diploid backcross population (The Potato Genome Sequence Consortium, 2011).

Chromosomes identified in the present study and the published map of Stirling and 12601ab1 (Bradshaw *et al.*, 2008) were presented in Table 7.4. Previously, the alignment of linkage maps and identification of chromosomes were based on co-migrating SSR and UHD-AFLP markers (Bradshaw *et al.*, 2008). With the alignment information of DArT markers on potato chromosomes, all the 12 chromosomes in both the parents were identified in this study (Figure 7.1).

Linkage groups Ia, II, VI and XI in Stirling and II, IV, V, VIII and XI in 12601ab1 had greater than 50 markers (Table 7.2). The 'ripple' option in TetraploidMap for marker ordering allows only 50 markers per LG and hence only 50 markers per LGs were used for linkage map construction. However, the addition of more markers into LGs will be possible in the near future, as measures to

update the TetraploidMap software is currently underway (Dr. Christine Hackett, BioSS, personal communication).

Table 7.4: Summary of the present and published (Bradshaw *et al.*, 2008) linkage maps of Stirling and 12601ab1

CHR	Updated map (present study)	Map length, cM	Publishedmap (Bradhaw *et al.*, 2008)	Map length, cM
	Stirling			
I	Ia	82	Ia	71
	Ib	125	Ib	144
II	II	120	II	105
III	III	120	III	146
IV	IV	132	IV	130
V	V	118	V	126
VI	VI	106	VI	122
VII	VII	50	-	-
VIII	VIII	126	VIII	110
IX	IX	120	A	98
X	X	119	XIa	147
XI	XI	106	XIb	114
XII	XII	128	B	139
	12601ab1			
I	Ia	99	Ib	91
	Ib	117	Ia	115
II	II	154	II	150
III	III	133	III	134
IV	IV (IV+E)^	108	IV	96
			E	27
V	V	122	V	126
VI	VI	132	VI	123
VII	VII	118	D	46
VIII	VIII	109	VIII	133
IX	IXa	88	A	112
	IXb	111	F	89
X	X	105	XIa	98
XI	XI (XIc +C)^	155	XI c	106
			C	119
XII	XII	94	B	94

CHR-chromosome; -not reported; ^Chromosomes with characters within the parentheses indicate the updated genetic maps were constructed by integrating/combining two linkage maps reported in the previous study.

Two linkage maps were constructed for chromosomes I and IX (Figure 7.1; Table 7.2). For chromosome IX, the markers in IXa and IXb of 12601ab1 showed no associaton between them and this might be due to lack of molecular markers covering the whole chromosome, or due to loss of a segment of the chromosome as a result of pericentric inversions followed by cross-over inside the inversion loop. In chromosome I, although the markers showed an association between Ia and Ib, due to size limit of 50 markers per linkage group in Tetraploidmap, the markers were clustered into two groups, Ia and Ib.

Several genetic maps were constructed for tetraploid potatoes to study different traits of interest (Table 7.5 and references therein). The relatively large population size used for construction of the genetic linkage map presented in this study (190 clones) as compared with other studies (Table 7.5) will be highly advantageous for further exploitation of this map. The sizes of tetraploid populations determine the sensitivity of the ability to detect the major QTLs. Hackett *et al.* (1998) reported that a minimum population size of 150 individuals should be used to develop a linkage map in an autotetraploid species and that a large population size (e.g., 250) would provide a better chance of identifying homologous chromosomes. In this study, with a population size of 190 clones and the addition of DArT markers covering all the 12 chromosomes, the existing maps were greatly improved (Figure 7.1).

The proportion of DArT markers that were in common between tetraploid and diploid genetic maps was relatively small (Table 7.3). This might be due to a lack of polymorphism in some genomic regions, and may also reflect the different genetic backgrounds of the diploid and tetraploid mapping parents. Furthermore, markers segregating in 11:1 (simplex x duplex) and 35:1 (duplex x duplex) ratios were not used for mapping as these markers are typically inherited by most of the progeny and are often uninformative about recombination between markers. Inevitably, this leads to the absence of

markers in certain genomic regions. However, the DArT markers in common between the maps allowed for the comparison of marker locations between tetraploid and diploid genetic maps. The shared DArT markers were mapped in the same order in both populations, with Stirling having near complete colinearity with the diploid map compared with 12601ab1 (Figure 7.3).

In summary, the addition of genome-linked DArT markers to the existing AFLP- and SSR-based linkage maps of 12601ab1 and Stirling greatly improved the maps. Using the information on the genomic location of DArT markers, all the 12 chromosomes in each parent were identified.

Table 7.5: Molecular maps constructed for potato chromosomes in tetraploid populations

Popn. name	Size and type of progeny	Marker type	Segreg. of mapped markers	Parental species	No. of marker loci	Map coverage, cM	Trait	Reference
-	94, F1 & 78, F1	AFLP	1:1, 3:1 & 5:1	Stirling	116	484.6	Late blight & PCN resistance	Meyer *et al.* (1998) & Bradshaw *et al.* (1998)
				12601ab1	229	990.9		
-	227, F1	AFLP & SSRs	1:1 & 5:1	Stirling	-	105 (LG IV) & 104 (LG V)	Late blight resistance, plant height and maturity	Bradshaw *et al.* (2004)
				12601ab1	-	-		
-	227, F1	AFLP & SSRs	1:1 & 5:1	Stirling	-	-	PCN resistance	Bryan *et al.* (2004)
				12601ab1	261	63 (LG IV) & 146 (LG XI)		
ND5873	93, F1*	AFLP	1:1	ND4382-19	320	1883.3	Leptine content	Sagredo *et al.* (2006, 2011)
				Chipeta	168	1021.4		
-	227, F1	AFLP & SSRs	1:1 & 5:1	Stirling	221	1234	Yield, agronomic & quality traits	Bradshaw *et al.*(2008)
				12601ab1	293	1202		
-	92, F1	AFLP, SSCP & SSR	1:1 & 5:1	PA95A33-1	-	2940	CRS	Khu *et al.* (2008)
				A9446-7		1929		
NDG116	99, F1*	AFLP	1:1	ND4382-19	266	1395.7	Colorado potato beetle	Sagredo *et al.* (2009)
				N142-72	196	1141.6		
AO1687	35, BC3	RFLP & CAPs	-	Etb 6-21-3	-	-	PLRV resistance	Kelley *et al.* (2009)

				GemStar Russet	-	-		
B2721	160, F1	AFLP & SSRs	1:1, 3:1 & 5:1	Atlantic	274; 274	1034.4; 1059.4	IHN & foliage maturity; agronomic traits	McCord *et al.* (2010), (2011)
				B1829-5	244 ;252	940.2; 940.5		
GenPop1	190, F1	AFLP, DArT & SSRs	1:1 & 5:1	Stirling	449	1451.3	Plant emergence, maturity, flower colour, tuber yield, DM and mineral concentrations	Present study (2011)
				12601ab1	546	1644.1		

*Half-sib family; -Information not available; Popn-population; Segreg-segregation ratio; PCN-potato cyst nematode; CRS-corky ringspot; PLRV-potato leafroll virus; IHN-internal heat necrosis.

CHAPTER 8 IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR MINERAL TRAITS IN POTATO TUBER

8.1 INTRODUCTION

Genetic linkage maps are essential for mapping and dissection of complex quantitative trait loci (QTL) such as the ones for mineral accumulation in plants, which is controlled by a number of genes. The movement of mineral elements from soil to edible plant parts involves a series of processes, including their mobilization from soil, uptake by roots, translocation to the shoot, redistribution within the plant and accumulation in edible parts (White and Broadley, 2009). QTL studies involving mineral concentrations/accumulation in food crops are limited to rice, wheat, maize, barley, brassica, common bean and soybean (see section 1.6, Table 1.1 and references therein). These reported studies indicate that the regulation of mineral accumulation in plants is genetically complex, with several genetic loci controlling this process. However, to date no QTL studies have been reported for tuber mineral concentrations in potatoes.

The objective of this study was to identify the QTL associated with tuber mineral concentrations in potatoes, using the linkage maps produced from Chapter 7 and tuber mineral data generated by ICP analysis (see Chapter 5). In addition, the SSR (GenPop1) and DArT (NTB population) markers, identified using bulked segregant analysis (BSA, see Chapter 6) that were associated with tuber yield, Ca, Fe and Zn concentrations, are discussed with particular reference to the QTL identified in this study. Attempts were also made to identify the potential candidate genes underlying mineral QTLs. Candidate genes that have been reported to be involved in mineral homeostasis and accumulation were subjected to BLAST analysis in the genome browser in the Potato Genome Sequencing Consortium (PGSC) database to find superscaffold hits, and the superscaffolds were then aligned to chromosomes in the mapping population.

8.2 SEARCH FOR CANDIDATE GENES

The PGSC web portal (www.potatogenome.net) provides access to the physical and genetic maps, bacterial artificial chromosome (BAC) sequences, genome browsers, gene expression data and annotation information. Reported genes pertaining to mineral accumulation in plants (or family members of such genes, section 1.2.3 and Appendix I, Chapter 1) were used to identify the superscaffolds containing these genes in the potato genome (not shown). Information on location of the DArT markers in the superscaffolds on the potato genome is now available from PGSC. Based on the DArT marker/superscaffold alignment to the GenPop1 genetic maps, the potential candidate genes influencing mineral accumulation were aligned to genetic maps (Figure 8.1). Arrows indicate the exact location of superscaffolds in the genetic maps and dotted lines indicate the assigned location of superscaffolds (Figure 8.1). The candidate gene and superscaffold search was done during June–July 2011. The candidate gene search for mineral traits includes the transporters, chelators, storage molecules, regulators of transporters or homeostasis and genes involved in metabolism (section 1.2.3 and Appendix I, Chapter 1).

8.3 RESULTS

The QTL analysis was performed using interval mapping procedure as described in section 2.7.5, Chapter 2. Figure 8.1 shows the location of the QTLs, with one-LOD support intervals. The results obtained from bulked segregant analysis (BSA) using SSR and DArT markers for tuber yield, Ca, Fe and Zn concentrations in GenPop1 and NTB populations (see Chapter 6) are also presented in Figure 8.1. The candidate genes associated with the identified QTLs are also shown.

QTLs were detected in one, two or all the three years of study (Figure 8.1, Appendix Table A8.1) and were well distributed on all twelve chromosomes of the potato genome (Table 8.1). Almost all the mineral QTLs were co-located with at least one other QTL. The co-location of QTLs was noted on most chromosomes (Figure 8.1). The positions, LOD scores and percentage phenotypic variance explained for the full

models of six QTL genotypes and for a simpler model, if there is one that fits the data, are presented in Appendix Table A8.1 and A8.2. The dominant or additive effect of QTLs governing mineral traits are presented in Appendix Table A8.1. Summary of the percentage variance explained by QTLs for tuber yield, Ca, Fe, Zn, Cu, Mg and K concentrations in 12 chromosomes of Stirling and 12601ab1 are presented in Tables 8.4 - 8.7 and for the rest of the traits, a synopsis is provided in Appendix Tables A8.3 - 8.5.

Table 8.1: Summary of the location of QTLs on different chromosomes identified for several plant and tuber traits in 12601ab1 x Stirling population

Trait	Chromosomes	
	Stirling	**12601ab1**
Plant traits		
Emergence	II, V, VII, IX & XII	II, V, VII, VIII, X, XI & XII
Foliage maturity	Ib, V, VI & VIII	Ia, V, VI, X & XII
Flower colour	-	X
Tuber traits		
Yield	Ia, II, V, VI & XII	Ia, II, III, IV, V, VII, VIII, XI & XII
Dry matter	II, V, VI & X,	Ia, Ib, V, VI, VII, VIII, XI & XII
Boron	Ia, II, IV, V, VI, VII, IX & XII	Ia, Ib, III, V, VI, VIII, X & XII
Nitrogen	II, V, VI, IX, X & XII	Ia, II, III,IV, VI, VII, VIII, X, XI & XII
Phosphorus	Ia, Ib, III, IV, V, VI, IX, X & XII	all 12 chromosomes
Sulphur	II, IV, V, VI, X & XII	Ia, Ib, II, IV, VI, VII, VIII, IXb, X, XI & XII
Calcium	Ia, Ib, II, III, V, VI, VIII, IX, X, XI & XII	Ia, II, III, IV, VIII, IXb & XII
Copper	Ia, Ib, II, III, V, VI, & XII	all 12 chromosomes
Iron	Ia, Ib, II, III, IV, VIII, IX, X, XI & XII	Ia, Ib, II, III, V, VI, VII, VIII, IXb & XII
Potassium	Ib, II, V, VI, IX & X	Ia, Ib, II, III,IV, V, VI, VII, VIII, IX & XII
Magnesium	Ib, II, IV, V, VI, IX, XI & XII	Ia, Ib, II, III, VI, VIII, IXb, X & XII
Manganese	Ib, II, III, IV, V, VI, VIII, X & XII	Ia, Ib, II, III, VI, VIII, X, XI & XI
Sodium	Ia, Ib, III, VI, VIII, IX & XI	Ia, III, IV, VI, VII, VIII & XI
Nickel	VIII & X	Ia, Ib, IV, VI, VIII & XI
Zinc	Ia, Ib, II, III, V, VI, X & XI	Ia, Ib, II, III, IV, VII, VIII, IX, X, XI & XII

8.3.1 Plant emergence

QTLs associated with plant emergence were mapped on Stirling chromosomes II, V, VII, IX and XII and on 12601ab1 chromosomes II, V, VII, VIII, X, XI and XII (Figure 8.1, Appendix Table A8.1). The variance explained by QTLs governing plant emergence ranged from 4.3 to 13.1% in 12601ab1 and from 5.3 to 13.0% in Stirling (Appendix Table A8.1, Figure 8.1). QTLs pertaining to late emergence were found on 12601ab1 chromosomes II (2007 & 2009), VII (2007) and XII (2009) and to early emergence on 12601ab1 chromosomes V (2007), VIII (2007) and Stirling chromosome VII (2007) (Appendix Table A8.1). The above mentioned QTLs displayed a dominant effect and all other QTLs showed an additive effect on plant emergence.

8.3.2 Foliage maturity

QTLs for foliage maturity were detected on Stirling chromosomes Ib, V, VI and VIII in either one or two of the study years (Figure 8.1, Appendix Table A8.1). All QTLs except for Stirling chromosomes V and VIII showed an additive effect (Appendix Table A8.1). The QTL for Stirling chromosome V was associated with early foliage maturity with a simplex dominant effect, whereas the QTL on Stirling chromosome VIII was associated with late maturity with a duplex dominant effect. The largest LOD score for maturity was found on Stirling chromosome V, where the presence of a simplex allele on homologous chromosome (C1) explained 47 and 45% variance in 2007 and 2009, respectively.

In 1260ab1, QTLs for foliage maturity were identified on chromosomes Ia, V, VI, X and XII. The variation explained by QTLs for foliage maturity ranged from 4.5 to 6.9% in 12601ab1 and from 6.2 to 50.4% in Stirling (Appendix Table A8.1, Figure 8.1). All QTLs, except for the one on Stirling chromosome VIII, were found in the same location in 2007 and 2009.

8.3.3 Flower colour

QTL with largest (simplex dominant) effect for flower colour was found on 12601ab1 chromosome X, explaining 68% of the total variation (Figure 8.1, Appendix Table A8.1). Two secondary QTLs were also found on 12601ab1 chromosome X for flower colour. However, no QTL with influence on flower colour was identified from Stirling.

8.3.4 Tuber yield

QTLs governing tuber yield were mapped on Stirling chromosomes Ia, II, V, VI and XII and on 12601ab1 chromosomes Ia, II, III, IV, V, VII, VIII, XI and XII either in one or two years (Figure 8.1, Appendix Table A8.1). These QTLs explained about 5.9 to 10.8% variance in Stirling and about 6.3 to 15.0% variance in 12601ab1. The only QTL that was detected for all three study years was found on 12601ab1 chromosome XII, which accounted for about 6.6 to 8.2% of total variation. This QTL displayed an additive effect in all the three years. QTLs with a dominant effect for high tuber yield were found on Stirling chromosomes Ia and V (2009), and for low yield on 12601ab1 chromosomes XI (2007) and VII (2009) (Appendix Table A8.1).

8.3.5 Tuber DM

In Stirling, QTLs for tuber DM content were identified on chromosomes II, V, VI and X, explaining about 5.2 to 10.1% of total variance (Figure 8.1, Appendix Table A8.1). All these QTLs were detected only in 2009 except for the one on Stirling chromosome X, for which the QTL was found in two years (2008 and 2009). In 12601ab1, QTLs for tuber DM were found in the chromosomes Ia, Ib, V, VI, VII, VIII, XI and XII, explaining about 5.1 to 13.9% of the variation (Figure 8.1, Appendix Table A8.1). Among these, QTLs with a dominant effect on tuber DM were found on Stirling chromosome X (2008) and on 12601ab1 chromosomes VII, VIII and XII (2008).

8.3.6 Mineral traits

8.3.6.1 Iron

QTLs for Fe concentration were identified on nine chromosomes each in 12601ab1 (Ia, Ib, II, III, V, VI, VII, VIII, IXb and XII) and in Stirling (Ia, Ib, II, III, IV, VIII, IX, X, XI and XII), explaining about 4.9 to 14.3% and about 4.3 to 14.7% of the total phenotypic variation, respectively. QTLs influencing tuber Fe concentration detected in all three years were located on chromosome Ia of 12601ab1 and on chromosome Ib of Stirling (Figure 8.1, Appendix Table A8.1).

8.3.6.2 Zinc

QTLs for Zn concentrations were identified on almost all chromosomes in 12601ab1 except for chromosomes V and VI. In Stirling, QTLs associated with Zn were found on seven chromosomes (Ia, Ib, II, III, V, VI, X and XI). The variance explained by these QTLs was about 4.0 to 11.0% in 12601ab1 and about 3.9 to 11.7% in Stirling. The chromosome VII of 12601ab1 exhibited Zn QTLs over the three years, explaining about 6.6 to 9.6% the total phenotypic variance (Figure 8.1, Appendix Table A8.1).

8.3.6.3 Calcium

QTLs for tuber Ca was found on seven chromosomes (Ia, II, III, IV, VIII, IXb and XII) in 12601ab1 and on 10 chromosomes (Ia, Ib, II, III, V, VI, VIII, IX, X, XI and XII) in Stirling (Figure 8.1, Appendix Table A8.1). These QTLs accounted for about 3.5 to 13.7% of total variation in 12601ab1 and about 4.4 to 15.7% variation in Stirling. QTLs with consistency over three years were found on chromosomes II, IV, VIII and XII in 12601ab1 and chromosomes V, VI, VIII, IX and XII in Stirling.

8.3.6.4 Potassium

QTLs associated with K concentration were found on almost all chromosomes of 12601ab1, except for chromosomes X and XI, explaining about 4.0 to 18.4% of the variance (Figure 8.1, Appendix Table A8.1). In Stirling, QTLs influencing K

concentrations were detected on six chromosomes (Ib, II, V, VI, IX and X), accounting for about 5.0 to 31.6% of the total variance. QTLs consistent over the three study years were found on chromosomes VIII, IXb and XII in 12601ab1 and on chromosomes V and X in Stirling.

8.3.6.5 Copper

QTLs for tuber Cu concentrations were found on all the 12 chromosomes of 12601ab1, explaining about 4.9 to 20.3% of the total phenotypic variation (Figure 8.1, Appendix Table A8.1). In Stirling, QTLs for tuber Cu concentration were detected on seven chromosomes (Ia, Ib, II, III, V, VI, and XII), accounting for about 4.1 to 14.2% of the total variation. QTLs with consistent presence over the three years were found only in 12601ab1 chromosomes II, VIII, XI and XII.

8.3.6.6 Magnesium

For tuber Mg concentration, QTLs were identified on eight chromosomes each in 12601ab1 (Ia, Ib, II, III, VI, VIII, IXb, X and XII) and in Stirling (Ib, II, IV, V, VI, IX, XI and XII) (Figure 8.1, Appendix Table A8.1). These QTLs explained about 5.0 to 15.3% and about 5.5 to 42.1% of variance in 12601ab1 and Stirling, respectively. Among these, the QTL on chromosome V of Stirling population was consistent over three years and accounted for a higher percent variation (10.4 to 42.1%) than other QTLs in this population. In 12601ab1, chromosomes II, III and XII had QTLs for tuber Mg concentration in all the three study years, with QTL on chromosome II alone explaining about 8.5 to 15.3% of the total variance.

8.3.6.7 Boron

QTLs for tuber B concentrations were identified on chromosomes Ia, II, IV, V, VI, VII, IX and XII in Stirling and on chromosomes Ia, Ib, III, V, VI, VIII, X and XII in 12601ab1 (Figure 8.1, Appendix Table A8.1). These QTLs explained about 4.7 to 14.4% of variance in Stirling and about 4.1 to 17.5% of variance in 12601ab1. QTLs with

presence in all the three years were found in Stirling chromosomes V, VI and IX and in 12601ab1 chromosome Ib.

8.3.6.8 Nitrogen

For tuber N concentrations, QTLs have been identified on 10 chromosomes in 12601ab1 (excluding chromosomes V and IX), and on six chromosomes (II, V, VI, IX, X and XII) in Stirling (Figure 8.1, Appendix Table A8.1). These QTLs accounted for about 4.7 to 13.8% of variance in 12601ab1 and about 5.3 to 16.4% of variance in Stirling. The QTL on Stirling chromosome V was detected in all the three study years.

8.3.6.9 Phosphorus

QTLs for tuber P concentrations was distributed across all the 12 chromosomes of 12601ab1, explaining about 4.3 to 14.3% of the total phenotypic variation (Figure 8.1, Appendix Table A8.1). In Stirling, QTLs for tuber P concentrations were located on eight chromosomes (Ia, Ib, III, IV, V, VI, IX, X and XII) accounting for about 3.8 to 28.1% of the total phenotypic variation. QTLs with presence in all the three years were found on chromosome IV in Stirling and on chromosomes Ib and VIII in 12601ab1.

8.3.6.10 Sulphur

For tuber S concentration, QTLs were distributed across 10 chromosomes (Ia, Ib, II, IV, VI, VII, VIII, IXb, X, XI and XII) in 12601ab1 and six chromosomes (II, IV, V, VI, X and XII) in Stirling (Figure 8.1, Appendix Table A8.1). The identified QTLs explained about 5.7 to 12.5% variation in 12601ab1 and about 5.1 to 28.7% variation in Stirling. The QTL on Stirling chromosome V alone explained about 22.8 to 28.7% of the total phenotypic variation. The QTL that was detected in all the three years was found on 12601ab1 chromosome XII.

8.3.6.11 Manganese

QTLs for tuber Mn concentrations were detected on eight chromosomes of 12601ab1 (Ia, Ib, II, III, VI, VIII, X, XI and XI) and on nine chromosomes of Stirling (Ib, II, III, IV, V, VI, VIII, X and XII) (Figure 8.1, Appendix Table A8.1), accounting for about 4.6 to 14.1% and about 4.7 to 20.4% of total variance, respectively. The QTLs on chromosomes X and XII in 12601ab1 and on chromosomes II, V and XII in Stirling were consistent over the three years.

8.3.6.12 Sodium

QTLs associated with tuber Na concentrations were found on seven chromosomes of 12601ab1 (Ia, III, IV, VI, VII, VIII and XI) and on six chromosomes of Stirling (Ia, Ib, III, VI, VIII, IX and XI) (Figure 8.1, Appendix Table A8.1). The identified QTLs explained about 5.9 to 17.4% and 4.3 to 14.7% of the total phenotypic variances in 12601ab1 and Stirling respectively. QTLs consistent over the three years were found on Stirling chromosomes Ib, VI, VIII and XI.

8.3.6.13 Nickel

Tuber Ni concentrations were measured only during 2007 (analysed at SCRI). The QTLs influencing tuber Ni concentrations were identified on five chromosomes (Ia, Ib, IV, VI, VIII and XI) in 12601ab1 and two chromosomes (VIII and X) in Stirling, accounting for about 7.4 to 10.1% and about 6.5 to 7.6% of the total variance, respectively (Figure 8.1, Appendix Table A8.1).

8.3.6.14 Zinc

QTLs for Zn concentrations were identified on almost all chromosomes in 12601ab1 except for chromosomes V and VI. In Stirling, QTLs associated with Zn were found on seven chromosomes (Ia, Ib, II, III, V, VI, X and XI). The variance explained by these QTLs was about 4.0 to 11.0% in 12601ab1 and about 3.9 to 11.7% in Stirling. The chromosome VII of 12601ab1 exhibited Zn QTLs over the three years, explaining about 6.6 to 9.6% the total phenotypic variance (Figure 8.1, Appendix Table A8.2).

8.3.7 Maturity corrected QTLs on Stirling chromosome V

The QTL for foliage maturity on Stirling V alone explained about 45 to 47% of the total phenotypic variance (Figure 8.1). The analysis of residuals, following the regression of all the phenotypic traits on maturity, showed no or few associations with this region. Additionally, the following changes were noted after regression: (1) QTLs affecting N, P, S, Ca, and Zn concentrations were not prominent/identified, (2) QTLs governing Mg, Mn and K disappeared at the maturity locus but appeared at different regions, (3) for emergence, B and Cu concentrations, the same QTL existed with low LOD values, (4) for tuber yield, the maturity loci associated with QTL for yield (for year 2009) disappeared, but appeared at a different region and (5) a new QTL was identified for over-year Na concentration, accounting for about 5.8% of the total phenotypic variation (Figure 8.2).

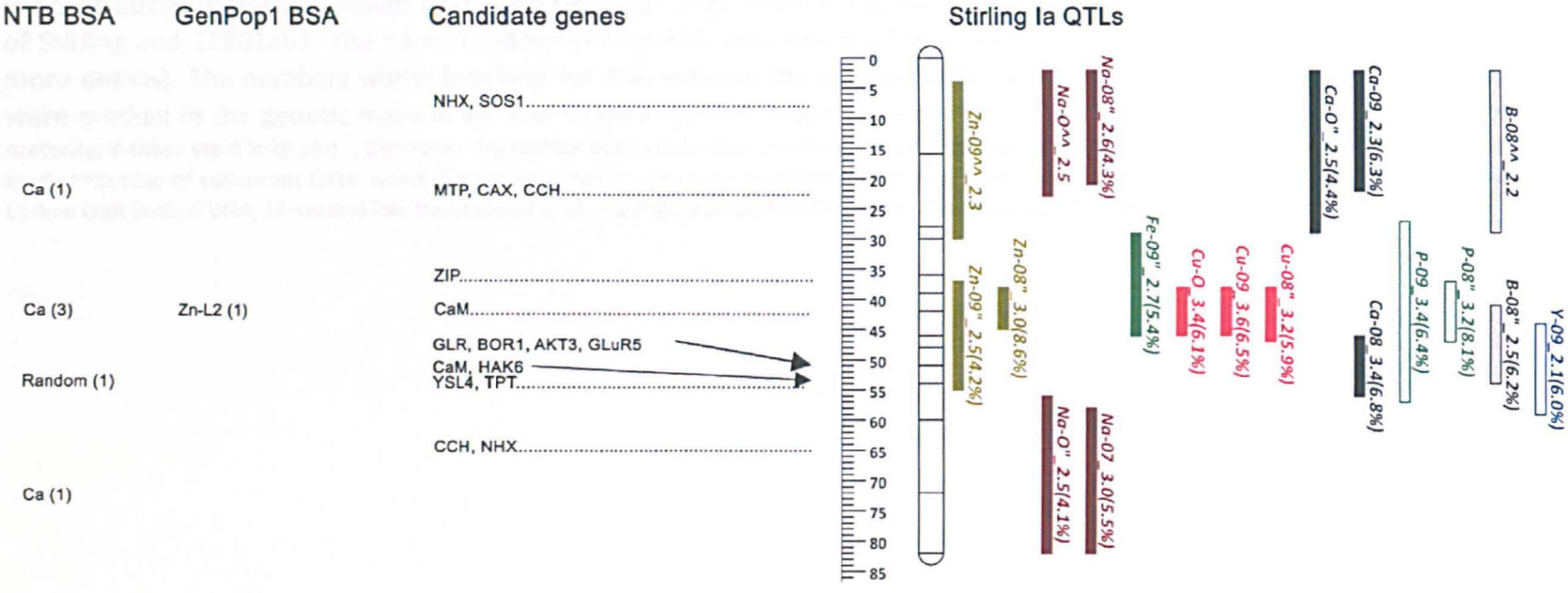

Figure 8.1 (continued on next page): Chromosomal location of the QTLs for plant emergence, maturity, tuber yield, dry matter content and mineral concentrations in tetraploid parents, Stirling and 12601ab1. The ruler in the centre gives the genetic distance in centiMorgans (cM). QTLs are shown at the right side in vertical bars with 1-LOD interval with different colours and fillings/shadings for each trait. The two-digit number following each abbreviation of the trait indicates the year in which QTL was detected (07-2007, HRI mineral analysis; 07a-2007, SCRI analysis; 08-2008; 09-2009). Numbers followed by abbreviation of the trait/year is the LOD score and the numbers in brackets represents the percentage of trait variation explained. Mineral-related candidate genes were aligned to the genetic maps with arrows (→), indicating the precise location of candidate genes (superscaffold, SS) based on mapped DArT markers in the mapping population. The dotted lines indicate the assigned location/estimated genetic location of candidate genes (SS). DArT and SSR markers identified for tuber yield, Ca, Fe and Zn

concentrations in Neotuberosum (NTB) and GenPop1 population using bulked segregant analysis (BSA) were also aligned to the genetic maps of Stirling and 12601ab1. The name random in NTB-BSA indicates the DArT markers that were identified in random bulks (see Chapter 6 for more details). The numbers within brackets for BSA indicate the number of DArT or SSR markers identified for each trait. The mapped DArTs were marked in the genetic maps in an oval-shaped symbol. Maps were drawn using MapChart (Voorrips 2002). Emer-plant emergence; Mat-plant maturity; Y-tuber yield in kg plot^{-1}; DM-tuber dry matter content; fc-flower colour; *ns; ^in case of significant QTLs, when the second peak is above or within the significant level; ^^in case of significant QTLs, when the second peak is below the significant level and/or in case of non-significant QTLs with more than one peak. For GenPop1 BSA, L1-low trait bulked DNA, L2-second low trait bulked DNA, H1-high trait bulked DNA and H2-second high trait bulked DNA.

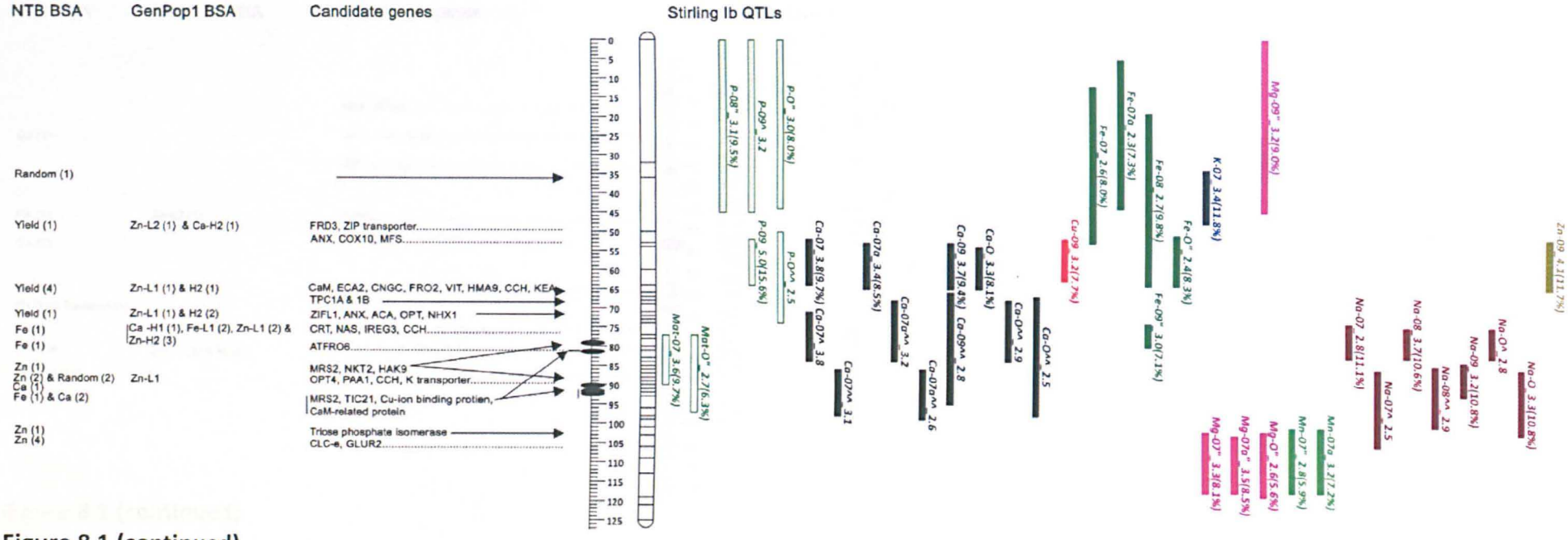

Figure 8.1 (continued)

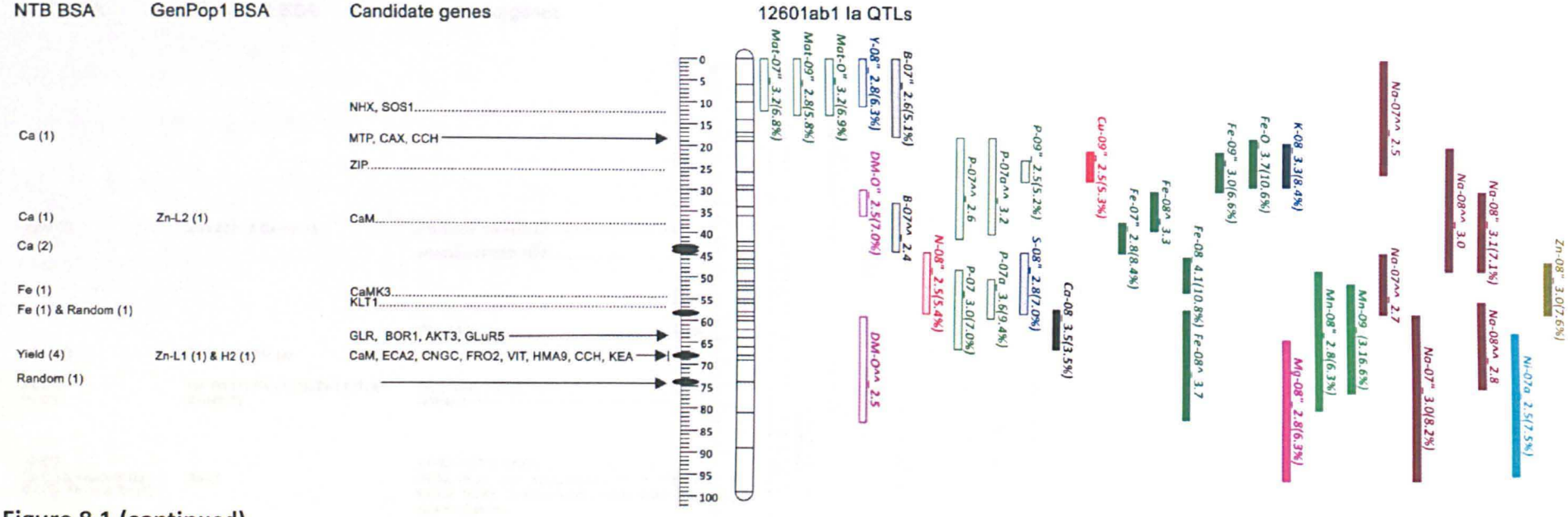

Figure 8.1 (continued)

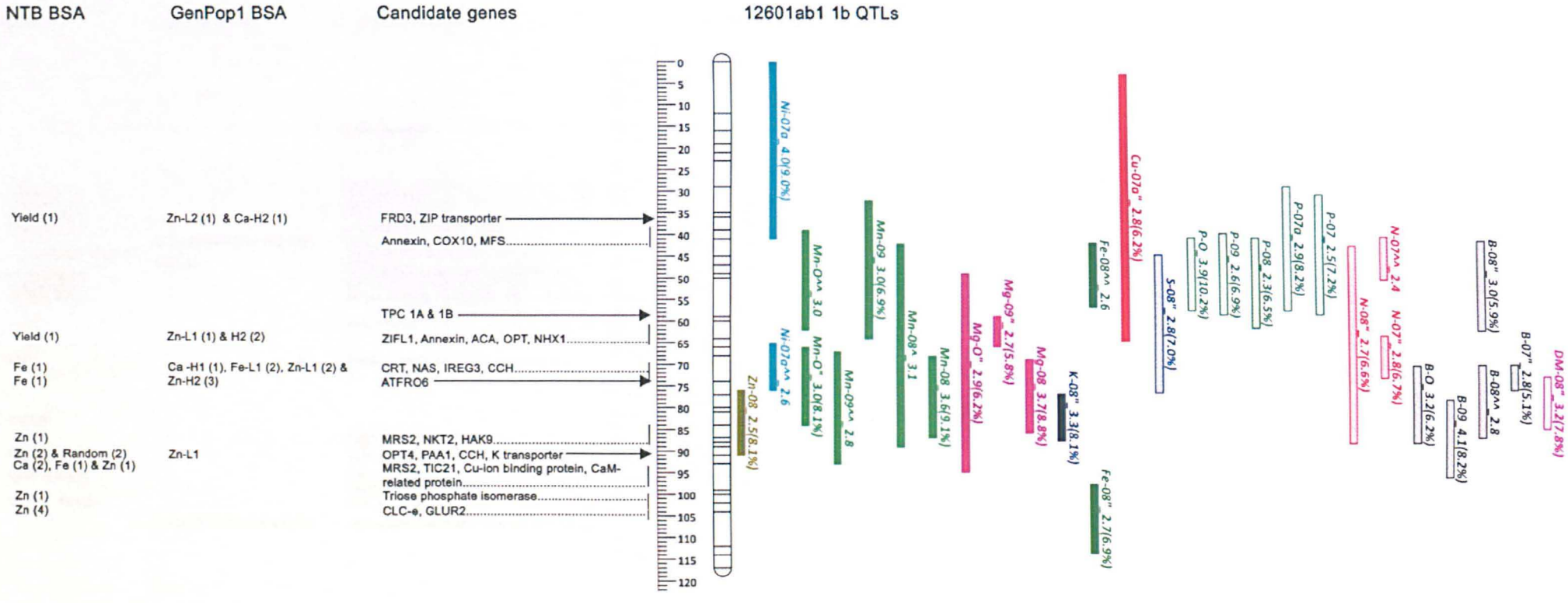

Figure 8.1 (continued)

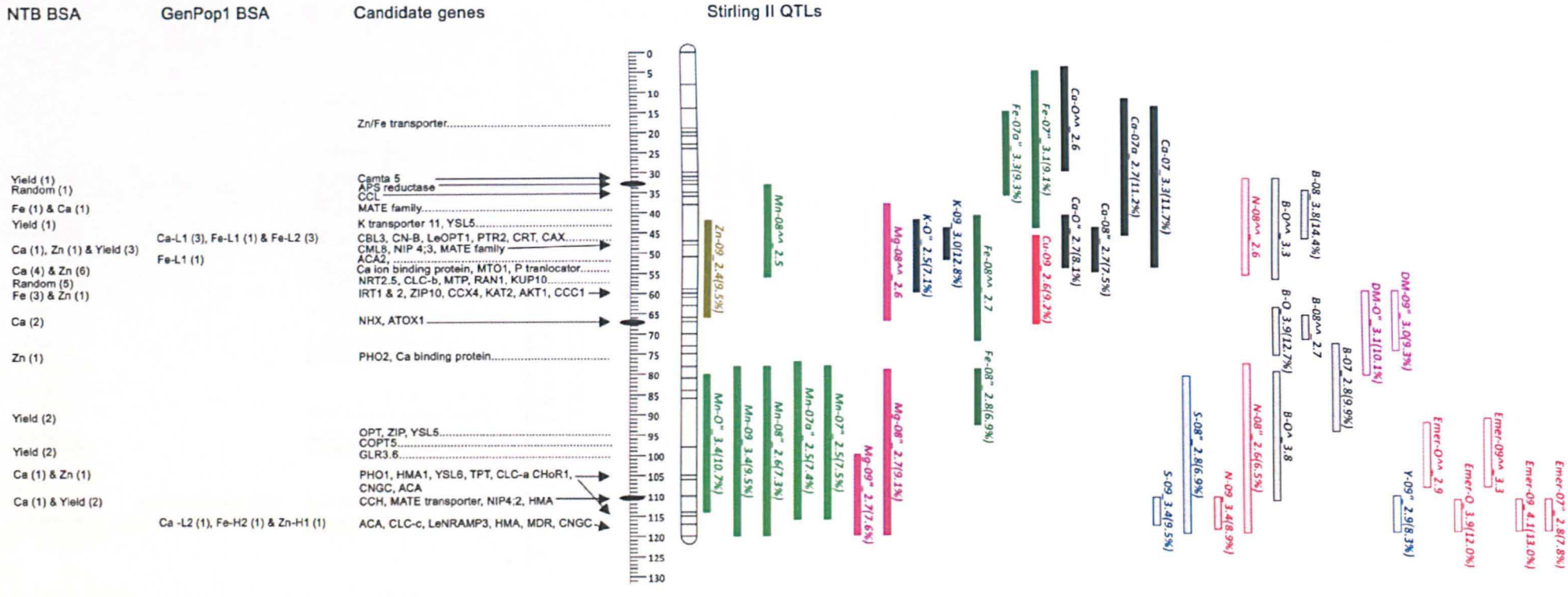

Figure 8.1 (continued)

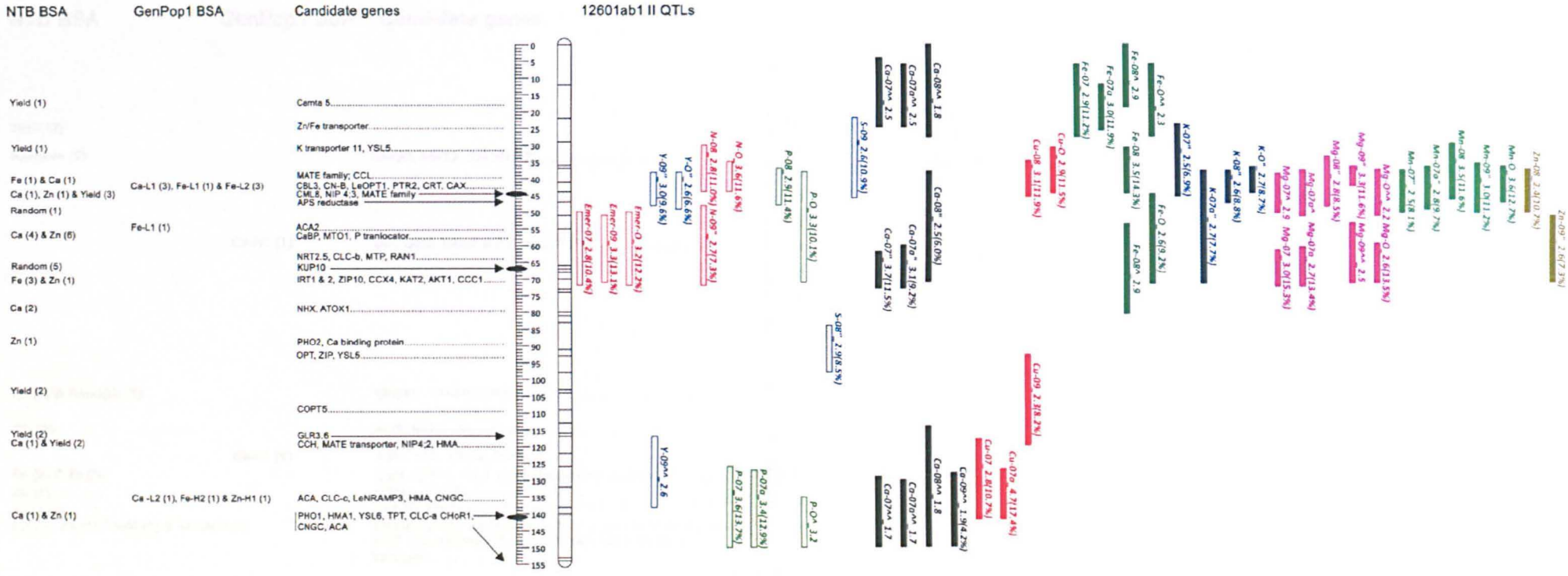

Figure 8.1 (continued)

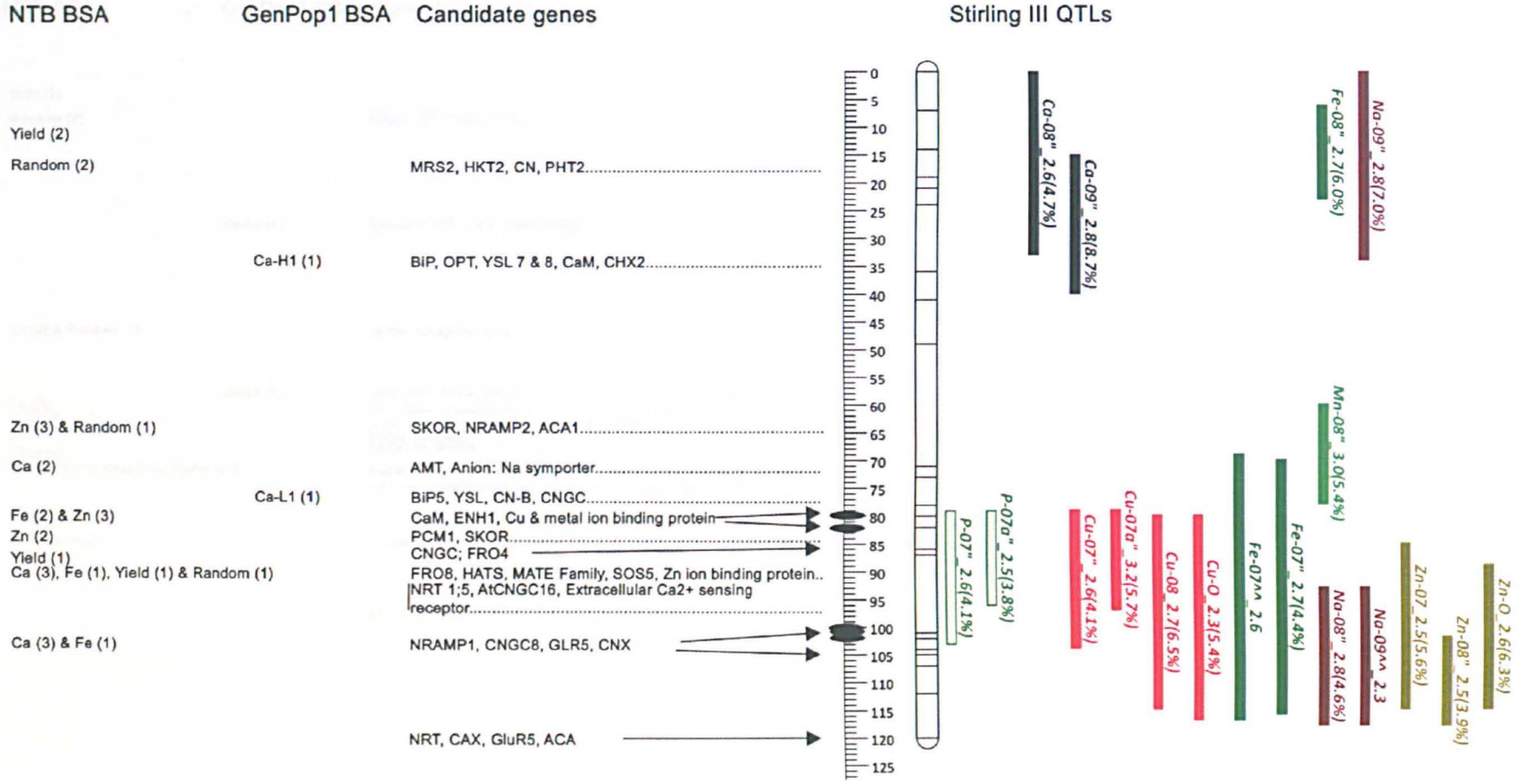

Figure 8.1 (continued)

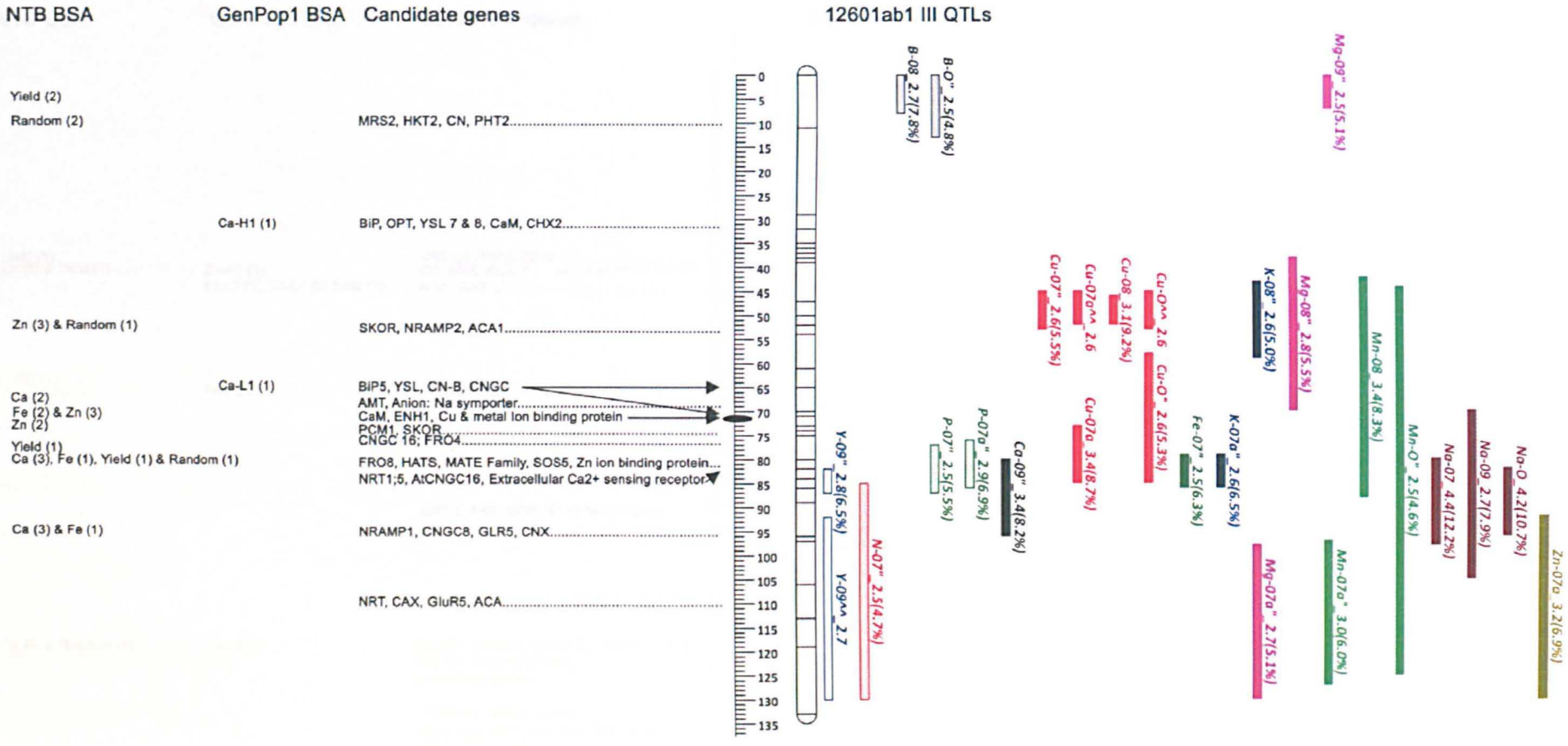

Figure 8.1 (continued)

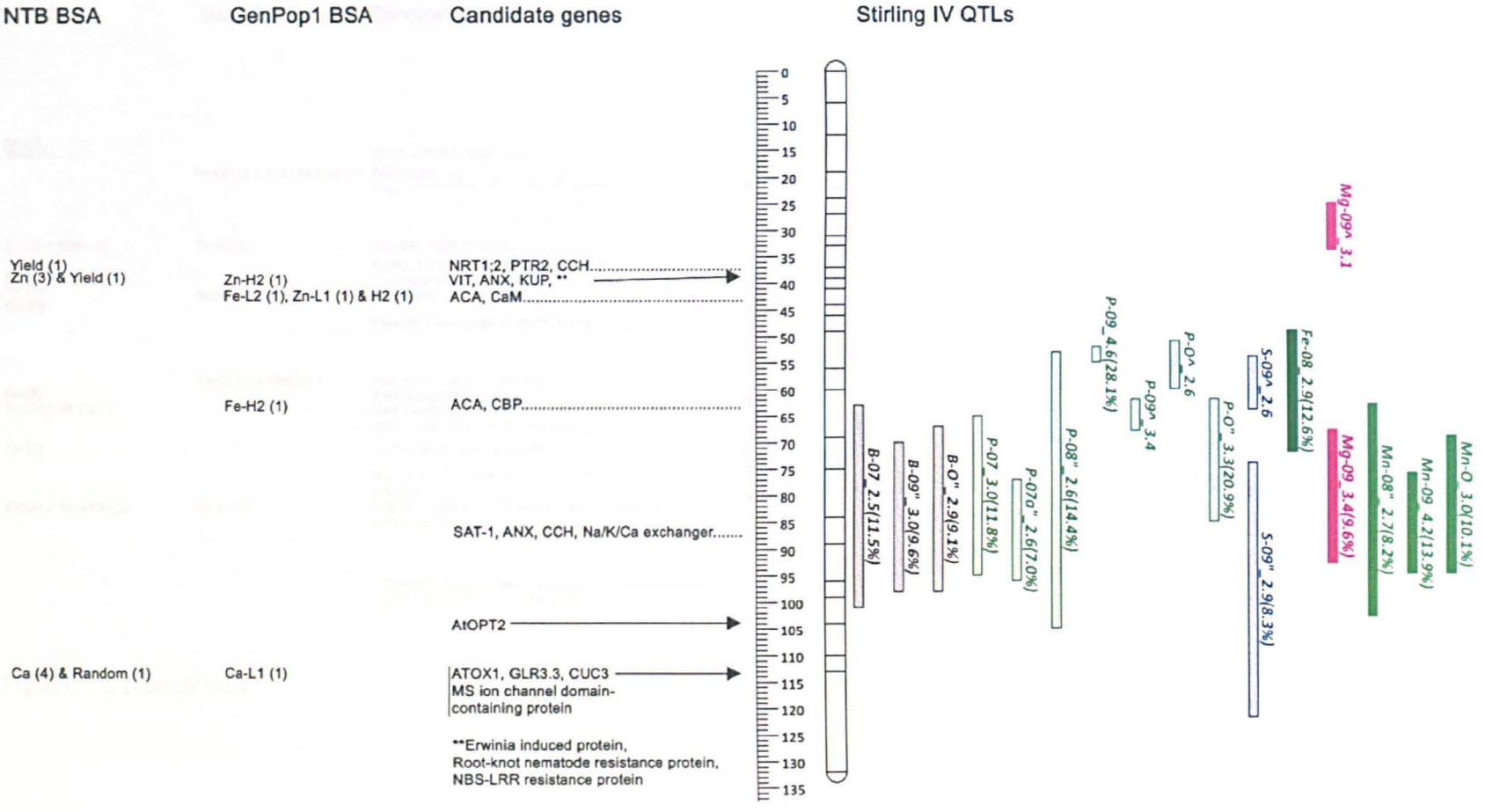

Figure 8.1 (continued)

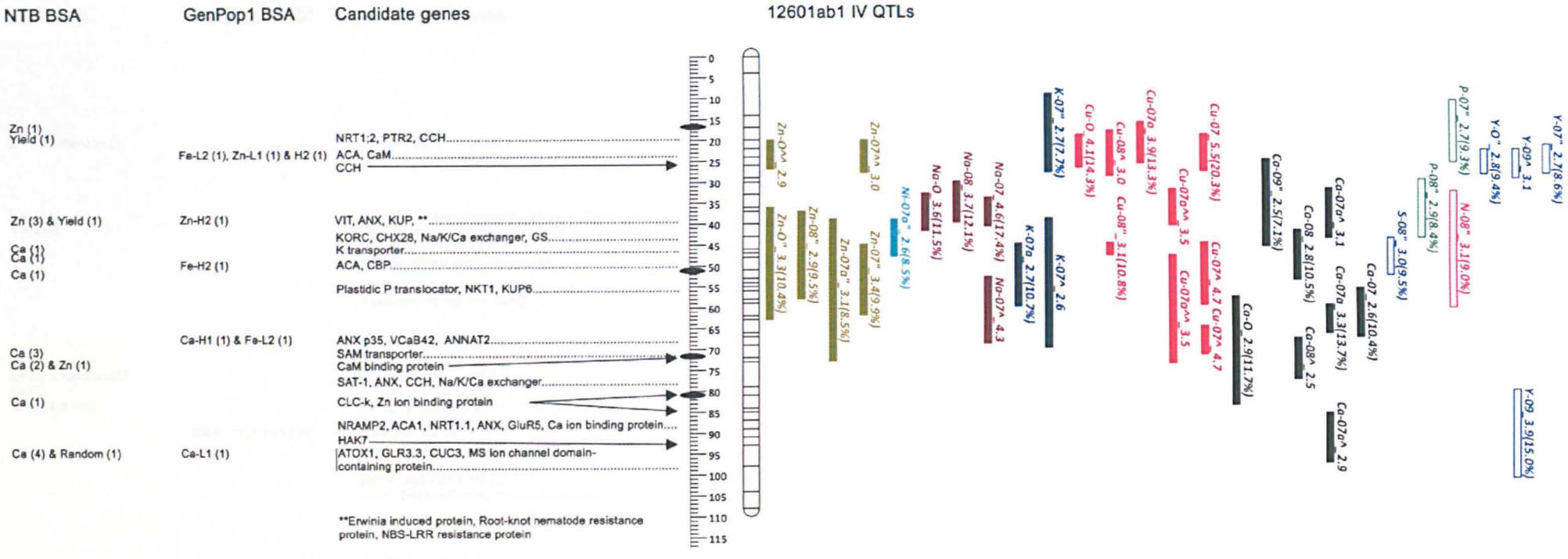

Figure 8.1 (continued)

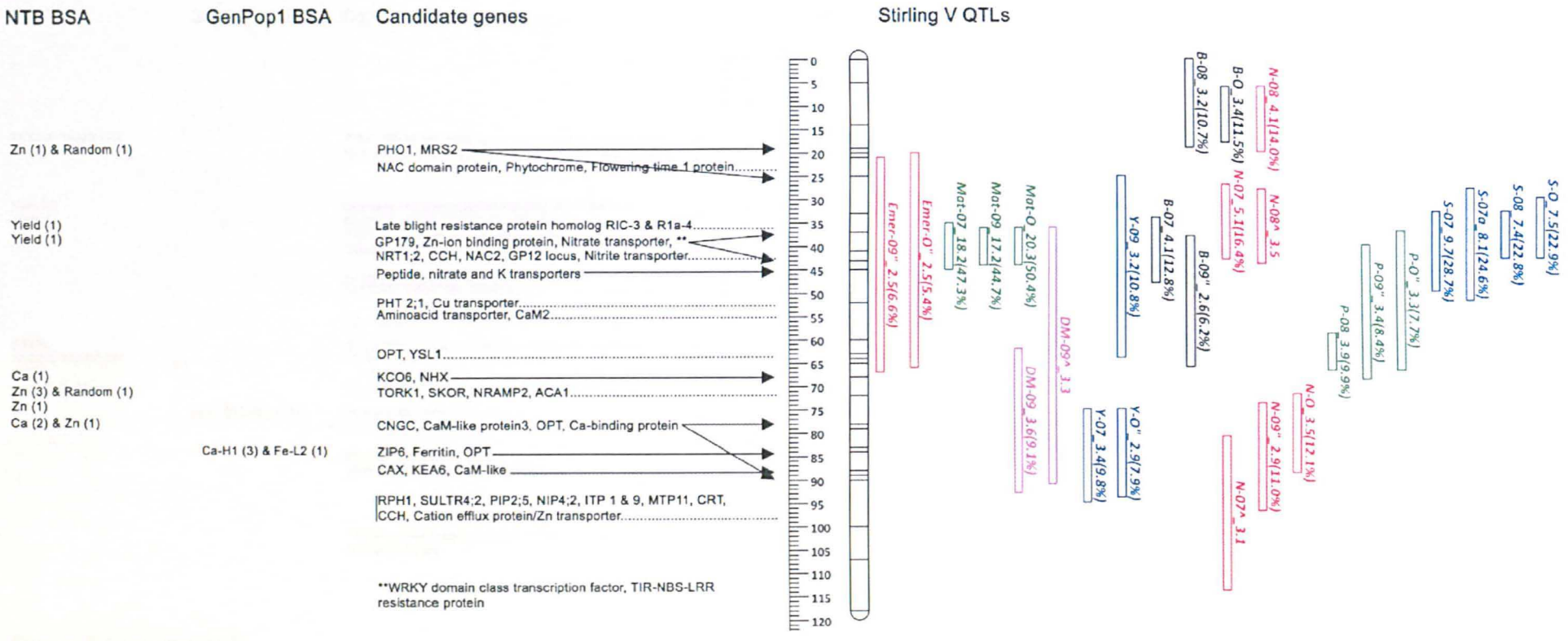

Figure 8.1 (continued)

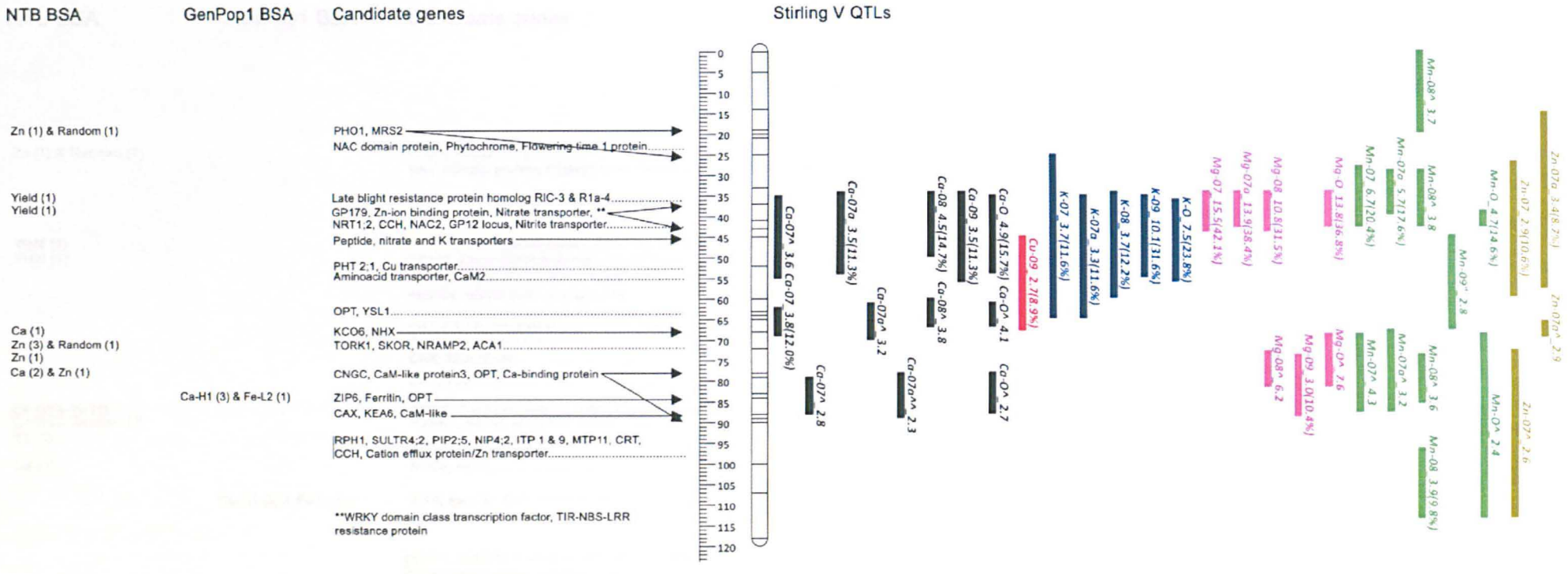

Figure 8.1 (continued)

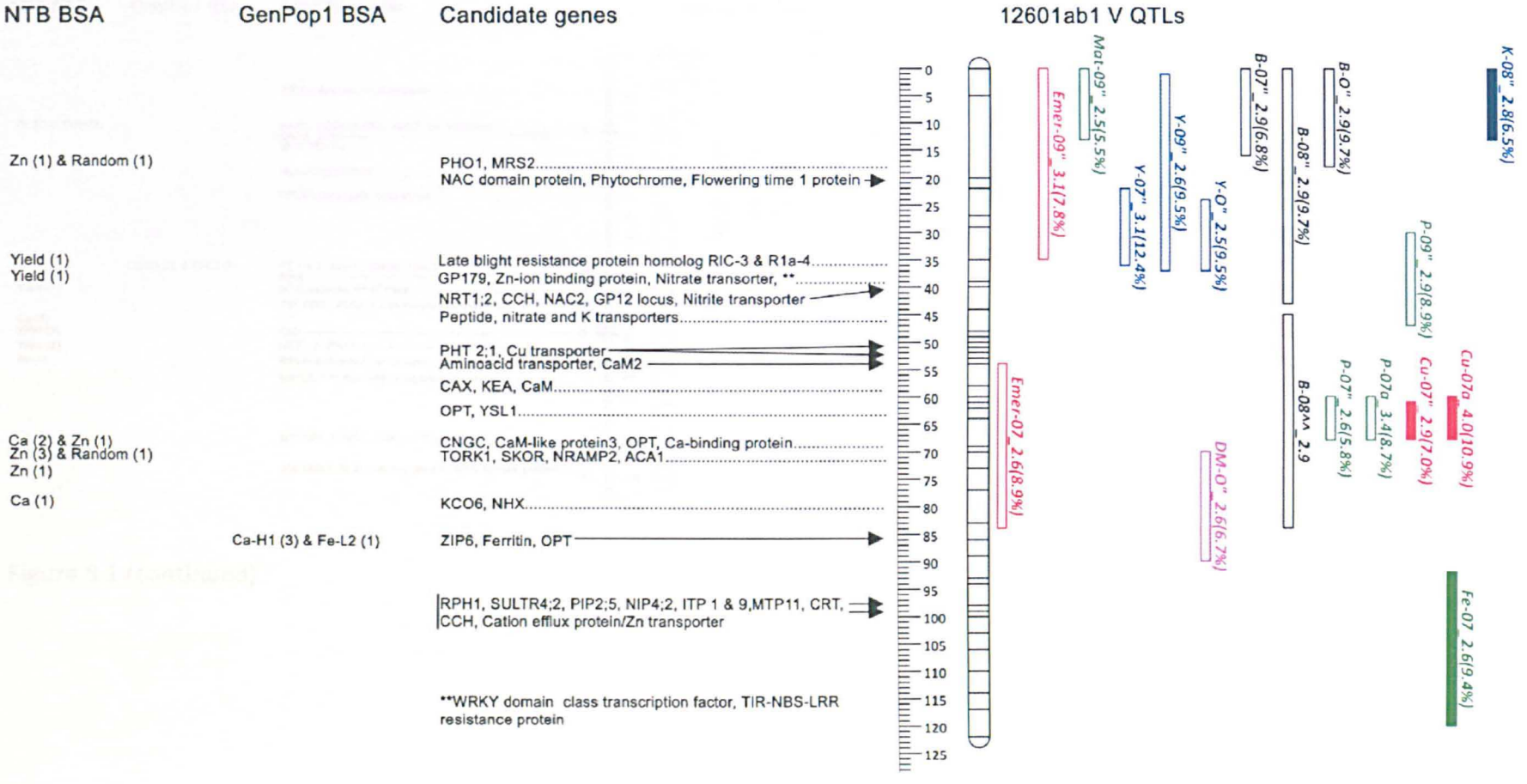

Figure 8.1 (continued)

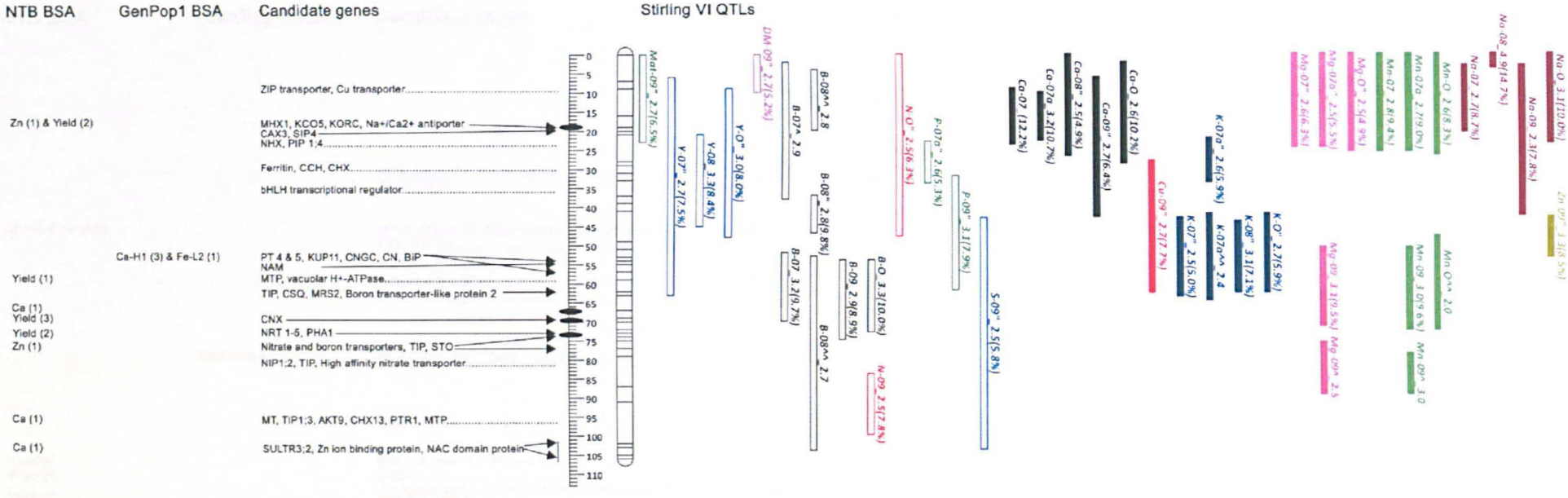

Figure 8.1 (continued)

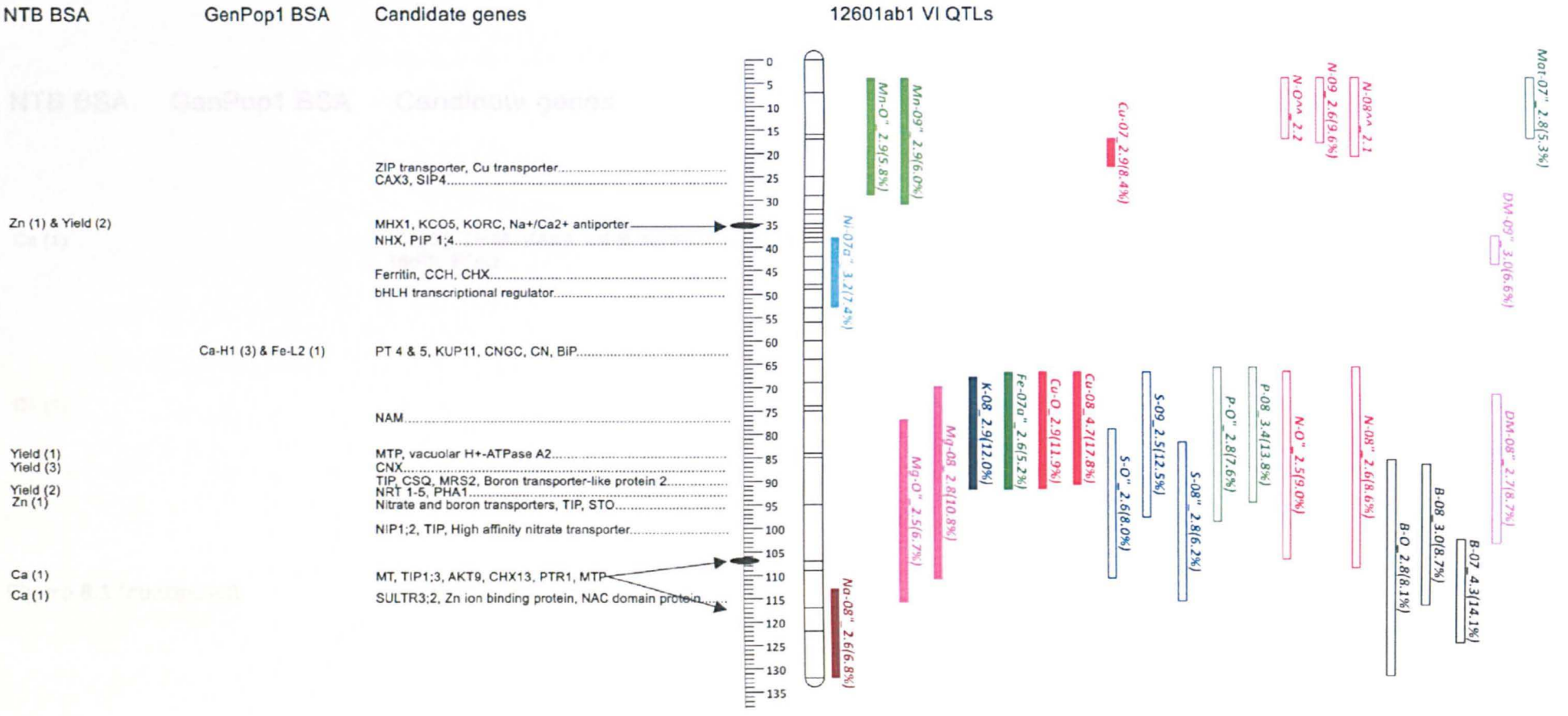

Figure 8.1 (continued)

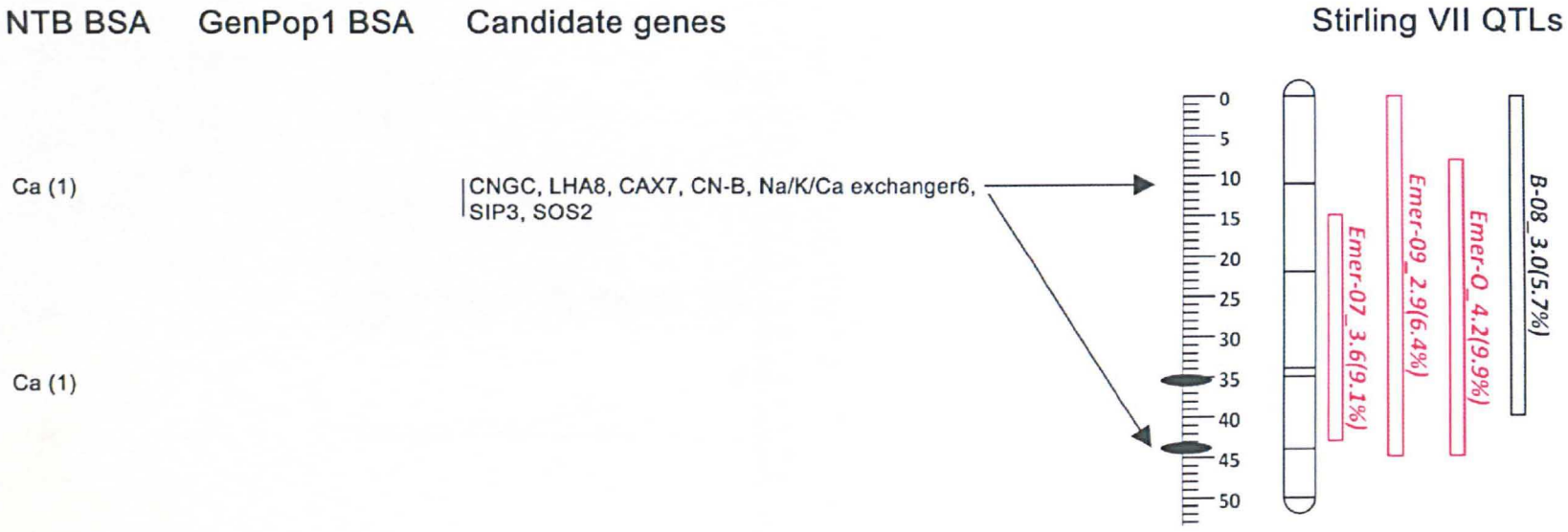

Figure 8.1 (continued)

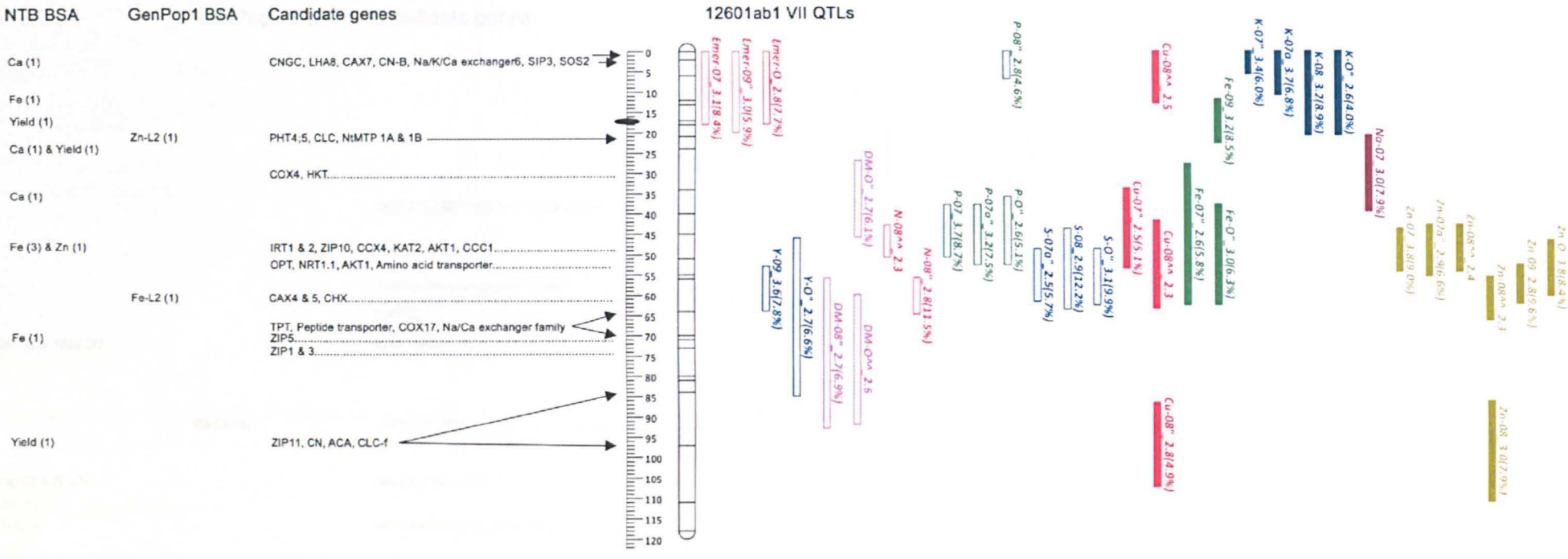

Figure 8.1 (continued)

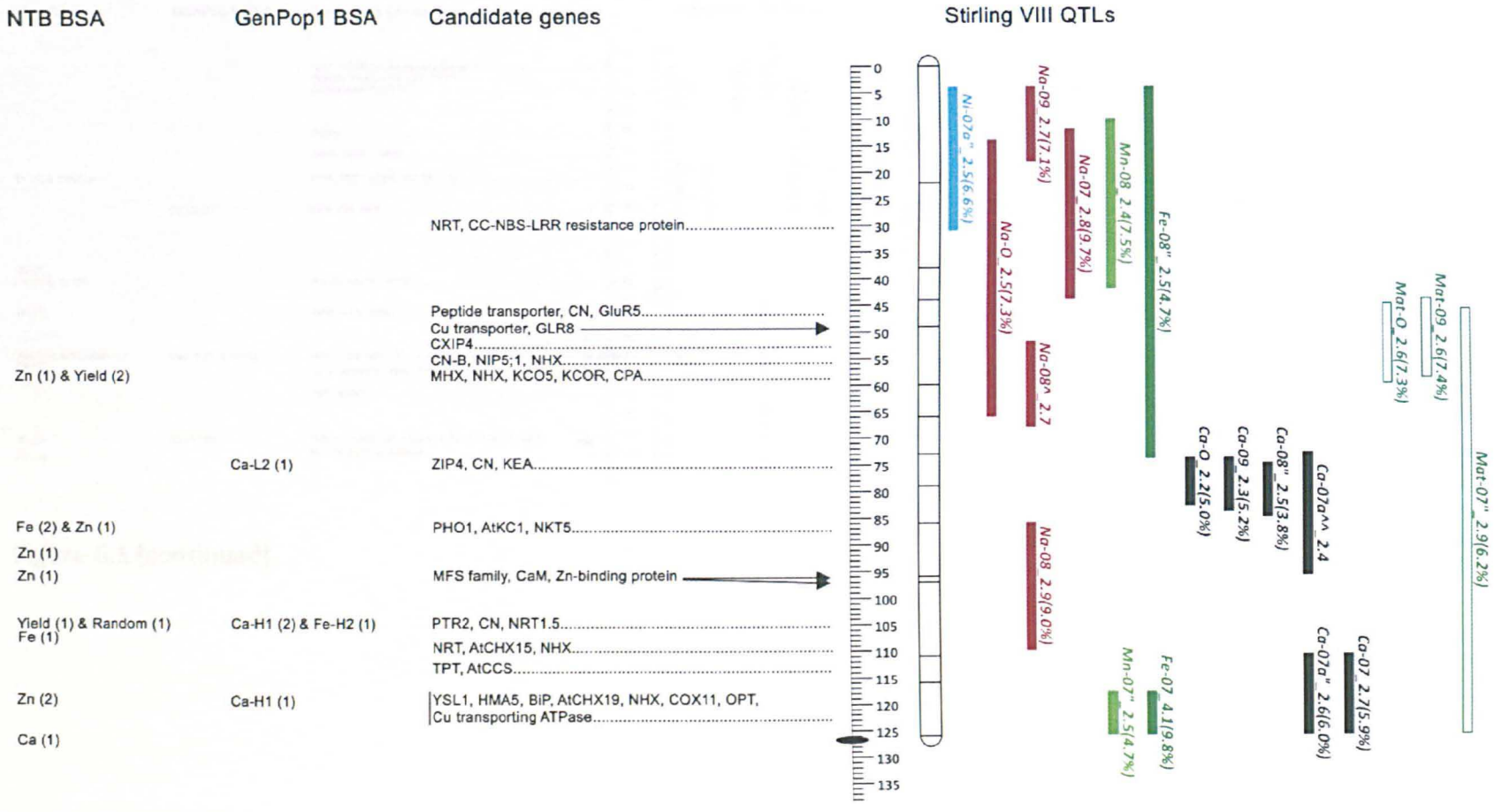

Figure 8.1 (continued)

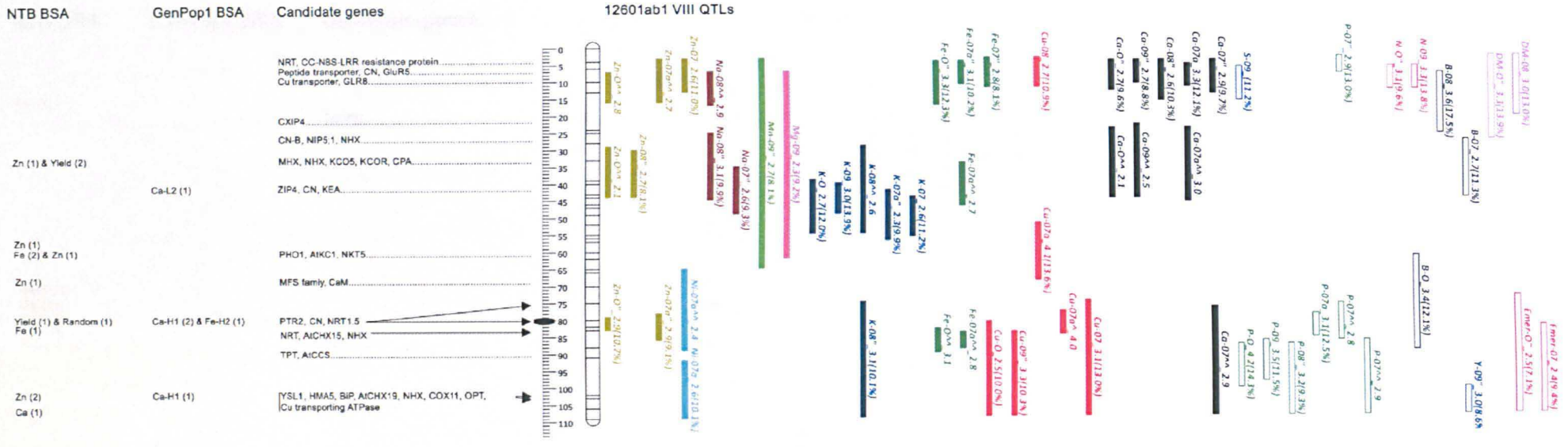

Figure 8.1 (continued)

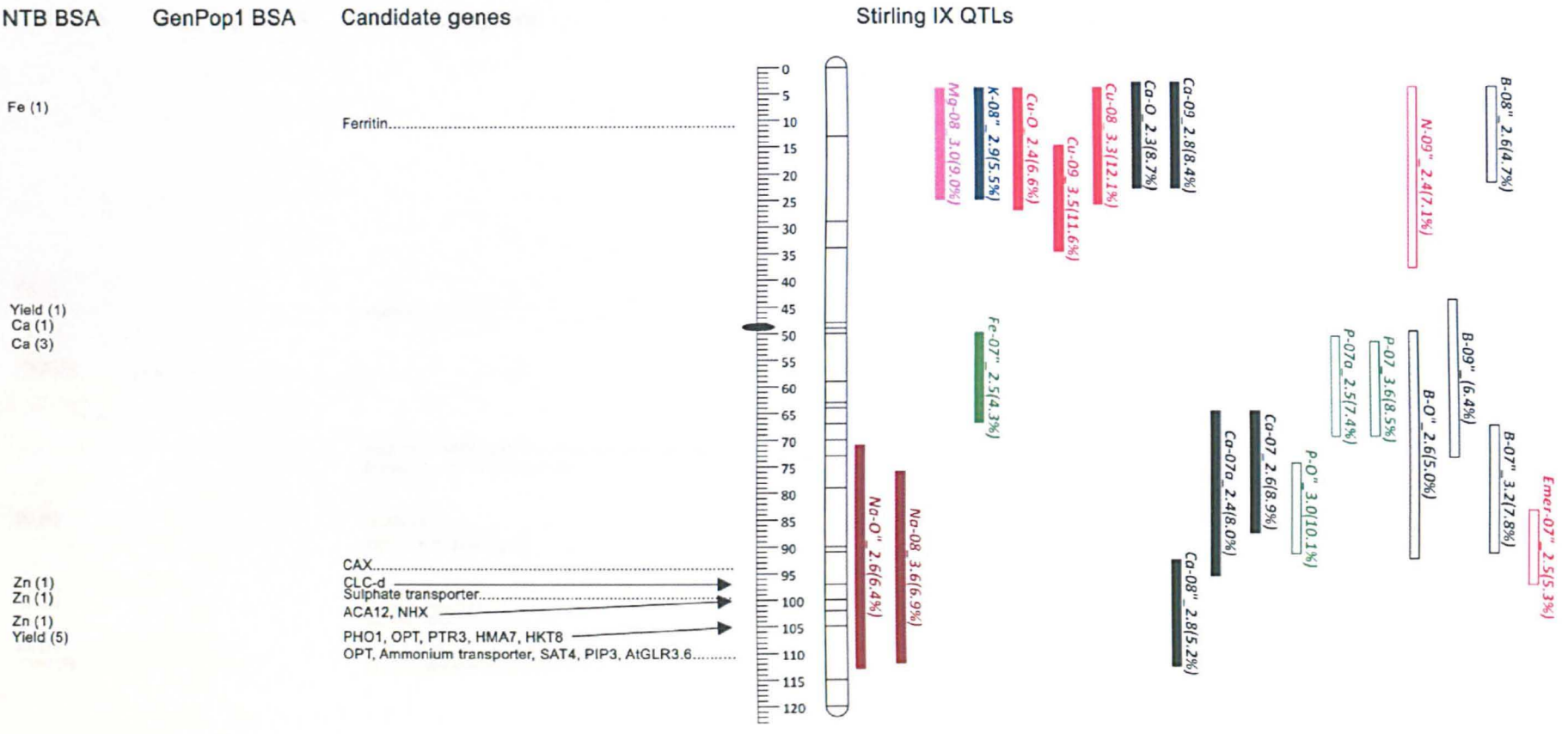

Figure 8.1 (continued)

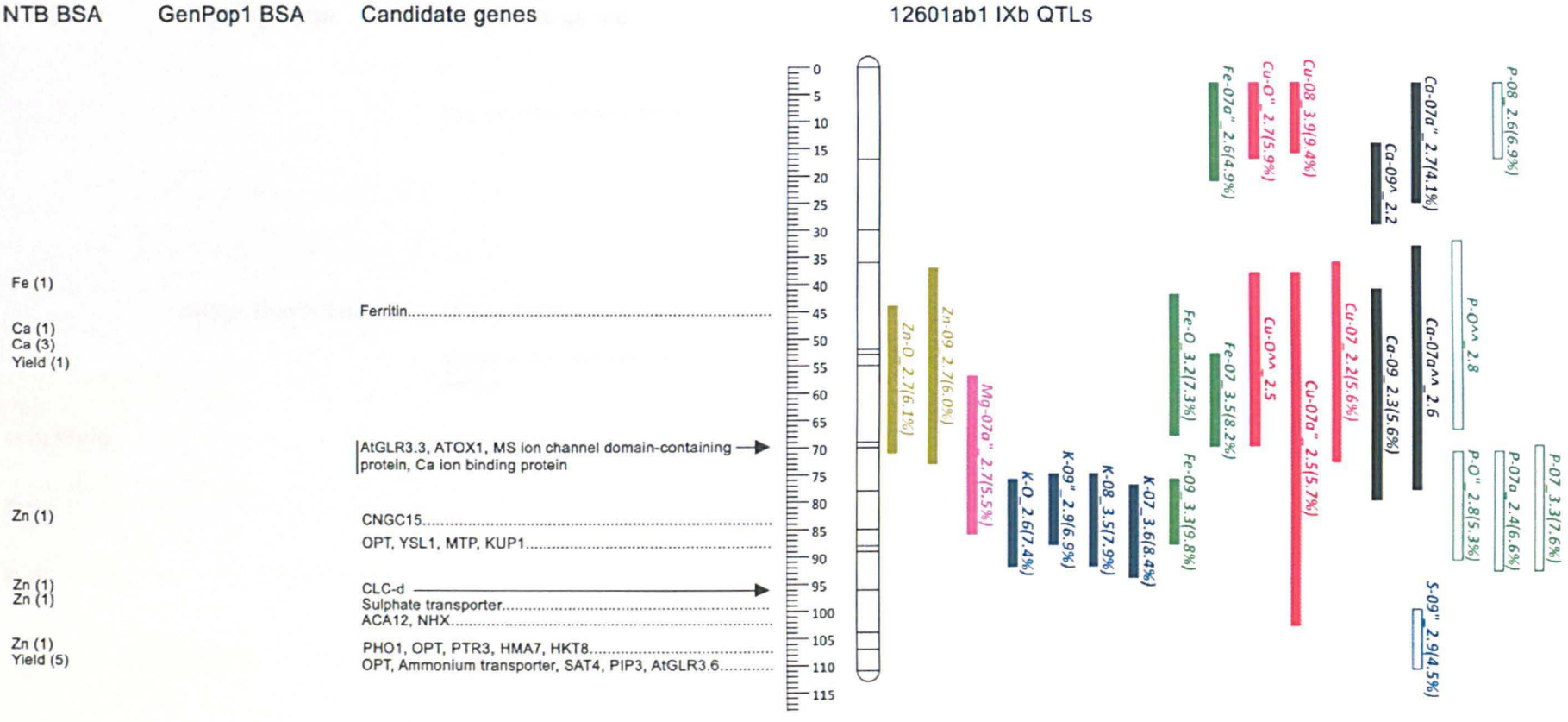

Figure 8.1 (continued)

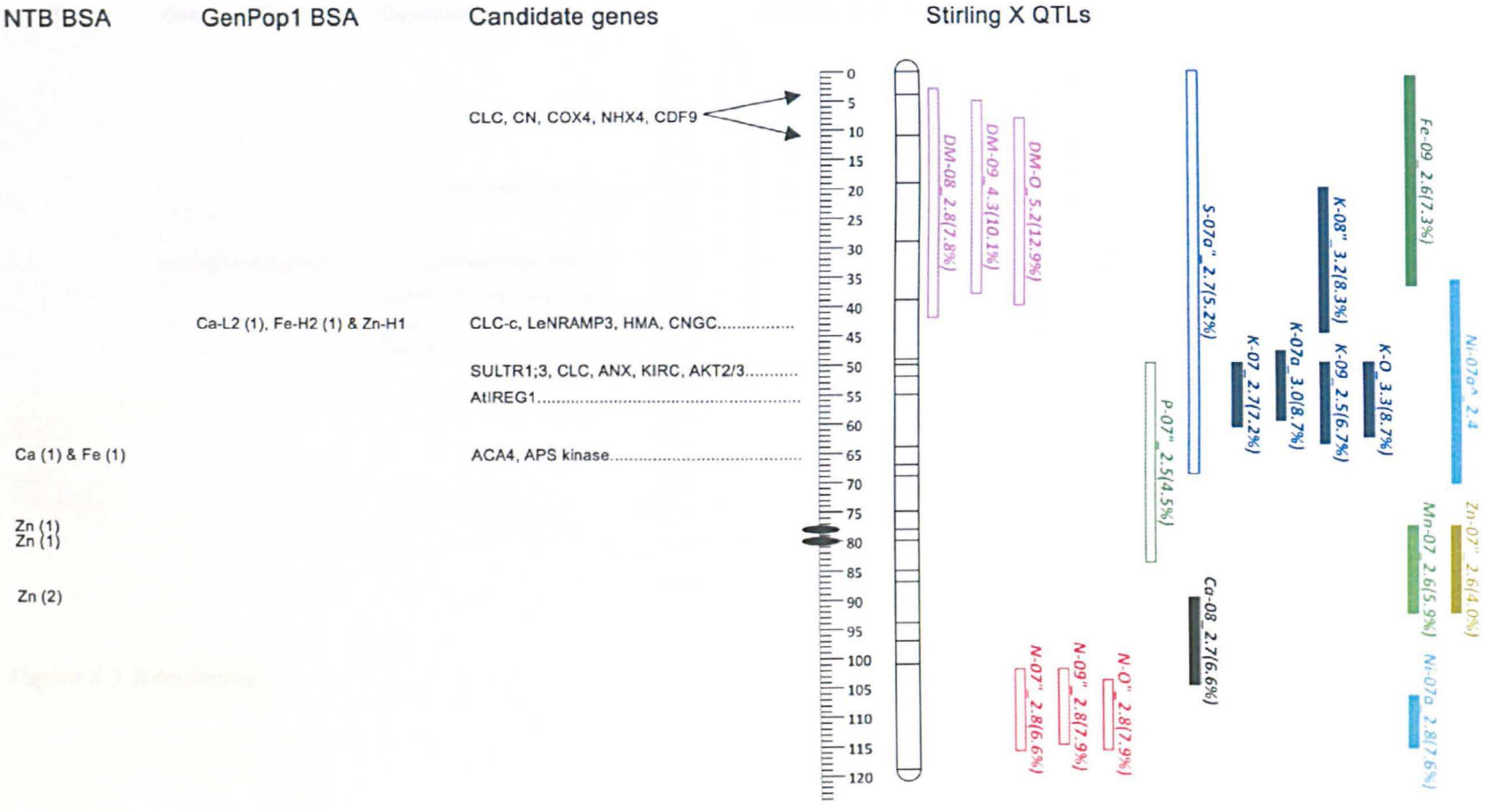

Figure 8.1 (continued)

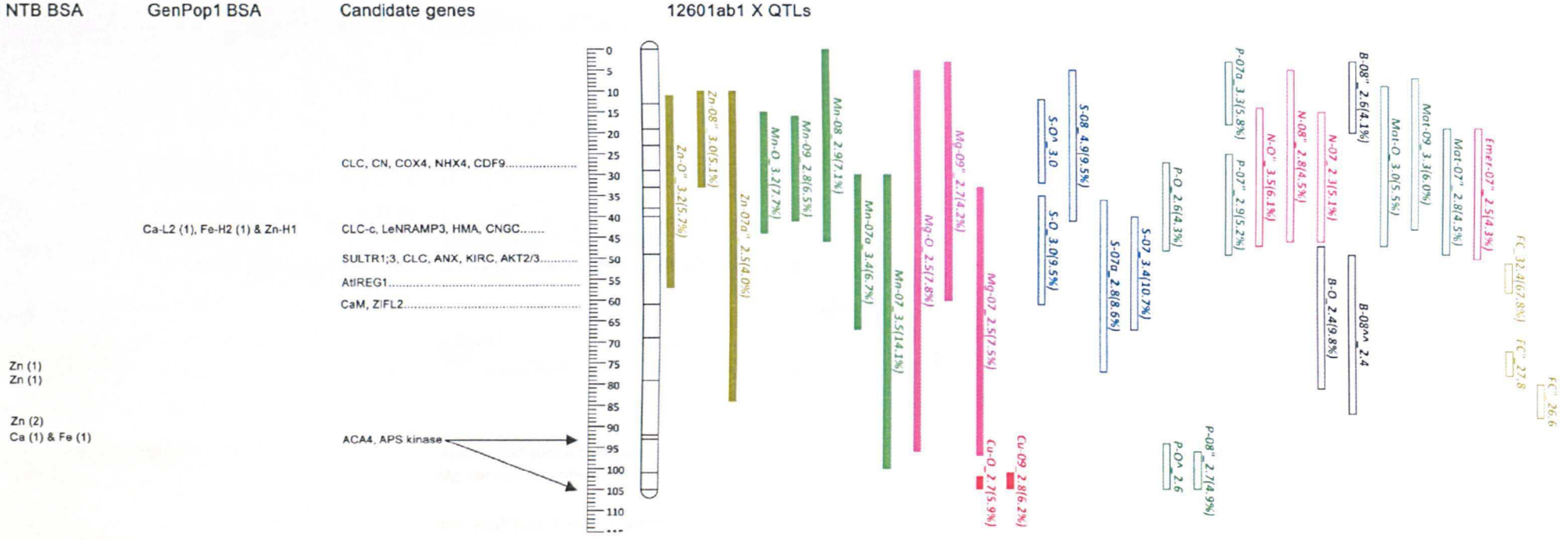

Figure 8.1 (continued)

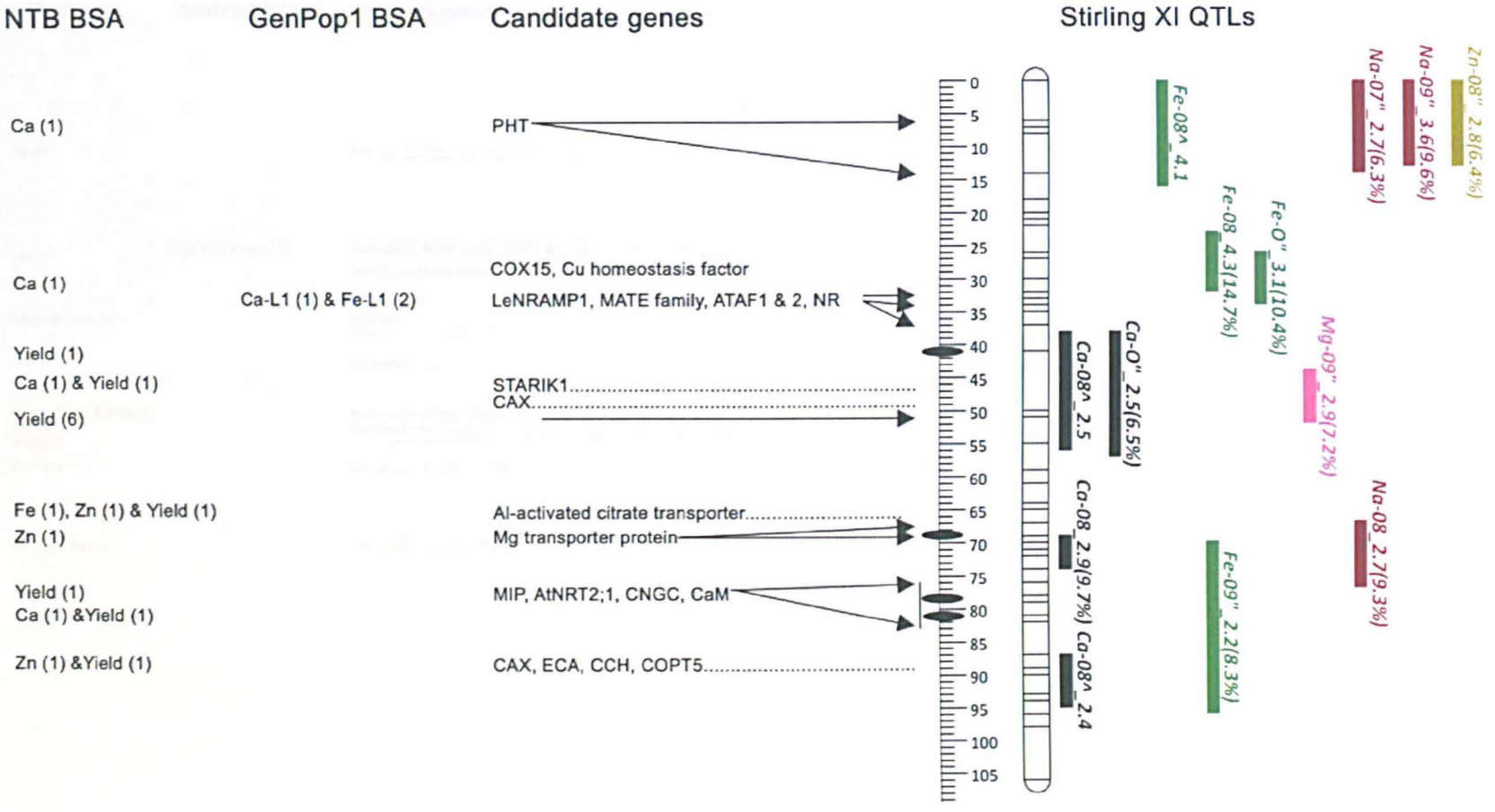

Figure 8.1 (continued)

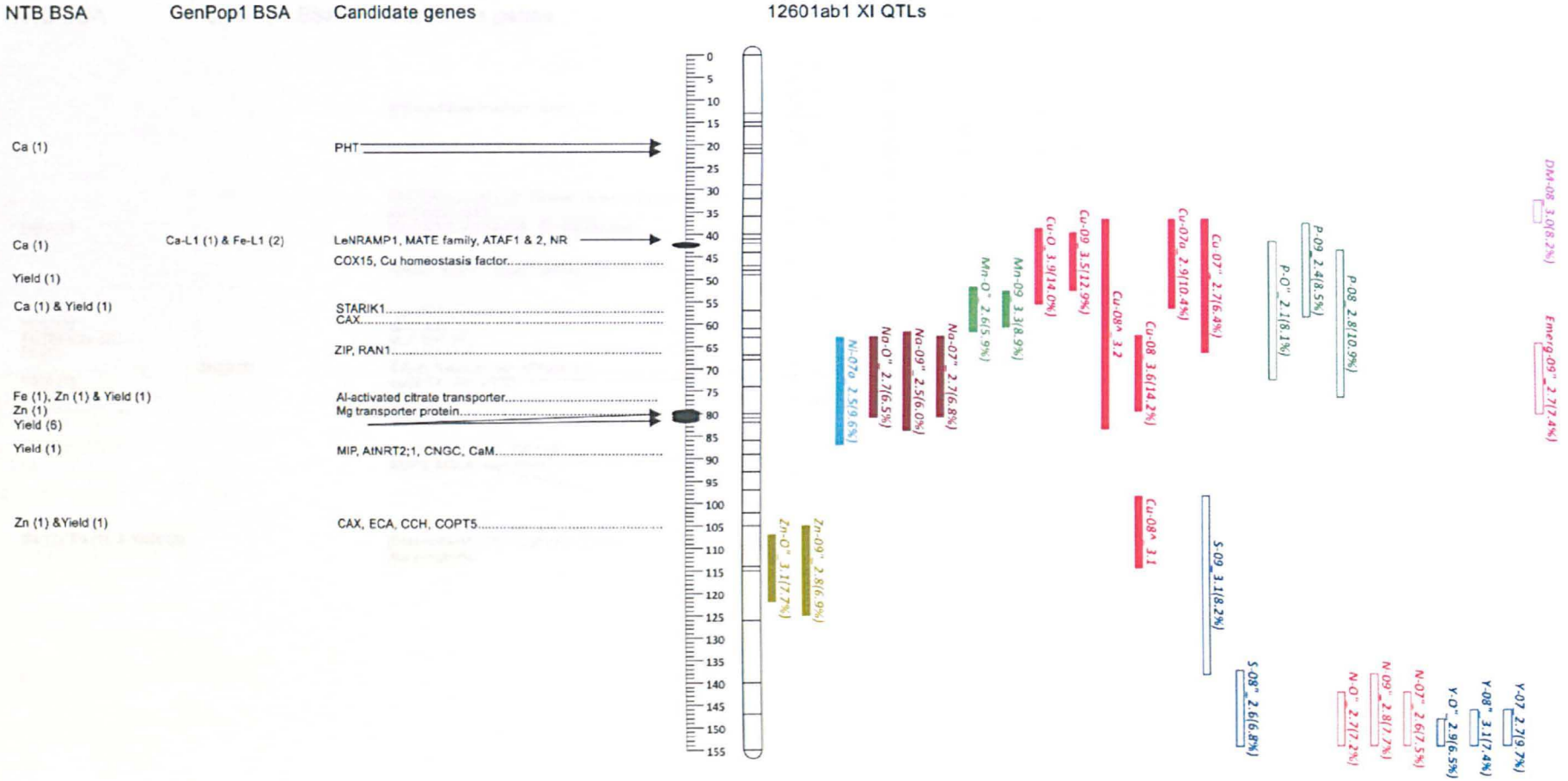

Figure 8.1 (continued)

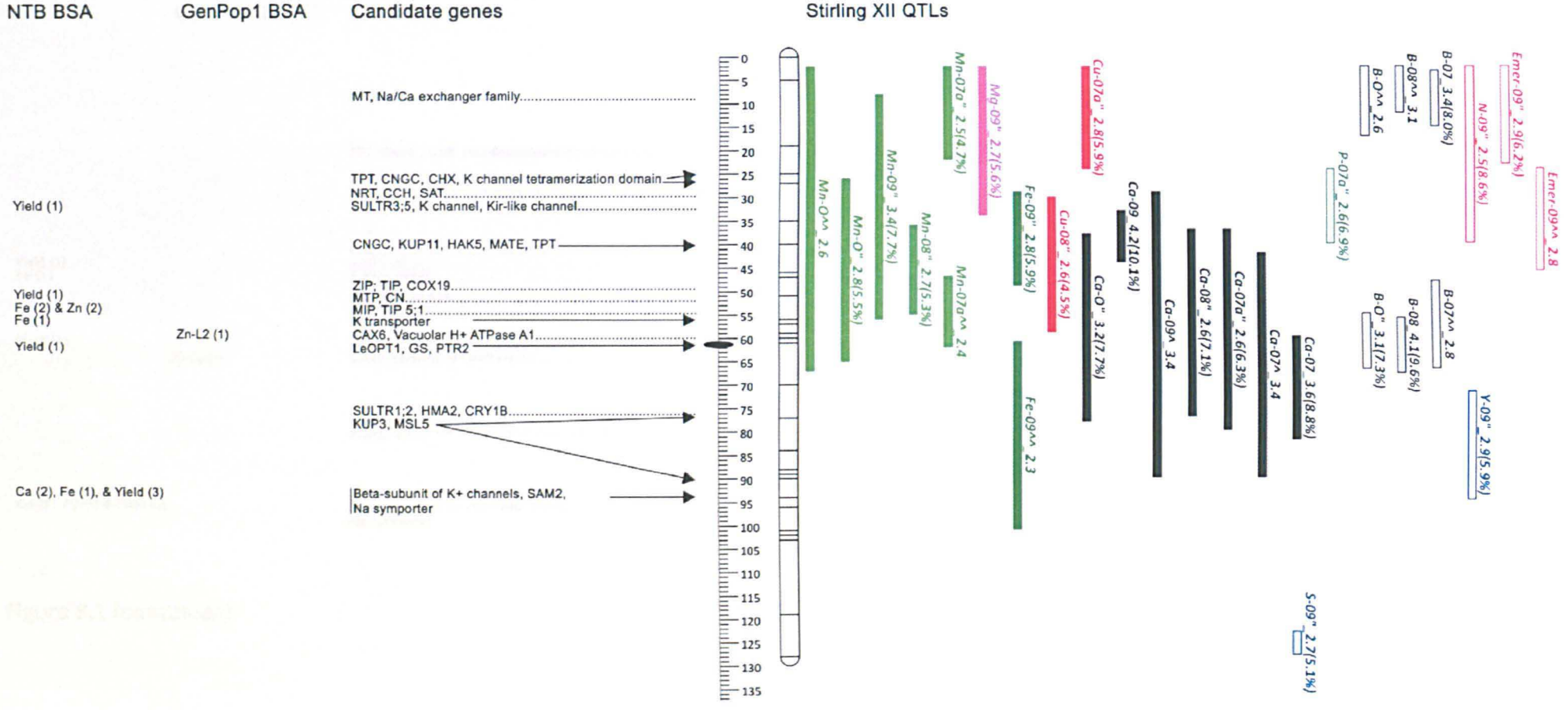

Figure 8.1 (continued)

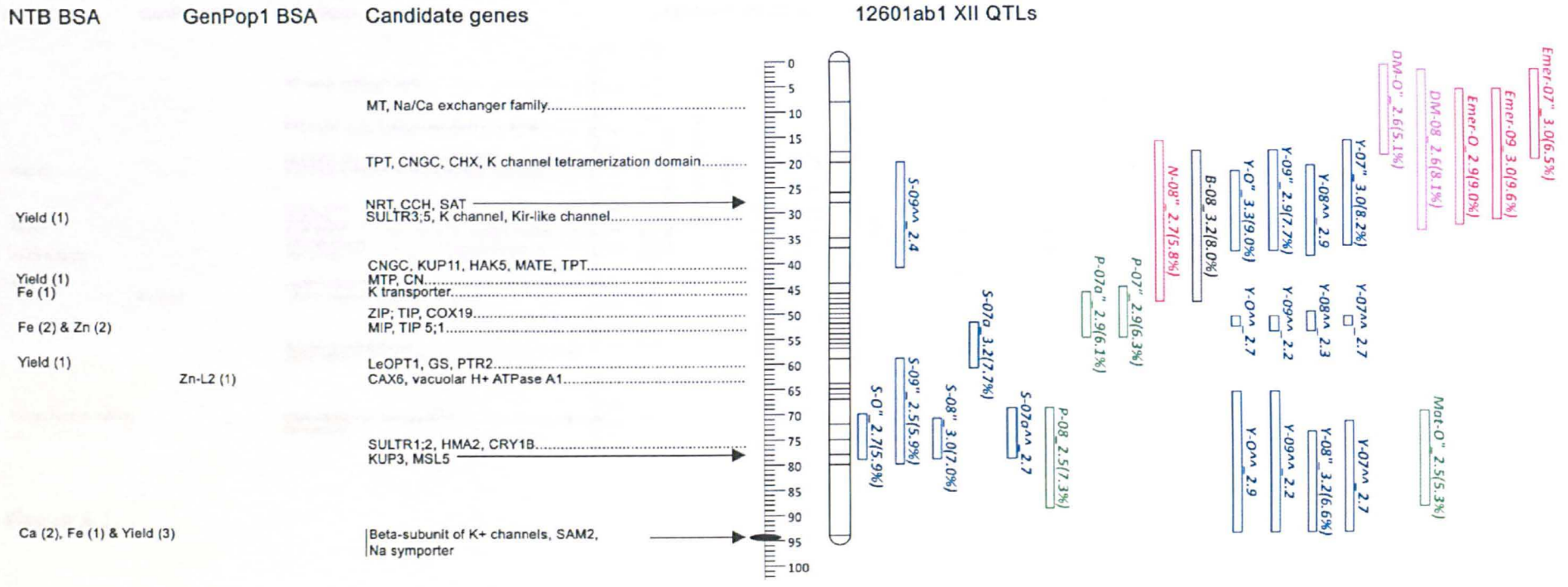

Figure 8.1 (continued)

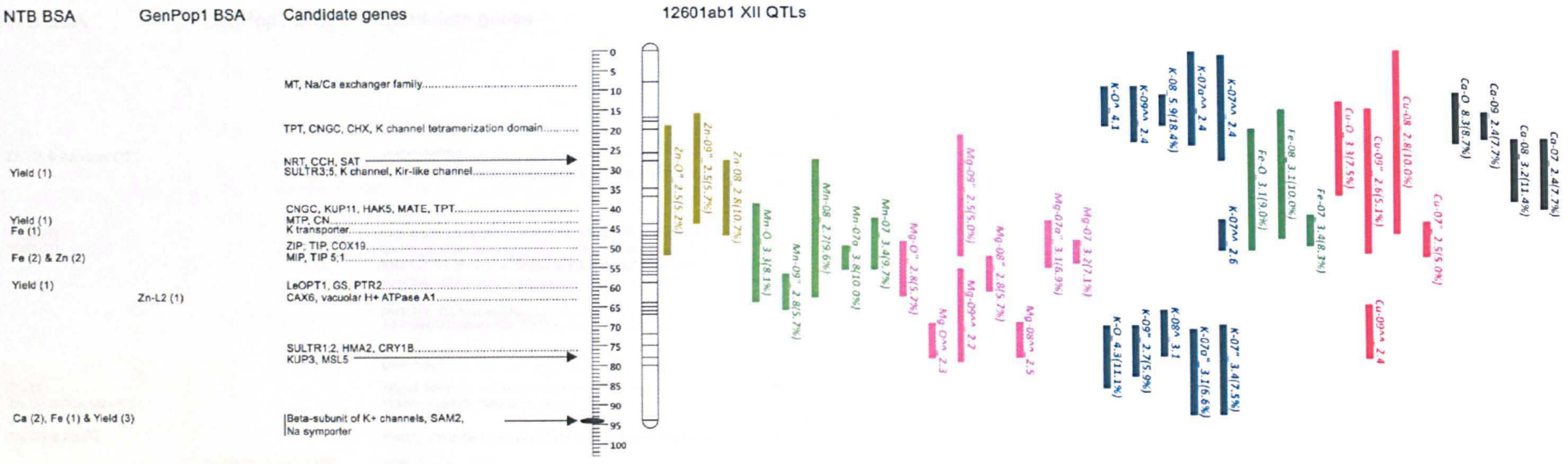
NTB BSA
GenPop1 BSA
Candidate genes
12601ab1 XII QTLs
MT, Na/Ca exchanger family
TPT, CNGC, CHX, K channel tetramerization domain
NRT, CCH, SAT
SULTR3;5, K channel, Kir-like channel
Yield (1)
CNGC, KUP11, HAK5, MATE, TPT
MTP, CN
K transporter
Yield (1)
Fe (1)
ZIP; TIP, COX19
MIP, TIP 5;1
Fe (2) & Zn (2)
Yield (1)
LeOPT1, GS, PTR2
CAX6, vacuolar H+ ATPase A1
Zn-L2 (1)
SULTR1;2, HMA2, CRY1B
KUP3, MSL5
Beta-subunit of K+ channels, SAM2, Na symporter
Ca (2), Fe (1) & Yield (3)
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
Ca-07 2.4(7.7%)
Ca-08 3.2(11.4%)
Ca-09 2.4(7.7%)
Ca-O 8.3(8.7%)
Cu-07^ 2.5(5.0%)
Cu-08 2.8(10.0%)
Cu-09^^ 2.6(5.1%)
Cu-O 3.3(7.5%)
Fe-07 3.4(8.3%)
Fe-08 3.1(10.0%)
Fe-O 3.1(9.0%)
K-07^^ 2.4
K-07^^ 2.6
K-07a^^ 2.4
K-08 5.9(18.4%)
K-09^^ 2.4
K-O^ 4.1
K-07" 3.4(7.5%)
K-07a" 3.1(6.6%)
K-08^ 3.1
K-09" 2.7(5.9%)
K-O 4.3(11.1%)
Mg-07 3.2(7.1%)
Mg-07a" 3.1(6.9%)
Mg-08^^ 2.5
Mg-08" 2.8(5.7%)
Mg-09^^ 2.2
Mg-09" 2.5(5.0%)
Mg-O^^ 2.3
Mn-07 3.4(9.7%)
Mn-07a 3.8(10.0%)
Mn-08 2.7(9.6%)
Mn-09" 2.8(5.7%)
Mn-O 3.3(8.1%)
Zn-08 2.8(10.7%)
Zn-09" 2.5(5.7%)
Zn-O" 2.5(5.2%)

Figure 8.1.

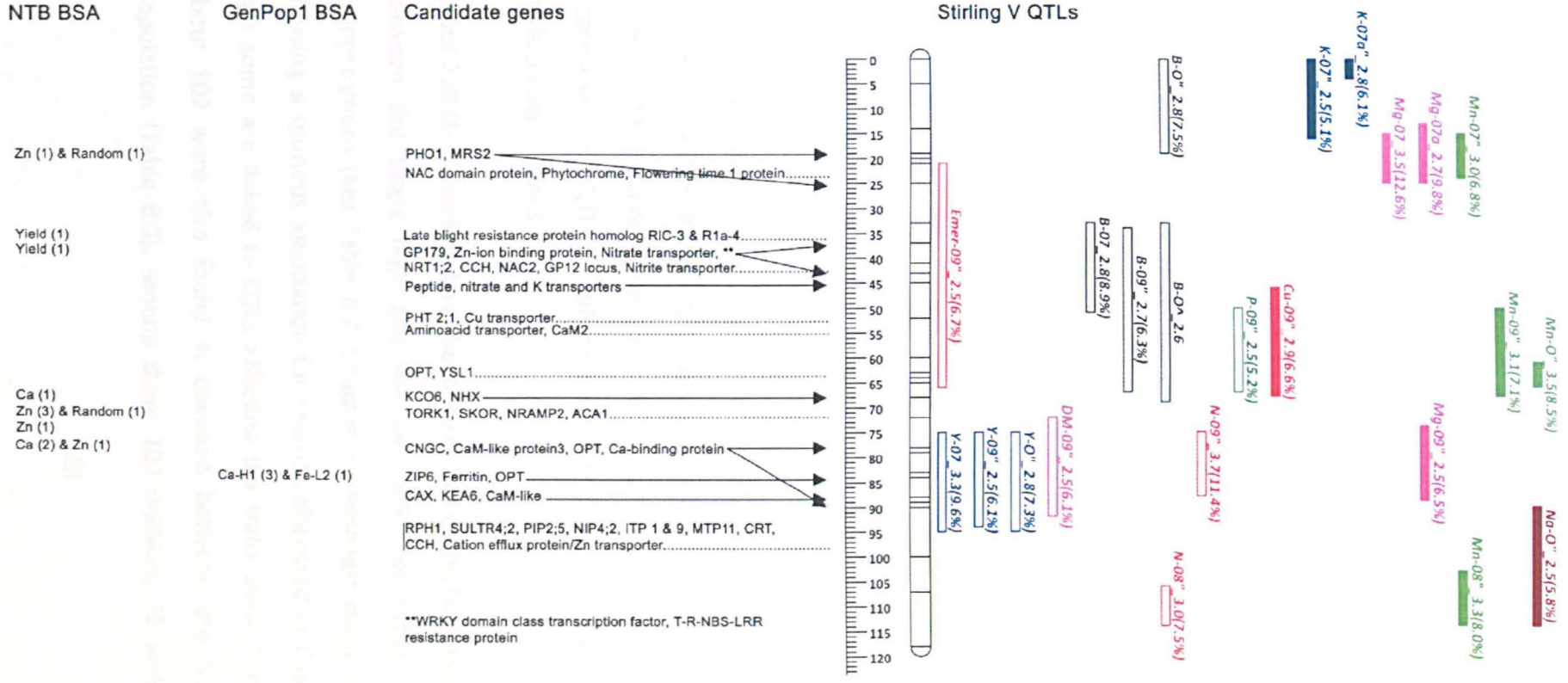

Figure 8.2: QTLs on chromosome V of Stirling corrected for foliage maturity.

8.3.8 Association of QTLs with putative mineral-related genes

Using the available potato genome sequence information and existing knowledge of mineral-related proteins, the potential candidate genes influencing tuber mineral concentrations were identified and assigned to the GenPop1 linkage maps (Figure 8.1). By anchoring the DArT markers used for map construction with known positions on the physical map, estimates were made to identify the candidate genes falling within the QTL intervals (Figure 8.1).

The candidate gene search also revealed the presence of mineral-related gene families (see section 1.2.3 in Chapter 1 and Appendix I) on all the chromosomes of potato genome (Figure 8.1). The candidate genes related to QTLs for minerals of dietary significance, notably Ca, Fe, Zn, Cu, Mg and K were discussed in this chapter and a summary for other minerals were given in Appendix IV.

8.3.9 Linking of DArT and SSR markers with QTLs for tuber yield, Ca, Fe and Zn in GenPop1

The DArT and SSR markers apparently associated with the three mineral traits using BSA in the NTB and GenPop1 populations (see Chapter 6) were aligned on the genetic maps of Stirling and 12601ab1 (Figure 8.1). The marker loci that were co-locating with the QTLs for tuber yield, Ca, Fe and Zn concentrations are discussed in forthcoming sections.

About 238 DArT markers have been identified in the NTB population which differed between the single high and the low bulks for tuber yield, Ca, Fe and Zn concentrations (see Table 6.7, Chapter 6). Amongst these markers, some will be showing a spurious association for reasons discussed in Chapter 6, but it is likely that some are linked to QTLs affecting the traits used. Out of the 238 markers, about 102 were also found in common between the NTB and the GenPop1 population (Table 8.2). Among these 102 markers, 30 and 28 were mapped in

Stirling and 12601ab1, respectively (Table 8.2), and their chromosomal distribution is given in Table 8.3.

Table 8.2: DArT markers found in common between the NTB (using BSA) and GenPop1 mapping populations

Trait	Stirling DArT Markers		12601ab1 DArT Markers		Stirling & 12601ab1 DArT Markers
	Identified	Mapped	Identified	Mapped	Identified (not used in mapping)
Fe	3	2	3	2	1
Zn	8	7	3	3	3
Ca	18	14	10	9	8
Yield	9	6	13	11	10
Random	4	1	8	3	1
Total	**42**	**30**	**37**	**28**	**23**

8.3.9.1 Tuber Yield

QTLs for tuber yield were identified on five chromosomes in Stirling and nine chromosomes in 12601ab1 (Figure 8.1, Table 8.4). A total of 16 and 29 marker loci apparently associated with the yield trait from BSA were aligned on chromosomes in Stirling and 12601ab1, respectively. However, only six and nine marker loci were co-located with yield QTLs on Stirling and 12601ab1 chromosomes, respectively (Table 8.4).

8.3.9.2 Iron

QTLs on chromosomes that were consistent over two or three years, and the ones explaining high percentage variance for tuber Fe concentration were found on chromosomes Ib, II, VIII and XI in Stirling and on chromosomes Ia, II, VIII, IX and XII in 12601ab1 (Table 8.5).

Table 8.3: Chromosomal distribution of DArT markers identified using BSA in the NTB population that were mapped in the GenPop1 mapping population

NTB-BSA / GenPop1	Fe		Zn		Ca		Yield		Random		Total		Shared marker between different bulks	
Chromosomes	Stir	ab1	Stir	ab1	Stir	ab1	Stir	ab1	Stir	ab1	Stir	ab1	Stir	ab1
Ia	-	1	-	-	-	2	-	1	-	2	0	6	-	1 (Fe/Random)
Ib	1	-	1	-	3	-	-	-	-	-	5	0	-	-
II	-	-	-	1	2	1	-	-	1	1	3	3	-	-
III	1	1	2	-	3	-	-	-	-	-	6	1	1 (Fe/Zn)	-
IV	-	-	-	1	-	3	-	-	-	-	0	4	-	-
V	-	-	-	-	-	-	-	-	-	-	0	0	-	-
VI	-	-	1	1	1	1	2	1	-	-	4	3	-	1 (Zn/Yield)
VII	-	-	-	-	2	-	-	1	-	-	2	1	-	-
VIII	-	-	-	-	1	-	-	2	-	-	1	2	-	-
IX	-	-	-	-	1	-	-	-	-	-	1	-	-	-
IXa	-	-	-	-	-	-	-	1	-	-	-	1	-	-
IXb	-	-	-	-	-	-	-	-	-	-	-	0	-	-
X	-	-	2	-	-	-	-	-	-	-	2	0	-	-
XI	-	-	1	-	1	1	3	4	-	-	5	5	1 (Ca/Yield)	-
XII	-	-	-	-	-	1	1	1	-	-	1	2	-	1 (Ca/Yield)
Total	**2**	**2**	**7**	**3**	**14**	**9**	**6**	**11**	**1**	**3**	**30**	**28**		

Stir- Stirling; ab1- 12601ab1.

Table 8.4: Overlapping of yield QTLs in the GenPop1 population with the marker loci identified in the NTB population using bulked segregant analysis for tuber yield

Yield QTLs in GenPop1 (Chrom. no.)	Phenotypic variance explained by QTLs (%)	No. of DArT marker loci aligned on GenPop1 chrom.	No. of yield QTLs overlapping with DArT marker loci	Variance explained by the yield QTLs overlapping with markers (%)
Stirling				
Ia	6.0	0	0	-
II	8.3	6	1	8.3
V	9.8 & 10.8	2	2	10.8
VI	7.5 & 8.4	4	2	7.5
XII	5.9	4	1	5.9
Total		**16**	**6**	
12601ab1				
Ia	6.3	1	0	-
II	9.6	6	2	9.6
III	6.5	3	2*	6.5
IV	8.6 & 15.0	2	1	8.6
V	9.5 & 12.4	2	2*	9.5 & 12.4
VII	7.8	3	0	-
VIII	8.6	2	0	-
XI	7.4 & 9.7	6	0	-
XII	6.6–8.2	4	2	6.6–8.2
Total		**29**	**9**	

Chrom-Chromosome; *Marker loci in close proximity to QTLs; & represents QTLs found in two seasons; '–' represents QTLs found over three years or over two years plus measurements carried out in SCRI.

Sixteen DArT marker loci that may have been associated with the Fe trait from BSA in NTB were aligned on Stirling genetic maps, and out of 16 markers, eight were found to be co-locating with QTLs for Fe on chromosomes Ib, II, III and XII (Figure 8.1, Table 8.5). Likewise, out of the nine SSR markers from BSA in GenPop1 that were aligned on Stirling, four markers co-localized with QTLs for Fe on chromosomes Ib, II, IV and XI (Table 8.5). In 12601ab1, eight DArT and four SSR marker loci overlapped with the QTLs for Fe on chromosomes Ia, II, III, VII, VIII and XII, and on chromosomes II, VII and VIII, respectively.

The candidate genes associated with the identified QTLs for Fe accumulation/concentration belong to several gene families, including ferric reductase oxidase (FRO), zinc-regulated transporter (ZRT)-IRT-like protein (ZIP), multi-drug and toxin efflux (MATE), nicotianamine synthase (NAS), yellow stripe-like transporters (YSL), vacuolar iron transporter (VIT), oligo-peptide transporters (OPT), natural resistance-associated macrophage protein (NRAMP) and iron regulated gene/ferroportin (IREG/FPN) (Table 8.5). Additionally, the candidate genes also include the iron transport protein (ITP) and storage (ferritin) protein.

8.3.9.3 Zinc

About 24 DArT marker loci that were apparently associated with tuber Zn concentrations from BSA in NTB were aligned on the Stirling chromosomes, and out of 24 markers 11 were found to be co-locating with QTLs for Zn on chromosomes II, III, V and X (Figure 8.1, Table 8.6). Out of eight SSR markers (from BSA in GenPop1) aligned on the Stirling genetic maps, only two markers co-localized with QTLs for Zn on chromosome I (Ia and Ib) (Table 8.6). In 12601ab1, 12 DArT and four SSR marker loci overlapped with the QTLs for Zn on chromosomes Ib, IV and X, and on chromosomes Ib, II, IV, VII, VIII, X, XI and XII, respectively. The candidate genes associated with the QTLs identified for Zn includes several gene families such as ZIP, zinc-induced facilitator (ZIF), Mg^{2+}/H^{+} antiporter (MHX), cation diffusion facilitator (CDF), NAS, YSL, OPT, NRAMP and VIT (Table 8.6).

QTLs on chromosomes that were consistent over two or three years, and explaining high percentage of the variance for tuber Zn concentrations were found on Ia and V in Stirling and on II, IV, VII, VIII and XII in 12601ab1 (Table 8.6).

8.3.9.4 Calcium

The DArT and SSR markers that were apparently associated with the tuber Ca concentration from BSA in NTB and GenPop1 populations were aligned on Stirling and 12601ab1 genetic maps (Figure 8.1, Table 8.7). Out of 29 DArT marker loci aligned on Stirling genetic maps, 10 were found to be co-locating with QTLs for Ca

on chromosomes I, II, V, VIII and XI (Figure 8.1, Table 8.7). Out of the 13 SSR markers aligned on Stirling, seven were co-localized with QTLs for Ca on chromosomes Ib, II, III, V, VIII and XI (Table 8.7). In 12601ab1, 17 DArT and seven SSR marker loci overlapped with the QTLs for Ca on chromosomes II, III, IV, VIII and IX, and on chromosomes II, IV and VIII, respectively. The candidate genes associated with the QTLs identified for Ca includes Ca^{2+}-permeable cation channels ((annexin (ANX), cyclic nucleotide gated channel (CNGC), glutamate receptor (GLR), two pore channel (TPC), Ca^{2+}-permeable outward-rectifying K^+ channels (KORC) and mechanosensitive (MS) Ca^{2+} channels)), gene families such as P_{2A}-ATPase (ECA), P_{2B}-ATPase (ACA) and Ca^{2+}/H^+ antiporter (CAX), Ca-binding proteins (calmodulin (CaM), calmodulin-related proteins, calcineurin-B-like proteins (CBLs), annexins, calreticulin (CRT), calsequestrin (CSQ), calnexin (CNX) and luminal binding proteins (BiP)) and regulators such as CAX-interacting protein (CXIP) (Table 8.7).

QTLs on chromosomes that were consistent over two or three years, and explaining high percentage variance for tuber Ca concentrations were found on chromosomes Ib, II, V, VI and XII in Stirling and on chromosomes II, IV, VIII and XII in 12601ab1 (Table 8.7).

Table 8.5: QTLs for iron (Fe) in the GenPop1 population overlapping with the DArT and SSR marker loci putatively associated with the traits in the NTB and GenPop1 populations using bulked segregant analysis

QTLs for Fe in GenPop1 (Chrom. No.)	Variance explained by QTLs (%)	Candidate genes underlying QTLs for Fe	DArT (SSR) marker loci aligned on GenPop1 chrom.^	QTLs for Fe overlapping with DArT (SSR) markers	Candidate genes putatively associated with marker loci	Variance explained by the Fe QTLs overlapping with markers (%)
Stirling						
Ia	5.4	ZIP	0 (0)	0 (0)	_	-
Ib	7.1–9.8	FRD3, ZIP, FRO2, VIT, IREG3, NAS	3 (1)	2 (1)	IREG3, NAS, FRO6, (IREG3, NAS)	7.1 (7.1)
II	6.9–9.3	Fe transporter, YSL5, IRT1 & 2, LeOPT1, ZIP10, MATE	2 (3)	2 (1)	MATE, IRT1 & 2, ZIP10; (LeOPT1)	9.1–9.3 (NE)
III	4.4 & 6.0	YSL, FRO4 & 8, NRAMP1	3 (0)	3 (0)	FRO8, NRAMP1 (_)	4.4 (-)
IV	12.6	_	0 (2)	0 (1)	_ (X)	12.6
VIII	4.7 & 9.8	ZIP4, YSL1, OPT	2 (1)	0 (0)	_	-
IX	4.3	_	1 (0)	0 (0)	_	-
X	7.3	_	1 (1)	0 (0)	_	-
XI	8.3 & 14.7	LeNRAMP1	1 (1)	0 (1)	_ (LeNRAMP1)	- (14.7)
XII	5.9	LeOPT1, ZIP,	3 (0)	1 (0)	X (_)	NE (-)
Total			**16 (9)**	**8 (4)**		
12601ab1						
Ia	6.6–10.8	ZIP, FRO2	2 (0)	2 (0)	X (_)	NE(-)
Ib	6.9	_	3 (1)	0 (0)	X (_)	-

II	9.2–14.3	Fe transporter, YSL5, MATE, LeOPT1, IRT1 & 2, ZIP10,	2 (3)	2 (2)	MATE, IRT1 & 2, ZIP10 (LeOPT1)	14.3 (9.2 & 14.3)
III	6.3	FRO4 & 8, MATE	3 (0)	1 (0)	FRO8, MATE (_)	6.3 (-)
V	9.4	ITP1 & 9	0 (1)	0 (0)	_	-
VI	5.2	-	0 (1)	0 (0)	_	-
VII	5.8 & 8.5	IRT1 & 2, ZIP10	3 (1)	1 (1)	IRT1 & 2, ZIP10 (X)	5.8 & 8.5 (5.8)
VIII	8.1 & 10.2	ZIP4	2 (1)	1 (1*)	X (X)	NE(NE)
IXb	4.9–9.8	Ferritin, YSL1	1 (0)	0 (0)	_	-
XII	8.3 & 10.0	MATE, ZIP	3 (0)	2 (0)	X (_)	8.3 & 10.0 (-)
Total			**19 (8)**	**10 (4)**		

Chrom-Chromosome; ^DArT and SSR markers identified in NTB and GenPop1 populations using BSA; *Marker loci in close proximity to QTLs; NE-% variance explained by QTL was not estimable particularly when a secondary QTL was found; Over-year QTLs were not considered for this table; X-no mineral candidate genes identified; &-represents QTLs found in two seasons; '–' represents QTLs found over three seasons or over two seasons plus measurements carried out in SCRI

Table 8.6: QTLs for zinc (Zn) in GenPop1 population overlapping with the DArT and SSR marker loci putatively associated with the traits in the NTB and Genpop1 populations using bulked segregant analysis[†]

QTLs for Zn in GenPop1 (Chrom. No.)	Variance explained by QTLs (%)	Candidate genes underlying QTLs for Zn	DArT (SSR) marker loci aligned on GenPop1 chrom.^	QTLs for Zn overlapping with DArT (SSR) markers	Candidate genes putatively associated with markers	Variance explained by the Fe QTLs overlapping with markers (%)
Stirling						
Ia	4.2–8.6	MTP, YSL4, ZIP	0 (1)	0 (1)	-(X)	4.2–8.6
Ib	11.7	ZIP, VIT, HMA9	4 (5)	0 (1)	-(VIT, HMA9)	11.7
II	9.5	LeOPT1, MTP, IRT1 &2, ZIP10	5 (1)	3 (0)	IRT1&2, ZIP10 (-)	9.5 (-)
III	3.9 & 6.3	Zn ion binding protein, NRAMP1	3 (0)	1 (0)	X (-)	5.6 (-)
V	8.7 & 10.6	Zn-ion binding protein, NRAMP2, OPT, ZIP6, MTP11, Cation efflux protein/Zn transporter	4 (0)	4 (0)	NRAMP2, OPT (-)	NE (-)
VI	8.5	-	2 (0)	0 (0)	-	-
X	4.0	-	3 (1)	3 (0)	X (-)	4.0 (-)
XI	6.4	-	3 (0)	0 (0)	-	-
Total			**24 (8)**	**11 (2)**		
12601ab1						
Ia	7.6	-	0 (2)	0 (0)	-	-
Ib	8.1	OPT4	5 (4)	2 (1)	OPT4 (OPT4)	8.1

II	7.3 & 10.7	LeOPT1, MTP, IRT1 & 2, ZIP10	5 (1)	3 (0)	IRT1 & 2, ZIP10 (-)	7.3 & 10.7 (-)
III	6.9	NRAMP1	3 (0)	0 (0)	-	-
IV	8.5–9.9	VIT	3 (2)	2 (2)	VIT (VIT)	8.5–9.9 (8.5–9.9)
VII	6.69.6	IRT1 &2, ZIP10, OPT, ZIP11	1 (1)	1 (0)	IRT1 &2, ZIP10 (-)	6.6-9.6 (-)
VIII	8.1–11.0	MHX, ZIP4	5 (0)	1 (0)	MHX (-)	8.1 (-)
IXb	6.0	-	4 (0)	0 (0)	-	-
X	4.0 & 5.7	CDF9, LeNRAMP3, HMA, ZIFL2	3 (1)	2 (1)	X (LeNRAMP3, HMA)	4.0 (4.0–5.7)
XI	6.9	-	3 (0)	1 (0)	X (-)	6.9
XII	5.7 & 10.7	MTP, ZIP	1 (1)	1* (0)	X (-)	-(-)
Total			**33 (12)**	**13 (4)**		

†See Table 8.5 for keywords.

Table 8.7: QTLs for calcium (Ca) in GenPop1 population overlapping with the DArT and SSR marker loci putatively associated in the NTB and Genpop1 populations using bulked segregant analysis[†]

QTLs for Ca in GenPop1 (Chrom. No.)	Variance explained by QTLs (%)	Candidate genes underlying QTLs for Ca	DArT (SSR) marker loci aligned on GenPop1 chrom^	QTLs for Ca overlapping with DArT (SSR) markers	Candidate genes putatively associated with markers	Variance explained by the Ca QTLs overlapping with markers (%)
Stirling						
Ia	4.4 & 6.8	CAX, GLR, GLuR5, CaM	3 (0)	1 (0)	CAX (-)	4.4 & 6.8 (-)
Ib	8.1–9.7	CaM, ECA2, CNGC, TPC1A & 1B, ANX, ACA, CRT, CaM-related protein	2 (2)	2 (1)	CaM-related protein (CRT)	NE (NE)
II	7.5–11.7	CBL3, CN-B, CRT, CAX, ACA2	6 (2)	3 (1)	Ca ion binding protein (CBL3, CN-B, CRT, CAX)	7.5 & 8.1 (7.5 & 8.1)
III	4.7 & 8.7	CN, BiP, CaM	3 (2)	0 (1)	X (BiP, CAM)	-(4.7–8.7)
V	11.3–14.7	CaM2, ACA1, CNGC, CaM-like protein3, Ca-binding protein	2 (1)	2 (1)	CNGC, CaM-like protein3, Ca-binding protein (X)	12.0 (NE)
VI	4.9–12.2	Na^+/Ca^{2+} antiporter, CAX3	3 (1)	0 (0)	-	-
VIII	3.8–6.0	CN, CaM, BiP	1 (3)	1 (2)	X (CN, BiP	6.0 (3.8–6.0)
IX	5.2–8.9	CAX, ACA12	2 (0)	0 (0)	-	-
X	6.6	-	1 (1)	0 (0)	-	-
XI	9.7	CAX, ECA	4 (1)	1 (1)	X (X)	NE (NE)

XII	6.3–10.1	CNGC, CN, CAX6, MSL5	1 (0)	0 (0)	-	-
Total			**29 (13)**	**10 (7)**		
12601ab1						
Ia	3.5	GLR, GLuR5	3 (0)	0 (0)	-	-
II	4.2–11.5	CBL3, CN-B, CRT, CAX, ACA, CaBP, CNGC, HMA1, CHoR1	6 (2)	5 (2)	CaBP, HMA1, CHoR1, CNGC, ACA (CBL3, CN-B, CRT, CAX, ACA, CNGC)	4.2–11.5 (4.2 & 6.0)
III	8.2	CNGC15 & 8, GLR5, CNX	3 (2)	2 (0)	CNGC8, GLR5, CNX (-)	8.2 (-)
IV	7.1–13.7	ANX, Na/K/Ca exchanger, ACA, CBP, ANX p35, VCaB42, GLR3.3, MS ion channel	7 (2)	7 (2)	ACA, CBP, GLR3.3, MS ion channel (ANX p35, VCaB42, GLR3.3, MS ion channel)	7.1–13.7 (NE)
VIII	9.7–12.1	CN, GLuR5, GLR8, CXIP4, BiP	1 (3)	1 (3)	X (CN, BiP)	NE (NE)
IXb	4.1 & 5.6	GLR3.3, MS ion channel, Ca ion binding protein	2 (0)	2 (0)	X (-)	5.6 (-)
XII	7.7–9.1	CNGC	1 (0)	0 (0)	-	-(-)
Total			**23 (9)**	**17 (7)**		

†See Table 8.5 for keywords.

8.3.10 QTLs and candidate genes for tuber Cu, Mg and K concentrations

8.3.10.1 Copper

QTLs on chromosomes that were consistent over two or three years, and explaining high percentage variance for tuber Cu concentrations were found on chromosome IX in Stirling and on chromosomes II, IV, V, VI, VIII, XI and XII in 12601ab1 (Table 8.8).

The candidate genes putatively associated with the identified QTLs for Cu belong to several gene families such as YSL, FRO, OPT, HMA, and Cu chaperones (Figure 8.1). QTLs for Cu on chromosome III overlap with the YSL and FRO loci in Stirling and 12601ab1, and on chromosome II with LeOPT1 in both parents. High-affinity Cu transporters (COPT5) were found in QTL regions for Cu on chromosomes II and XI in 12601ab1. Heavy metal P1b-ATPase (HMA) family genes were located within QTLs for tuber Cu on chromosomes II, VIII, XI and XII in 12601ab1 and on chromosome II in Stirling. Copper chaperone protein genes such as copper chaperone (CCH), Cu chaperone for Cu/ZnSOD (CCS) and Cu chaperone for cytochrome C oxidase (COX) were found in QTL regions on chromosomes Ib, VI and XII in Stirling, and on chromosomes Ib, II, IV, VII, VIII, XI and XII in 12601ab1. The Cu binding protein metallothioneins (MTs) was found in QTL regions for Cu on chromosome XII in both Stirling and 12601ab1. The YSL gene family was located in QTL regions of chromosome V in Stirling and chromosome II, III, V, VIII and IX in 12601ab1.

8.3.10.2 Magnesium

The QTL for Mg in Stirling chromosome V explained the highest variance for the tuber Mg concentration, ranging from 10.4 to 42.1% (Table 8.8). When corrected for maturity effects, QTLs for Mg explained about 6.5 to 12.6% of the total phenotypic variance (Figure 8.2, Table 8.8). In 12601ab1, the QTL on chromosome II explained the highest variance for Mg, ranging from 8.5 to 15.3%.

Table 8.8: Total variance explained by potassium (K), copper (Cu) and magnesium (Mg) QTLs in 12 chromosomes of Stirling and 12601ab1[†]

	Variability explained by mineral QTLs (%)					
	Stirling			12601ab1		
Chrom. no.	K	Cu	Mg	K	Cu	Mg
Ia	-	5.9 & 6.5	-	8.4	5.3	-
Ib	11.8	7.7	8.1–9.0	8.1	6.2	5.8 & 8.8
II	12.8	9.2	7.6 & 9.1	6.9–8.8	8.2–17.4	8.5–15.3
III	-	4.1–6.5	-	8.5 & 6.5	5.5–9.2	5.1–5.5
IV	-	-	9.6	7.7 & 10.7	10.8–20.3	-
V	11.6–31.6	8.9	10.4– 42.1	6.5	7.0 & 10.9	-
V*	5.1 & 6.1	6.6	6.5–12.6			
VI	5.0–7.1	7.7	5.5–9.5	12.0	8.4 & 17.8	10.8
VII	-	-	-	6.0– 8.9	4.9 & 5.1	-
VIII	-	-	-	9.9–13.9	10.3– 13.6	9.2
IX	5.5	11.6 & 12.0	9.0	6.9–8.4	5.6–9.4	5.5
X	6.7–8.7	-	-	-	6.2	4.2 & 7.5
XI	-	-	7.2	-	6.4–14.2	-
XII	-	4.5 & 5.9	5.6	5.9–18.4	5.0–10.0	5.0–7.1

*Maturity corrected QTL.

QTLs on chromosomes V (maturity corrected QTL) and VI in Stirling and chromosomes Ib, III and VI in 12601ab1 were found to be associated with mitochondrial RNA splicing2 (MRS2) gene family (Figure 8.1). Further, Mg^{2+}/H^{+} antiporters (MHX1) were associated with QTLs for Mg on chromosome VI in Stirling and on chromosome VIII in 12601ab1.

8.3.10.3 Potassium

The QTL for K on chromosome V in Stirling explained the highest variance for tuber K concentration, ranging from 11.6 to 31.6% (Table 8.8). When corrected for maturity effects, the QTLs for K explained only between 5.1 and 6.1% of the phenotypic variance (Figure 8.2). In 12601ab1, QTLs on chromosomes IV, VIII and XII contributed to high variance for tuber K concentrations (Table 8.8).

The candidate genes within the identified QTLs for K on different chromosomes include cation transporters such as cyclic-nucleotide gated channels (CNGC), outward rectifying K channel (KORC), Arabidopsis K^+ transporter (AKT), high-affinity K^+ transporter (HAK), K^+ uptake permease (KUP), high-affinity K^+ transporter (HKT), stelar K^+ outward-rectifier (SKOR), K^+ transporter (KT), K^+ channel outward-rectifier (KCO), vacuolar transporters, cation/H^+ exchanger (CHX), cation exchanger (CCX), Na^+/H^+ exchanger (NHX), K^+ exchange/efflux antiporter (KEA) and KIR-like channels (Figure 8.1).

8.4 DISCUSSION

In this study, several QTLs affecting mineral concentrations in potato tubers were identified in a F1 mapping population (12601ab1 x Stirling), in an effort to understand the genetic complexity of tuber mineral accumulation in potatoes. In addition to tuber mineral traits, other important tuber (yield and DM content as well as plant emergence and maturity) traits were also studied. Due to space constraints, only the key outcomes were discussed. Further, the function of candidate genes that were aligned to the genetic maps was not discussed (see section 1.2.3, Chapter 1).

8.4.1 Plant traits

8.4.1.1 Plant maturity

In this study, a major QTL for plant (foliage) maturity was detected on chromosome V, corroborating the results of earlier studies conducted in independent mapping populations (Table 8.9). QTLs with minor effect on plant maturity have also been found in other chromosomes in this study (Table A8.1), validating earlier findings (Table 8.9). In addition to hosting the maturity QTL, the chromosome V also has QTLs for other useful traits such as mineral traits (this study), resistance to tuber blight and foliage blight, plant height and tuber shape, size and DM content (Bradshaw *et al.*, 2004, 2008). Further in the present study, the flowering time

candidate gene (phytochrome) and a flowering time protein, blight resistance protein, WRKY transcription factor and NAC domain were assigned to the proximal end of maturity locus on chromosome V (Figure 8.1). The timing of flowering and plant maturity plays an important role in plant development and growing periods. This perhaps suggests that gene(s) with pleiotrophic effects might be controlling these traits in potato chromosome V.

Table 8.9: Reported QTLs for plant emergence, maturity, tuber dry matter content and yield in potatoes

Trait	Chromosome(s) associated with the QTL	Reference
Diploid populations		
Yield (well-watered & drought stressed)	V	Anithakumari (2011)
Yield	I, II, V, VI, VII, VIII, X & XII	Schäfer-Pregl *et al.* (1998)
Plant maturity	V	Anithakumari (2011)
Plant maturity	I, II, IV, V, VI, VII & VIII	Collins *et al.* (1999)
Plant maturity	V; III & V	Visker *et al.* (2003, 2005)
Plant maturity	V&VI	Oberhagemann *et al.* (1999)
Plant maturity	V, VI, VIII & X	Simko *et al.* (2006)
Plant maturity	IV, V, VIII, IX & X; V & VII	Sliwka *et al.* (2006, 2007)
Tetraploid populations*		
Yield	Ia & VI (Stirling)	Bradshaw *et al.* (2008)
Yield	XII (Atlantic) & VII, VI, V & VIII (B1829-5)	McCord *et al.*, (2011a)
Plant Emergence	A (IX, Stirling) & II (12601ab1)	Bradshaw *et al.* (2008)
Plant maturity	IV, V, VI, VIII, IX, XI & XII	Bormann *et al.* (2004)
Plant maturity	V (Stirling)	Bradshaw *et al.* (2004, 2008)
Plant maturity	II, III, V & XII (Atlantic); III & V (B1829-5)	McCord *et al.* (2011a, b)
Plant maturity	V	Schäfer-Pregl *et al.* (1998)
Dry matter	V (Stirling)	Bradshaw *et al.* (2008)
Dry matter	II, III, V, VII (Atlantic) & II, V & VIII (B1829-5)	McCord *et al.* (2011a)

*For tetraploid populations, QTL found for the respective parent is given in brackets.

8.4.1.2 Flower colour

Flower colour is a qualitative trait controlled by a single gene with major phenotypic impact. Previously, the flower colour locus assumed as P for blue flower colour (van Eck *et al.*, 1993), was mapped to linkage group (LG) XIa in 12601ab1 (Bradshaw *et al.*, 2008). Based on the alignment of DArT markers to the potato genome, the previously reported LG XIa was now identified as LG X (Table 7.4, Chapter 7). In the present study, a significant QTL was detected for blue flower colour on chromosome X of 12601ab1 (Figure 8.1), explaining about 68% of the total phenotypic variance. The F locus on chromosome X is reported to be involved in flower-specific expression of pigmentation (van Eck *et al.*, 1993). The F locus behaves in a dominant fashion, as does the locus uncovered in this study for flower colour (Appendix Table A8.1).

8.4.2 Tuber traits

8.4.2.1 Tuber yield

Previously, two yield-related QTLs were detected in this population on chromosomes Ia and VI of Stirling, accounting for about 13 and 7% of the phenotypic variance, respectively (Bradshaw *et al.*, 2008). With an updated map in the present study, we were able to identify QTLs governing tuber yield on 10 chromosomes (Figure 8.1). Results from this study and previous studies (Table 8.9) indicate that yield is a complex trait controlled by several genes and is typically influenced by environmental variables. Further, this study found that the tuber yield was not affected by plant maturity, suggesting that it is possible to select/breed for high tuber yield along with preferred maturity characteristics.

8.4.2.2 Tuber dry matter content

QTLs influencing tuber dry matter content were identified in potatoes. The QTLs identified in this study confirm the results of the previous studies (Table 8.9 and

references therein). Further, we revealed the presence of additional QTLs for tuber dry matter content on chromosomes I, VI, X, XI and XII (Table 8.1).

8.4.2.3 Tuber mineral concentrations

The QTLs for tuber mineral concentrations were distributed across all the 12 chromosomes of the potato genome (Figure 8.1, Table 8.1), indicating that accumulation of mineral elements in potato tubers involves a large number of genes. Several significant QTLs were discovered in this study, even with the use of an F1 population whose parents were not highly variable for mineral traits (Table 5.3, Chapter 5). This suggests that populations developed for non-mineral traits (e.g., yield, disease resistance, abiotic stress tolerance, etc.) could also be exploited for the identification of tuber mineral QTLs. In this study, majority of the QTLs were consistently found in the same genomic location for two or three years (Figure 8.1, Tables 8.4-8.8). In addition to the major QTLs identified for the mineral traits measured in this study, secondary QTLs were also observed on some chromosomes (eg., QTLs for Ca accumulation on chromosomes II, IV, VIII and IXb in 12601ab1), indicating the influence of multiple genes and environmental factors on QTLs for tuber mineral accumulation.

8.4.3 Co-location of QTLs for plant- and tuber-related traits

8.4.3.1 Plant emergence and tuber yield

Bradshaw *et al.* (2008) have reported QTLs for plant emergence on chromosomes II in 12601ab1 and on LG A (IX) in Stirling (Table 8.9). Results from the current study support the previous findings and additional QTLs were also identified on other chromosomes (Table 8.1). A correlation analysis between plant emergence and tuber yield showed a significant positive association between them (r=0.49, Table 5.5, Chapter 5). In addition, QTL analysis showed the co-location of yield and emergence QTLs on chromosomes II and V in Stirling and on chromosomes V, VIII and XII in 12601ab1 (Figure 8.1). The QTL model for these two traits confirmed the

result from correlation analysis in that early plant emergence was positively associated with high tuber yield (Appendix Table A8.1).

8.4.3.2 Plant maturity and tuber mineral concentrations

The correlation analysis showed a significant association between plant maturity and concentrations of minerals in tubers (Figure 5.1, Table 5.5). Early maturity was associated with high concentrations of tuber Mg, S, Mn, N, B and Zn, and low concentrations of Ca, K and Na. These correlations were consistent with the results of QTL analysis (Appendix Table A8.1). Significant QTLs were found for plant maturity and tuber mineral concentrations on chromosome V of Stirling (Figure 8.1). The effects of QTL were found to be simplex dominant contributed by homologous chromosome 1 on chromosome V of Stirling. However, when the residuals from the regressions on maturity were analysed, no or less significant effect was observed (Figure 8.2). Therefore, it appears that maturity of plants affects the mineral concentration in tubers. In wheat, the *Gpc-B1/NAM-B1* locus, which encodes a NAC transcription factor, was reported to be associated with high grain protein, Fe and Zn concentrations (Uauy *et al.*, 2006). The *Gpc-B1* locus in wheat controls the nutrient remobilization from leaves to the developing grains.

In potatoes, however, NAC genes were found to be induced in response to wounding or by infection of *Phytophthora infestans* (Collinge and Boller, 2001). In the present study, a NAC gene was aligned to the maturity locus (Figure 8.1). Moreover, transporters for Mg, P, Cu, nitrate, nitrite and K were located near the maturity locus, suggesting the role of these transporters in remobilization of mineral elements to tubers during senescence.

8.4.4 Co-location of QTLs for tuber mineral traits and candidate genes

Most of the mineral-related candidate genes that were aligned to the GenPop1 genetic maps overlapped with the identified QTLs for tuber mineral concentration (Figure 8.1). Some of the candidate genes have an assigned location on the genetic

map, yet the available results provide a great deal of information about the chromosomal location of the genes underlying the identified QTLs for mineral traits.

Several QTLs co-localized together for different mineral elements (Figure 8.1), suggesting a possible relationship among the mineral elements at the molecular level. The assessment of the aligned candidate genes for mineral accumulation to the GenPop1 genetic maps indicates that the co-localization of mineral QTLs is due to close physical location of genes for different elements, sharing of common pathways or to common transporters controlling the uptake and movement of the mineral elements in tubers. For example, the co-localization of Mn, Ni and Zn on chromosome Ib in 12601ab1 is associated with nicotianamine synthase (NAS), a key enzyme involved in the synthesis of nicotianamine (NA). Nicotianamine is an important metal chelator involved in intra- and inter-cellular trafficking of Fe, Zn, Mn and Ni in plants. The NAS gene was mapped on the chromosome 1 in tomato (Ling *et al.*, 1996), and in the present study, NAS gene was aligned to chromosome Ib in Stirling and 12601ab1.

The co-localization of QTLs for Zn, Mn, Fe and Cu on chromosome II in 12601ab1 suggests a common transporter LeOPT1, which belongs to the oligopeptide transporter (OPT) gene family from tomato. In addition to the transport of peptides in phloem, the oligo-peptide transporters OPT2 and OPT3 in Arabidopsis were proposed to transport the mineral elements Cu and Zn (OPT2), and Cu, Mn and Fe (OPT3) from phloem to the developing seeds (reviewed in Waterworth and Bray, 2006). Hence, it is possible that LeOPT1 might transport metal (Cu, Fe, Mn and Zn)–chelator complex in phloem and is likely a candidate gene for the above mentioned QTLs for mineral traits. Moreover, the QTLs for each of these mineral elements on chromosome II in 12601ab1 accounted for about 8.1 to 14.3% of the total phenotypic variance (Figure 8.1).

8.4.5 Chromosomal location of mineral-related candidate genes in the Solanaceae

Only three studies have investigated the chromosomal location of mineral-related (Fe-related and salt-tolerance) genes in the species of *Solanaceae* (Table 8.10). In potatoes, the chromosomal location of the mineral-related genes that were assigned to the mapping population (present study) was given in Table 8.10.

From Table 8.10, it is evident that (1) a candidate gene was assigned to two chromosomes based on the superscaffold alignment in the pseudomolecule (e.g., IRT1 & 2, LeNRAMP3), (2) a candidate gene hit was found in different superscaffolds and was assigned to different chromosomes (e.g., NRAMP1) and (3) variants of a gene were found in different superscaffolds and were assigned to the same chromosome (e.g., FRO2 & 6, FRO4 & 8). This perhaps suggests that more copies of these candidate genes might be present in the potato genome. This information can be retrieved from PGSC, however, due to time constraints the number of copies of these candidate genes has not been ascertained.

Table 8.10: Reports on chromosomal location of candidate genes for iron accumulation and salt tolerance in tomato and their alignment to potato chromosomes in the present study

Gene family	Fe- and Na-related genes identified in tomato	Chr	Reference	Candidate gene(s) identified in the present study	Potato chromosome No. (superscaffolds, SS)	Co-location of genes with Fe/Na QTLs identified in this study[&]
Fe accumulation						
IRT	LeIRT1 & 2	II	(1)	IRT1 & 2	II (SS141); VII (SS141)	Yes; Yes
FRO	LeFRO1 & LeFROTC129233	I	(1)	FRO2 & 6	I (SS10 & SS277)	Yes
	LeFRO-TC124302	III	(1)	FRO4 & 8	III (SS159 & SS55)	Yes
NRAMP	LeNRAMP1	XI	(1)	NRAMP1	III (26); XI (SS133)	Yes
	LeNRAMP3	II	(1)	LeNRAMP3	II (12); X (12)	No; No
NAS	LeNAS	I	(2)	NAS	I (SS60)	Yes
bHLH transcriptional regulator	LeFER	VI	(2)	bHLH transcriptional regulator	VI (SS5)	No
Salt tolerance^						
SOS	SOS1	I	(3)	SOS1	I (SS469)	Yes
	SOS2	XII	(3)	SOS2-like protein kinase	VII (SS47)	No
	SOS3	III	(3)	Enhancer of SOS3-1 (ENH1) & SOS3-interacting protein 3 & 4 (SIP3 &4)	I (SS93); VI (SS52; VII(SS47)	Yes; Yes; No
NHX	LeNHX1 & LeNHX3	I& VI	(3)	NHX	I (SS469); II (204); V (204); VI (SS411); VIII (SS147; 208, 201, 48); IX (SS137)	Yes; No; No; Yes; Yes; Yes; No
				NHX1	I (SS92)	Yes
				NHX4	X (SS149)	No

Chr-Chromosome; Chromosome and superscaffold number of the candidate genes in column ; (1) Bauer *et al.* (2004); (2) Ling *et al.* (1999); (3) Villalta *et al.* (2008); ^Although salinity tolerance was not examined in this study, QTLs found for tuber Na concentrations were reported here; [&] QTLs identified either in one or both parents of the mapping population

8.4.6 Co-location of DArT and SSR marker loci with identified QTLs for mineral traits

Thirty seven SSR and 238 DArT markers were found to be putatively associated with traits such as tuber yield, Ca, Fe and Zn concentrations using the two variants of BSA (see Chapter 6). These markers were aligned to the GenPop1 genetic maps (Figure 8.1) and most of the aligned marker loci overlapped with the identified QTLs for mineral traits either in one or both of the parents. This indicates that BSA is an efficient and rapid method for identifying molecular markers linked to the traits of interest in potato diversity sets and genetic mapping populations. Due to time constraints, the identified molecular markers were not tested in the individual clones within the bulk. However, the overlapping of the mineral QTLs and marker loci indicates that, despite the likely presence of some spurious associations, the identified markers can be considered as candidates that could be further validated and used in breeding programmes for enhancing mineral concentration in tubers for human nutrition.

8.4.7 QTLs for tuber mineral concentrations and their implications for biofortification

Although QTLs for tuber mineral concentrations on chromosome V of Stirling explained a high percentage variance initially (Figure 8.1), the variance explained was greatly reduced after correcting for maturity effects (Figure 8.2). However other QTLs that were not associated with maturity can be used to select for genotypes with greater concentrations of essential mineral elements for human nutrition within the same maturity class. The QTLs on some of the individual chromosomes explained about 6 to 15% of the variance for essential mineral concentrations, suggesting that it is possible to select genes for enhancing mineral nutrients in all maturity classes.

For tuber Ca, Fe and Zn concentrations, no major QTL explaining a high percentage of variance was found (Table 8.5-8.8), demonstrating the additive effect of several genes for the final trait value. For example, among the QTLs identified for tuber Ca, Fe and Zn concentrations on most chromosomes in either one or two of the parents, the variance explained ranged from 4.0 to 15% (Table 8.5), the QTLs for tuber K and Mg also behaved in a similar fashion (Table 8.8). For Cu, QTLs on chromosomes II, IV, VI, VIII and XI in 12601ab1 explained a high variance (ranging from 6.4 to 20.3%) compared to other chromosomes (Table 8.8). This indicates that several genes may need to be targeted simultaneously for enhancing the concentration/accumulation of a nutritionally important mineral element (biofortification) in potato tubers.

However, the QTLs for the essential mineral elements, notably Ca, Fe, Zn, Cu, K and Mg were co-locating with at least three or more minerals on chromosomes II, IV, VIII and XII in 12601ab1, accounting for a high variance (Tables 8.5–8.8). This indicates that targeting candidate genes in those genomic regions may allow for the enhancement of multiple mineral elements in potato tubers.

8.5 CONCLUSION

In addition to the traditional interventions of mineral supplementation and agronomic management, biofortification through molecular breeding can be a viable option to increase dietary mineral supply. The ultimate solution is dietary diversification, but this is not immediately practical. As such, biofortification of edible crops (preferably staples) is the better alternative for micronutrient supplementation. The present study has provided a useful resource of genome-linked DArTs and candidate gene-based SSRs for enhancing tuber Ca, Fe and Zn concentrations. This is expected to be of potential use in genetic and association mapping, molecular marker-assisted selection and in the development of transgenic plants with enhanced micronutrient accumulation in potato tubers. In addition, the identified QTLs and the underlying candidate genes reveal the complex

phenomenon of uptake, transport and accumulation of mineral elements in potato tubers. The information generated in this study provided a better understanding of the genomic regions and candidate genes involved in the accumulation of mineral elements in potato tubers. Further, the findings from this study serve as a platform for selecting genes for further characterization and for planning strategies for functional genomic approaches for tuber mineral improvement in potato tubers. Additionally, the knowledge developed in this study could be applied to other similar traits and to other crops of similar physiology.

CHAPTER 9 GENERAL DISCUSSION

Mineral malnutrition is one of the most serious challenges to human health, and is widespread worldwide. It is estimated that up to two-thirds of the world's population might be at risk of deficiency for one or more mineral elements. The elements that are most commonly lacking in human diet are iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), copper (Cu), potassium (K), iodine (I) and selenium (Se). Concentration of these elements can be improved in plant produces through a number of approaches, including mineral fertilization and breeding for crop genotypes that are efficient in mineral acquisition from soil and distribution to edible organs (i.e., biofortification).

Potato is one of the world's most important food crops and is of increasing importance in developing countries because of its high yield potential, nutritional quality and economic viability. Potatoes have high mineral bioavailability in humans because of high concentrations of organic compounds that stimulate the absorption of mineral elements in the human gut and low concentrations of anti-nutritional compounds such as phytate and oxalate that typically inhibit mineral absorption. Potatoes are excellent sources for providing dietary mineral nutrition in humans and biofortification is a novel approach to improve the mineral concentrations in potatoes. Understanding the mechanisms involved in plant mineral acquisition and homeostasis and identification of genes involved in mineral accumulation are prerequisites for biofortification. The aim of this study was to understand the genetics of mineral accumulation in potato tubers in order to facilitate plant breeders to select potato genotypes for enhanced human nutritional value. For this purpose, genetic and molecular tools were employed to identify the genetic factors/genomic regions affecting the accumulation of essential mineral elements in potato tubers.

The first part of the project, described in Chapter 3, was focused on gaining a detailed knowledge of the distribution of mineral elements in potato tubers that were stored for short or long periods. Following a short-term storage, a distinct distribution pattern was observed for mineral elements within the tubers of potato cultivar Stirling. Further, mineral variation exists between the skin and flesh parts of the tuber, with the skin containing about 17, 34 and 55% of the total tuber Zn, Ca and Fe concentration respectively. Within the flesh region (on a fresh weight basis), Ca and phosphorus (P) content decreased towards the centre of the tuber. The elements Fe, Mg, Zn, manganese (Mn), sulphur (S) and chlorine (Cl) were mostly concentrated at the stem end, while K levels were higher at the bud end. The inability of mineral elements to enter the maturing tuber from the soil solutes after suberization of periderm, together with differences in the mobility of mineral elements in the phloem, results in the distinct spatial distribution of mineral elements within the tuber. This experiment provided an understanding of the patterns of mineral accumulation within a potato tuber. In addition, this study emphasised the dietary significance of tuber skin with reference to mineral elements as the skin is often peeled off before food preparation or is not readily consumed.

Mineral distribution patterns within tubers of two potato genotypes, cv. Stirling and clone 12601ab1, that were grown in field and stored at 4°C for six months (Experiment 2), were subsequently investigated to elucidate the genotypic differences for mineral variation. Significant differences in the distributions of several minerals were observed between the genotypes, suggesting that genotype plays an important role in mineral mobilisation and redistribution in tubers. The difference in the distribution of minerals observed in this study could be linked to cold-induced sweetening of potatoes and/or to general tuber metabolism and preparation of tubers for sprouting (growth), the processes that are known to be associated with hydrolysis of

starch and subsequent accumulation of reducing sugars. However, further studies are needed to establish the link between tuber mineral dynamics and hydrolysis of tuber starch under cold storage.

The second part of the project examined the mineral variation in diverse potato germplasm to establish the extent of genetic variability for tuber mineral concentrations in potatoes (Chapter 4). Genetic diversity lies in the core of plant breeding programmes and that a more extensive investigation was carried out using diverse genetic resources to understand the extent of mineral variation in potato tubers. Three diverse germplasm collections maintained at JHI, namely the Commonwealth Potato Collection (CPC) comprising wild accessions, the Core Collection comprising *Solanum tuberosum* Group *Phureja* and *Solanum tuberosum* Group *Tuberosum,* and the Neotuberosum Population (NTB) were studied. This study provided an opportunity to evaluate the extent of genetic variability for tuber mineral concentrations, defining a baseline for its improvement through breeding and selection.

The wild accessions of species in the CPC from diverse geographical origins exhibited a greater variability for nutritionally important minerals (Ca, Fe and Zn with 6.7-, 3.6-, and 4.5-fold variation, respectively) than several other minerals important for primary plant metabolism (e.g., K, P and S, which were typically within 3-fold variability). A key observation in this experiment was that accessions with low tuber DM content showed high concentrations of Ca and K and *vice versa*.

The Core Collection also exhibited a significant variation for mineral elements within and between the diploid Phureja and the tetraploid Tuberosum groups, with diploid Phureja group having lower tuber yield and higher mineral concentrations than the tetraploid Tuberosum group. The multivariate (PCA) analysis of tuber mineral data showed a distinct grouping

of Phureja and Tuberosum groups. Further, the PCA plots also showed distinct patterns of clustering of mineral elements, suggesting that some groups of minerals share the mechanisms of accumulation in tubers. The variation for tuber mineral concentrations observed between Phureja clones and Tuberosum cultivars might be attributable to inherent genetic differences for mineral uptake, partitioning and accumulation and for differences in rooting and maturing characteristics. Some of this difference might be driven by mineral transporter genes, whereas others may be influenced by interactions in the route of transport, means of deposition and duration of life cycle. The heritability of these two groups for different mineral elements was generally high (except for Fe), ranging from 52.2 to 91.1% for Phureja and 67.6 to 89.7% for Tuberosum.

The third diverse collection studied was the tetraploid Neotubersoum (NTB) population, which was derived by recurrent selection from the Andean tetraploids for early tuber production and high tuber yield in the climatic conditions of UK. The NTB clones showed a diverse mineral profile, accumulating greater concentrations of mineral elements in tuber tissues compared with Phureja and Tuberosum genotypes. For example, for tuber Ca concentrations, a 2.0-, 3.0- and 5.0-fold variation was found in Tuberosum, Phureja and Neotuberosum genotypes, respectively. This variation could be attributed to the existence of high levels of genetic differences within this population. Except for Fe, the heritability scores for tuber mineral concentrations were high, ranging from 59.7 to 80.7%.

The observed variations in mineral concentrations indicate ample genetic diversity that might be exploited in breeding programmes seeking to increase levels of these minerals in the human diet. Furthermore, the PCA clustering of minerals and correlation analysis provides an insight into the mineral accumulation patterns in potato tubers, suggesting shared uptake and transport pathways for certain minerals.

The third part of the project (Chapter 5) explored mineral variations in a F1 tetraploid mapping population, 12601ab1 x Stirling (GenPop1), comprising of 190 clones. The potato germplasm analyzed in this study showed a wide variation for tuber yield and mineral concentrations. In order to understand the genetic control of mineral accumulation in potato tubers, the mineral variations in GenPop1 were studied. While the parents did not vary greatly for tuber mineral concentrations, the progenies from this population displayed a transgressive segregation for tuber mineral concentrations, displaying a 1.5- to 3.0-fold variation for tuber mineral concentrations. The heritability (H^2) scores for all traits were generally high (≥75% except for Fe, where H^2= 56.2%), indicating that this mapping population is ideal for selection as well as identification of the QTLs associated with mineral traits in potatoes.

The extensive genetic variation for tuber mineral concentrations discovered in potato germplasms and mapping population (Chapters 4 and 5), will be instrumental in improving this trait through plant breeding efforts. In addition, significant positive associations were found among different mineral elements in each of the three diverse (CPC, Core Collection and NTB) populations and the mapping population. This positive association indicates the existence of one or more common genetic/physiological mechanisms involved in mineral uptake by the root system, translocation and redistribution within the plant tissues or remobilization and accumulation in tubers. A QTL analysis was carried out to further identify the genomic regions associated with tuber mineral concentrations in potatoes (Chapter 8).

The fourth part of this project was aimed at identifying molecular markers that were closely associated with tuber yield, Fe, Ca and Zn concentrations, using the bulked segregant analysis (BSA) approach (Chapter 6). Two variants of BSA were used: (1) BSA using candidate gene approach in the GenPop1 mapping population for tuber Ca, Fe and Zn, using simple sequence repeat (SSR) markers, and (2) a genome-wide approach using diversity arrays

technology (DArT) in the diverse NTB population for tuber yield, Ca, Fe and Zn concentrations. By the BSA approach, 37 polymorphic SSRs and 161 polymorphic DArT markers associated with tuber Ca, Fe and Zn concentrations were identified in the mapping population and in the diverse NTB population, respectively. The alignment of potato sequence superscaffolds to pseudo-chromosomes is now available from the Potato Genome Sequencing Consortium (PGSC). Using the superscaffold number for the identified polymorphic SSR and DArT markers, it was found that these molecular markers were distributed on all 12 chromosomes of the potato genome, suggesting the complexity of tuber yield and mineral traits and their control by multiple genes. However the identified markers need to be validated for their linkage to the trait. If a marker identified in BSA exhibited a tight linkage to the QTL/gene, then the potential marker needs to be validated in potato populations or germplasms to determine if they are expressed within other genetic backgrounds. The results from BSA provide sufficient grounds for further analysis of the genetic basis of mineral accumulation in potato tubers.

A good quality, moderate to high density linkage map is an important requirement for genetic dissection of a complex trait such as the tuber mineral concentration. The fifth part of this project was therefore focused on including more molecular markers to the existing linkage maps of the F1 tetraploid mapping population, 12601ab1 x Stirling (GenPop1) (Chapter 7). Previously, this mapping population was genotyped using AFLP and SSR markers; only the markers for 10 of the 12 chromosomes were identified. However, linkage mapping in species with polysomic (tetrasomic) inheritance requires many more markers to include the entire genome than in a diploid organism. Thus, to increase the marker intensity and coverage of the genome, this population was genotyped with genome-linked DArT markers. In total, 464 DArT markers specific to 12601ab1, 428 markers specific to Stirling and

380 markers common to both parents were identified. Linkage maps for each parent were constructed using the TetraploidMap software based on AFLP, SSR and DArT marker scores. For the majority of the DArT markers, their genomic location is already known, allowing for the identification of markers across all the 12 chromosomes in each of the parent. In addition, this work updated the previously published map for these parents by Bradshaw *et al.*, (2008).

In this study, only a few of the double-simplex (3:1) markers were found to be linked to the simplex (1:1) markers in both parents (not shown). Therefore, considering the time constraints, the double-simplex (3:1) markers were not included in linkage mapping. However, they will be included in future, which will perhaps provide a better alignment of the genetic maps of Stirling and 12601ab1. Further, the availability of co-dominant markers such as SSRs or SNPs, where the allele dosage is known, greatly favors the generation of linkage maps and identification of QTLs.

Developing molecular markers will facilitate marker-assisted breeding and is a prerequistie for adopting molecular biological techniques in plant improvement. QTL analysis has provided unprecedented opportunities to identify chromosome regions linked to a trait of interest. The last part of the project was focused on interval mapping of quantitative trait loci (QTL) on the 12601ab1 and Stirling chromosomes (Chapter 8). Single markers were tested in cases where interval mapping was not possible. QTLs for plant traits (such as plant emergence, plant maturity), tuber traits (such as tuber yield, DM content) and mineral traits were identified. The identified QTLs were spread across the 12 chromosomes of the potato genome, indicating that these traits are controlled by multiple genes. No major QTL was detected for the essential mineral elements (Fe, Zn, Ca, Cu, Mg and K) in this study, indicating that the genetic improvement of these traits by breeding will require combination of many genes contributing to the traits.

QTL analysis only identifies the genomic regions associated with the trait investigated; however this information can be used as a baseline for identification and cloning of candidate genes for the trait (Paran and Zamir, 2003). Using a large recombinant population, near isogenic line or other introgression lines (back-cross), the identified QTL intervals can be fine mapped and the candidate gene(s) can be assigned to the QTL after determining the genomic sequence of the fine mapped locus. With the use of a fine map, marker-assisted selection/breeding can be very precise.

In addition to QTL mapping of minerals, a great leap forward taken in this study was the narrowing of the QTL location using BSA and the suggested candidate genes. BSA can be a useful approach for identifying the approximate location of marker(s) near a target gene. The candidate gene search was carried out for mineral-related genes (13 mineral elements) using the genome browser in the PGSC database. The candidate-gene containing superscaffolds and the DArT and SSR markers associated with tuber yield, Ca, Fe and Zn concentrations from BSA in NTB and GenPop1 (Chapter 6), were aligned to the genetic maps of 12601ab1 and Stirling. The identified SSR and DArT markers using BSA co-located with the identified QTLs, suggesting that BSA is a rapid and efficient method for identifying genomic regions associated with the traits of interest. For example, the QTL for tuber Zn concentrations on chromosome IV in 12601ab1 explained about 8.5 to 10.4% of total phenotypic variance and were consistent over the two years. The putative candidate gene underlying the Zn QTL on chromosome IV was found to be vacuolar Fe transporter (VIT). In addition, the three DArTs and one SSR marker were identified in mineral bulks of Zn in NTB and GenPop1 respectively using BSA and these markers were from the superscaffold 189 where the VIT gene was aligned. The VIT was proposed to transport Fe (Kim *et al.*, 2006) and Zn (Tauris *et al.*, 2009) into the protein storage vacuoles in grains. Although the identified DArT and SSR markers were not mapped in this

study, the alignment of VIT with Zn QTLs on chromosome IV in 12601ab1 indicates that VIT might be a putative candidate gene for the transport of Zn into vacuoles in tubers, and the identified DArT and SSR markers might be potential markers associated with Zn concentration in tubers.

Mineral accumulation in tubers could be greatly influenced by their mobility in phloem tissues. Recent study by White *et al.* (2012) suggests that biofortification of tubers with Zn is limited by the mobility of Zn in the phloem. Thus, biofortification of minerals with intermediate or less mobility in phloem should consider strategies to increase the movement of such minerals in the phloem tissues.

Intake of potatoes with improved mineral concentrations will help improve dietary mineral uptake in human body. Additionally, it was shown that improved mineral concentrations (such as Ca) also increase the quality of potato tubers (reviewed by Palta, 2010). For instance, an adequate supply of Ca is a critical aspect of the mineral nutrition of potatoes because Ca is involved in the structure and function of plant cell walls and membranes. An inadequate Ca can thus increase the risks of internal defects such as internal brown spot and hollow heart in tubers (Palta, 2010) and also increase their susceptibility to soft rot bacteria (McGuire and Kelman, 1984). This suggests enhancing Ca concentration in tubers might improve the physical quality of tubers, in addition to improving its nutritional quality.

In addition to tuber mineral traits, the mapping population (12601ab1 x Stirling) used in this study also segregates for a number of important traits, including resistance to blight and PCN, foliage maturity and other agronomic and tuber quality traits (see Table 7.5 and references therein). As such, co-dominant markers such as SNPs would greatly improve mapping for these traits. Recently, this mapping population has been genotyped using the Potato SolCap 8300 Infinium Chip, which could measure up to 8300 SNPs

(Illumina Golden Gate system). However due to time constraints and to the unavailability of a more robust software for tetraploids, SNP data analysis was not included in the thesis. Out of 8300 SNPs, 5586 SNP markers were found to be polymorphic between the parents (Dr. Christine Hackett, personal communication). Using an updated map with SNPs would narrow down the QTL regions for tuber mineral concentrations and other measured traits in this population.

Most quantitative approaches used to study complex traits such as mineral accumulation have been conducted in a limited number of mapping populations, which harbour a very small part of the existing allelic variation. An association mapping approach is well suited for this because it scrutinizes the results of thousands of generations of recombination and selection (Syvanen, 2005). A higher mapping resolution may be reached as many more meiotic recombination events are sample compared to a bi-parental segregating mapping population. Furthermore, the main advantages of association mapping are exploitation of allelic diversity from a collection of various more or less related cultivars and breeding materials, and providing generic results.

In potatoes, association mapping has been successfully applied for tuber quality traits (D'hoop *et al.*, 2008; Urbany *et al.*, 2011), resistance to late blight (Gebhardt *et al.*, 2004; Malosetti *et al.*, 2007; Pajerowska-Mukhtar *et al.*, 2009), resistance to *Verticillium* (Simko *et al.*, 2004a, b) and for cold-induced sweetening (Baldwin *et al.*, 2011; Li *et al.*, 2005). Along with the evaluation of tuber mineral concentrations of the tetraploid mapping population GenPop1, the germplasm collections were analysed at JHI. The germplasm collections including Core Collection and NTB population displayed high levels of genetic variation for tuber mineral concentrations, which can be explored and possibly utilized with an association mapping approach.

Overall, a wide variation for tuber mineral traits was observed in germplasm collections. Chromosomal regions associated with the tuber mineral concentrations were identified by QTL mapping. In addition, DArT and SSR markers associated with tuber Fe, Zn and Ca concentrations were identified using the BSA approach. Further exploration of the data collected in this thesis and additional focus on specific mineral trait QTLs and associated genes will allow the identification of specific genes and alleles that can be exploited and used in breeding programs. The present study is the first detailed work in *Solanaceae* family to identify the genomic regions associated with mineral traits. The findings obtained and knowledge gained from this study help to make a great leap towards developing potato cultivars with improved mineral qualities and ultimately towards alleviating mineral malnutrition in humans.

REFERENCES

ABDEL-GHANY, S.E., MULLER-MOULE, P., NIYOGI, K.K., PILON, M. and SHIKANAI, T. (2005) Two P-type ATPases are required for copper delivery in *Arabidopsis thaliana* chloroplasts. *Plant Cell,* 17, 1233 - 1251.

ALLEN, E.J. and WURR, D.C.E. (1976) The relationships between tuber size and tuber concentrations of nitrogen, phosphorus and potassium. *The Journal of Agricultural Science,* 86, 503 - 505.

AL-SAIKHAN, M.S., HOWARD, L.R. and MILLER, JR, J.C. (1995) Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum* L.). *Journal of Food Science,* 60, 341 - 343.

ALSCHER, R.G., ERTURK, N. and HEATH, L.S. (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *Journal of Experimental Botany,* 53, 1331 - 1341.

ANDERSON, K.A., MAGNUSON, B.A., TSCHIRGI, M.L. and SMITH, B. (1999) Determining the geographic origin of potatoes with trace metal analysis using statistical and neural network classifiers. *Jou rnal of Agricultural and Food Chemistry,* 47, 1568 - 1575.

ANDRE, C.M., GHISLAIN, M., BERTIN, P., OUFIR, M., DEL ROSARIO HERRERA, M., HOFFMANN, L., HAUSMAN, J.F., LARONDELLE, Y. and EVERS D. (2007) Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. *Journal of Agricultural and Food Chemistry,* 55, 366 - 378.

ANITHAKUMARI, A.M. (2011) Genetic dissection of drought tolerance in potato. PhD thesis, University of Wageningen, Netherlands.

ALTSCHUL, S.F., MADDEN, T.L., SCHAFFER, A.A., ZHANG, J., ZHANG, Z., MILLER, W. and LIPMAN, D.J. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research,* 25, 3389 - 3402.

APSEA, M.P. and BLUMWALD, E. (2007) Na^+ transport in plants. *FEBS Letters,* 581, 2247 - 2254.

ARIZA-NIETO, M., BURGOS, G., BONIERBALE, M., GLAHN, R.P. and WELCH, R.M. (2007) Fe bioavailability in potato (*Solanum tuberosum*). *The Journal of the Federation of American Societies for Experimental Biology (FASEB),* 21, 858.3.

ARRIVAULT, S., SENGER, T. and KRAMER, U. (2006) The *Arabidopsis* metal tolerance protein AtMTP3 maintains metal homeostasis by mediating Zn exclusion from the shoot under Fe deficiency and Zn oversupply. *Plant Journal,* 46, 861 - 879.

ARTECA, R.N., POOVAIAH, B.W. and HILLER, L.K. (1980) Electron microprobe and neutron activation analysis for the determination of elemental distribution in hollow heart potato tubers. *American Potato Journal,* 57, 241 - 247.

ARTSCHWAGER, E. (1918) Anatomy of the potato plant with special reference to the ontogeny of the vascular system. *Journal of Agricultural Research,* 14, 221 - 252.

ARTSCHWAGER, E. (1924) Studies on the potato tuber. *Journal of Agricultural Research,* 27, 809 - 835.

ASSUNÇÃO, A.G.L., HERRERO, E., LIN, Y.F., HUETTEL, B., TALUKDAR, S., SMACZNIAK, C., IMMINK, R.G.H., VAN ELDIK, M., FIERS, M., SCHAT, H. and AARTS, M.G.M. (2010) *Arabidopsis thaliana* transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency. *Proceedings of the National Academy of Sciences USA,* 107, 10296 - 10301.

BAIJAL, B.D. and VAN VLIET, W.F. (1966) The chemical composition in different parts of the potato tuber during storage. *European Potato Journal,* 9, 179 - 192.

BAJAJ, Y.P.S. (1987) Potato. In: *Biotechnology in Agriculture and Forestry,* version 3, p. 509. Springer-Verlag, Berlin, New York.

BAKKER, E., ACHENBACH, U., BAKKER, J., VAN VLIET, J., PELEMAN, J., *et al.,* (2004) A high-resolution map of the H1 locus harbouring resistance to the potato cyst nematode *Globodera rostochiensis. Theoretical and Applied Genetics,* 109, 146 - 152.

BALDWIN, S.J., DODDS, K.G., AUVRAY, B., GENET, R.A., MACKNIGHT, R.C. and JACOBS, J.M.E. (2011) Association mapping of cold-induced sweetening in potato using historical phenotypic data. *Annals of Applied Biology,* 158, 248 - 256.

BAMBERG, J.B., MARTIN, M.W. and J. PALTA. (2008) Variation in *Solanum* species tuber potassium accumulation and its implications for human nutrition. *American Journal of Potato Research,* 85, 2.

BAMBERG, J.B., PALTA, J.P., PETERSON, L.A., MARTIN, M. and KRUEGER, A.R. (1993) Screening tuber bearing *Solanum* (potato) species germplasm for efficient accumulation of tuber calcium. *American Journal of Potato Research,* 70, 219 - 226.

BAMBERG, J.B., PALTA, J.P., PETERSON, L.A., MARTIN, M. and KRUEGER, A.R. (1998). Fine screening potato (*Solanum*) species germplasm for tuber calcium. *American Journal of Potato Research,* 75, 181 - 186.

BANGERTH, F. (1979) Ca-related physiological disorders. *Annual Reviews in Phytopathology,* 17, 97 - 122.

BARBER, S.A. (1995) Soil nutrient bioavailability: a mechanistic approach, 2nd edition, Wiley & Sons Publishers, New York.

BAREL, G. and GINZBERG, I. (2008) Potato skin proteome is enriched with plant defence components. *Journal of Experimental Botany,* 59, 3347 - 3357.

BAUER, P., THIEL, T., KLATTE, M., BERCEZKY, Z., BRUMBAROVA, T., HELL, R. and GROSSE, I. (2004) Analysis of sequence, map position, and gene expression reveals conserved essential genes for iron uptake in Arabidopsis and tomato. *Plant Physiology,* 136, 4169 - 4183.

BAXTER, I., TCHIEU. J., SUSSMAN. M.R., BOUTRY, M., PALMGREN, M.G., GRIBSKOV, M., HARPER, J.F. and AXELSEN, K.B. (2003) Genomic comparison of P-type ATPase ion pumps in *Arabidopsis* and rice. *Plant Physiology,* 132, 618 - 628.

BEEBE, S., GONZALEZ, A.V. and RENGIFO, J. (2000) Research on trace minerals in the common bean. *Food and Nutrition Bulletin,* 21, 387 - 391.

BENDAHMANE, A., KANYUKA, K. and BAULCOMBE D.C. (1997) High-resolution genetical and physical mapping of the *Rx* gene for extreme resistance to potato virus *X* in tetraploid potato. *Theoretical and Applied Genetics,* 95, 153 - 162.

BENTSINK, L., YUAN, K., KOORNNEEF, M. and VREUGDENHIL, D. (2003) The genetics of phytate and phosphate accumulation in seeds and leaves of *Arabidopsis thaliana,* using natural variation. *Theoretical and Applied Genetics,* 106, 1234 - 1243.

BLAIR, M.W., ASTUDILLO, C., GRUSAK, M., GRAHAM, R. and BEEBE, S. (2009) Inheritance of seed iron and zinc content in common bean (*Phaseolus vulgaris* L.). *Molecular Breeding,* 23, 197 - 207.

BLAIR, M.W., KNEWTSON, S.J.B., ASTUDILLO, C., LI, C-M., FERNANDEZ, A.C. and GRUSAK, M.A. (2010b) Variation and inheritance of iron reductase activity in the roots of common bean (*Phaseolus vulgaris* L.) and association with seed iron accumulation QTL. *BMC Plant Biology*, 10, 215.

BLAIR, M.W., MEDINA, J.I. ASTUDILLO, C., RENGIFO, J., BEEBE, S.E., MACHADO, G. and GRAHAM, R. (2010a) QTL for seed iron and zinc concentration and content in a Mesoamerican common bean (*Phaseolus vulgaris* L.) population. *Theoretical and Applied Genetics,* 121, 1059 - 1070.

BONAS, U., SCHMITZ, K., RENNENBERG, H. and BERGMANN, L. (1982) Phloem transport of sulfur in *Ricinus*. *Planta*, 155, 82 - 88.

BONIERBALE, M.W., PLAISTED, R.L. and TANKSLEY, S.D. (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. *Genetics,* 120, 1095 - 1103.

BOLUARTE-MEDINA, T. and VEILLEUX, R. (2002) Phenotypic characterization and bulk segregant analysis of anther culture response in two backcross families of diploid potato. *Plant Cell, Tissue and Organ Culture*, 68, 277 - 286.

BORMANN, C.A., RICKERT, A.M., CASTILLO RUIZ, R.A., PAAL. J., LÜBECK. J., STRAHWALD, J., BUHR, K. and GEBHARDT, C. (2004) Tagging quantitative trait loci for maturity-corrected late blight resistance in tetraploid potato with PCR-based candidate gene markers. *Molecular Plant-Microbe Interactions*, 17, 1126 - 1138.

BOUIS, H. (1996) Enrichment of food staples through plant breeding: a new strategy for fighting micronutrient malnutrition. *Nutrition Reviews*, 54, 131 - 137.

BOUIS, H.E. and WELCH, R.M. (2010) Biofortification – a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science*, 50, S20 - S32.

BOURGIS, F., ROJE, S., NUCCIO, M.L., FISHER, D.B., TARCZYNSKI, M.C., LI, C., HERSCHBACH, C., RENNENBERG, H., PIMENTA, M.J., SHEN, T.L., GAGE, D.A., and HANSON, A.D. (1999) S-methylmethionine plays a major role in phloem sulfur transport and is synthesized by a novel type of methyltransferase. *The Plant Cell*, 11, 1485 - 1498.

BRADSHAW, J.E. and RAMSAY, G. (2005) Utilisation of the Commonwealth Potato Collection in potato breeding. *Euphytica,* 146, 9 - 19.

BRADSHAW, J.E., BRYAN, G.J. and RAMSAY, G. (2006) Genetic resources (including wild and cultivated *Solanum* species) and progress in their utilisation in potato breeding. *Potato Research,* 49, 49 - 65.

BRADSHAW, J.E., HACKETT, C.A., PANDE, B., WAUGH, R. and BRYAN, G.J. (2008) QTL mapping of yield, agronomic and quality traits in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*). *Theoretical and Applied Genetics,* 116, 193 - 211.

BRADSHAW, J.E., PANDE, B., BRYAN, G.J., HACKETT, C.A., MCLEAN, K., STEWART, H.E. and WAUGH, R. (2004) Interval mapping of quantitative trait loci for resistance to late blight [*Phytophthora infestans* (Mont.) de Bary], height and maturity in a tetraploid population of potato (*Solanum tuberosum* subsp. *tuberosum*). *Genetics,* 168, 983 - 995.

BRADSHAW, J.E., STEWART, H.E., WASTIE, R.L., DALE, M.F.B. and PHILLIPS, M.S. (1995) Use of seedling progeny tests for genetical studies as part of a potato (*Solanum tuberosum* subsp. *tuberosum*) breeding programme. *Theoretical and Applied Genetics,* 90, 899 - 905.

BRADSHAW, J.E., HACKETT, C.A., MEYER, R.C., MILBOURNE, D., MCNICOL, J.W., PHILLIPS, M.S. AND WAUGH, R. (1998) Identification of AFLP and SSR markers associated with quantitative resistance to *Globodera pallida* (Stone) in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*) with a view to marker-assisted selection. *Theoretical and Applied Genetics,* 97, 202 - 210.

BRETZLOFF, C.W. (1971) Calcium and magnesium distribution in potato tubers. *American Potato Journal,* 48, 97 - 104.

BRETZLOFF, C.W. and MCMENAMIN, J. (1971) Some aspects of potato appearance and texture. III. Sampling tubers for cation analysis. *American Potato Journal,* 48, 246 - 254.

BROADLEY, M.R. and WHITE, P.J. (2010) Eat roots and leaves. Can edible horticultural crops address dietary calcium (Ca), magnesium (Mg) and potassium (K) deficiencies in humans? *Proceedings of the Nutrition Society,* 69, 601 - 612.

BROADLEY, M.R., HAMMOND, J.P., KING, G.J., ASTLEY, D., BOWEN, H.C., MEACHAM, M.C., MEAD, A., PINK, D.A.C., TEAKLE, G.R., HAYDEN, R.M., SPRACKLEN, W.P. and WHITE, P.J. (2008) Shoot calcium and magnesium concentrations differ between subtaxa, are highly heritable, and associate with potentially pleiotropic loci in *Brassica oleracea. Plant Physiology,* 146, 1707 - 1720.

BROADLEY, M.R., HAMMOND, J.P., KING, G.J., BOWEN, H.C., HAYDEN, R., SPRACKLEN, W.P., Ó LOCHLAINN, S. and WHITE, P.J. (2009) Biofortifying *Brassica* with calcium (Ca) and magnesium (Mg) (September 15, 2009), in *The Proceedings of the International Plant Nutrition Colloquium XVI,* Paper 1256.

BROADLEY, M.R., Ó LOCHLAINN, S., HAMMOND, J.P., BOWEN, H.C., CAKMAK, I., EKER, S., ERDEM, H., KING, G.J. and WHITE, P.J. (2010) Shoot zinc (Zn) concentration varies widely within *Brassica oleracea* L. and is affected by soil Zn and phosphorus (P) levels. *Journal of Horticultural Science & Biotechnology,* 85, 375 - 380.

BROADLEY, M.R., WHITE, P.J., BRYSON, R.J., MEACHAM, M.C., BOWEN, H.C., JOHNSON, S.E., HAWKESFORD, M.J., McGRATH, S.P., ZHAO, F.J., BREWARD, N. *et al.* (2006) Biofortification of UK food crops with selenium. *Proceedings of the Nutrition Society,* 65, 169 - 181.

BROADLEY, M.R., WHITE, P.J., HAMMOND, J.P., ZELKO, I. and LUX, A. (2007) Zinc in plants. *New Phytologist,* 173, 677 - 702.

BROWN, C.R. (2008) Breeding for phytonutrient enhancement of potato. *American Journal of Potato Research,* 85, 298 - 307.

BROWN, P.H. AND HU, H. (1996) Phloem mobility of boron is species dependent: evidence for phloem mobility in sorbitol rich species. *Annals of Botany,* 77, 497 - 505.

BROWN, C.R., HAYNES, K.G., MOORE, M., PAVEK, M.J., HANE, D.C., LOVE, S.L., NOVY, R.G. and MILLER JR, J.C. (2010) Stability and broad-sense heritability of mineral content in potato: iron. *American Journal of Potato Research,* 87, 390 - 396.

BROWN, C.R., HAYNES, K.G., MOORE, M., PAVEK, M.J., HANE, D.C., LOVE, S.L., NOVY, R.G. and MILLER JR, J.C. (2011) Stability and broad-sense heritability of mineral content in potato: zinc. *American Journal of Potato Research,* 88, 238 - 244.

BRYAN, G.J. (2010) The potato genome sequence. Proceedings of EAPR-EUCARPIA congress. Potato breeding after completion of the DNA sequence of the Potato Genome. P. No. 30, Wageningen, The Netherlands, 27-30 June 2010.

BRYAN, G.J. and HEIN, I. (2008) Genomic resources and tools for gene function analysis in potato. *International Journal of Plant Genomics,* Article ID216513.

BRYAN, G.J., MCLEAN, K., PANDE, B., PURVIS, A., HACKETT, C.A., BRADSHAW, J.E. and WAUGH, R. (2004) Genetical dissection of H3-mediated polygenic PCN resistance in a heterozygous autotetraploid potato population. *Molecular Breeding*, 14, 105 - 116.

BUCHER, M. (2007) Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Phytologist*, 173, 11 - 26.

BURGOS, G., AMOROS, W., MOROTE, M., STANGOULIS, J. and BONIERBALE, M. (2007) Iron and zinc concentration of native Andean potato cultivars from a human nutrition perspective. *Journal of Agricultural and Food Chemistry*, 87, 668 - 675.

BURGOS, G., SALAS, E., AMOROS, W., AUQUI, M., MUNOA, L., KIMURA, M. and BONIERBALE, M. (2008) Total and individual carotenoid profiles in the phureja group of cultivated potatoes: I. concentrations and relationships as determined by spectrophotometry and high performance liquid chromatography (HPLC). *Journal of Food Composition and Analysis*, 22, 503 - 508.

BURTON, W.G. (1989) The distribution and composition of the dry matter in the potato tuber. In: *The Potato*. pp. 286 - 335, Third edition. Longman Scientific and Technical, Harlow.

BURTON, W.G., VAN ES, A. and HARTMANS, K.J. (1992) The physics and physiology of storage. *In: The Potato Crop,* pp. 608 - 727, Second edition, (Ed. P. M. Harris), Chapman and Hall, London.

BUSHWAY, R.J., BUREAU, J.L. and MCGANN, D.F. (1984) Determinations of organic acids in potatoes by high performance liquid chromatography. *Journal of Food Science,* 49, 75 - 81.

BUSSE, J.S. and PALTA, J.P. (2006) Investigating the *in vivo* calcium transport path to developing potato tuber using ^{45}Ca: a new concept in potato tuber calcium nutrition. *Physiologia Plantarum,* 128, 313 - 323.

CAKMAK, I. (2008) Enrichment of cereal grains with zinc: agronomic or genetic biofortification? *Plant and Soil*, 302, 1 - 17.

CAKMAK, I., GRAHAM, R. and WELCH, R.M. (2002) Agricultural and molecular genetic approaches to improving nutrition and preventing micronutrient malnutrition globally. In: *Encyclopedia of Life Support Systems*. pp 1075 - 1099 (Eds. Cakmak and R. M. Welch), UNESCOEOLSS Publishers, Oxford.

CAKMAK, I., PFEIFFER, W.H. and McCLAFFERTY, B. (2010) Bioforitification of durum wheat with zinc and iron. *Cereal Chemistry*, 87, 10 - 20.

CAMPBELL, R. (2010). Determining the genetic and molecular control of carotenoids in potato tubers, PhD Thesis, University of Dundee.

CASAÑAS-RIVERO, R., SUÁREZ-HERNÁNDEZ, P., RODRÍGUEZ-RODRÍGUEZ, E.M., DARIAS-MARTÍN, J. and DÍAZ-ROMERO, B.C. (2003) Mineral concentrations in cultivars of potatoes. *Food Chemistry*, 83, 247 - 253.

CHEN, C., HUANG, D. and LIU, J. (2009) Functions and toxicity of nickel in plants: recent advances and future prospects. *Clean*, 37, 304 - 313.

CHEN, S., SANCHEZ-FERNANDEZ, R., LYVER, E.R., DANCIS, A. and REA, P.A. (2007) Functional characterization of AtATM1, AtATM2, and AtATM3, a subfamily of *Arabidopsis* half-molecule ATP-binding cassette transporters implicated in iron homeostasis. *Journal of Biological Chemistry*, 282, 21561 - 21571.

CHEN, J.J. and LIAO, Y.J. (1993) Nitrogen-induced changes in the growth and metabolism of cultured potato-tubers. *Journal of the American Society for Horticultural Science*, 118, 831 - 834.

CICHY, K.A., CALDAS, G.V., SNAPP, S.S. and BLAIR, M.W. (2009) QTL analysis of seed iron, zinc, and phosphorus levels in an Andean bean population. *Crop Science*, 9, 1742 - 1750.

CLEMENS, S. (2001) Molecular mechanisms of plant metal tolerance and homeostasis. *Planta*, 212, 475 - 486.

COLANGELO, E.P. and GUERINOT, M.L. (2004) The essential basic helix-loop-helix protein FIT1 is required for the iron deficiency response. *Plant Cell*, 16, 3400 - 3412.

COLANGELO, E.P. and GUERINOT, M.L. (2006) Put the metal to the petal: metal uptake and transport throughout plants. *Current Opinion in Plant Biology*, 9, 322 - 330.

COLE, C.S. (1975) Variation in dry matter between and within potato tubers. *Potato Research*, 18, 28 - 37.

COLLINGE, M. and BOLLER, T. (2001).Differential induction of two potato genes, Stprx2 and StNAC, in response to infection by *Phytophtora infestans* and to wounding. *Plant Molecular Biology*, 46, 521 - 529.

CONTE, S., STEVENSON, D., FURNER, I. and LLOYD, A. (2009) Multiple antibiotic resistance in Arabidopsis is conferred by mutation in a chloroplast-localized transport protein. *Plant Physiology*, 151, 559 - 573.

CONTE, S.S. and LLOYD, A.M. (2010) The MAR1 transporter is an opportunistic entry point for antibiotics. *Plant Signal Behavior*, 5, 49 - 52.

COLLINS, A., MILBOURNE, D., RAMSAY, L., MEYER, R., CHATOT-BALANDRAS, C., OBERHAGEMANN, P., DE JONG, W., GEBHARDT, C., BONNEL, E. and WAUGH, R. *(1999)* QTL for field resistance to late blight potato are strongly correlated with maturity and vigour. *Molecular Breeding*, 5, 387 - 398.

COPENHAGEN CONSENSUS (2008) Hunger and malnutrition – challenge paper. http://www.copenhagenconsensus.com/Files/Filer/CC08/Papers/0%20Challenge%20Papers/CP_Malnutrition_and_Hunger_-_Horton.pdf. Date visited: 18 August 2011.

CRAWFORD, N.M. (1995) Nitrate: nutrient and signal for plant growth. *The Plant Cell*, 7, 859 - 868.

CURIE, C., CASSIN, G., COUCH, D., DIVOL, F., HIGUCHI, K., JEAN, M., MISSON, J., SCHIKORA, A., CZERNIC, P. and MARI, S. (2009) Metal movement within the plant: contribution of nicotianamine and yellow stripe 1-like transporters. *Annals of Botany*, 103, 1 - 11.

CURIE, C., PANAVIENE, Z., LOULERGUE, C., DELLAPORTA, S.L., BRIAT, J.F. and WALKER, E.L. (2001) Maize yellow stripe1 encodes a membrane protein directly involved in Fe (III) uptake. *Nature*, 409, 346 - 349.

CUTTER, E.G. (1992) Structure and development of the potato plant. In: *The Potato Crop: The Scientific Basis for Improvement*, pp.65 - 161, Second edition (Ed.P. M. Harris), Chapman & Hall, London, UK.

CVITANICH, C., PRZYBYLOWICZ, W.J., URBANSKI, D.F., JURKIEWICZ, A.M., MESJASZ-PRZYBYLOWICZ, J., BLAIR, M.W., ASTUDILLO, C., JENSEN, E.O. and STOUGAARD, J. (2010) Iron and ferritin accumulate in separate cellular locations in *Phaseolus* seeds. *BMC Plant Biology*, 10, 26.

CZEMBOR, P.C., RADECKA-JANUSIK, M., PIETRUSIŃSKA, A. and CZEMBOR, H.J. (2008) Mapping resistance gene to leaf rust in wheat line KS91WGRC11 using quantitative bulked segregant analysis and DArT platform. In: The 11th International Wheat Genetic Symposium, Proceedings, Sydney University Press (http://ses.library.usyd.edu.au/bitstream/2123/3301/1/P117.pdf).

D'HOOP, B.B., PAULO, M.J., MANK, R.A., VAN ECK, H.J. and VAN EEUWIJK, F.A. (2008) Association mapping of quality traits in potato (*Solanum tuberosum* L.). *Euphytica*, 161, 47 - 60.

DALE, M.F.B. and MACKAY, G.R. (1994) Inheritance of table and processing quality. In: *Potato Genetics*, pp. 285 - 315, (Eds. J.E. Bradshaw and G.R. Mackay), CAB International, Wallingford, UK.

DAVID-ASSAEL, O., BEREZIN, I., SHOSHANI-KNAANI, N., SAUL, H., MIZRACHY-DAGRI, T., CHEN, J., BROOK, E. and SHAUL, O. (2006) AtMHX is an auxin and ABA regulated transporter whose expression pattern suggests a role in metal homeostasis in tissues with photosynthetic potential. *Functional Plant Biology*, 33, 661 - 672.

DAVIES, B.E. and CREWS, H.M. (1983) The contribution of heavy metals in potato peel to dietary intake. *Science of the Total Environment*, 30, 261 - 264.

DAVIES, H.V. and MILLARD, P. (1985) Fractionation and distribution of calcium in sprouting and non-sprouting potato tubers. *Annals of Botany*, 56, 745 - 754.

DAVIS, D. (2009) Declining fruit and vegetable nutrient composition: what is the evidence? *HortScience*, 44, 1519.

DE ANGELI, A., MONACHELLO, D., EPHRITIKHINE, G., FRACHISSE, J.M., THOMINE, S., GAMBALE, F. and BARBIER-BRYGOO, H. (2006) The nitrate/proton antiporter AtCLCa mediates nitrate accumulation in plant vacuoles. *Nature*, 442, 939 - 942.

DE JONG, W., FORSYTH, A., LEISTER, D., GEBHARDT, C. and BAULCOMBE, D.C. (1997) A potato hypersensitive resistance gene against potato virus X maps to a resistance gene cluster on chromosome 5. *Theoretical and Applied Genetics*, 95, 246 - 252.

DEKOCK, P.C., HALL, A. and INKSON, R.H.E. (1979) Nutrient distribution in the potato tuber in relation to soil pH. *Annals of Botany*, 43, 299 - 304.

DE MAINE, M.J., LEES, A.K., MUIR, D.D., BRADSHAW J.E. and MACKAY, G.R. (2000) Long-day adapted phureja as a resource for potato breeding and genetic research. In *Potato, Global Research and Development*, Khurana, S.M.P., Shekhawat, G.S., Singh, B.P., Pandey, S.K., Eds., Indian Potato Association, Shimla, India, 134 - 137.

DEEKEN, R., GEIGER, D., FROMM, J., KOROLEVA, O., ACHE, P., LANGENFELD-HEYSER, R., SAUER, N., MAY, S.T. and HEDRICH, R. (2002) Loss of the AKT2/3

potassium channel affects sugar loading into the phloem of *Arabidopsis*. *Planta*, 216, 334 - 344.

DELHAIZE, E., GRUBER, B.D., PITTMAN, J.K., WHITE, R.G., LEUNG, H., MIAO, Y., JIANG,L., RYAN, P.R. and RICHARDSON, A.E. (2007) A role for the AtMTP11 gene of Arabidopsis in manganese transport and tolerance. *Plant Journal*, 51, 198 - 210.

DELHAIZE, E., KATAOKA, T., HEBB, D.M., WHITE, R.G. and RYAN, P.R. (2003) Genes encoding proteins of the cation diffusion facilitator family that confer manganese tolerance. *Plant Cell*, 15, 1131 - 1142.

del POZO, T., CAMBIAZO, V. and GONZALEZ, M. (2010) Gene expression profiling analysis of copper homeostasis in *Arabidopsis thaliana*. *Biochemical and Biophysical Research Communications*, 393, 248 - 252.

DEMIDCHIK, V. and MAATHUIS, F.J.M. (2007) Physiological roles of nonselective cation channels in plants: from salt stress to signaling and development. *New Phytologist*, 175, 387 - 404.

DENG, W., LUO, K., LI, D., ZHENG, X., WEI, X., SMITH, W., THAMMINA, C., LU, L., LI, Y. and PEI, Y. (2006) Overexpression of an Arabidopsis magnesium transport gene, AtMGT1, in *Nicotiana benthamiana* confers Al tolerance. *Journal of Experimental Botany*, 57, 4235 - 4243.

DESBROSSES-FONROUGE, A.G., VOIGT, K., SCHRÖDER, A., ARRIVAULT, S., THOMINE, S. and KRÄMER, U. (2005) *Arabidopsis thaliana* MTP1 is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf Zn accumulation. *FEBS Letters*, 579, 4165 - 4174.

DIDONATO, R.J., ROBERTS, L.A., SANDERSON, T., EISLEY, R.B. and WALKER, E.L. (2004) Arabidopsis *Yellow Stripe-Like2* (*YSL2*): a metal-regulated gene encoding a plasma membrane transporter of nicotianamine-metal complexes. *Plant Journal*, 39, 403 - 414.

DINESH, K. and EZEKIEL R. (2004) Distribution of dry matter and sugars within a tuber of potato cultivars grown under short day conditions. *Potato Journal*, 31, Online ISSN : 0973-5909.

DING, G., YANG, M., HU, Y., LIAO, Y., SHI, L., XU, F. and MENG, J. (2010) Quantitative trait loci affecting seed mineral concentrations in *Brassica napus* grown with contrasting phosphorus supplies. *Annals of Botany*, 105, 1221 - 1234.

DISTELFELD, A., CAKMAK, I., PELEG, Z., OZTURK, L., YAZICI, A.M., BUDAK, H., SARANGA, Y. and FAHIMA, T. (2007) Multiple QTL effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. *Physiologia Plantarum,* 129, 635 - 643.

DODDS, K.S. (1962) Classification of cultivated potatoes. *In*: *The potato and its wild relatives.* D.S. Correll (ed.), pp. 517 - 539, Contributions from Texas Research Foundation, Botanical Studies.

DONG, B., RENGEL, Z. and DELHAIZE, E. (1998) Uptake and translocation of phosphate by pho2 mutant and wild-type seedlings of *Arabidopsis thaliana. Planta,* 205, 251 - 256.

DOUCHKOV, D., GRYCZKA, C., STEPHAN, U.W., HELL, R. and BÄUMLEIN, H. (2005) Ectopic expression of nicotianamine synthase genes results in improved iron accumulation and increased nickel tolerance in transgenic tobacco. *Plant, Cell & Environment,* 28, 365 - 374.

DRAAISTRA, J, (2006) Genetic analysis of root-knot nematode resistance in potato. Ph.D Thesis, Wageningen University and Research Centre, Wageningen.

DRUMMOND, R.S.M., TUTONE, A., LI, Y.C. and GARDNER, R.C. (2006) A putative magnesium transporter AtMRS2-11 is localized to the plant chloroplast envelope membrane system. *Plant Science,* 170, 78 - 89.

DURRETT, T.P., GASSMANN, W. and ROGERS, E.E. (2007) The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. *Plant Physiology,* 144, 197 - 205.

DUY, D., WANNER, G., MEDA, A.R., von WIRÉN, N., SOLL, J. and PHILIPPAR, K. (2007) PIC1, an ancient permease in Arabidopsis chloroplasts, mediates iron transport. *Plant Cell,* 19, 986 - 1006.

EKIN, Z. (2011) Some analytical quality characteristics for evaluating the utilization and consumption of potato (*Solanum tuberosum* L.) tubers. *African Journal of Biotechnology,* 10, 6001 - 6010.

ELBAZ, B., SHOSHANI-KNAANI, N., DAVID-ASSAEL, O., MIZRACHY-DAGRI, T., MIZRAHI K, SAUL, H., BROOK, E., BEREZIN, I. and SHAUL, O. (2006) High expression in leaves of the zinc hyperaccumulator *Arabidopsis halleri* of *AhMHX,* a homolog of an *Arabidopsis thaliana* vacuolar metal/proton exchanger. *Plant, Cell and Environment,* 29, 1179 - 1190.

EREIFEJ, E.I., SHIBLI, R.A., AJIOUNI, M.M. and HUSSEIN, A. (1998) Mineral contents of whole tubers and selected tissues of ten potato cultivars grown in Jordan. *Journal of Food Science and Technology*, 35, 55 - 58.

EWING, E.E. and STRUIK, P.C (1992) Tuber formation in potato: Induction, initiation and growth. *Horticultural Reviews,* 14, 89 - 198.

FAGERIA, N.K., BALIGAR, V.C. and CLARK, R.B. (2002) Micronutrients in crop production. *Advances in Agronomy*, 77, 185 - 268.

FAIRWEATHER-TAIT, S.J. (1983). Studies on the availability of iron in potatoes. *The British Journal of Nutrition,* 50, 15 - 23.

FAN, S.C., LIN, C.S., HSU, P.K., LIN, S.H. and TSAY, Y.F. (2009) The Arabidopsis nitrate transporter NRT1.7, expressed in phloem, is responsible for source-to-sink remobilization of nitrate. *The Plant Cell*, 21, 2750 - 2761.

FAO (1998) The state of the world's plant genetic resources for food and agriculture. FAO, Rome.

FAO (2010) http://www.fao.org/biodiversity/components/plants/en/. Date visited: 9 Oct 2010.

FAOSTAT (2005). Rome, FAO. http://faostat.fao.org. Date visited 9 Oct 2010.

FAOSTAT (2010) Food and agricultural commodities production, Potatoes, from http://faostat.fao.org/site/339/default.aspx, Date visited 18 July 2011.

FERNANDEZ-DEL-CARMEN, A., CELIS-GAMBOA, C., VISSER, R.G.F. and BACHEM, C.W.B. (2007) Targeted transcript mapping for agronomic traits in potato. *Journal of Experimental Botany*, 58, 2761 - 2774.

FLIS, B., HENNIG, J., STRELCZYK-ZYTA, D., GEBHARDT, C. and MARCZEWSKI, W. (2005) The *Ry-fsto* gene from *Solanum stoloniferum* for extreme resistance to potato virus Y maps to potato chromosome XII and is diagnosed by PCR marker $GP122_{718}$ in PVY resistant cultivars. *Molecular Breeding*, 15, 95 - 101.

FORSTER, H. and BERINGER, H. (1983) Starch content of potato tubers in relation to potassium nutrition and tuber development. *Journal of Plant Nutrition and Soil Science*, 146, 572 - 582.

FRANCESCHI, V.R. and NAKATA, P.A. (2005) Calcium oxalate in plants: formation and function. *Annual Reviews in Plant Biology*, 56, 41 - 71.

FRIDMAN E., PLEBAN, T. and ZAMIR, D. (2000) A recombinant hotspot delimits a wild QTL for tomato sugar content to 484 bp within an invertase gene. *Proceedings of the National Academy of Sciences USA*, 97, 4718 - 4723.

FRIEDMAN M. (1997) Chemistry, biochemistry, and dietary role of potato polyphenols. A review*Journal of Agricultural and Food Chemistry*, 45, 1523 - 1540.

FROSSARD, E., BUCHER, M., MACHLER, F., MOZAFAR, A., HURRELL R. (2000) Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *Journal of the Science of Food and Agriculture,* 80, 861 - 879.

GAMBALE, F. and UOZUMI, N. (2006) Properties of Shaker-type potassium channels in higher plants. *Journal of Membrane Biology*, 210, 1 - 19.

GAO, L., IORIZZO, M. MANN, H., CARPUTO, D. and BRADEEN, J.M. (2009) DArT based maps of wild potato species facilitate genome structure comparisons in the genus *Solanum*. Proceedings of the Plant and Animal Genomes XVII Conference, San Diego, CA, 10 - 14 January 2009.

GARCIA-OLIVEIRA, A.L., TAN, L., FU, Y. and SUN, C. (2009) Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. *Journal of Integrative Plant Biology,* 51, 84 - 92.

GARDNER, R.C. (2003) Genes for magnesium transport. *Current Opinion in Plant Biology*, 6, 263 - 267.

GAYMARD, F., PILOT, G., LACOMBE, B., BOUCHEZ, D., BRUNEAU, D., BOUCHEREZ, J., MICHAUX-FERRIÈRE, N., THIBAUD. J.B. and SENTENAC, H. (1998) Identification and disruption of a plant shaker-like outward channel involved in K^+ release into the xylem sap. *Cell,* 94, 647 - 655.

GEBHARDT, C. (2007) Molecular markers, maps, and population genetics. *In: Potato Biology and Biotechnology: Advances and Perspectives.* D. Vreugdenhil, Ed., pp. 77 - 89, Elsevier, Amsterdam, The Netherlands.

GEBHARDT, C., BALLVORA, A., WALKEMEIER, B., OBERHAGEMANN, P. and SCHULER. K. (2004) Assessing genetic potential in germplasm collections of crop plants by marker-trait association: a case study for potatoes with quantitative variation of resistance to late blight and maturity type. *Molecular Breeding,* 13, 93 - 102.

GEBHARDT, C., RITTER E., DEBENER, T., SCHACHTSCHABEL, U., WALKEMEIER, B., UHRIG H. and SALAMINI, F. (1989) RFLP analysis and linkage mapping in *Solanum tuberosum*. *Theoretical and Applied Genetics*, 78, 65 - 75.

GEBHARDT, C., RITTER, E., BARONE, A., DEBENER, T., WALKEMEIER, B., SCHACHTSCHABEL, U., KAUFMANN, H., THOMPSON, R.D., BONIERBALE, M.W., GANAL, M.W., TANKSLEY, S.D. and SALAMINI, F. (1991) RFLP maps of potato and their alignment with the homoeologous tomato genome. *Theoretical and Applied Genetics*, 83, 49 - 57.

GELIN, J.R., FORSTER, S., GRAFTON, K.F., MCCLEAN, P. and ROJAS-CIFUENTES, G.A. (2007) Analysis of seed-zinc and other nutrients in a recombinant inbred population of navy bean (*Phaseolus vulgaris* L.). *Crop Science*, 47, 1361 - 1366.

GENC, Y., VERBYLA, A.P., TORUN, A.A., CAKMAK, I., WILLSMORE, K., WALLWORK, H. and MCDONALD, G.K. (2009) Quantitative trait loci analysis of zinc efficiency and grain zinc concentration in wheat using whole genome average interval mapping. *Plant and Soil*, 314, 49 - 66.

GENDRE, D., CZERNIC, P., CONEJERO, G., PIANELLI, K., BRIAT, J.F., LEBRUN, M. and MARI, S. (2007) TcYSL3, a member of the YSL gene family from the hyper-accumulator *Thlaspi caerulescens*, encodes a nicotianamine-Ni/Fe transporter. *Plant Journal*, 49, 1 - 15.

GEORGE, L., ROMANOWSKY, S.M., HARPER, J.F., SHARROCK, R.A. (2008) The ACA10 Ca^{2+}ATPase regulates adult vegetative development and inflorescence architecture in Arabidopsis. *Plant Physiology*, 146, 716 - 728.

GHANDILYAN, A., BARBOZA, L., TISNE, S., GRANIER, C., REYMOND, M., KOORNNEEF, M., SCHAT, H. and AARTS, M.G.M. (2009) Genetic analysis identifies quantitative trait loci controlling rosette mineral concentrations in *Arabidopsis thaliana* under drought. *New Phytologist*, 184, 180 - 192.

GIERTH, M. and MÄSER, P. (2007) Potassium transporters in plants – involvement in K^+ acquisition, redistribution and homeostasis. *FEBS Letters*, 581, 2348 - 2356.

GLENDINNING, D.R. (1975a) Neo-Tuberosum: new potato breeding material. 1. The origin, composition, and development of the Tuberosum and Neo-Tuberosum gene pools. *Potato Research*, 18, 256 - 261.

GLENDINNING, D.R. (1975b) Neo-Tuberosum: new potato breeding material. 3. Characteristics and variability of Neo-Tuberosum, and its potential value in breeding. *Potato Research* 18, 351 - 362.

GLYNNE, M.D. and JACKSON, V. G. (1919) The distribution of dry matter and nitrogen in the potato tuber, variety King Edward. *Journal of Agricultural Science*, 9, 237 - 258.

GOLACHOWSKI, A. (1985) Properties of starch obtained from potato tubers influenced by various temperatures. *Starch*, 37, 263 - 266.

GOLEGAONKAR, P.G., KARAOGLU, H. and PARK, R.F. (2009) Molecular mapping of leaf rust resistance gene *Rph14* in *Hordeum vulgare*. *Theoretical and Applied Genetics*, 119, 1281 - 1288.

GRAHAM, R.D., WELCH, R.M., SAUNDERS, D.A., *et al.* (2007) Nutritious subsistence food systems. *Advances in Agronomy*, 92, 1 - 74.

GREGORIO, G.B., SENADHIRA, D., HTUT, H. and GRAHAM R.D. (2000) Breeding for trace mineral density in rice. *Food and Nutrition Bulletin*, 21, 382 - 386.

GREEN, L.S. AND ROGERS, E.E. (2004) FRD3 controls iron localization in Arabidopsis. *Plant Physiology*, 136, 2523 - 2531.

GROTZ, N. and GUERINOT, M.L. (2006) Molecular aspects of Cu, Fe and Zn homeostasis in plants. *Biochimica et Biophysica Acta*, 1763, 595 - 608.

GRZEBISZ, W. (2011) Magnesium – Food and human health. *Journal of Elementology*, 16, 299 - 323.

GUO, B., JIN, Y., WUSSLER, C., BLANCAFLOR, E.B., MOTES, C.M. and VERSAW, W.K. (2008b) Functional analysis of the Arabidopsis PHT4 family of intracellular phosphate transporters. *New Phytologist*, 177, 889 - 898.

GUO, W.J., BUNDITHYA, W. and GOLDSBROUGH, P.B. (2003) Characterization of the *Arabidopsis thaliana* metallothionein gene family: tissue-specific expression and induction during senescence and in response to copper. *New Phytologist*, 159, 369 - 381.

GUO, W.J., MEETAM, M. and GOLDSBROUGH, P.B. (2008a) Examining the specific contributions of individual *Arabidopsis* metallothioneins to copper distribution and metal tolerance. *Plant Physiology*, 146, 1697 - 1706.

GUSTIN, J.L., LOUREIRO, M.E., KIM,D., NA, G., TIKHONOVA, M. and SALT, D.E. (2009) MTP1-dependent Zn sequestration into shoot vacuoles suggest dual roles in Zn tolerance and accumulation in Zn-hyperaccumulating plants. *Plant Journal*, 57, 1116 - 1127.

GUZMAN-MALDONADO, S.H., MARTÍNEZ, O., ACOSTA-GALLEGOS, J., GUEVARA-LARA, F.J. and PAREDES-LOPEZ, O. (2003) Putative quantitative trait loci for physical and chemical components of common bean. *Crop Science*, 43, 1029 - 1035.

HABIB, A. and DONNELLY, D.J. (2002) Calcium translocation and accumulation into potato tubers. *Potato Research*, 45, 17 - 24.

HACKETT, C.A., BRADSHAW, J.E., MEYER, R.C., MCNICOL, J.W., MILBOURNE, D.M. and WAUGH, R. (1998) Linkage analysis in tetraploid potato: a simulation study. *Genetical Research*, 71, 143 - 154.

HACKETT, C.A., MILNE, I., BRADSHAW, J.E. and LUO, Z. (2007) TetraploidMap for Windows: linkage map construction and QTL mapping in autotetraploid species. *Journal of Heredity*, 98, 727 - 729.

HALL, J.L. and WILLIAMS, L.E. (2003) Transition metal transporters in plants. *Journal of Experimental Botany*, 54, 2601 - 2613.

HAMBURGER, D., REZZONICO, E., MACDONALD-COMBER PETÉTOT, J., SOMERVILLE, C. and POIRIER, Y. (2002) Identification and characterization of the Arabidopsis PHO1 gene involved in phosphate loading to the xylem. *The Plant Cell*, 14, 889 - 902.

HAMPTON, C.R., BOWEN, H.C., BROADLEY, M.R., HAMMOND, J.P., MEAD, A., PAYNE, K.A., PRITCHARD, J. and WHITE, P.J. (2004) Cesium toxicity in Arabidopsis. *Plant Physiology*, 136, 3824 - 3837.

HANSCH, R. and MENDEL, R.R. (2009) Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Current Opinion in Plant Biology*, 12, 259 - 266.

HARADA, H. and LEIGH, R.A. (2006) Genetic mapping of natural variation in potassium concentrations in shoots of *Arabidopsis thaliana*. *Journal of Experimental Botany*, 57, 953 - 960.

HARRIS, P.M. (1978) Mineral nutrition, *In*: *The Potato Crop: The scientific basis for improvement*, pp 195 - 243, First edition (Ed. P.M. Harris), Chapman and Hall, London, UK.

CIP and HARVESTPLUS (2011) https://research2.cip.cgiar.org/confluence/display/cipqnl/Iron+and+Zinc. Date visited 9/8/2011.

HASHIMOTO, K., SAITO, M., IIDA, H. and MATSUOKA, H. (2005) Evidence for the plasma membrane localization of a putative voltage dependent Ca^{2+} channel, OsTPC1, in rice. *Plant Biotechnology*, 22, 235 - 239.

HASSE, N.U. and PLATE, J. (1996) Properties of potato starch in relation to varieties and environmental factors. *Starch,* 48, 167 - 171.

HAWKES, J.G. (1990) The potato: evolution, biodiversity and genetic resources. Smithsonian Institution Press, Washington, D.C.

HAWKES, J.G. (1994) Origins of the cultivated potato and species relationships. *In: Potato Genetics*. pp 3 - 43, Eds J.E. Bradshaw and G.R. Mackay. CAB International, Wellingborough.

HAWKESFORD, M.J. (2003) Transporter gene families in plants: the sulfate transporter gene family – redundancy or specialization? *Physiologia Plantarum*, 117, 155 - 163.

HAWKESFORD, M.J. and DE KOK, L.J. (2006) Managing sulphur metabolism in plants. *Plant Cell and Environment*, 29, 382 - 395.

HAYDON, M.J. and COBBETT, C.S. (2007) A novel major facilitator superfamily protein at the tonoplast influences Zn tolerance and accumulation in Arabidopsis. *Plant Physiology*, 143, 1705 - 1719.

HAYNES, K.G., YENCHO, G.C., CLOUGH, M.E., HENNINGER, M.R. and STERRETT, S.B. (2012) Genetic variation for potato tuber micronutrient content and implications for biofortification of potatoes to reduce micronutrient malnutrition. *American Journal of Potato Research*, DOI 10.1007/s12230-012-9242-7.

HEARD, J. (2004) Nutrient uptake and partitioning by potatoes in Manitoba. *In:* Proceedings of Manitoba Agronomists conference. P. No. 169.

HEISLER, E.G., SICILANO, J., TREADWAY, R.H. and WOODWARD, C.F. (1963) After-cooking discoloration of potatoes. Iron content in relation to blackening tendency of tissue. *Journal of Food Science,* 28, 453 - 459.

HELDAK, J., BEZO, M., STEFUNOVA, V. and GALLIKOVA, A. (2007) Selection of DNA markers for detection of extreme resistance to potato virus Y in tetraploid potato (*Solanum tuberosum* L.) F1 progenies. *Czech Journal of Genetics and Plant Breeding*, 43, 125 - 134.

HELDAK, J., BRUTOVSKA, E. and GALLIKOVA, A. (2009). Selection of valuable potato genotypes with introduced resistance genes derived from wild species. *Agriculture*, 55, 133 - 139.

HELL, R. and STEPHAN, U.W. (2003) Iron uptake, trafficking and homeostasis in plants. *Planta,* 216, 541 - 551.

HIGGINS, C.F. and PAYNE, J.W. (1980) Transport and utilization of amino acids and peptides by higher plants. *In: Microorganisms and Nitrogen Sources.* Pp 609 - 637. J.W. Payne, Ed. John Wiley and Sons, New York.

HIGGINS, C.F. and PAYNE, J.W. (1982) Plant peptides. *In: Nucleic Acids and Proteins in Plants, Encyclopedia of Plant Physiology,* pp. 438 - 458, D. Boulter, B. Parthier, eds, Vol 14A. Springer, Berlin.

HIRAYAMA, T., KIEBER, J.J., HIRAYAMA, N., KOGAN, M., GUZMAN, P., NOURIZADEH, S., ALONSO, J.M., DAILEY, W.P., DANCIS, A. and ECKER, J.R. (1999) RESPONSIVE-TO-ANTAGONIST1, a Menkes/Wilson disease-related copper transporter, is required for ethylene signaling in Arabidopsis. *Cell,* 97, 383 - 393.

HIRSCHI, K. (2001) Vacuolar H^+/Ca^{2+} transport: who's directing the traffic? *Trends in Plant Science,* 6, 100 - 104.

HIRSCHI, K. D. (2009) Nutrient biofortification of food crops. *Annual Review of Nutrition*, 29, 401 - 421.

HO, L. and WHITE, P.J. (2005) A cellular hypothesis for the induction of blossom end rot in tomato fruit. *Annals of Botany,* 95, 571 - 581.

HORIGUCHI, T. and NISHIHARA, T. (1981) Heavy metals associated with the major constituents of potato tubers and peanut seeds. *Memoirs of the Faculty of Agriculture, Kagoshima University,* 17, 95 - 101.

HORTON, D. (1980) The potato as a food crop for the developing world. A bulletin of the International Potato Centre, Lima, Peru, p. no.1.

HORTON, D. and SAWYER, R.L. (1985) The potato as a world food crop, with special reference to developing areas. *In: Potato Physiology.* Li, P.H. (ed.) pp. 1 - 34, Academic Press, London.

HOSAKA, K., HOSAKA, Y., MORI, M., MAIDA, T. and MATSUNAGA, H. (2001) Detection of a simplex RAPD marker linked to resistance to potato virus Y in a tetraploid potato. *American Journal of Potato Research*, 78, 191 - 196.

HOUGHLAND, G.V.C. (1930) Sampling potatoes for starch analysis. *American Potato Journal*, 7, 219 - 222.

HUFFMAN, D.L. and O'HALLORAN, T.V. (2001) Function, structure, and mechanism of intracellular copper trafficking proteins. *Annual Review of Biochemistry*, 70, 677 - 701.

HUGHES, J.C. and SWAIN, T. (1962) After-cooking blackening in potatoes. II. Core experiments. *Journal of the Science of Food and Agriculture*, 13, 229 - 236.

HUSSAIN, D., HAYDON, M.J., WANG, Y., WONG, E., SHERSON, S.M., YOUNG, J.,CAMAKARIS, J., HARPER, J.F. and COBBETT, C.S. (2004) P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in Arabidopsis. *Plant Cell*, 16, 1327 - 1339.

INTERNATIONAL YEAR OF THE POTATO (2008) - http://www.potato2008.org/en/. Date visited: 24 December 2008.

IORIZZO, M., MANN, H., D'AGOSTINO, N., CHIUSANO, M., VALERIA, M., BRADEEN, J.M. and CARPUTO, D. (2009) Comparative structural genomics between incongruent wild potato species. Proceedings of the 53rd Italian Society of Agricultural Genetics Annual Congress, Torino, Italy, 16–19 September 2009.

ISHIMARU, Y., SUZUKI, M., TSUKAMOTO, T., SUZUKI, K., NAKAZONO, M., KOBAYASHI, T., WADA, Y., WATANABE, S., MATSUHASHI, S., TAKAHASHI, M., NAKANISHI, H., MORI, S. and NISHIZAWA, N.K. (2006) Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. *Plant Journal*, 45, 335 - 346.

JACOBS, J.M.E., ECK, H.J., HORSMAN, K., ARENS, P.F.P., VERKERK-BAKKER, B., JACOBSEN, E., PEREIRA, A. and STIEKEMA, W.J. (1996) Mapping of resistance to the potato cyst nematode *Globodera rostochiensis* from the wild potato species *Solanum vernei*. *Molecular Breeding*, 2, 51 - 60.

JACCOUD, D., PENG, K., FEINSTEIN, D. and KILIAN, A. (2001) Diversity arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research*, 29, e25.

JAKOBY, M., WANG, H.Y., REIDT, W., WEISSHAAR, B. and BAUER, P. (2004) *FRU* (*BHLH029*) is required for induction of iron mobilization genes in *Arabidopsis thaliana*. *FEBS Letters*, 577, 528 - 534.

JAVOT, H., PUMPLIN, N. and HARRISON, M.J. (2007) Phosphate in the arbuscular mycorrhizal symbiosis: transport properties and regulatory roles. *Plant, Cell and Environment*, 30, 310 - 322.

JEGADEESAN, S., YU, K., POYSA, V., GAWALKO, E., MORRISON, M.J., SHI, C. and COBER, E. (2010) Mapping and validation of simple sequence repeat markers linked to a major gene controlling seed cadmium accumulation in soybean [*Glycine max* (L.) Merr]. *Theoretical and Applied Genetics*, 121, 283 - 294.

JEONG, J. and GUERINOT, M.L. (2009) Homing in on iron homeostasis in plants. *Trends in Plant Science*, 14, 280 - 285.

JEONG, J., COHU, C., KERKEB, L., PILON, M., CONNOLLY, E.L. and GUERINOT, M.L. (2008) Chloroplast Fe (III) chelate reductase activity is essential for seedling viability under iron limiting conditions. *Proceedings of the National Academy of Sciences USA*, 105, 10619 - 10624.

JOHNSTON, F.B., HOFFMAN, I. and PETRASOVITS, A. (1968) Distribution of mineral constituents and dry matter in the potato tuber. *American Potato Journal*, 45, 287 - 292.

KAPLAN, B., SHERMAN, T. and FROMM, H. (2007) Cyclic nucleotide-gated channels in plants. *FEBS Letters*, 581, 2237 - 2246.

KÄRENLAMPI, S. and WHITE, P.J. (2009) Potato proteins, lipids and minerals. In: *Advances in potato chemistry and technology.* pp. 99-126, (Ed. J. Singh), Elsevier, Oxford.

KARLEY, A.J. and WHITE P.J. (2009) Moving cationic minerals to edible tissues: potassium, magnesium, calcium. *Current Opinion in Plant Biology*, 12, 291 - 298.

KARLSSON, M.E. and ELIASSON, A.C. (2003) Gelatinization and retrogradation of potato (*Solanum tuberosum*) starch *in situ* as assessed by differential scanning calorimetry (DSC). *LWT- Food Science and Technology*, 36, 735 - 741.

KATAOKA, T., HAYASHI, N., YAMAYA, T. and TAKAHASHI, H. (2004a) Root to-shoot transport of sulfate in Arabidopsis. Evidence for the role of SULTR3;5 as a component of low-affinity sulfate transport system in the root vasculature. *Plant Physiology*, 136, 4198 - 4204.

KATAOKA, T., WATANABE-TAKAHASHI, A., HAYASHI, N., OHNISHI, M., MIMURA, T., BUCHNER, P., HAWKESFORD, M.J., YAMAYA, T. and TAKAHASHI, H. (2004b) Vacuolar sulfate transporters are essential determinants

controlling internal distribution of sulfate in Arabidopsis. *The Plant Cell,* 16, 2693 - 2704.

KAZUNORI, H., AKIRA, S., HIROSHI, K., KAZUO, Y., TADATOSHI, S., MICHIYUKI, K., MOTOYUKI, M. and SHOGO, T. (2008) Unequal distribution of pH, polyphenol content and polyphenol oxidase activity within Japanese processing potato tubers. *Research bulletin Obihiro University,* 29, 18 - 23.

KELLEY, K.B., WHITWORTH, J.L. and R.G. NOVY. (2009) Mapping of the potato leafroll virus resistance gene, *Rlretb,* from *Solanum etuberosum* identifies interchromosomal translocations among its E-genomes 4 and 9 relative to the A-genome of *Solanum* L. sect. *Petota. Molecular Breeding,* 23, 489 - 500.

KHOSHGOFTARMANESH, A. H., SCHULIN, R., CHANEY, R. L., DANESHBAKHSH, B. and AFYUNI, M. (2010) Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture - A review. *Agronomy for Sustainable Development,* 30, 83 - 107.

KHU, D., LORENZEN, J., HACKETT, C.A., and LOVE, S.L. (2008) Interval mapping of quantitative trait loci for corky ringspot disease resistance in a tetraploid population of potato (*Solanum tuberosum* subsp. *tuberosum*). *American Journal of Potato Research,* 85, 129 - 139.

KILIAN, A., HUTTNER, E., WENZL, P., JACCOUD, D., CARLING, J., CAIG, V., EVERS M., HELLER-USZYNSKA, K., CAYLA, C., PATARAPUWADOL, S., XIA, L., YANG, S. and THOMSON, B. (2005) The fast and the cheap: SNP and DArT-based whole genome profiling for crop improvement. *In* Proceedings of the International Congress "In the Wake of the Doube Helix: From the green revolution to the gene revolution". Tuberosa R., Phillips, R.I., Galem M. (eds). Bologna, Italy, Avenue media, 443 - 461.

KIM, S.A. and GUERINOT, M.L. (2007) Mining iron: iron uptake and transport in plants. *FEBS Letters,* 581, 2273 - 2280.

KIM, S.A., PUNSHON, T., LANZIROTTI, A., LI, L.T., ALONSO, J.M., ECKER, J.R., KAPLAN, J. and GUERINOT, M.L. (2006) Localization of iron in Arabidopsis seed requires the vacuolar membrane transporter VIT1. *Science,* 314, 1295 - 1298.

KIRBY, E.J.M. and RYMER, J.L. (1975) The vascular anatomy of the barley spikelet. *Annals of Botany,* 39, 205 - 211.

KISPAL, G., CSERE, P., PROHL, C. and LILL, R. (1999) The mitochondrial proteins Atm1p and Nfs1p are essential for biogenesis of cytosolic Fe/S proteins. *EMBO Journal,* 18, 3981 - 3989.

KLEIN, L.B., CHANDRA, S. and MONDY, N.I. (1982) Sprouting of potatoes: compositional changes in light and dark. *Journal of Food Biochemistry*, 6, 97 - 109.

KLOOSTERMAN, B., OORTWIJN, M., UITDEWILLIGEN, J., AMERICA, T., DE VOS, R., VISSER, R.G.F. and BACHEM, C. (2010) From QTL to candidate gene: genetical genomics of simple and complex traits in potato using a pooling strategy. *BMC Genomics*, 11, 158.

KNAPPE, S., FLÜGGE, U.I. and FISCHER, K. (2003) Analysis of the plastidic phosphate translocator gene family in Arabidopsis and identification of new phosphate translocator-homologous transporters, classified by their putative substrate-binding site. *Plant Physiology*, 131, 1178 - 1190.

KOBAE, Y., UEMURA, T, SATO, M.H., OHNISHI, M., MIMURA, T., NAKAGAWA, T., and MAESHIMA, M. (2004) Zinc transporter of *Arabidopsis thaliana* AtMTP1 is localized to vacuolar membranes and implicated in zinc homeostasis. *Plant Cell Physiology*, 45, 1749 - 1758.

KOH, S., WILES, A.M., SHARP, J.S., NAIDER, F.R., BECKER, J.M. and STACEY, G. (2002) An oligopeptide transporter gene family in Arabidopsis. *Plant Physiology*, 128, 21 - 29.

KOIKE, S., INOUE, H., MIZUNO, D., TAKAHASHI, M., NAKANISHI, H., MORI, S. and NISHIZAWA, N.K. (2004) OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *Plant Journal*, 39, 415 - 424.

KOLBE, H. and STEFAN-BECKMANN, S. (1997a) Development, growth and chemical composition of the potato crop (*Solanum tuberosum* L.). I. Leaf and stem. *Potato Research*, 40, 111 - 130.

KOLBE, H. and STEFAN-BECKMANN, S. (1997b) Development, growth and chemical composition of the potato crop (*Solanum tuberosum* L.). II. Tuber and whole plant. *Potato Research*, 40, 135 - 153.

KORSHUNOVA, Y.O., EIDE, D., CLARK, W.G., GUERINOT, M.L. and PAKRASI, H.B. (1999) The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad substrate range. *Plant Molecular Biology, 40*, 37 - 44.

KRAMER, U., TALKE, I.N. and HANIKENNE, M. (2007) Transition metal transport. *FEBS Letters*, 581, 2263 - 2272.

KRAUSS, A. and MARSCHNER, H. (1971) Influence of direct supply of calcium to potato tubers on the yield and the calcium content. *Zeitschrift für Pflanzenernärhung und Bodenkunde*, 129, 1 - 9.

KRATZKE M.G. and PALTA J.P. (1985) Evidence for the existence of functional roots on potato tubers and stolons: significance of water transport to the tuber. *American Potato Journal* 62, 227 - 236.

KRATZKE M.G. and PALTA J.P. (1986) Calcium accumulation in potato tubers: role of the basal roots. *Hortscience,* 21, 1022 - 1024.

KRISHNAN, S. and DAYANANDAN, P. (2003) Structural and histochemical studies on grain-filling in the caryopsis of rice (*Oryza sativa* L.). *Journal of Bioscience*, 28, 455 - 469.

KRUGER, C., BERKOWITZ, O., STEPHAN, U. W. and HELL, R. (2002) A metal-binding member of the late embryogenesis abundant protein family transports iron in the phloem of *Ricinus communis* L. *Journal of Biological Chemistry,* 277, 25062 - 25069.

KUZUHARA, Y., ISOBE, A., AWAZUHARA, M., FUJIWARA, T. and HAYASHI, H. (2000) Glutathione levels in phloem sap of rice plants under sulphur deficient conditions. *Soil Science and Plant Nutrition*, 46, 265 - 270.

LACHMAN, L. and HAMOUZ, K. (2005) Red and purple coloured potatoes as a significant antioxidant source in human nutrition – a review. *Plant Soil and Environment*, 51, 477 - 482.

LANQUAR, V., LELIÈVRE, F., BOLTE, S., HAMÈS, C., ALCON, C., NEUMANN, D., VANSUYT, G., CURIE, C., SCHRÖDER, A., KRÄMER, U., BARBIER-BRYGOO, H., and THOMINE, S. (2005) Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. *EMBO Journal,* 24, 4041 - 4051.

LEBAUDY, A., VÉRY, A.A. and SENTENAC, H. (2007) K^+ channel activity in plants: genes, regulations and functions. *FEBS Letters*, 581, 2357 - 2366.

LEFEVRE, I., ZIEBEL, J., GUIGNARD, C., HAUSMAN, J.-F., GUTIERREZ ROSALES, R.O., BONIERBALE, M., HOFFMANN, L., SCHAFLEITNER, R. and EVERS, D. (2012). Drought impacts mineral contents in Andean potato cultivars. *Journal of Agronomy and Crop Science*, DOI: 10.1111/j.1439-037X.2011.00499.x.

LEHMENSIEK, A., BOVILL, J., SUTHERLAND, M.W. and MCNAMARA, R.B. (2010) Genomic regions associated with common root rot resistance in the barley variety Delta. *Australasian Plant Pathology*, 39, 241 - 246.

LERICHE, E.L., WANG-PRUSKI, G. and ZHELJAZKOV, V.D. (2006) Mineral concentration and distribution in tubers of fertilized and unfertilized potato cultivars Shepody and Russet Burbank as determined by VP-SEM/EDS. *Canadian Journal of Plant Science*, 86, 1349 - 1353.

LERICHE, E.L., WANG-PRUSKI, G. and ZHELJAZKOV, V.D. (2009) Distribution of elements in potato (*Solanum tuberosum* L.) tubers and their relationship to after-cooking darkening. *HortScience*, 44, 1866 - 1873.

LEVITT, J. and TODD, G.W. (1952) Metal-protein complexes in the potato. *Physiologia Plantarum*, 5, 419 - 429.

LEWANDOWSKA M, and SIRKO A. (2008) Recent advances in understanding plant response to sulfur-deficiency stress. *Acta Biochimica Polonica*, 55, 457 - 471.

LI, L., STRAHWALD, J., HOVERBERT, H.R., LUBECK, J., TACKE, E., JUNGHANS, H., WUNDER, J. and GEBHARDT, C. (2005) DNA variation at the invertase locus invGE/GF is associated with tuber quality traits in populations of potato breeding clones. *Genetics*, 170, 813 - 821.

LI, X., van ECK, H.J., ROUPPE van der VOORT, J.N.A.M., HUIGEN, D.J. STAM, P. and JACOBSEN, E. (1998) Autotetraploids and genetic mapping using common AFLP markers: the R2 allele conferring resistance *to Phytophthora infestans* mapped on potato chromosome 4. *Theoretical and Applied Genetics*, 96, 1121 - 1128.

LILL, R. and KISPAL, G. (2000) Maturation of cellular Fe-S proteins: an essential function of mitochondria. *Trends in Biochemical Science*, 25, 352 - 356.

LIN, K.H., LO, H.F., LEE, S.P., KUO, C.G., CHEN J.T. and YEH W.L. (2006) RAPD markers for the identification of yield traits in tomatoes under heat stress via bulked segregant analysis. *Hereditas*, 143, 142 - 154.

LIN, S.H., KUO, H.F., CANIVENC, G., LIN, C.S., LEPETIT, M., HSU, P.K., TILLARD, P., LIN, H.L., WANG, Y.Y., TSAI, C.B., GOJON, A. and TSAY, Y.F. (2008) Mutation of the Arabidopsis NRT1.5 nitrate transporter causes defective root-to-shoot nitrate transport. *Plant Cell*, 20, 2514 - 2528.

LINDHAUER, M.G. and DE FEKETE M.A.R. (1990) Starch synthesis in potato (*Solanum tuberosum*) tubers: Activity of selected enzymes in dependence of potassium content in storage tissue. *Plant and Soil*, 124, 291 - 295.

LING, H.Q., BAUER, P., BERECZKY, Z., KELLER, B. and GANAL, M. (2002) The tomato *fer* gene encoding a bHLH protein controls iron-uptake responses in

roots. *Proceedings of the National Academy of Sciences USA,* 99, 13938 - 13943.

LING, H.Q., KOCH, G., BAUMLEIN, H. and GANAL, M.W. (1999) Map-based cloning of chloronerva, a gene involved in iron uptake of higher plants encoding nicotianamine synthase. *Proceedings of the National Academy of Sciences USA,* 96, 7098 - 7103.

LING, H.Q., PICH, A., SCHOLZ, G., and GANAL, M.W. (1996) Genetic analysis of two tomato mutants affected in the regulation of iron metabolism. *Molecular and General Genetics,* 252, 87 - 92.

LIU, B.H. (1998) Statistical genomics – linkage, mapping and QTL analysis. CRC Press LLC, Boca Raton, Florida. P. No. 611.

LIU, K.H. and TSAY, Y.F. (2003) Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation. *EMBO Journal,* 22, 1005 - 1013.

LIU, K.H., HUANG, C.Y. and TSAY, Y.F. (1999) CHL1 is a dual affinity nitrate transporter of Arabidopsis involved in multiple phases of nitrate uptake. *Plant Cell,* 11, 865 - 874.

LONERGAN, P.F., PALLOTTA, M.A., LORIMER, M., PAULL, G.J., BARKER, S.J. and GRAHAM, R.D. (2009) Multiple genetic loci for zinc uptake and distribution in barley (*Hordeum vulgare*). *New Phytologist,* 184, 168 - 179.

LOQUE, D., LUDEWIG, U., YUAN, L. and von WIRÉN, N. (2005) Tonoplast intrinsic proteins AtTIP2;1 and AtTIP2;3 facilitate NH3 transport into the vacuole. *Plant Physiology,* 137, 671 - 680.

LOUDET, O., CHAILLOU, S., MERIGOUT, P., TALBOTEC, J. and DANIEL-VEDELE, F. (2003) Quantitative Trait Loci analysis of nitrogen use efficiency in Arabidopsis. *Plant Physiology,* 131, 345 - 358.

LOUDET, O., SALIBA-COLOMBANI, V., CAMILLERI, C., CALENGE, F., GAUDON, V., KOPRIVOVA, A., NORTH, K.A., KOPRIVA, S. and DANIEL-VEDELE, F. (2007) Natural variation for sulfate content in *Arabidopsis thaliana* is highly controlled by APR2. *Nature Genetics,* 39, 896 - 900.

LU, K.Y., LI, L.Z., ZHENG, X.F., ZHANG, Z.H., MOU, T.M. and HU, Z.L. (2008) Quantitative trait loci controlling Cu, Ca, Zn, Mn and Fe content in rice grains. *Journal of Genetics,* 87, 305 - 310.

LUIS, G., RUBIO, C., GONZALEZ-WELLER, D., GUTIERREZ, A., REVERT, C. and HARDISSON, A. (2011) Comparative study of the mineral composition of several varieties of potatoes (*Solanum tuberosum* L.) from different countries cultivated in Canary Islands (Spain). *International Journal of Food Science and Technology*, 46, 774 - 780.

LUNG'AHO, M.G., MWANIKI, A.M., SZALMA, S.J., HART, J.J., RUTZKE, M.A., KOCHIAN, V., GLAHN, R.P. and HOEKENGA, O.A. (2011) Genetic and physiological analysis of iron biofortification in maize kernels. *PLoS ONE*, 6, e20429.

LULAI, E.C. (2001) Tuber periderm and disease resistance. *In: Compendium of Potato Diseases.* pp. 3 - 6, Stevenson, W.R., Loria, R., Franc, G.D. and Weingartner, D.P. eds. APS Press, St Paul, Minnesota, USA.

MAATHUIS, F.J.M. (2009) Physiological functions of mineral macronutrients. *Current Opinion in Plant Biology*, 12, 250 - 258.

MAATHUIS, F.J.M. AND SANDERS, D. (1994) Mechanism of high affinity potassium uptake in roots of *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences USA*, 91, 9272 - 9276.

MABERLY, G.F., TROWBRIDGE, F.L., YIP, R., SULLIVAN, K.M. and WEST, C.E. (1994) Programs against micronutrient malnutrition: ending hidden hunger. *Annual Reviews in Public Health*, 15, 277 - 301.

MACKLON, A.E.S. and DEKOCK, P.C. (1967) Physiological gradients in the potato tuber. *Physiologia Plantarum,* 20, 421 - 429.

MALOSETTI, M., VAN DER LINDEN, C.G., VOSMAN, B. and VAN EEUWIJK, F.A. (2007) A mixed-model approach to association mapping using pedigree information with an illustration of resistance to *Phytophthora infestans* in potato. *Genetics,* 175, 879 - 889.

MARCZEWSKI, W., OSTROWSKA, K. and ZIMNOCH-GUZOWSKA, E. (1998) Identification of RAPD markers linked to the *Ns* locus in potato. *Plant Breeding,* 117, 88 - 90.

MARCZEWSKI, W., STRZELCZYK-ZYTA, D., HENNIG, J., WITEK, K. and GEBHARDT, C. (2006) Potato chromosomes IX and XI carry genes for resistance to potato virus M. *Theoretical and Applied Genetics,* 112, 1232 - 1238.

MARI, S., GENDRE, D., PIANELLI, K., OUERDANE, L., LOBINSKI, R., BRIAT, J.F., LEBRUN, M. and CZERNIC, P. (2006) Root-to-shoot long-distance circulation of

nicotianamine and nicotianamine-nickel chelates in the metal hyperaccumulator *Thlaspi caerulescens*. *Journal of Experimental Botany*, 57, 4111 - 4122.

MARMAGNE, A., VINAUGER-DOUARD, M., MONACHELLO, D., FALCON, D.E., LONGEVIALLE, A., CHARON, C., ALLOT, M., RAPPAPORT, F., WOLLMAN, F.A., BARBIER-BRYGOO, H. and EPHRITIKHINE, G. (2007) Two members of the Arabidopsis CLC (chloride channel) family, AtCLCe and AtCLCf, are associated with thylakoid and Golgi membranes, respectively. *Journal of Experimental Botany*, 58, 3385 - 3393.

MARSCHNER, H. (1995) Mineral nutrition of higher plants. Second edition. Academic Press, London.

MARSCHNER, H. and KRAUSS, A. (1980) Correlations between potassium content and quality of potatoes. *Kartoffelbau*, 31, 65 - 67.

MÄSER, P., GIERTH, M. and SCHROEDER, J.I. (2002) Molecular mechanisms of potassium and sodium uptake in plants. *Plant and Soil*, 247, 43 - 54.

MAATHUIS, F.J. and SANDERS, D. (1994) Mechanism of high-affinity potassium uptake in roots of *Arabidopsis thaliana*. *Proccedings of the National Academy of Sciences USA*, 91, 9272 - 9276.

MAXWELL, J.J., LYERLY, J.H., SRNIC, G., PARKS, R., COWGER, C., MARSHALL, D., BROWN-GUEDIRA, G. and MURPHY, J.P. (2010) MlAB10: a *Triticum turgidum* subsp. *dicoccoides* derived powdery mildew resistance gene identified in common wheat. *Crop Science*, 50, 2261 - 2267.

MAYER, C. (2009) Phobos 3.3.10, 2006 - 2009, http://www.rub.de/spezzoo/cm/cm_phobos.htm

MAYER, J.E., PFEIFFER, W.H. and BEYER, P. (2008) Biofortified crops to alleviate micronutrient malnutrition. *Current Opinion in Plant Biology*, 11, 166 - 170.

MCCANN, L.C., BETHKE, P.C. and SIMON, P.W. (2010) Extensive variation in fried chip color and tuber composition in cold-stored tubers of wild potato (*Solanum*) germplasm. *Journal of Agriculture and Food Chemistry*, 58, 2368 - 2376.

MCCAY, C. M., MCCAY, J. B., SMITH, O. and TALBURT, W. F. (1987) The nutritive value of potatoes. *In: Potato Processing*, pp 287 - 331, Van Nostrand Reinhold, New York.

MCCORD, P.H., SOSINSKI, B.R., HAYNES, K.G., CLOUGH, M.E. and YENCHO, G.C. (2010) QTL mapping of internal heat necrosis in tetraploid potato. *Theoretical and Applied Genetics*, 122, 129 - 142.

MCCORD, P.H., SOSINSKI, B.R., HAYNES, K.G., CLOUGH, M.E. and YENCHO, G.C. (2011) Linkage mapping and QTL analysis of agronomic traits in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*). *Crop Science*, 51, 771 - 785.

MCGUIRE, R.G. and KELMAN, A. (1984) Reduced severity of Erwinia soft rot in potato tubers with increased calcium content. *Phytopathology*, 74, 1250 - 1256.

MENGEL, K., KIRKBY, E.A., KOSEGARTEN, H. and APPEL, T. (2001) Principles of plant nutrition. Kluwer Academic Publishers, Dordrecht, The Netherlands.

MEYER, R. C., MILBOURNE, D., HACKETT, C.A., BRADSHAW, J. E., MCNICOL, J.W. and WAUGH, R. (1998) Linkage analysis in tetraploid potato and associations of markers with quantitative resistance to late blight (*Phytophthora infestans*). *Molecular and General Genetics*, 259, 150 - 160.

MICA, B. (1976) Characteristics of starch of selected potato varieties. II. Phosphorus, potassium and calcium content in the starch. *Starch*, 28, 410 - 413.

MICHELMORE, R.W., PARAN, I. and KESSELI, R.V. (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations (random amplified polymorphic DNA/restriction fragment length polymorphism). *Proceedings of the National Academy of Sciences USA*, 88, 9828 - 9832.

MIEDEMA, H., DEMIDCHIK, V., VÉRY, A.A., BOTHWELL, J.H.F., BROWNLEE, C., DAVIES, J.M. (2008) Two voltage-dependent calcium channels co-exist in the apical plasma membrane of *Arabidopsis thaliana* root hairs. *New Phytologist*, 179, 378 - 385.

MILLER, A.J., SHEN, Q., XU, G. (2009) Freeways in the plant: transporters for N, P and S and their regulation. *Current Opinion in Plant Biology*, 12, 284 - 290.

MILLS, R.F., DOHERTY, M.L, LOPEZ-MARQUES, R.L., WEIMAR, T., DUPREE, P., PALMGREN, M.G., PITTMAN, J.K. and WILLIAMS, L.E. (2008) ECA3, a Golgi-localized P2A-type ATPase, plays a crucial role in manganese nutrition in Arabidopsis. *Plant Physiology*, 146, 116 - 128.

MIWA, K., TAKANO, J., OMORI, H., SEKI, M., SHONOZAKI, K. and FUJIWARA, T. (2007) Plants tolerant of high boron levels. *Science,* 318, 1417.

MIWA, K., KAMIYA, T. and FUJIWARA, T. (2009) Homeostasis of the structurally important micronutrients, B and Si. *Current Opinion in Plant Biology,* 12, 307 - 311.

MIZUNO, T., USUI, K., HORIE, K., NOSAKA, S., MIZUNO, N. and OBATA, H. (2005) Cloning of three ZIP/Nramp transporter genes from a Ni hyperaccumulator plant *Thlaspi japonicum* and their Ni^{2+}- transport abilities. *Plant Physiology and Biochemistry,* 43, 793 - 801.

MORENO, I., NORAMBUENA, L., MATURANA, D., TORO, M., VERGARA, C., ORELLANA, A., ZURITA-SILVA, A. and ORDENES, V.R. (2008) AtHMA1 is a thapsigargin-sensitive Ca^{2+}/heavy metal pump. *Journal of Biological Chemistry,* 283, 9633 - 9641.

MORRIS, J., TIAN, H., PARK, S., SREEVIDYA, C.S., WARD, J.M., HIRSCHI, K.D. (2008) AtCCX3 is an Arabidopsis endomembrane H^+-dependent K^+ transporter. *Plant Physiology,* 148, 1474 - 1486.

MORRISSEY, J. and GUERINOT, M.L. (2009) Iron uptake and transport in plants: the good, the bad, and the ionome. *Chemical Reviews,* 109, 4553 - 4567.

MORRISSEY, J., BAXTER, I.R., LEE, J., LI, L.T., LAHNER, B., GROTZ, N., KAPLAN, J., SALT, D.E. and GUERINOT, M.L. (2009) The ferroportin metal efflux proteins function in iron and cobalt homeostasis in Arabidopsis. *Plant Cell,* 21, 3326 - 3338.

MORTIMER, J.C., LAOHAVISIT, A., MACPHERSON, N., WEBB, A., BROWNLEE, C., BATTEY, N.H. and DAVIES, J.M. (2008) Annexins: multifunctional components of growth and adaptation. *Journal of Experimental Botany,* 59, 533 - 544.

MUÑOZ, O., DIAZ, O.P., LEYTON, I., NUÑEZ, N., DEVESA, V., SÚÑER, M.A., VÉLEZ, D. and MONTORO, R. (2002) Vegetables collected in the cultivated Andean area of northern Chile: total and inorganic arsenic contents in raw vegetables.*Journal of Agricultural and Food Chemistry,* 50, 642 - 647.

MUNSHI, C.B., CARY, E.E. and MONDY, N.I. (1993) The effect of soil entrapment by the periderm on the mineral element composition of potatoes. *Journal of Food Quality,* 16, 45 - 55.

NA, G. and SALT, D.E. (2010). The role of sulfur assimilation and sulfur-containing compounds in trace element homeostasis in plants. *Environmental and Experimental Botany*, 1, 18 - 25.

NITSOS, R.E. and EVANS, H.J. (1969) Effects of univalent cations on the activity of particulate starch synthetase. *Plant Physiology,* 44, 1260 - 1266.

NODA, T., TSUDA, S., MORI, M., TAKIGAWA, S., MATSUURA-ENDO, C., HASHIMOTO, N. and YAMAUCHI, H. (2004a) Properties of starches from several potato varieties grown in Hokkaido. *Journal of Applied Glycoscience,* 51, 241 - 246.

NODA, T., TSUDA, S., MORI, M., TAKIGAWA, S., MATSUURA-ENDO, C., SAITO, K., MANGALIKA, W.H.A., HANAOKA, A., SUZUKI, Y. and YAMAUCHI, H. (2004b) The effect of harvest dates on the starch properties in various potato cultivars. *Food Chemistry*, 86, 119 - 125.

NORTON, G. J., DEACON, C.M., XIONG, L., HUANG, S., MEHARG, A.A. and PRICE, A.H. (2010) Genetic mapping of the rice ionome in leaves and grain: identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. *Plant and Soil,* 329, 139 - 153.

NOURIAN, F., RAMASWAMY, H.S. and KUSHALAPPA, A.C. (2003) Kinetics of quality change associated with the potatoes stored at different temperatures. *LWT- Food Science and Technology*, 36, 49.

NYQUIST, W.E. (1991) Estimation of heritability and prediction of selection response in plant populations. *Critical Reviews in Plant Sciences*, 10, 235 - 322.

OBERHAGEMANN, P., CHATOT-BALANDRAS, C., SCHÄFER-PREGL, R., WEGENER, D., PALOMINO, C., SALAMINI, F., BONNEL, E. and GEBHARDT, C. (1999) A genetic analysis of quantitative resistance to late blight in potato: towards marker-assisted selection. *Molecular Breeding* 5, 399 - 415.

O'HALLORAN, T.V. and CULOTTA, V.C. (2000) Metallochaperones, an intracellular shuttle service for metal ions. *Journal of Biological Chemistry,* 275, 25057 - 25060.

OPARKA, K.J. (1986) Phloem unloading in the potato tuber. Pathways and sites of ATPase. *Protoplasma,* 131, 201 - 210.

OPARKA, K.J. and WRIGHT, K.M. (1988) Osmotic regulation of starch synthesis in potato tubers. *Planta,* 174, 123 - 126.

ORTIZ-MEDINA, E. and DONNELLY, D.J. (2003) Concentration and distribution of total soluble protein in fresh and stored potato tubers. *ISHS Acta Horticulturae*, 619, 323 - 328.

ORTIZ-MEDINA, E., SOSLE, V., RAGHAVAN, V. and DONNELLY, D.J. (2009) A method for intercultivar comparison of potato tuber nutrient content using specific tissue weight proportions. *Journal of Food Science*, 74, S177 - S181.

OZGEN, S., KARLSSON, B.H. and PALTA, J.E. (2006) Response of potatoes (cv Russet Burbank) to supplemental calcium applications under field conditions: Tuber calcium, yield, and incidence of internal brown spot. *American Journal of Potato Research,* 83, 195 - 204.

OZKAN, H., BRANDOLINI, A., TORUN, A., ALTINTAS, S., EKER, S., KILIAN, B., BRAUN, H. J., SALAMINI, F. and CAKMAK, I. (2007) Natural variation and identification of microelements content in seeds of einkorn wheat (*Triticum monococcum*). *In*: H.T. Buck, J.E. Nisi, and N. Salomon, eds. *Wheat production in stressed environments*. Springer, Berlin, Germany, 455 - 462.

ÖZTÜRK, E., ATSAN, E., POLAT, T. and KARA, K. (2011) Variation in heavy metal concentrations of potato (*Solanum tuberosum* L.) cultivars. *The Journal of Animal and Plant Sciences*, 21, 235 - 239.

PAJEROWSKA-MUKHTAR, K., STICH, B., ACHENBACH, U., BALLVORA, A., LÜBECK, J., STRAHWALD, J., TACKE, E., HOFFERBERT, H-R., ILARIONOVA, E., BELLIN, D., WALKEMEIER, B., BASEKOW, R., KERSTEN, B. and GEBHARDT, C. (2009) Single nucleotide polymorphisms in the *Allene Oxide Synthase 2* gene are associated with field resistance to late blight in populations of tetraploid potato cultivars. *Genetics,* 181, 1115 - 1127.

PALMER, C. and GUERINOT, M.L. (2009) Facing the challenges of Cu, Fe and Zn homeostasis in plants. *Nature Chemical Biology*, 5, 333 - 340.

PALMGREN, M.G., CLEMENS, S., WILLIAMS, L.E., KRAEMER, U., BORG, S., SCHJORRING, J.K. and SANDERS, D. (2008) Zinc biofortification of cereals: problems and solutions. *Trends in Plant Science*, 13, 464 - 473.

PALTA, J.P. (1996) Role of calcium in plant responses to stresses: Linking basic research to the solution of practical problems. Proceedings of Colloquium: Recent advances in plant responses to stress: bridging the gap between science and technology. *HortScience,* 31, 51 - 57.

PALTA, J.P. (2010). Improving Potato Tuber Quality and Production by Targeted Calcium Nutrition: the Discovery of Tuber Roots Leading to a New Concept in Potato Nutrition. *Potato Research*, 53, 267 - 275.

PANDE, B. (2002) The genetic analysis of traits of economic importance in the principal cultivated potato, *Solanum tuberosum* ssp. *tuberosum*. PhD thesis, University of Dundee, UK.

PANDEY, S. K., and KAUSHIK, S. K. (2003) Origin, evolution, history and spread of potato. In: *The Potato - Production and Utilization in Sub-Tropics,* pp. 15 - 24, S. M. P. Khurana, J. S. Minhas, S. K. Pandey (Eds.)., Mehta Publishers, New Delhi.

PARAN, I. and ZAMIR, D. (2003). Quantitative traits in plants: beyond the QTL. *Trends in Genetics,* 19, 303 - 306.

PARK, S., KANG, T., KIM, C., HAN, J., KIM, S., SMITH, R.H., PIKE, L.M. and HIRSCHI, K.D. (2005) Genetic manipulation for enhancing calcium content in potato tuber. *Journal of Agricultural and Food Chemistry,* 53, 5598 - 5603.

PATERSON, L.J., WILD, P. and WILLIAMS, E.R. (1988) An algorithm to generate designs for variety trials. *The Journal of Agricultural Science*, 111, 133 - 136.

PATTERSON, J., FORD, K., CASSIN, A., NATERA, S. AND BACIC, A. (2007) Increased abundance of proteins involved in phytosiderophore production in boron-tolerant barley. *Plant Physiology,* 144, 1612 - 1631.

PAYNE, K.A., BOWEN, H.C., HAMMOND, J.P., HAMPTON, C.R., LYNN, J.R., MEAD, A., SWARUP, K., BENNETT, M.J., WHITE, P.J. and BROADLEY, M.R. (2004) Natural genetic variation in caesium (Cs) accumulation by *Arabidopsis thaliana. New Phytologist,* 162, 535 - 548.

PEITER, E., MONTANINI, B., GOBERT, A., PEDAS, P., HUSTED, S., MAATHUIS, F.J.M., BLAUDEZ, D., CHALOT, M. and SANDERS, D. (2007) A secretory pathway-localized cation diffusion facilitator confers plant manganese tolerance. *Proceedings of the National Academy of Sciences USA*, 104, 8532 - 8537.

PELEG, Z., CAKMAK, I., OSTURK, L., YAZICI, A., JUN, Y., BUDAK, H., KOROL, A.B., FAHIMA, T. and SARANGA, Y. (2009) Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat × wild emmer wheat RIL population. *Theoretical and Applied Genetics*, 119, 353 - 369.

PENNER, G.A., CLARKE, J., BEZTE, L.J. AND LEISLE, D. (1995) Identification of RAPD markers linked to a gene governing cadmium uptake in durum wheat. *Genome*, 38, 543 - 547.

PERRENOUD, S. (1993) Fertilizing for High Yield Potato. *IPI Bulletin 8.*2nd Edition. International Potash Institute, Basel, Switzerland.

PETERSON, R.L. and BARKER, W.G. (1979) Early tuber development from explanted stolon nodes of *Solanum tuberosum* var. Kennebec. *Botanical Gazette,* 140, 398 - 406.

PETT, L.B. (1936) Changes in the ascorbic acid and glutathione contents of stored and sprouting potatoes. *Biochemical Journal,* 30, 1228 - 1232.

PFEIFFER, W.H. and McCLAFFERTY, B. (2007) HarvestPlus: breeding crops for better nutrition. *Crop Science,* 47, S88 - S105.

PFLIEGER, S., LEFEBVRE, V. and CAUSSE, M. (2001) The candidate gene approach in plant genetics: a review. *Molecular Breeding,* 7, 275 - 291.

PHILLIPPY, B.Q., LIN, M. and RASCO, B. (2004) Analysis of phytate in raw and cooked potatoes. *Journal of Food Composition and Analysis,* 17, 217 - 226.

PITTMAN, J.K. (2005). Managing the manganese: molecular mechanisms of manganese transport and homeostasis. *New Phytologist,* 167, 733 - 742.

PITTMAN, J.K., SHIGAKI, T., MARSHALL, J.L., MORRIS, J.L., CHENG, N.H. and HIRSCHI, K.D. (2004) Functional and regulatory analysis of the *Arabidopsis thaliana* CAX2 cation transporter. *Plant Molecular Biology,* 56, 959 - 971.

POIRIER, Y., THOMA, S., SOMERVILLE, C. and SCHIEFELBEIN, J. (1991) A mutant of Arabidopsis deficient in xylem loading of phosphate. *Plant Physiology,* 97, 1087 - 1093.

POTTOSIN, I.I. and SCHONKNECHT, G. (2007) Vacuolar calcium channels. *Journal of Experimental Botany,* 58, 1559 - 1569.

PRITCHARD, M.K. and SCANLON, M.G. (1997) Mapping dry matter and sugars in potato tubers for prediction of whole tuber processing quality. *Canadian Journal of Plant Science,* 77, 461 - 467.

PUIG, S., ANDRÉS-COLÁS, N., GARCÍA-MOLINA, A. and PEÑARRUBIA, L. (2007) Copper and iron homeostasis in *Arabidopsis*: responses to metal deficiencies, interactions and biotechnological applications. *Plant, Cell and Environment,* 30, 271 - 290.

PUIG, S. and PEÑARRUBIA, L. (2009) Placing metal micronutrients in context: transport and distribution in plants. *Current Opinion in Plant Biology,* 12, 299 - 306.

QUARRIE, S.A., LAZIĆ-JANČIĆ, V., KOVAČEVIĆ, D., STEED, A. and PEKIĆ, S. (1999) Bulk segregant analysis with molecular markers and its use for

improving drought resistance in maize. *Journal of Experimental Botany*, 50, 1299 - 1306.

QUEIROLO, F., STEGEN, S., RESTOVIC, M., PAZ, M., OSTAPCZUK, P., SCHWUGER, M.J. and MUÑOZ, L. (2000) Total arsenic, lead, and cadmium levels in vegetables cultivated at the Andean villages of northern Chile. *Science of the Total Environment*, 255, 75 - 84.

QUICK, W.A. and LI, P.H. (1976) Phosphorus balance in potato tubers. *Potato Research*, 19, 305 - 312.

QUICK, W.A. and LI, P.H. (1980) The effect of edaphic and genetic factors on the phosphorus components in potato tubers. *American Potato Journal*, 57, 249 - 257.

RAUSCH, C. and BUCHER, M. (2002) Molecular mechanisms of phosphate transport in plants. *Planta*, 216, 23 - 37.

RAUSER, W.E. (1999) Structure and function of metal chelators produced by plants: the case for organic acids, amino acids, phytin and metallothioneins. *Cell Biochemistry and Biophysics*, 31, 19 - 48.

REED, T. (1910) On the anatomy of some tubers. *Annals of Botany*, 24, 537 - 548.

REEVE, R.M., TIMM, H. and WEAVER, M.L. (1971) Cell size in Russet Burbank potato tubers grown with various levels of nitrogen and soil moisture tensions. *American Potato Journal*, 48, 450 - 456.

REEVE, R.M. (1974) Relevance of immature tuber periderm to high commercial peeling loss. *American Potato Journal*, 51, 254 - 262.

REEVE, R.M., HAUTALA, E. and WEAVER, M.L. (1969a) Anatomy and compositional variation within potatoes. I. Developmental histology of the tuber. *American Potato Journal*, 46, 361 - 373.

REEVE, R.M., HAUTALA, E. and WEAVER, M. L. (1969b) Anatomy and compositional variation within the potatoes. II. Phenolics, enzymes and other minor components. *American Potato Journal*, 46, 374 - 386.

REEVE, R.M., HAUTALA, E. and WEAVER, M. L. (1970) Anatomy and compositional variation within the potatoes. III. Gross compositional gradients. *American Potato Journal*, 47, 148 - 162.

REEVE, R.M., TIMM, H. and WEAVER, M.L. (1973) Parenchyma cell growth in potato tubers. I. Different tuber regions. *American Potato Journal,* 50, 49 - 57.

REID, R.J., DUNBAR, K.R. and MCLAUGHLIN, M.J. (2003) Cadmium loading into potato tubers: the roles of the periderm, xylem and phloem. *Plant, Cell and Environment,* 26, 201 - 206.

RENGEL, Z., BATTEN, G.D. and CROWLEY, D.E. (1999) Agronomic approaches for improving the micronutrient density in edible portions of field crops. *Field Crops Research,* 60, 27 - 40.

RENNENBERG, H., SCHMITZ, K. and BERGMANN, L. (1979) Long-distance transport of sulfur in *Nicotiana tabacum. Planta,* 147, 57 - 62.

RENTSCH, D., SCHMIDT, S. and TEGEDER, M. (2007) Transporters for uptake and allocation of organic nitrogen compounds in plants. *FEBS Letters,* 581, 2281 - 2289.

ROY, S.J., GILLIHAM, M., BERGER, B., ESSAH, P.A., CHEFFINGS, C., MILLER, A.J., DAVENPORT, R.J., LIU, L.H., SKYNNER, M.J., DAVIES, J.M. RICHARD, P., LEIGH, R.A. and TESTER, M. (2008) Investigating glutamate receptor-like gene co-expression in *Arabidopsis thaliana. Plant, Cell Environment,* 31, 861 - 871.

SABINIANO, N.S., ISHIBASHI, K. and HIRONAKA, K. (1995) Effect of low-temperature storage on some properties of potato starch. *Journal of the Japenese Society for Food Science and Technology,* 42, 815 - 819.

SADEGHZADEH, B., RENGEL, Z., LI, C. and YANG, H. (2010) Molecular marker linked to a chromosome region regulating seed Zn accumulation in barley. *Molecular Breeding,* 25, 167 - 177.

SAGREDO, B., BALBYSHEV, N., LAFTA, A., CASPER, H. and LORENZEN, J. (2009) A QTL that confers resistance to Colorado potato beetle (*Leptinotarsa decemlineata* [Say]) in tetraploid potato populations segregating for leptine. *Theoretical and Applied Genetics,* 119, 1171 - 1181.

SAGREDO, B., LAFTA, A., CASPER, H. and LORENZEN, J. (2006) Mapping of genes associated with leptine content in tetraploid potato. *Theoretical and Applied Genetics,* 114, 131 - 142.

SAGREDO, B., LORENZEN, J., CASPER, H. and LAFTA, A. (2011) Linkage analysis of a rare alkaloid present in a tetraploid potato with *Solanum chacoense* background. *Theoretical and Applied Genetics,* 122, 471 - 478.

SALVI, S. and TUBEROSA, R. (2005) To clone or not to clone plant QTLs: present and future challenges. *Trends in Plant Science*, 10, 297 - 304.

SAMOTUS, B. (1965) Role of phytic acid in potato tuber. *Nature*, 206, 1372 - 1373.

SAMOTUS, B. and SCHWIMMER, S. (1962) Effect of maturity and storage on distribution of phosphorus among starch and other components of potato tuber. *Plant Physiology*, 37, 519 - 522.

SANCENÓN, V., PUIG, S., MATEU-ANDRÉS, I., DORCEY, E., THIELE, D.J. and PEÑARRUBIA L. (2004) The *Arabidopsis* copper transporter COPT1 functions in root elongation and pollen development. *Journal of Biological Chemistry*, 279, 15348 - 15355.

SCHAAF, G., HONSBEIN, A., MEDA, A.R., KIRCHNER, S., WIPF, D. and von WIRÉN, N. (2006) *AtIREG2* encodes a tonoplast transport protein involved in iron-dependent nickel detoxification in *Arabidopsis thaliana* roots. *Journal of Biological Chemistry*, 281, 25532 - 25540.

SCHACHTMAN, D.P., KUMAR, R., SCHROEDER, J.I. and MARSH, E.L. (1997) Molecular and Functional Characterization of a Novel Low-Affinity Cation Transporter (LCT1) in Higher Plants. *Proceedings of the National Academy of Sciences USA*, 94, 11079 - 11084.

SCHÄFER-PREGL, R., RITTER, E. CONCILIO, L., HESSELBACH, J., LOVATTI, L., WALKEMEIER, B., THELEN, H., SALAMINI, F. and GEBHARDT, C. (1998) Analysis of quantitative trait loci (QTLs) and quantitative trait alleles (QTAs) for potato tuber yield and starch content. *Theoretical and Applied Genetics*, 97, 834 - 846.

SCHOCH, T.J. (1942) Non-carbohydrate substances in the cereal starches. *Journal of the American Chemical Society*, 64, 2954 - 2956.

SCHULZE, W., FROMMER, W.B. and WARD, J.M. (1999) Transporters for ammonium, amino acids and peptides are expressed in pitchers of the carnivorous plant *Nepenthes*. *The Plant Journal*, 17, 637 - 646.

SHAUL, O. (2002) Magnesium transport and function in plants: the tip of the iceberg. *Biometals*, 15, 309 - 323.

SHAUL, O., HILGEMANN, D.W., DE-ALMEIDA-ENGLER, J., VAN MONTAGU, M., INZÉ, D. and GALILI, G. (1999) Cloning and characterization of a novel Mg^{2+}/H^{+}exchanger. *EMBO Journal*, 18, 3973 - 3980.

SHASHIDAR, H.E., VINOD, M.S., SUDHIR, G.V., SHARMA, N. and KRISHNAMURTHY, K. (2005). Markers linked to grain yield using bulked segregant analysis approach in rice (*Oryza sativa* L.). *Rice Genetics Newsletter*, 22, 73 - 76.

SHEKHAR, V.C. and IRITANI, W.M. (1978) Starch to sugar interconversion in *Solanum tuberosum* L. I. Influence of inorganic ions. *American Journal of Potato Research*, 55, 345 - 350.

SHEPHERD, L.V.T., BRADSHAW, J.E., DALE, M.F.B., MCNICOL, J.W., PONT, S.D.A., MOTTRAM, D.S. and DAVIES, H.V. (2010) Variation in acrylamide producing potential in potato: Segregation of the trait in a breeding population. *Food Chemistry*, 123, 568 - 573.

SHI, H., QUINTERO, F.J., PARDO, J.M. and ZHU, J.K. (2002) The putative plasma membrane Na^+/H^+ antiporter SOS1 controls long-distance Na^+ transport in plants. *Plant Cell*, 14, 465 - 477.

SHI, R., LI, H., TONG, Y., JING, R., ZHANG, F. and ZOU, C. (2008) Identification of quantitative trait locus of zinc and phosphorus density in wheat (*Triticum aestivum* L.) grain. *Plant and Soil*, 306, 95 - 104.

SHIGAKI, T. and HIRSCHI, K.D. (2006) Diverse functions and molecular properties emerging for CAX cation/H^+ exchangers in plants. *Plant Biology*, 8, 419 - 429.

SHIKANAI, T., MÜLLER-MOULÉ, P., MUNEKAGA, Y., NIYOGI, K.K. and PILON, M. (2003) PAA1, a P-type ATPase of *Arabidopsis*, functions in copper transport in chloroplasts. *Plant Cell*, 15, 1333 - 1346.

ŠIMIĆ, D., MLADENOVIĆ DRINIĆ, S., ZDUNIĆ, Z., JAMBROVIĆ, A., LEDENČAN, T., BRKIĆ, J., BRKIĆ, A. and BRKIĆ, I. (2011) Quantitative trait loci for biofortification traits in maize grain. *Journal of Heredity*, doi:10.1093/jhered/esr122.

SILLANPÄÄ, M. (1982) Micronutrients and the nutrient status of soils: a global study. Food and agriculture organization of the United Nations. *FAO Soil Bulletin*, 48.

SILLANPÄÄ, M. (1990) Micronutrient assessment at the country level: an international study. Food and Agriculture Organization of the United Nations in cooperation with the Finnish International Development Agency (FINNIDA). *FAO Soil Bulletin*, 63.

SIMKO, I., COSTANZO, S., HAYNES, K.G., CHRIST, B.J., and JONES, R.W. (2004a) Linkage disequilibrium mapping of a *Verticillium dahliae* resistance quantitative trait locus in tetraploid potato (*Solanum tuberosum*) through a candidate gene approach. *Theoretical and Applied Genetics,* 108, 217 - 224.

SIMKO, I., COSTANZO, S., RAMANJULU, V., CHRIST, B.J. and HAYNES, K.G. (2006) Mapping polygenes for tuber resistance to late blight in a diploid *Solanum phureja* x *S. stenotomum* hybrid population. *Plant Breeding,* 125, 385 - 389.

SLIWKA, J., JAKUCZUN, H., LEBECKA, R., MARCZEWSKI, W., GEBHARDT, C. and ZIMNOCH-GUZOWSKA, E. (2007) Tagging QTLs for late blight resistance and plant maturity from diploid wild relatives in a cultivated potato (*Solanum tuberosum*) background. *Theoretical and Applied Genetics,* 115, 101 - 112.

SIMKO, I., HAYNES, K.G., EWING, E.E., COSTANZO, S., CHRIST, B.J. and JONES R.W. (2004b) Mapping genes for resistance to *Verticillium albo-atrum* in tetraploid and diploid potato populations using haplotype association tests and genetic linkage analysis. *Molecular Genetics and Genomics,* 271, 522 - 531.

SIMKO, I., van den BERG, J.H., VREUGDENHIL, D. and EWING, E.E. (20 08) Mapping loci for chlorosis associated with chlorophyll b deficiency in potato. *Euphytica,* 162, 99 - 107.

SLIWKA, J., JAKUCZUN, H., LEBECKA, R., MARCZEWSKI, W., GEBHARDT, C. and ZIMNOCH-GUZOWSKA, E. (2006) A novel late blight resistance gene *Rpi-phu1* mapped to potato chromosome IX is not related to long vegetation period. *Theoretical and Applied Genetics,* 113, 685 - 695.

SLIWKA, J., WASILEWICZ-FLIS, I., JAKUCZUN, H. and GEBHARDT, C. (2008) Tagging quantitative trait loci for dormancy, tuber shape, regularity of tuber shape, eye depth and flesh colour in diploid potato originated from six *Solanum* species. *Plant Breeding,* 127, 49 - 55.

SONG, Y-S. (2004). Genetic marker analysis in potato for extreme resistance (*RYsto*) to PVY and for chip quality after long term storage at 4°C. Ph.D. Thesis. Technical University, Munich.

SORENSEN, K., KIRK, H., OLSSON, K., LABOURIAU, R. and CHRISTIANSEN, J. (2008) A major QTL and an SSR marker associated with glycoalkaloid content in potato tubers from *Solanum tuberosum* × *S. sparsipilum* located on chromosome I. *Theoretical and Applied Genetics,* 117, 1 - 9.

SPOONER, D.M., MCLEAN, K., RAMSAY, G., WAUGH, R. and BRYAN, G.J. (2005). A single domestication for potato based on multilocus AFLP genotyping. *Proceedings of the National Academy of Sciences USA,* 102, 14694 - 14699.

STACEY, M.G., OSAWA, H., PATEL, A., GASSMANN, W. and STACEY, G. (2006) Expression analyses of Arabidopsis oligopeptide transporters during seed germination, vegetative growth and reproduction. *Planta,* 223, 291 - 305.

STANGOULIS, J.C.R., HUYNH, B., WELCH, R.M,, CHOI, E. and GRAHAM, R.D. (2007) Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica,* 154, 289 - 294.

STEFANOVIC, A., RIBOT, C., ROUACHED, H., WANG, Y., CHONG, J., BELBAHRI, L., DELESSERT, S. and POIRIER, Y. (2007) Members of the PHO1 gene family show limited functional redundancy in phosphate transfer to the shoot, and are regulated by phosphate deficiency via distinct pathways. *Plant Journal,* 50, 982 - 994.

STEIN, A.J. (2010) Global impacts of human mineral malnutrition. *Plant and Soil,* 335, 133 - 154.

STEGEN, S., QUEIROLO, F., CARRASCO, C., OSTAPCZUK, P. and SCHWUGER, M.J. (2002) Concentrations of Ni and Co in crop plants cultivated in northern Chile. *Journal of the Chilean Chemical Society,* 47, 279 - 287.

STOMPH, T.J., JIANG, W. and STRUIK, P.C. (2009) Zinc biofortification of cereals: rice differs from wheat and barley. *Trends in Plant Science,* 14, 123 - 124.

STOREY, M. (2007) The harvested crop. *In: Potato Biology and Biotechnology,* D. Vreugdenhil, Editor, pp. 441 - 470, Elsevier, Amsterdam.

SU, Z., CHAI, M.F., LU, P.L., AN, R., CHEN, J. and WANG, X.C. (2007) AtMTM1, a novel mitochondrial protein, may be involved in activation of the manganese-containing superoxide dismutase in Arabidopsis. *Planta,* 226, 1031 - 1039.

SUBEDI, P.P. and WALSH, K.B. (2009) Assessment of potato dry matter concentration using short-wave near-infrared spectroscopy. *Potato Research,* 52, 67 - 77.

SUBRAMANIAN, N.K., WHITE, P.J., BROADLEY, M.R. and RAMSAY, G. (2011) The three dimensional distribution of minerals in potato tubers. *Annals of Botany,* 107, 681 - 691.

SUGIURA, M., GEORGESCU, M.N. and TAKAHASHI, M. (2007) A nitrite transporter associated with nitrite uptake by higher plant chloroplasts. *Plant and Cell Physiology*, 48, 1022 - 1035.

SULAIMAN, M.I. (2005) Effect of calcium fertilization on the quality of potato tubers (*Solanum tuberosum* L.) cv. Saturna. Doctoral Thesis, Georg-August-Universität Gottingen, Germany.

SUZUKI, M., TAKAHASHI, T., TSUKAMOTO, T., WATANABE, S., MATSUHASHI, S., YAZAKI, J., KISHIMOTO, N., KIKUCHI, S., NAKANISHI, H., MORI, S. and NISHIZAWA, N.K. (2006) Biosynthesis and secretion of mugineic acid family phytosiderophores in zinc-deficient barley. *Plant Journal*, 48, 85 - 97.

SYVANEN, A.C. (2005) Toward genome-wide SNP genotyping. *Nature Genetics*, 37, S5 - S10.

TAKAHASHI, H., WATANABE-TAKAHASHI, A., SMITH, F.W., BLAKE-KALFF, M., HAWKESFORD, M.J. and SAITO, K. (2000) The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in *Arabidopsis thaliana*. *Plant Journal*, 23, 171 - 182.

TAKANO, J., NOGUCHI, K., YASUMORI, M., KOBAYASHI, M., and GAJDOS, Z. (2002) Arabidopsis boron transporter for xylem loading. *Nature*, 420, 337 - 340.

TAKANO, J., WADA, M., LUDEWIG, U., SCHAAF, G., von WIRÉN, N. and FUJIWARA T. (2006) The Arabidopsis major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation. *The Plant Cell*, 18, 1498 - 1509.

TANAKA, M., WALLACE, I.S., TAKANO, J., ROBERTS, D.M. and FUJIWARA, T. (2008) NIP6;1 is a boric acid channel for preferential transport of boron to growing shoot tissues in Arabidopsis. *The Plant Cell*, 20, 2860 - 2875.

TANHUANPAA, P., KALENDAR, R., SCHULMAN, A. H. and KIVIHARJU, E. (2007) A major gene for grain cadmium accumulation in oat (*Avena sativa* L.). *Genome*, 50, 588 - 594.

TAURIS, B., BORG, S., GREGERSEN, P.L. and HOLM, P.B. (2009) A roadmap for zinc trafficking in the developing barley grain based on laser capture microdissection and gene expression profiling. *Journal of Experimental Botany*, 60, 1333 - 1347.

TEJADA-JIMENEZ, M., GALVAN, A., FERNANDEZ, E. and LIAMAS, A. (2009) Homeostasis of the micronutrients Ni, Mo and Cl with specific biochemical functions. *Current Opinion in Plant Biology*, 12, 358 - 363.

THE POTATO GENOME SEQUENCING CONSORTIUM (2011). Genome sequence and analysis of the tuber crop potato. *Nature*, 475, 189 - 195.

THEIL, E.C., BURTON, J.W. and BEARD, J.L. (1997) A sustainable solution for dietary iron deficiency through plant biotechnology and breeding to increase seed ferritin control. *European Journal of Clinical Nutrition*, 51, S28 - S31.

THOMINE, S., LELIÈVRE, F., DEBARBIEUX, E., SCHROEDER, J. I. and BARBIER-BRYGOO, H. (2003) AtNRAMP3, a multispecific vacuolar metal transporter involved in plant responses to iron deficiency. *Plant Journal*, 34, 685 - 695.

TIWARI, V.K., RAWAT, N., CHHUNEJA, P., NEELAM, K., AGGARWAL, R., RANDHAWA, G. S., DHALIWAL, H. S., KELLER, B. and SINGH, K. (2009) Mapping of quantitative trait loci for grain iron and zinc concentration in diploid A genome wheat. *Journal of Heredity*, 100, 771 - 776.

TOURAINE, B. (2004) Nitrate uptake by roots – Transport and root development. *In: Nitrogen Acquisition and Assimilation in Higher Plants.* pp. 1 – 34, Amâncio S. and Stulen I. (eds.), Kluwer Academic Publishers, Dordrecht, The Netherlands.

TREHAN, S.P. and SHARMA, R.C. (1996) Mineral nutrient composition in peels and flesh of tubers of potato genotypes. *Journal of the Indian Potato Association*, 23, 139 - 143.

TSAY, Y.F., CHIU, C.C., TSAI, C.B., HO, C.H. and HSU, P.K. (2007) Nitrate transporters and peptide transporters. *FEBS Letters*, 581, 2290 - 2300.

TSUCHIYA, T., MATSUDA, T. and CHONAN, T. (1993) Distribution and connection of vascular bundles in relation to the accumulation of starch grains in the potato tuber. *Japanese Journal of Crop Science*, 62, 172 - 182.

TURAKAINEN, M., HARTIKAINEN, H. and SEPPANEN, M.M. (2004) Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. *Journal of Agricultural and Food Chemistry*, 52, 5378 - 5382.

TYNER, D.N., HOCART, M.J., LENNARD, J.H. and GRAHAM, D.C. (1997) Periderm and lenticle characterization in relation to potato cultivar, soil moisture and maturity. *Potato Research*, 40, 181 - 190.

UAUY, C., DISTELFELD, A., FAHIMA, T., BLECHL, A. and DUBCOVSKY, J. (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science*, 314, 1298 - 1301.

URBANY, C., STICH, B., SCHMIDT, L., SIMON, L., BERDING, H., JUNGHANS, H., NIEHOFF, K.H., BRAUN, A., TACKE, E., HOFFERBERT, H.R., LÜBECK, J., STRAHWALD, J. and GEBHARDT, C. (2011) Association genetics in *Solanum tuberosum* provides new insights into potato tuber bruising and enzymatic tissue discoloration. *BMC Genomics*, 12, 7.

van den BERG, J.H., EWING, E.E., PLAISTED, R.L., MCMURRY, S. and BONIERBALE, M.W. (1996a) QTL analysis of potato tuberization. *Theoretical and Applied Genetics*, 93, 307 - 316.

van den BERG, J.H., EWING, E.E., PLAISTED, R.L., MCMURRY, S. and BONIERBALE, M.W. (1996b) QTL analysis of potato tuber dormancy. *Theoretical and Applied Genetics*, 93, 317 - 324.

VAN ECK, H. J., JACOBS, J.M.E., VAN DIJK, J., STIEKEMA, W.J. and JACOBSEN. E. (1993) Identification and mapping of three flower colour loci of potato (*S. tuberosum* L.) by RFLP analysis. *Theoretical and Applied Genetics*, 86, 295 - 300.

van LOON, C.D. and MULLER, K. (1984) Einflüsse des Bodens und der Düngung auf den Ernährungswert der Kartoffel. *Proceedings of the ninth Triennial Conference EAPR*, Interlaken, Switzerland, 71 - 79.

van der LEE, T., ROBOLD, A., TESTA, A., van't KLOOSTER, J.W. and GOVERS, F. (2001). Mapping of avirulence genes in *Phytophthora infestans* with amplified fragment length polymorphism markers by bulked segregant analysis. *Genetics*, 157, 949 - 956.

VENUPRASAD, R., DALID, C.O., DEL VALLE, M., ZHAO, D., ESPIRITU, M., STA CRUZ, M.T., AMANTE, M., KUMAR, A. and ATLIN, G.N. (2009) Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theoretical and Applied Genetics*, 120, 177 - 190.

VERT, G., GROTZ, N., DÉDALDÉCHAMP, F., GAYMARD, F., GUERINOT, M.L., BRIAT, J.F. and CURIE, C. (2002) IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. *Plant Cell*, 14, 1223 - 1233.

VILLALTA, I., REINA-SANCHEZ, A., BOLARÍN, M.C., CUARTERO, J., BELVER, A., VENEMA, K., CARBONELL, E.A. and ASINS, M.J. (2008) Genetic analysis of Na^+

and K^+ concentrations in leaf and stem as physiological components of salt tolerance in tomato. *Theoretical and Applied Genetics,* 116, 869 - 880.

VISKER, M.H.P.W., HEILERSIG, H.J.B., KODDE, L.P., VAN DE WEG, W.E., VOORRIPS, R.E., STRUIK, P.C. and COLON, L.T. (2005) Genetic linkage of QTLs for late blight resistance and foliage maturity type in six related potato progenies. *Euphytica*, 143, 189 - 199.

VISKER, M.H.P.W., KEIZER, L.C.P., VAN ECK, H.J., JACOBSEN, E., COLON, L.T. and STRUIK, P.C. (2003) Can the QTL for late blight resistance on potato chromosome 5 be attributed to foliage maturity type? *Theoretical and Applied Genetics*, 106, 317 - 325.

VISSER, R.G.F., BACHEM, C.W.B., DE BOER, J.M., BRYAN, G.J., CHAKRABATI, S.K., FEINGOLD, S., GROMADKA, R., VAN HAM, R.C.H.J., HUANG, S., JACOBS, J.M.E., KUZNETSOV, B., DE MELO, P.E., MILBOURNE, D., ORJEDA, G., SAGREDO, B. and TANG, X. (2009) Sequencing the Potato genome: outline and first results to come from the Elucidation of the sequence of the world's third most important food crop. *American Journal of Potato Research*, 86, 417 - 429.

von WIRÉN, N. and MERRICK, M. (2004) Regulation and function of ammonium carriers in plants, yeast and bacteria. *In: Topics in current genetics: molecular mechanisms controlling transmembrane transport.* Pp. 95 - 120. Boles, E., Krämer R., eds. Springer Verlag, Berlin.

von WIRÉN, N., KLAIR, S., BANSAL, S., BRIAT, J.F., KHODR, H., SHIOIRI, T., LEIGH, R.A. and HIDER, R.C. (1999) Nicotianamine chelates both Fe^{III} and Fe^{II}. Implications for metal transport in plants. *Plant Physiology,* 119, 1107 - 1114.

von WIRÉN, N., MARSCHNER, H. and RÖMHELD, V. (1996) Roots of iron-efficient maize also absorb phytosiderophore-chelated zinc. *Plant Physiology,* 111, 1119 - 1125.

VOORRIPS, R.E. (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, 93, 77 - 78.

VOS, P., HOGERS, R., BLEEKER, M., REIJANS, M., VAN DE LEE, T., HORNES, M., FRIJTERS, A., POT, J., PELEMAN, J., KUIPER, M. and ZABEAU, M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, 23, 4407 - 4414.

VREUGDENHIL, D., AARTS, M.G.M., KOORNNEEF, M., NELISSEN, H. and ERNST, W.H.O. (2004) Natural variation and QTL analysis for cationic mineral content in seeds of *Arabidopsis thaliana*. *Plant Cell and Environment*, 27, 828 - 839.

WAGER, H.G. (1963) The role of phytin in the texture of cooked potatoes. *Journal of the Science of Food and Agriculture*, 14, 583 - 586.

WANG, G.L. and PATERSON, A.H. (1994) Assessment of DNA pooling strategies for mapping of QTLs. *Theoretical and Applied Genetics*, 88, 355 - 361.

WATERS, B.M. and GRUSAK, M.A. (2008a) Whole-plant mineral partitioning throughout the life cycle in *Arabidopsis thaliana* ecotypes Columbia, *Landsberg erecta*, Cape Verde Islands, and the mutant line *ysl1ysl3*. *New Phytologist*, 177, 389 - 405.

WATERS, B.M. and GRUSAK, M.A. (2008b) Quantitative trait locus mapping for seed mineral concentrations in two *Arabidopsis thaliana* recombinant inbred lines. *New Phytologist*, 179, 1033 - 1047.

WATERWORTH, W.M. and BRAY, C.M.(2006) Enigma variations for peptides and their transporters in higher plants. *Annals of Botany*, 98, 1 - 8.

WEAVER, M.L., TIMM, H., NONAKA, M., SAYRE, R.N., REEVE, R.M., MCCREADY, R.M. and WHITEHAND, L.C. (1978b) Potato composition: II. Tissue selection and its effects on total sugar, total reducing sugar, glucose, fructose and sucrose contents. *American Potato Journal*, 55, 83 - 93.

WEAVER, M.L., TIMM, H., NONAKA, M., SAYRE, R.N., REEVE, R.M., MCCREADY, R.M. and WHITEHAND, L.C. (1978a) Potato composition: I. Tissue selection and its effects on solids content and amylose/amylopectin ratios. *American Potato Journal*, 55, 73 - 82.

WEAVER, M.L., TIMM, H., NONAKA, M., SAYRE, R.N., NG, K.C. and WHITEHAND, L.C. (1978c) Potato composition: III. Tissue selection and its effects on total nitrogen, free amino acid nitrogen and enzyme activity (polyphenolase, monophenolase, peroxidase and catalase). *American Potato Journal*, 55, 319 - 331.

WENZL, P., RAMAN, H., WANG, J., ZHOU, M., HUTTNER, E. and KILIAN, A. (2007) A DArT platform for quantitative bulked segregant analysis. *BMC Genomics*, 8, 196 - 205.

WELCH, R.M. (1995) Micronutrient nutrition of plants. *Critical Reviews in Plant Sciences*, 14, 49 - 82.

WELCH, R.D. and GRAHAM, R.D. (1999) A new paradigm for world agriculture: meeting human needs: Productive, sustainable, nutritious. *Field Crops Research*, 60, 1 - 10.

WERIJ, J.S., KLOOSTERMAN, B., CELIS-GAMBOA, C., VOS, C.H.R., AMERICA, T., VISSER, R.G.F. and BACHEM, C.W.B. (2007) Unravelling enzymatic discoloration in potato through a combined approach of candidate genes, QTL, and expression analysis. *Theoretical and Applied Genetics,* 115, 245 - 252.

WESTERMANN, D.T. (2005) Nutritional requirement of potatoes. *American Journal of Potato Research,* 82, 301 - 308.

WESTERMANN, D.T., JAMES, D.W., TINDALL, T.A. and HURST, R.L. (1994) Nitrogen and potassium fertilization of potatoes: Sugars and starch. *American Potato Journal,* 71, 432 - 452.

WHEELER, G.L. and BROWNLEE, C. (2008) Ca^{2+} signaling in plants and green algae - changing channels. *Trends in Plant Science,* 13, 506 - 514.

WHITE, P.J. and BROWN, P.H. (2010) Plant nutrition for sustainable development and global health. *Annals of Botany,* 105, 1073 - 1080.

WHITE, P.J. (2000) Calcium channels in higher plants. *Biochimica et Biophysica Acta (Biomembranes),* 1465, 171 - 189.

WHITE, P.J. (2001) The pathways of calcium movement to the xylem. *Journal of Experimental Botany,* 52, 891 - 899.

WHITE, P.J. (2005) Calcium. *In: Plant Nutritional Genomics,* pp. 66–86. Broadley, M.R., White, P.J., eds. Blackwell: Oxford. ISBN 1405121149.

WHITE, P.J. (1998) Calcium channels in plasma membrane of root cells. *Annals of Botany,* 81, 173 - 183.

WHITE, P.J. and BROADLEY, M.R. (2000) Mechanisms of caesium uptake by plants. *New Phytologist,* 147, 241 - 256.

WHITE, P.J. and BROADLEY, M.R. (2001) Chloride in soils and its uptake and movement within the plant: A review. *Annals of Botany,* 88, 967 - 988.

WHITE, P.J. and BROADLEY, M.R. (2003) Calcium in plants. *Annals of Botany,* 92, 487 - 511.

WHITE, P.J. and BROADLEY, M.R. (2005) Biofortifying crops with essential mineral elements. *Trends in Plant Science,* 10, 586 - 593.

WHITE, P.J. and BROADLEY, M.R. (2009) Biofortification of crops with seven mineral elements often lacking in human diets: iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist*, 182, 49 - 84.

WHITE, P.J. and KARLEY, A.J. (2010) Potassium. In: *Plant Cell Monographs 17, Cell Biology of Metals and Nutrients.* Eds: R. Hell and R-R. Mendel, pp. 199 - 224, Springer, Berlin. ISBN 978-3-642-10612-5.

WHITE, P.J., BOWEN, H.C., DEMIDCHIK, V., NICHOLS, C. and DAVIES, J.M. (2002) Genes for calcium-permeable channels in the plasma membrane of plant root cells. *Biochimica et Biophysica Acta (Biomembranes),* 1564, 299 - 309.

WHITE, P.J., BRADSHAW, J.E., DALE, M.F.B., RAMSAY, G., HAMMOND, J.P. and BROADLEY, M.R. (2009) Relationships between yield and mineral concentrations in potato tubes. *HortScience,* 44, 6 - 11.

WHITE, P.J., BROADLEY, M.R., HAMMOND, J.P., RAMSAY, G., SUBRAMANIAN, N.K., THOMPSON, J. and WRIGHT, G. (2012) Biofortification of potato tubers using foliar zinc-fertilisers. *Journal of Horticultural Science and Biotechnology,* 87, 123 - 129.

WHITE, P.J., BROADLEY, M.R., SUBRAMANIAN, N.K., RAMSAY, G., BRADSHAW, J.E., BRYAN, G., HACKETT, C.A., HAMMOND, J.P., THOMPSON, J. and WRIGHT, G. (2011) Biofortification of potato tubers with zinc. Abstract for the Second Research Coordination Meeting (RCM) on "Improving nutritional quality by altering concentrations of enhancing factors using induced mutation and biotechnology in crops", 11 - 15 April 2011, Pretoria, South Africa.

WHITE, P.J., HAMMOND, J.P., KING, G.J., BOWEN, H.C., HAYDEN, R.M., MEACHAM, M.C., SPRACKLEN, W.P. and BROADLEY, M.R. (2010) Genetic analysis of potassium use efficiency in *Brassica oleracea. Annals of Botany,* 105, 1199 - 1210.

WHITTAKER, A., MAROTTI, I., DINELLI, G., CALAMAI, L., ROMAGNOLI, S., MANZELLI, M., PALCHETTI, E., VECCHIO, V. and BENEDETTELLI, S. (2010) The influence of tuber mineral element composition as a function of geographical location on acrylamide formation in different Italian potato genotypes. *Journal of the Science of Food and Agriculture,* 90, 1968 - 1976.

WHITTENBERGER, R.T. and NUTTING, G.C. (1950) Observations on sloughing of potatoes. *Journal of Food Science,* 15, 331 - 339.

WHO/FAO (2004) Vitamin and mineral requirements in human nutrition, Second edition, World Health Organization, Geneva.

WICKRAMASINGHE, W.M.D., QU, X.S.S., COSTANZO, S., HAYNES, K.G. and CHRIST, B.J. (2009) Development of PCR-based markers linked to quantitative resistance to late blight in a diploid hybrid potato population of *Solanum phureja* x *S. stenotomum*. *American Journal of Potato Research*, 86, 188 - 195.

WILKINSON, S.R., WELCH, R.M., MAYLAND, H.F. and GRUNES, D.L. (1990) Magnesium in plants: Uptake, distribution, function, and utilization by man and animals. *Metal Ions in Biological Systems,* 26, 33 - 56.

WILLIAMS, L.E. and MILLS, R.F. (2005) P1B-ATPases – an ancient family of transition metal pumps with diverse functions in plants. *Trends in Plant Science,* 10, 491 - 502.

WINTZ, H., FOX, T., WU, Y.Y., FENG, V., CHEN, W., CHANG, H.S., ZHU, T. and VULPE, C. (2003) Expression profiles of *Arabidopsis thaliana* in mineral deficiencies reveal novel transporters involved in metal homeostasis. *Journal of Biological Chemistry* 278, 47644 - 47653.

WISHART, J., GEORGE, T.S., BROWN, L.K., THOMPSON, J.A., RAMSAY, G., BRADSHAW, J.E., WHITE, P.J. and GREGORY, P.J. (2009) Variation in rooting habit of potatoes: potential for improving resource capture. Proceedings of 7th ISRR symposium on 'Root research and applications (RootRAP)', Vienna, Austria, 2-4 September 2009, pp. 76.

WISHART, J., GEORGE, T.S., BROWN, L.K., RAMSAY, G., BRADSHAW, J.E., WHITE, P.J. and GREGORY, P.J. (in preparation). Variation in rooting habit of potatoes: potential for improving resource capture.

WOOLFE, J. A. (1987) Potato in the human diet, Cambridge University Press, UK.

WSZELAKI, A.L., DELWICHE, J.F., WALKER, S.D., LIGGETT, R.E., SCHEERENS, J.C. and KLEINHENZ, M.D. (2005) Sensory quality and mineral and glycoalkaloid concentrations in organically and conventionally grown redskin potatoes (*Solanum tuberosum*). *Journal of the Science of Food and Agriculture*, 85, 720 - 726.

WU, J., YUAN, Y.X., ZHANG, X.W., ZHAO, J., SONG, X., LI, Y., LI, X., SUN, R., KOORNNEEF, M., AARTS, M.G.M. and WANG, X.W. (2008) Mapping QTLs for mineral accumulation and shoot dry biomass under different Zn nutritional conditions in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *Plant and Soil*, 310, 25 - 40.

WU, Z.Y., LIANG, F., HONG, B.M., YOUNG, J.C., SUSSMAN, M.R., HARPER, J.F. and SZE, H. (2002) An endoplasmic reticulum-bound Ca^{2+}/Mn^{2+} pump, ECA1,

supports plant growth and confers tolerance to Mn^{2+} stress. *Plant Physiology,* 130, 128 - 137.

WURSTER, R.T. and SMITH, O. (1963) Potato quality XVIII: The distribution of radioiron in the potato tuber and its significance in after-cooking darkening. *American Potato Journal,* 40, 415 - 420.

WURSTER, R.T. and SMITH, O. (1965) Potato quality XX: After cooking darkening in potato as related to the distribution of radioiron. *American Potato Journal,* 42, 37 - 44.

XU, X., VREUGDENHIL, D. and VAN LAMMEREN, A.A.M. (1998) Cell division and cell enlargement during potato tuber formation. *Journal of Experimental Botany,* 49, 573 - 582.

YOKOSHO, K., YAMAJI, N., UENO, D., MITANI, N. and MA, J.F. (2009) OsFRDL1 is a citrate transporter required for efficient translocation of iron in rice. *Plant Physiology,* 149, 297 - 305.

YONEYAMA, T. and TAKEBA, G. (1984) Compartment analysis of nitrogen flows through mature leaves. *Plant Cell Physiology,* 25, 39 - 48.

YOSHIMOTO, N., INOUE, E., SAITO, K., YAMAYA, T. and TAKAHASHI, H. (2003) Phloem-localizing sulfate transporter, Sultr1;3, mediates re-distribution of sulfur from source to sink organs in Arabidopsis. *Plant Physiology,* 131, 1511 - 1517.

YOSHIMOTO, N., TAKAHASHI, H., SMITH, F.W., YAMAYA, T. and SAITO, K. (2002) Two distinct high-affinity sulfate transporters with different inducibilities mediate uptake of sulfate in Arabidopsis root. *The Plant Journal,* 29, 465 - 473.

YUAN, Y.X., ZHANG, J., WANG, D.W. and LING, H.Q. (2005) AtbHLH29 of *Arabidopsis thaliana* is a functional ortholog of tomato FER involved in controlling iron acquisition in strategy I plants. *Cell Research,* 15, 613 - 621.

YUASA, K. and MAESHIMA, M. (2000) Purification, properties, and molecular cloning of a novel Ca^{2+}-binding protein in radish vacuoles. *Plant Physiology,* 124, 1069 - 1978.

YUSUPH, M., TESTER, R.F., ANSELL, R., and SNAPE, C.E. (2003) Composition and properties of starches extracted from tubers of different potato varieties grown under the same environmental conditions. *Food Chemistry,* 82, 283 - 289.

ZEE, S.Y. (1972) Vascular tissue and transfer cell distribution in the rice spikelet. *Australian Journal of Biological Science*, 25, 411 - 414.

ZEE, S.Y. and O'BRIEN, T.P. (1970) A special type of tracheary element associated with 'xylem discontinuity' in the floral axis of wheat. *Australian Journal of Biological Science*, 23, 783 - 791.

ZGÓRSKA, K. and FRYDECKA-MAZURCZYK, A. (2003) Dry matter and sugars content of different parts within potato tubers at harvest and storage time. *Acta Horticulturae*, 600, 711 - 715.

ZHANG, B., CHEN, P., SHI, A., HOU, A., ISHIBASHI, T. and WANG, D. (2009) Putative quantitative trait loci associated with calcium content in soybean seed. *Journal of Heredity*, 100, 263 - 269.

ZHANG, J.L., FLOWERS, T.J. and WANG, S.M. (2010) Mechanisms of sodium uptake by roots of higher plants. *Plant and Soil*, 326, 45 - 60.

ZHANG, X., ZHANG, G., GUO, L., WANG, H., ZENG, D., DONG, G., QIAN, Q. and XUE, D. (2011) Identification of quantitative trait loci for Cd and Zn concentrations of brown rice grown in Cd-polluted soils. *Euphytica*, 180, 173 - 179.

ZHAO, J., CHENG, N.H., MOTES, C.M., BLANCAFLOR, E.B., MOORE, M., GONZALES, N., PADMANABAN, S., SZE, H., WARD, J.M. and HIRSCHI, K.D. (2008) AtCHX13 is a plasma membrane K^+ transporter. *Plant Physiology*, 148, 796 - 807.

ZHAO, F.J. and SHEWRY, P. (2011) Recent developments in modifying crops and agronomic practice to improve human health. *Food Policy*, 36, S94 - S101.

ZHU, C., NAQVI, S., GOMEZ-GALERA, S., PELACHO, A.M., CAPELL, T. and CHRISTOU, P. (2007) Transgenic strategies for the nutritional enhancement of plants. *Trends in Plant Sciences*, 12, 548 - 555.