Genetic Resources and Crop Evolution Genetic Variation for Tuber Mineral Concentrations in Accessions of the **Commonwealth Potato Collection**

--Manuscript Draft--

Manuscript Number:	GRES-D-16-00114						
Full Title:	Genetic Variation for Tuber Mineral Concer Commonwealth Potato Collection	ntrations in Accessions of the					
Article Type:	Regular research paper						
Keywords:	Potato (Solanum tuberosum); Commonwea minerals; ICP-MS; biofortification; altitude						
Corresponding Author:	Nithya K Subramanian, PhD Texas A&M University College Station College Station, Texas UNITED STATES						
Corresponding Author Secondary Information:							
Corresponding Author's Institution:	Texas A&M University College Station						
Corresponding Author's Secondary Institution:							
First Author:	Nithya K Subramanian, PhD						
First Author Secondary Information:							
Order of Authors:	Nithya K Subramanian, PhD						
	Philip J White, PhD						
	Martin R Broadley, PhD						
	Gavin Ramsay, PhD						
Order of Authors Secondary Information:							
Funding Information:	Rural and Environment Science and Analytical Services Division (RESAS)of the Scottish Government	Professor Philip J White					
Abstract:	The variation in tuber mineral concentration Solanum species in the Commonwealth Po greenhouse conditions. Selected CPC acce distribution of wild potatoes, were grown to controlled conditions. Tubers from five plan bulked and their mineral composition analys there was a greater range in tuber concentr significance to both plants and animals, suc respectively) than others, such as (K, P and correlations were found between mean altit Cu and Mg concentrations. The amount of indicates the existence of wide differences different potato accessions. This might be u improvement of potato tubers.	essions, representing the eco-geographical maturity in peat-based compost under ts of each accession were harvested, sed. Among the germplasm investigated, rations of some elements of nutritional ch as (Ca, Fe and Zn; 6.7, 3.6, and 4.5-fold d S; all <3-fold). Significant positive rude of the species' range and tuber P, K, diversity observed in the CPC collection in tuber mineral accumulation among					

±

Genetic Variation for Tuber Mineral Concentrations in Accessions of the Commonwealth Potato Collection

Nithya K. Subramanian, Philip J. White, Martin R. Broadley and Gavin Ramsay

N. K. Subramanian, G. Ramsay

Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK Email: <u>nithya@tamu.edu</u>; Telephone: 979-845-6498.

P. J. White

Ecological Sciences, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK and Distinguished Scientist Fellowship Program, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia

N. K. Subramanian, M. R. Broadley

Division of Plant and Crop Sciences, University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD, UK

Present Address:

N. K. Subramanian

Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas-77843, USA.

Abstract

The variation in tuber mineral concentrations amongst accessions of wild tuber-bearing *Solanum* species in the Commonwealth Potato Collection (CPC) was evaluated under greenhouse conditions. Selected CPC accessions, representing the eco-geographical distribution of wild potatoes, were grown to maturity in peat-based compost under controlled conditions. Tubers from five plants of each accession were harvested, bulked and their mineral composition analysed. Among the germplasm investigated, there was a greater range in tuber concentrations of some elements of nutritional significance to both plants and animals, such as (Ca, Fe and Zn; 6.7, 3.6, and 4.5-fold respectively) than others, such as (K, P and S; all <3-fold). Significant positive correlations were found between mean altitude of the species' range and tuber P, K, Cu and Mg concentrations. The amount of diversity observed in the CPC collection indicates the existence of wide differences in tuber mineral accumulation among different potato accessions. This might be useful in breeding for nutritional improvement of potato tubers.

Key words: Potato (*Solanum tuberosum*); Commonwealth Potato Collection (CPC); tuber; minerals; ICP-MS; biofortification; altitude

Introduction

At least 25 mineral elements are considered essential for the normal functioning of human body and mineral malnutrition is considered to be one of the most serious challenges facing the everincreasing global population (WHO/FAO 2004; White and Brown 2010). 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 (White and Broadley 2009). The bioavailability of minerals in potato tubers is potentially high, because of the presence of high concentrations of β -carotene, protein cysteine, ascorbate and other organic and amino acids that stimulate micronutrient absorption (White et al. 2009). In addition, potato tubers contain low concentrations of anti-nutritional factors such as phytates (Frossard et al. 2000; Phillippy et al. 2004) and oxalates (Bushway et al. 1984). For these reasons, potato tubers make an excellent candidate for biofortification with mineral elements that are lacking in the human diet in populations with this as their staple food. 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).

Potato germplasm collections are maintained worldwide (Bradshaw 2000), including at the International Potato Centre (CIP, Lima, Peru), the Dutch-German Potato Collection (CGN, Wageningen, the Netherlands), the Groß Lusewitz Potato Collection (GLKS, IPK, Groß Lusewitz, Germany), the Potato Collection of the Vavilov Institute (VIR, St Petersburg, Russia), the US Potato Genebank (NRSP-6, Sturgeon Bay, USA), in addition to the Commonwealth Potato Collection (CPC) which is now held at the James Hutton Institute (JHI), Dundee, Scotland. Wild potato species represent a diverse gene pool which might be utilized in breeding programmes as sources of valuable genes (reviewed by Bradshaw and Ramsay 2006).

There are good prospects for significant improvements using this diverse potato germplasm for many traits (Bradshaw et al. 2006), including, for example, tuber calcium concentrations (Bamberg et al. 1993, 1998; Paget et al. 2014), iron and zinc concentrations (Paget et al. 2014). Therefore, it is useful to explore the genetic resources of the wild relatives of potatoes as a source for the genetic enhancement of tuber minerals, because they may harbour an allelic richness for useful traits including tuber mineral concentration. Identifying genes controlling these traits in diverse wild and cultivated populations will allow researchers to extend the range of variation found in modern cultivars.

The CPC is one of the major genebanks containing around 1500 potato accessions, of which about two-thirds are wild potato species and the rest are cultivated types from South America (Bradshaw and Ramsay 2005). The objective of this study was to evaluate the variability of tuber mineral concentrations among CPC accessions representing the eco-geographical distribution of wild potatoes under greenhouse conditions.

Materials and Methods

Plant material and mineral analyses

Forty nine selected accessions of potato species (Table 1) from the CPC, originating from different habitats and altitudes, were grown in a greenhouse (15 to 20 plants per accession) in 2007, and tuber samples were obtained from this material for mineral analyses. The individual seedlings obtained from true potato seeds (TPS) of selected CPC accessions were sown in 15 cm diameter pots containing standard peat-based potting compost in April 2007 and the plants were grown to maturity in the greenhouse under natural daylight. The compositions of the potting compost and the base fertilizer Sincrostart (William Sinclair, Lincoln, UK) are given in Supplementary Tables 1 and 2.

Tubers from five randomly chosen CPC plants of each accession were harvested in January 2008 and stored at 4°C for two days. As the tubers from wild species were smaller than selected cultivated genotypes, the whole tubers (with periderm) were used for mineral analyses. Five medium-sized healthy tubers, one each from the five representative plants, were selected and bulked for mineral analyses. The tubers were washed thoroughly under running tap water, rinsed in deionised water, and briefly air-dried. The air-dried whole tubers were then chopped into pieces measuring less than 1cm across and were frozen at -20°C prior to freeze-drying. 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 weighed to determine the dry matter content and then ground in a coffee blender (De'Longhi, Treviso, Italy) and were stored in re-sealable, air-tight polyethylene bags at -20°C until analysed for their mineral concentrations were determined on acid-digested material using inductively coupled plasma mass spectrometry (ICP-MS; ELAN DRCe; PerkinElmer, Waltham, MA, USA), following the method reported by Subramanian et al. (2011).

Data Analyses

Differences among taxonomic groups were not tested for statistical significance because some of the series and species used in this study contained only a few accessions. Principal component analysis (PCA, based on correlation matrix) and Spearman's correlation analysis were performed in GenStat version13.2 (VSN International Ltd, Hemel Hempstead, UK) to determine the correlations between tuber dry matter content and mineral concentrations. In the PCA plot, the accessions were coded according to molecular groupings of *S. acaule, S. demissum*, series Tuberosa (northern species), series Tuberosa (southern species) and others, and taxonomic series according to Hawkes (1990).

Results

The CPC accessions grown in the greenhouse showed a wide variation for tuber dry matter (DM) and mineral concentrations (Table 2). A detailed summary of the mineral composition of 44 wild accessions and five cultivated taxa is presented in Supplementary Table 3. Among the germplasm investigated, there was a greater range in the concentration of some mineral nutrients

of nutritional significance (Ca, Fe and Zn; 6.7, 3.6, and 4.5-fold respectively) than others (e.g. K, P and S all < 3-fold, Table 2).

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 nine 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 (Fig. 1). The contributions of each variable (tuber DM and mineral concentrations) to the first two PC scores are shown in the biplot in Fig. 2A.

The PC1 was positively associated with mineral elements (Mn, Cu, Fe, Zn, S, Mg, P, K and Ca), and negatively with DM. 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 (Table 3). This trend was clearly evident when the accessions that fall within 15% of low and high extreme values for tuber Ca, K and DM were sorted (Supplementary Table 4).

In addition, PCA analysis was also performed among the nine mineral elements excluding DM (Fig. 2B and 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 (Fig. 2B). The loadings plot indicated that all minerals were positively associated with PC1, whereas PC2 was positively associated with 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 are similar with the exception of Ca and Cu which showed an exchange of positions with each other. Correlations coefficient matrix (Table 3) show a negative relationship between Ca and DM, which may explain the shift in the loadings plot position of Ca. Weak separation of groups of accessions was observed in the PCA plots constructed using all mineral traits with (Fig. 1) and without (Fig. 3) DM as a variable. There was a strong 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 (Fig. 3).

No clear groupings of accessions were observed in the PCA plot for tuber mineral concentrations based on altitude (Fig. 4). However, significant positive correlations between altitude and tuber P, K, Cu and Mg concentrations were evident in the Spearman's correlation analysis (Table 4). The highest correlation between tuber mineral concentration and altitude was for phosphorus.

Discussion

The CPC accessions used in this study represented different taxonomic series and diverse geographical origins within South and Central America (Table 1), making them valuable resources for investigating the genetic variation in mineral accumulation in wild potatoes adapted to different environments. The greenhouse study demonstrated considerable variability among the wild CPC accessions for tuber DM and mineral concentrations (Table 2). The range of values (Table 2) shows a relatively narrow spread for some minerals, such as Mg, K, P and S, and a greater spread for others, such as Ca, Fe and Zn. This suggests that breeding for high tuber Ca, Fe and Zn concentrations might have potential. Furthermore, the heritability of tuber mineral

concentrations in potato were found to be moderately high (Brown et al. 2010, 2011, 2012, 2013 and 2014; Haynes et al. 2012; Paget et al. 2014; Zorrilla et al. 2014), indicating the possibility of mineral biofortification by plant breeding.

Principal component analysis biplots of the accessions for tuber DM and mineral traits (Fig. 1) showed much overlap of accessions based on molecular groups and taxonomic series. However, when PCA analysis was performed using only mineral elements (Fig. 3), although there was some overlap among accessions based on molecular grouping, some groups of accessions did form separate clusters. The Mexican hexaploids in series Demissa (DMS) and series Acaulia (ACL) accessions formed separate groups. Also, the two main molecular groups within series Tuberosa *sensu* Hawkes (1990), the Peruvian species (*Solanum* series Tuberosa Northen group, TBR-N) and the Bolivian and Argentinian species (*Solanum* series Tuberosa Southern group, TBR-S), also occupied different areas in the biplot of PC1 and PC2.

Genetic variation was observed in the CPC germplasm collection for tuber DM, Ca and K, and these data corroborate those of McCann et al. (2010), Bamberg et al. (1993, 2008) and Paget et al. (2014). In the present study, tuber DM content varied from 17-48% (Table 2), which is in agreement with the range reported by McCann et al. (2010) in wild Solanum species (18-35% DM) and by Paget et al. (2014) in a breeding population of Andean landrace cultivars (13-36% DM). Research by Bamberg et al. (1993) suggests that there is a great genetic variation within Solanum germplasm for the ability to accumulate Ca in tubers. Bamberg et al. (1993) screened wild Solanum species at adequate (solution Ca concentrations-80 mg/kg) and high (800 mg/kg) Ca levels in 21 Solanum species (three accessions per species) in a greenhouse study. They found that the tuber Ca concentrations ranged from 0.16–0.74 mg/g DW with an adequate supply of Ca, which is similar to the range reported in this study (0.10-0.67 mg/g DW, Table 2) with a comparable Ca supply, and Paget et al. (2014) (0.04-0.78 mg/g DW). Among the Solanum species investigated in the current study, S. bulbocastanum (CPC 7638) and S. chacoense (CPC 3504) exhibited the highest and the lowest tuber Ca concentrations, respectively (Supplementary Table 3). Considering the species that were common between the present study and those evaluated by Bamberg et al. (1993), the ranking of genotypes were similar such that S. chacoense and S. kurtzianum had low tuber Ca concentrations and S. gourlayi and S. tubersoum Group Stenotomum (as S. stenotomum) showed high tuber Ca concentrations (Supplementary Table 4; Bamberg et al. 1993). Recently, Chung et al. (2016) reported SSR markers associated with high tuber Ca content in an F₂ population derived from a high- Ca accumulating wild relative, S. *microdontum* and a low-Ca accumulating, *S. kurtzianum*.

With regard to K accumulation, *S. capsicibaccatum* (CPC 3554) exhibited the highest tuber K concentrations, whereas *S. chacoense* (CPC 3504) showed the lowest K concentration (Supplementary Table 3), a 1.8-fold range for tuber K concentrations (Table 1). In a study assaying tuber K concentrations in different *Solanum* species, Bamberg et al. (2008) found large tuber K concentrations in *S. acaule, S. chacoense, S. okadae* and *S. pinnatisectum*.

In general, accessions with small tuber DM content had large concentrations of Ca and K and *vice versa* (Supplementary Table 4). A negative correlation between tuber dry matter content and Ca, Fe, and Zn concentrations, expressed on a dry weight basis, was reported by Paget et al.

(2014). About 65–75% of DM content in tubers is made up of starch (Burton 1989). Tuber mineral concentrations (including Ca and K) have been found to decrease following DM (starch) accumulation during tuber bulking (Kolbe and Stephan-Beckmann 1997). Within the tuber, Ca is concentrated around the periphery and decreases towards the centre (Subramanian et al. 2011). As Ca is relatively immobile in the phloem (Westermann 2005; Kärenlampi and White 2009), its concentration tends to decrease with increasing DM accumulation. On the other hand, K is mobile in the phloem and its concentration was found to be higher at the bud end than the stem end of the tuber (Subramanian et al. 2011). Within cells K is required for many physiological functions, including osmoregulation, enzyme activation and membrane transport processes (White and Karley 2010). Nitsos and Evans (1969) first observed that starch synthesizing enzymes have a specific requirement for K and about 1.8% of K (as a proportion of DM) is critical 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 could be explained in terms of an osmotic optimum for starch synthesis (Oparka and Wright 1988).

The association of S and Zn, as evident from PCA plots, might be attributed to the similar phloem mobility of these elements (Kärenlampi and White, 2009). On the other hand, neither Fe nor Ca have high phloem mobility (Kärenlampi and White, 2009), and the association of Fe and Ca might be due to the high entrapment of these minerals by periderm of the potato tuber, since the surface layers of potato tubers contain 55% and 34% of total tuber Fe and Ca concentrations, respectively (Subramanian et al. 2011).

Potatoes are adapted to grow in different habitats including high altitudes. There are no studies reported on mineral concentrations in potato tubers grown at different altitudes. Results from this study showed that tuber P, K, Mg and Cu concentrations increased as the altitude of the habitat of a species increased, and that phosphorus is the mineral found to be most strongly linked with altitude. Kitayama and Aiba (2002) suggest that trees on Mount Kinabalu, Borneo, maintain net assimilation rate with increasing altitude by increasing foliar N and P, where soil P deficiency does not inhibit this process. Work in the Andes suggests that P availability may not be strongly linked to altitude (Fisher et al. 2013), which would support a mechanism for P accumulation which is not driven by adaptation to low P availability. However, whatever the adaptive forces driving this trend, the higher concentrations of tuber minerals in high altitude species provide useful variation for breeding for these traits. These minerals include those of greatest importance to human nutrition (Ca, Fe and Zn) as well as P which has importance for productivity and sustainable production.

Acknowledgements

We thank Dr Colin Alexander (BioSS) for his advice on multivariate data analysis, and Gaynor Mackenzie (JHI) for assistance with maintaining the CPC accessions in the greenhouse. We also thank Jacqueline Thompson (JHI) for assistance with ICP-MS analysis. N.K.S. gratefully acknowledges the financial support from the International Office, University of Nottingham for a Research Scholarship and JHI for an SCRI–Universities PhD Scholarship. This work was supported by the Rural and Environment Science and Analytical Services Division (RESAS) of the Scottish Government.

Compliance with Ethical Standards

Conflict of interest

The authors declare no conflict of interest.

References

Bamberg JB, Palta JP, Peterson LA, Martin M, Krueger AR (1993) Screening tuber bearing *Solanum* (potato) species germplasm for efficient accumulation of tuber calcium. Am J Potato Res 70: 219–226

Bamberg JB, Palta JP, Peterson LA, Martin M, Krueger AR (1998) Fine screening potato (*Solanum*) species germplasm for tuber calcium. Am J Potato Res 75:181–186

Bamberg JB, Martin MW, Palta J (2008) Variation in Solanum species' tuber potassium accumulation and its implications for human nutrition. Am J Potato Res 85:2

Bradshaw JE (2000) Conventional breeding in potatoes: Global Achievements. In: Khurana SMP, Shekhawat GS, Singh BP, Pandey SK (Eds.) Potato, Global Research & Development, Indian Potato Association, Shimla, India, Vol. 1, pp 41–51

Bradshaw JE, Ramsay G (2005) Utilisation of the Commonwealth Potato Collection in potato breeding. Euphytica 146:9–19

Bradshaw JE, Bryan GJ, Ramsay G (2006) Genetic resources (including wild and cultivated *Solanum* species) and progress in their utilisation in potato breeding. Potato Res 49:49–65

Brown CR, Haynes KG, Moore M, Pavek MJ, Hane DC, Love SL, Novy RG, Miller Jr JC (2010) Stability and broad-sense heritability of mineral content in potato: iron. Am J Potato Res 87: 390–396

Brown CR, Haynes KG, Moore M, Pavek MJ, Hane DC, Love SL, Novy RG, Miller Jr JC (2011) Stability and broad-sense heritability of mineral content in potato: zinc. Am J Potato Res 88: 238–244

Brown CR, Haynes KG, Moore M, Pavek MJ, Hane DC, Love SL, Novy RG, Miller Jr JC (2012) Stability and broad-sense heritability of mineral content in potato: calcium and magnesium. Am J Potato Res 89: 255–261

Brown CR, Haynes KG, Moore M, Pavek MJ, Hane DC, Love SL, Novy RG (2013) Stability and broad-sense heritability of mineral content in potato: Potassium and phosphorus. Am J Potato Res 90: 516–523

Brown CR, Haynes KG, Moore M, Pavek MJ, Hane DC, Love SL, Novy RG, Miller Jr JC (2014) Stability and broad-sense heritability of mineral content in potato: copper and sulfur. Am J Potato Res 91: 618–624

Burton WG (1989) The distribution and composition of the dry matter in the potato tuber. In: Harris PM (ed), The Potato, 3rd edn. Longman Scientific and Technical, Harlow, Great Britain pp 286–335

Bushway RJ, Bureau JL, Mcgann DF (1984) Determinations of organic acids in potatoes by high performance liquid chromatography. J Food Sci 49:75–81

Chung YS, Palta P, Bamberg J, Jansky S (2016) Potential molecular markers associated with tuber calcium content in wild potato germplasm. Crop Sci 56:576–584

Fisher JB, Malhi Y, Torres IC, Metcalfe DB, van de Weg MJ, Meir P, Silva-Espejo JE, Huasco WH (2013) Nutrient limitation in rainforests and cloud forests along a 3,000-m elevation gradient in the Peruvian Andes. Oecologia 172:889–902

Forster H, Beringer H (1983) Starch content of potato tubers in relation to potassium nutrition and tuber development. J Plant Nutr Soil Sc 146:572–582

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. J Sci Food Agr 80:861–879

Hawkes JG (1990) The potato: evolution, biodiversity and genetic resources. Smithsonian Institution Press, Washington, D.C.

Haynes KG, Yencho GC, Clough ME, Henninger MR, Sterrett SB (2012) Genetic variation for potato tuber micronutrient content and implications for biofortification of potatoes to reduce micronutrient malnutrition. Am J of Potato Res 89:192–198

Heřmanová V, Bárta J, Čurn V (2007) Wild potato species: characterization and biological potential for potato breeding. Czech J Genet Plant 43:73–81

Jansen G, Flamme W, Schüler K, Vandrey M (2001) Tuber and starch quality of wild and cultivated potato species and cultivars. Potato Res 44:137–146

Kärenlampi SO, White PJ (2009) Potato proteins, lipids and minerals. In: Singh J and Kaur L (eds) Advances in Potato Chemistry and Technology. Academic Press, London, pp. 99–126

Kitayama K, Aiba SI (2002) Ecosystem structure and productivity of tropical rain forests along altitudinal gradients with contrasting soil phosphorus pools on Mount Kinabalu, Borneo. J Ecol 90:37–51

Kolbe H, Stefan-Beckmann S (1997) Development, growth and chemical composition of the potato crop (*Solanum tuberosum* L.) II. Tuber and whole plant. Potato Res 40:135–153

Lindhauer MG, De Fekete MAR (1990) Starch synthesis in potato (*Solanum tuberosum*) tubers: Activity of selected enzymes in dependence of potassium content in storage tissue. Plant Soil 124:291–295

Marschner H, Krauss A (1980) Correlations between potassium content and quality of potatoes. Kartoffelbau 31:65–67

McCann LC, Bethke PC, Simon PW (2010) Extensive variation in fried chip color and tuber composition in cold-stored tubers of wild potato (*Solanum*) germplasm. J Agric Food Chem 58:2368–2376

Nitsos RE, Evans HJ (1969) Effects of univalent cations on the activity of particulate starch synthetase. Plant Physiol 44:1260–1266

Oparka KJ, Wright KM (1988) Osmotic regulation of starch synthesis in potato tubers. Planta 174:123-126

Paget M, Amoros W, Salas E, Eyzaguirre R, Alspach P, Apiolaza L, Noble A, Bonierbale M (2014) Genetic evaluation of micronutrient traits in diploid potato from a base population of Andean landrace cultivars. Crop Sci 54:1–11

Phillippy BQ, Lin M, Rasco B (2004) Analysis of phytate in raw and cooked potatoes. J Food Compos Anal 17:217–226

Smillie RM, Hetherington SE, Ochoa C, Malagamba P (1983) Tolerances of wild potato species from different altitudes to cold and heat. Planta 159:112–118

Subramanian NK, White PJ, Broadley MR, Ramsay G (2011) The three dimensional distribution of minerals in potato tubers. Ann Bot 107:681–691

Wegener C, Jansen G (2010) Antioxidant capacity in cultivated and wild *Solanum* species: The effect of wound stress. Food Funct 1:209–218

White PJ, Karley AJ (2010) Potassium. In: Hell R, Mendel RR (eds) Plant Cell Monographs 17, Cell Biology of Metals and Nutrients. Springer, Berlin, pp. 199–224

White PJ, Brown PH (2010) Plant nutrition for sustainable development and global health. Ann Bot 105:1073–1080

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets: iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182:49–84

White PJ, Bradshaw JE, Dale MFB, Ramsay G, Hammond JP, Broadley MR (2009) Relationships between yield and mineral concentrations in potato tubers. Hortscience 44:6–11

WHO/FAO (2004) Vitamin and mineral requirements in human nutrition. Second edition, World Health Organization, Geneva

Woolfe JA (1987) Potato in the human diet. Cambridge University Press, UK

Zorrilla C, Navarro F, Vega S, Bamberg J, Palta J (2014) Identification and selection for tuber calcium, internal quality and pitted scab in segregating 'Atlantic' x 'Superior' reciprocal tetraploid populations. Am J Potato Res 91:673–687

2 used in the study

Series	Species	CPC number	Country of origin	Altitude (m
Acaulia	S. acaule	2109	Bolivia	4100
Acaulia	S. acaule	2113	Bolivia	4100
Acaulia	S. acaule	2456	Argentina	4100
Bulbocastana	S. bulbocastanum	7638	Mexico	1900
Bulbocastana	S. bulbocastanum ssp. partitum	7650	Unknown	1900
Circaeifolia	S. capsicibaccatum	3554	Bolivia	3000
Conicibaccata	S. violaceimarmoratum	7782	Bolivia	3300
Cuneolata	S. infundibuliforme	2477	Argentina	4100
Demissa	S. brachycarpum	2922	Mexico	2500
Demissa	S. brachycarpum	7031	Mexico	2500
Demissa	S. brachycarpum	7027	Mexico	2500
Demissa	S. demissum	1126	Mexico	3250
Demissa	S. demissum	1345	Mexico	3250
Tuberosa	S. demissum S. demissum	4630	Unknown	3250
Demissa	S. demissum S. demissum	7524	Mexico	3250
Demissa	S. hougasii	7049	Mexico	2300
Demissa	S. hougasii	7048	Mexico	2300
Longipedicellata	S. fendleri	7048	Mexico	2200
Longipedicellata	S. fendleri	2605	USA	2200
Longipedicellata		2603	USA	2200
	S. fendleri S. hiartingii	2001 5697	Mexico	2200
Longipedicellata	S. hjertingii			2150
Longipedicellata	S. polytrichon	3987 2620	Mexico	
Longipedicellata	S. stoloniferum	2639	Mexico	2400
Megistacroloba	S. megistacrolobum	3273	Bolivia	4000
Megistacroloba	S. megistacrolobum	2482	Argentina	3600
Pinnatisecta	S. cardiophyllum	5908	Mexico	2250
Pinnatisecta	S. trifidum	7124	Mexico	2250
Tuberosa	S. canasense	3059	Peru	3500
Tuberosa	S. gourlayi	7161	Argentina	3500
Tuberosa	S. kurtzianum	6065	Unknown	1950
Tuberosa	S. kurtzianum	3783	Argentina	1800
Tuberosa	S. kurtzianum	5890	Unknown	1950
Tuberosa	S. microdontum	3764	Argentina	3080
Tuberosa	S. microdontum	3757	Argentina	2550
Tuberosa	S. microdontum	3740	Argentina	1730
Tuberosa	S. multidissectum	7180	Peru	4050
Tuberosa	S. multidissectum	7171	Peru	4050
Tuberosa	S. marinasense	6020	Peru	3300
Tuberosa	S. marinasense	7739	Peru	3250
Tuberosa	S. neocardenasii	7612	Unknown	1400
Tuberosa	S. neorossii	7628	Unknown	3000
Tuberosa	S. okadae	7775	Unknown	3100
Tuberosa*	S. tuberosum Group Phureja	3672	Peru	-
Tuberosa*	S. tuberosum Group Andigena	61	Bolivia	-
Tuberosa*	S. tuberosum Group Andigena	573	Peru	-
Tuberosa*	S. tuberosum Group Andigena	7617	Unknown	-
Tuberosa*	<i>S. tuberosum</i> Gp Stenotomum	7699	Peru	_
Yungasensa	S. chacoense	3732	Argentina	850
Yungasensa	S. chacoense	3504	Unknown	1200

- Table 2 Mean values and ranges for tuber dry matter (DM) and mineral concentrations observed 3
- in CPC accessions 4

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

5 6 ¹Mineral concentrations presented on a DW basis; ²Maximum/minimum trait value; ³Excluding one outlier (S.

bulbocastanum CPC 7650) with a value 3.8-fold higher than the preceding highest value.

- 8 Table 3 Spearman's correlation coefficients among the DM content and nine mineral elements
- 9 for 49 accessions of CPC potatoes

	DM	Ca	K	Mg	Р	S	Cu	Fe	Mn	Zn
DM	-									
Ca	-0.44***	-								
K	-0.37**	0.38**	-							
Mg	-0.13ns	0.51***	0.62***	-						
Р	-0.10ns	0.34**	0.60***	0.60***	-					
S	-0.22*	0.28*	0.50***	0.32**	0.41***	-				
Cu	0.15ns	0.16ns	0.49***	0.62***	0.61***	0.34**	-			
Fe	-0.02ns	0.54***	0.38**	0.63***	0.48***	0.51***	0.61***	-		
Mn	0.05ns	0.16ns	0.15ns	0.28*	0.12ns	0.33**	0.15ns	0.46***	-	
Zn	-0.09ns	0.44***	0.40***	0.53***	0.53***	0.53***	0.50***	0.72***	0.44***	-

10 Significance of the effects is given in three levels: * $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$; ns-non-significant.

11

12 Table 4 Spearman's correlation coefficients between the altitude (m) and nine mineral elements

13 for wild species CPC accessions

	Ca	К	Mg	Р	S	Cu	Fe	Mn	Zn
Altitude	0.35**	0.54***	0.51***	0.65***	0.29**	0.52***	0.37**	-0.10 ^{ns}	0.37**

14 Significance of the effects is given in three levels: * $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$; ns-non-significant.

15

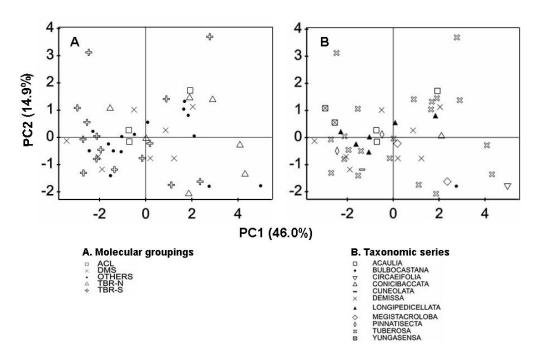


Fig. 1 PCA plots for 49 CPC accessions based on tuber DM together with mineral concentrations. The accessions are coded based on (A) molecular groupings, and (B) taxonomic series, as per Hawkes (1990). Abbreviations: TBR-S, *Solanum* series Tuberosa Southern Group (Argentina and Bolivia); TBR-N, *Solanum* series Tuberosa Northern Group (Peru); DMS, Mexican hexaploids in series Demissa; ACL, series Acaulia. Mineral concentrations based on dry weight basis.

8

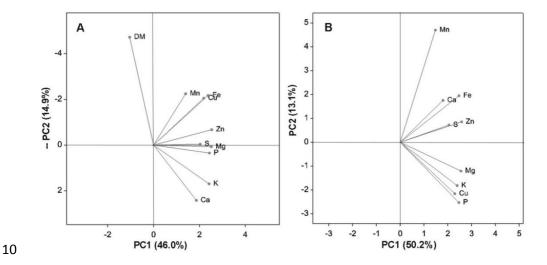
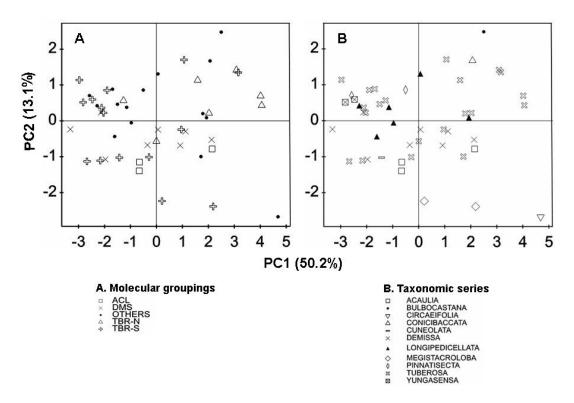


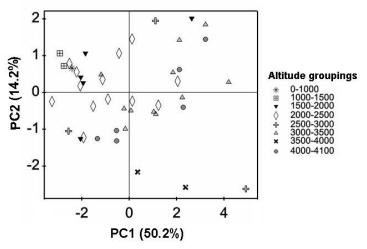
Fig. 2 Biplots from PCA analysis for 49 CPC accessions based on (A) tuber DM and mineral
traits, and (B) mineral traits alone.



14

Fig. 3 PCA plots for 49 CPC accessions based on tuber mineral concentrations alone. The
 accessions are coded based on (A) molecular groupings and (B) taxonomic series, as per Hawkes

17 (1990). Abbreviations as in Figure 1.



19 Fig. 4 PCA plots for wild species CPC accessions based on tuber mineral concentrations. The

- accessions are grouped on the altitude of the site of collection or the mean altitude of the rangefor the species.

≛

1 Genetic Variation for Tuber Mineral Concentrations in Accessions of the Commonwealth

- 2 **Potato Collection**
- 3 Genetic Resources and Crop Evolution
- 4 Nithya K. Subramanian, Gavin Ramsay, Philip J. White and Martin R. Broadley
- 5 N. K. Subramanian, G. Ramsay
- 6 Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK
- 7 Email: <u>nithya@tamu.edu</u>.
- 8 P. J. White
- 9 Ecological Sciences, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK and
- 10 Distinguished Scientist Fellowship Program, King Saud University, Riyadh 11451, Kingdom of
- 11 Saudi Arabia
- 12
- 13 N. K. Subramanian, M. R. Broadley
- 14 Division of Plant and Crop Sciences, University of Nottingham, Sutton Bonington Campus,
- 15 LE12 5RD, UK
- 16
- 17 *Present Address*:
- 18 N. K. Subramanian
- 19 Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas-77843,
- 20 USA.
- 21

23	Supplementary Table	1 Composition of the	potting compost used	in the glasshouse experiment
----	----------------------------	-----------------------------	----------------------	------------------------------

Material	Quantity	
Sphagnum moss peat	12001	
Sand	100 1	
Perlite	100 1	
Mg limestone	2.5 kg	
Ca limestone	2.5 kg	
Sincrostart fertilizer ¹	1.5 kg	
Polycon ²	1.5kg	
Celcote water retaining gel	1 kg	
Intercept insecticide	390 g	

¹Base fertiliser, see Supplementary Table 2; ²Controlled release fertilizer (three to four months) blend 17-10-16 plus sulphur trioxide 6% plus trace elements

Supplementary Table 2 Nutritional composition of the Sincrostart base fertilizer

Mineral element	Composition (%)	
Total nitrogen	12.0	
Ammonium nitrogen	5.2	
Nitrate nitrogen	6.8	
Phosphorus pentoxide	14.0 (6.1% P)	
Potassium	19.9	
Potassium oxide	24.0 (19.9% K)	
Magnesium oxide	3.0 (1.8% Mg)	
Boron	0.03	
Copper	0.12	
Iron chelated by EDTA	0.23	
Manganese	0.16	
Molybdenum	0.19	
Zinc	0.04	

EDTA-Ethylenediaminetetraacetic acid

No	Series/Species	Code*	СРС	DM	Minerals								
	-		accession		Ca	K	Mg	Р	S	Cu	Fe	Mn	Zn
			no.	%	mg/g	mg/g	mg/g	mg/g	mg/g	μg/g	µg/g	μg/g	µg/g
	Acaulia												
1	S. acaule	ACL	2109	31.78	0.19	18.09	1.33	3.99	1.03	5.8	25.34	4.41	13.32
2	S. acaule	ACL	2113	31.19	0.21	17.74	1.46	3.99	1.23	5.6	21.34	3.95	13.40
3	S. acaule	ACL	2456	34.49	0.19	19.41	1.87	4.37	1.51	8.7	29.67	7.37	18.58
	Bulbocastana												
4	S. bulbocastanum	BLB	7638	20.34	0.67	18.03	1.88	3.94	1.46	3.8	28.47	9.96	21.08
5	S. bulbocastanum	BLB	7650	28.71	0.11	17.20	1.18	2.80	1.18	3.6	(166.04)	7.70	7.93
	Circaeifolia												
6	S. capsicibaccatum	CAP	3554	17.32	0.34	26.86	2.15	5.18	2.03	10.0	29.83	4.35	23.91
	Conicibaccata												
7	S. violaceimarmoratum	VIO	7782	26.78	0.31	25.45	1.79	2.89	2.00	4.3	40.23	9.30	11.87
	Cuneolata												
8	S. infundibuliforme	IFD	2477	27.81	0.19	21.05	1.51	3.57	1.03	3.3	16.05	5.31	9.48
-	Demissa												
9	S. brachycarpum	BCP	2922	27.58	0.18	17.77	1.29	2.78	1.70	3.8	13.06	6.73	8.05
10	S. brachycarpum	BCP	7031	25.25	0.29	21.77	1.64	3.68	2.29	6.0	27.09	6.25	10.63
11	S. brachycarpum	BCP	7027	24.25	0.16	21.80	1.38	3.71	2.04	5.0	20.72	7.15	12.01
12	S. demissum	DMS	1126	30.07	0.17	20.65	1.48	4.63	2.14	8.9	25.70	8.41	17.43
13	S. demissum	DMS	1345	30.26	0.18	20.81	1.55	4.11	1.79	6.8	17.60	7.39	18.22
14	S. demissum	DMS	4630	30.63	0.18	19.49	1.56	4.57	2.09	7.7	27.89	9.74	12.96
15	S. demissum	DMS	7524	34.02	0.22	16.98	1.28	3.92	1.46	8.0	23.69	6.22	9.33
16	S. hougasii	HOU	7049	34.87	0.14	15.57	0.86	3.10	1.80	2.7	12.15	4.43	7.64
17	S. hougasii	HOU	7048	28.13	0.12	20.97	1.06	3.96	1.78	2.8	14.78	5.07	5.95
	Longipedicellata												
18	S. fendleri	FEN	7214	26.67	0.17	18.47	1.12	4.28	1.50	3.3	14.66	8.89	10.80
19	S. fendleri	FEN	2605	29.94	0.18	16.97	1.34	3.27	1.60	4.8	16.57	5.56	9.42
20	S. fendleri	FEN	2601	29.50	0.13	15.57	1.24	2.71	1.32	4.6	17.89	6.78	9.50
21	S. hjertingii	HJT	5697	31.96	0.19	21.14	1.26	3.49	1.17	4.1	20.50	7.07	11.38
22	S. polytrichon	PLT	3987	27.16	0.21	18.12	1.45	3.07	1.68	5.6	27.62	9.15	12.48
23	S. stoloniferum	STO	2639	30.71	0.20	21.81	1.69	3.48	2.43	7.4	26.10	7.78	18.48
	Megistacroloba	510	2007	50.71	0.20	21.01	1.07	5.10	2.10	<i></i>	20.10	,.,0	10.10

Supplementary Table 3 Tuber dry matter (DM) and mineral concentration for 49 accessions of CPC potatoes (DW basis)

24	S. megistacrolobum	MGA	3273	30.09	0.15	21.27	1.95	4.13	1.15	7.2	15.68	5.50	10.83
25	S. megistacrolobum	MGA	2482	22.99	0.30	26.55	1.92	4.80	1.33	8.2	17.37	6.09	14.95
	Pinnatisecta												
26	S. cardiophyllum	CPH	5908	24.90	0.14	15.34	1.22	2.53	1.25	3.7	16.65	6.87	10.63
27	S. trifidum	TRF	7124	28.40	0.18	17.29	0.93	3.18	2.40	4.3	23.05	6.05	20.43
	Tuberosa												
	Peru (TBR-N)												
28	S. canasense	CAN	3059	28.26	0.12	22.36	1.26	3.94	1.90	5.9	20.34	7.39	11.14
29	S. multidissectum	MLT	7180	33.40	0.45	20.74	1.55	4.36	1.89	9.1	29.84	11.56	15.55
30	S. multidissectum	MLT	7171	31.17	0.13	20.91	1.46	3.95	2.41	7.9	27.07	8.68	20.57
31	S. marinasense	MRN	6020	19.39	0.38	26.29	1.74	4.36	2.36	6.1	30.50	8.28	26.89
32	S. marinasense	MRN	7739	39.15	0.21	19.38	1.25	2.90	1.32	4.3	17.99	7.43	13.37
	Bolivia and Argentina (TBR-S)												
33	S. gourlayi	GRL	7161	24.79	0.47	21.28	1.56	3.92	1.85	5.6	20.85	5.97	12.76
34	S. kurtzianum	KTZ	6065	29.70	0.18	15.81	1.17	3.18	1.02	4.1	18.57	6.81	10.77
35	S. kurtzianum	KTZ	3783	48.43	0.11	15.55	1.28	2.76	1.05	4.9	19.96	8.16	12.14
36	S. kurtzianum	KTZ	5890	26.33	0.22	15.52	1.23	2.91	1.04	4.7	20.69	5.79	10.59
37	S. microdontum	MCD	3740	29.26	0.15	20.61	1.45	2.84	1.05	4.7	13.93	5.01	6.66
38	S. microdontum	MCD	3757	31.38	0.12	17.26	1.14	3.04	1.00	5.4	15.45	4.26	8.54
39	S. microdontum	MCD	3764	24.35	0.22	20.13	1.43	3.77	1.32	6.6	23.69	5.48	9.73
40	S. neocardenasii	NCD	7612	20.75	0.17	16.45	0.96	2.63	1.18	3.1	12.71	8.10	9.50
41	S. neorossii	NRS	7628	33.57	0.23	15.94	1.66	3.54	2.11	4.2	29.13	9.08	19.82
42	S. okadae	OKA	7775	38.08	0.17	20.28	1.77	4.06	1.29	9.2	43.61	11.73	18.92
	<u>Cultivated</u>												
43	S. tuberosum Group Phureja	PHU	3672	19.99	0.50	25.11	1.48	3.55	1.56	5.4	21.29	9.54	15.46
44	S. tuberosum Group Andigena	TBRAD	61	26.09	0.19	15.25	1.16	3.17	1.69	3.6	20.20	5.59	16.08
		G											
45	S. tuberosum Group Andigena	TBRAD	573	29.53	0.15	21.57	1.33	3.51	2.22	10.9	24.02	6.09	21.81
		G											
46	S. tuberosum Group Andigena	TBRAD	7617	24.77	0.31	18.29	1.07	3.04	1.39	2.8	17.24	6.68	12.89
	1 0	G											
47	S. tuberosum Gp Stenotomum	STN	7699	25.26	0.32	26.58	1.58	4.50	2.89	6.7	26.76	10.03	23.76
	Yungasensa												
48	S. chacoense	CHC	3732	32.10	0.11	16.75	1.28	2.56	1.02	4.2	15.68	7.77	9.86
49	S. chacoense	CHC	3504	34.05	0.10	15.05	1.11	2.45	1.06	5.2	16.52	7.15	9.60

*Accession code; (166.04)-extreme value.

Trait	Code	CPC number	Species	Ca (mg/g)	K (mg/g)	DM (%)
Low Ca	CHC	3504	S. chacoense	0.10	15.05	34.05
	BLB	7650	S. bulbocastanum	0.11	17.20	28.71
	CHC	3732	S. chacoense	0.11	16.75	32.10
	KTZ	3783	S. kurtzianum	0.11	15.55	48.43
	MCD	3757	S. microdontum	0.12	17.26	31.38
	HOU	7048	S. hougasii	0.12	20.97	28.13
	CAN	3059	S. canasense	0.12	22.36	28.26
High Ca	STN	7699	S. tuberosum Gp Stenotomum	0.32	26.58	25.26
	CAP	3554	S. capsicibaccatum	0.34	26.86	17.32
	MRN	6020	S. marinasense	0.38	26.29	19.39
	MLT	7180	S. multidissectum	0.45	20.74	33.40
	GRL	7161	S. gourlayi	0.47	21.28	24.79
	PHU	3672	S. tuberosum Gp Phureja	0.50	25.11	19.99
	BLB7	7638	S. bulbocastanum	0.67	18.03	20.34
Trait	Code	CPC number	Species	K (mg/g)	DM_(%)	Ca (mg/g ¹)
Low K	CHC	3504	S. chacoense	15.05	34.05	0.10
	TBRADG	61	S. tuberosum Gp Andigena	15.25	26.09	0.19
	CPH	5908	S. cardiophyllum	15.34	24.90	0.14
	KTZ	5890	S. kurtzianum	15.52	26.33	0.22
	KTZ	3783	S. kurtzianum	15.55	48.43	0.11
	FEN	2601	S. fendleri	15.57	29.50	0.13
	HOU	7049	S. hougasii	15.57	34.87	0.14
High K	CAN	3059	S. canasense	22.36	28.26	0.12
	PHU	3672	S. tuberosum Gp Phureja	25.11	19.99	0.50
	VIO	7782	S. violaceimarmoratum	25.45	26.78	0.31
	MRN	6020	S. marinasense	26.29	19.39	0.38
	MGA	2482	S. megistacrolobum	26.55	22.99	0.30
	STN	7699	S. tuberosum Gp Stenotomum	26.58	25.26	0.32
	CAP	3554	S. capsicibaccatum	26.86	17.32	0.34
Trait	Code	CPC number	Species	DM (%)	Ca (mg/g)	K (mg/g)
Low DM	CAP	3554	S. capsicibaccatum	17.32	0.34	26.86
	MRN	6020	S. marinasense	19.39	0.38	26.29
	PHU	3672	S. tuberosum Gp Phureja	19.99	0.50	25.11
	BLB	7638	S. bulbocastanum	20.34	0.67	18.03

Supplementary Table 4 Accessions that fall under 15% of low and high extreme values for tuber calcium (Ca) and potassium (K) concentrations and dry matter (DM) content

	NCD	7612	S. neocardenasii	20.75	0.17	16.45	
	MGA	2482	S. megistacrolobum	22.99	0.30	26.55	
	BCP	7027	S. brachycarpum	24.25	0.16	21.80	
High DM	DMS	7524	S. demissum	34.02	0.22	16.98	
	CHC	3504	S. chacoense	34.05	0.10	15.05	
	ACL	2456	S. acaule	34.49	0.19	19.41	
	HOU	7049	S. hougasii	34.87	0.14	15.57	
	OKA	7775	S. okadae	38.08	0.17	20.28	
	MRN	7739	S. marinasense	39.15	0.21	19.38	
	KTZ	3783	S. kurtzianum	48.43	0.11	15.55	