Development of high yielding potato (*Solanum tuberosum* L.) genotypes with resistance to bacterial wilt (*Ralstonia solanacearum)* for the Kenyan highlands

by

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Thesis abstract

Bacterial wilt caused by *Ralstonia solanacearum* race 3 (R3bv2A), is an important disease contributing to low potato yields in temperate areas and tropical highlands. In Kenya, the disease is widespread in most potato growing areas causing yield losses between 50 and 100%. Host plant resistance could be the best option for controlling the disease because other measures are costly, ineffective or impractical to deploy. The overall objective of this study was to contribute to improved food security in Kenya by developing potato cultivars that are resistant to bacterial wilt. The specific objectives of the study were to: (1) document farmers' practices, key potato production and marketing constraints, and to determine farmers' potato cultivar preferences, the prevalence of bacterial wilt in the major potato growing areas and farmers' management practices of bacterial wilt, (2) determine the response of the potato genotypes currently grown by farmers in Kenya as well as other clones from the international Potato Center (CIP) to bacterial wilt, (3) determine the genetic relationships among potato clones,(4) determine the combining ability effects for yield and yield related traits and bacterial wilt resistance of selected potato varieties and clones and their crosses, and (5) to estimate the magnitude of genotype x environment interaction (GEI) for potato tuber yield and bacterial wilt resistance.

At the beginning, a participatory rural appraisal (PRA) was conducted in three major potato growing counties involving 253 potato growers in Kenya. Farmers varied in cultivar and trait preferences; in Bomet district the red-skinned Dutch Robyjn is widely grown. In Molo district, the white-skinned Cangi is prominent while in Meru Central, the red-skinned Asante is predominantly grown by farmers. The cultivar preferences are mostly dictated by availability of markets, yield potential and taste. Over 75% of respondents indicated that the major production constraints are diseases with bacterial wilt being the most prominent. Farmers use different methods in managing the disease in the field such as spraying with fungicides, roguing and burning the wilting plants, and burying of the rotten tubers after harvest.

Field experiments were conducted to evaluate 36 potato genotypes for their response to bacterial wilt for three consecutive seasons between November 2011 and February 2013. The potato genotypes varied in their susceptibility to bacterial

wilt and the most resistant genotypes were Kenya Karibu followed by Kenya Sifa. Twenty selected potato genotypes were evaluated for genetic variability using 24 SSR primer pairs selected based on high polymorphism. The SSR markers identified 160 alleles.The 20 potato clones were grouped into 3 clusters. Cluster I was composed of Meru Mugaruro, cluster II had CIP materials while local materials were in cluster III. Therefore, the SSR markers generated useful information that will assist in identifying parents to include in the breeding programme.

Fourteen potato genotypes were identified as promising parents for further breeding based on their resistance to bacterial wilt. These parents were crossed in a North Carolina II mating design to generate 48 families for determining combining ability. Parents with highest general combining ability for bacterial wilt resistance were Ingabire, Meru Mugaruro, 391919.3, 394895.7 and 394903.5. These parents were selected for future crosses. In addition, nine crosses with the highest SCA effects for total tuber yield (TTW) at Kenya Agricultural Research Institute, National Research Laboratories (KARI-NARL) were 394905.8 x Kihoro (31.94), 394903.5 x Kenya Karibu (31.46), 394904.9 x Meru Mugaruro (25.73), 394895.7 x Bishop Gitonga (15.37), 394905.8 x Cangi (13.06), 394895.7 x Tigoni (12.23), 394904.9 x Sherekea (11.44), 394895.7 x Sherekea (10.92) and 391919.3 x Tigoni (10.32) in that order. At Kinale, the nine crosses with the highest SCA effects for TTW were 394905.8 x Kihoro (27.13), 394903.5 x Kenya Karibu (24.37), 394904.9 x Meru Mugaruro (19.59), 394895.7 x Cangi (15.69), 3948957 x Bishop Gitonga (15.35), 394895.7 x Tigoni (11.93), 394904.9 x Sherekea (9.36), 392278.19 x Meru Mugaruro (9.10) and 391919.3 x Cangi (7.64) in that order. These crosses were selected for high tuber yield and will be evaluated in future.

The GEI effects on 48 potato families were evaluated at two sites for two consecutive seasons (making a total of four environments). The potato families were ranked differently in terms of resistance against bacterial wilt across the four environments. The additive main effects and multiplicative interaction (AMMI) 1 and genotype and genotype x environment (GGE) biplot models were used to determine yield stability. In terms of yield stability, family 20 (394905.8 x Kihoro) was closest to the ideal genotype; it was the highest yielding (104.7 t ha^{-1}) and

most stable; it was closely followed by family 43 (394903.5 x Kenya Karibu) which yielded 98.3 t ha^{-1}. The environment ENVI 1(short rains of 2013 at Kinale) was the closest to ideal environment and therefore the most desirable of the four test environments.

In general, the study identified valuable potato genotypes with high combining ability for tuber yield and bacterial wilt resistance. It also generated novel families which will be further evaluated.

Declaration

I, Jane Muthoni Mbugua, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other University.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a. Their words have been re-written but the general information attributed to them has been referenced.
    b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed

……..

Jane Muthoni Mbugua

As the candidate's supervisors, we agree to the submission of this thesis:

……

Prof. Shimelis Hussein (Supervisor)

……

Prof. Rob Melis (Co-Supervisor)

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May God bless you all and reward you abundantly. Amen.

Dedication

This thesis is dedicated to the Almighty God, from whom all good things come; to my sons, Fidel Mburu and Frank Mbugua, and to my late parents, Nancy Wairimu and Alex Mbugua.

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Publications pertaining to this thesis

Chapter 1

Muthoni Jane, Hussein Shimelis, Rob Melis and Jackson Kabira. 2012. Reproductive biology and early generations' selection in conventional potato breeding. Australian Journal of Crop Science 6(3): 488— 497.

Muthoni Jane, Hussein Shimelis and Rob Melis. 2012. Management of Bacterial Wilt [*Rhalstonia solanacearum* Yabuuchi et al., 1995] of Potatoes: Opportunity for host resistance in Kenya. Journal of Agricultural Science 4(9): 64— 78.

Chapter 2

Muthoni Jane, Hussein Shimelis and Rob Melis. 2013. Potato Production in Kenya: Farming systems and production constraints. Journal of Agricultural Science 5(5): 182—197.

Chapter 3

Muthoni Jane, Hussein Shimelis, Rob Melis and Z. M. Kinyua. 2014. Response of potato genotypes to bacterial wilt caused by *Ralstonia solanacearum* (Smith) (Yabuuchi et al.) in the tropical highlands. American Journal of Potato Research 91: 215—232.

Chapter 4

Muthoni Jane, Hussein Shimelis and Rob Melis. 2014**.** Study of genetic relationship among Kenyan cultivated potato clones using SSR markers**.** Australian Journal of Crop Science 8(4): 502—508.

Chapter 5

Muthoni Jane, Hussein Shimelis and Rob Melis. 2014. Combining ability analysis of tuber yield and related traits and bacterial wilt resistance in potato. Accepted by Australian Journal of Crop Science.

Chapter 6

Muthoni Jane, Hussein Shimelis and Rob Melis. 2014. Genotype x environment interaction and stability of potato tuber yield and bacterial wilt resistance in Kenya Accepted by American Journal of Potato Research.

Thesis Introduction

Economic importance of potato

Potato (*Solanum tuberosum* L. 2n=4x=48) is a crop of major economic importance worldwide (Tsegaw, 2005; FAO, 2008). On a global scale, potato is the third most important food crop after rice and wheat (FAO, 2008,2013; CIP, 2014); more than a billion people worldwide eat potato (CIP, 2014). Potato is the most important root and tuber crop, with an annual production of approximately 365 million tonnes grown on about 19.7 million ha (FAO, 2010; CIP, 2014; FAO, 2014); it is followed by cassava, sweetpotato, and yam (FAO, 2004, 2008). Potato is grown in more than 150 countries worldwide from latitudes 65^0N to 50^0S (Acquaah, 2007; FAO, 2014) and can grow from sea level up to 4 700 metres above sea level; from Southern Chile to Greenland (CIP, 2014). The world average potato production is 17 t ha^{-1}, while direct consumption as human food is 31.3 kg per capita (kg yr^{-1})(FAO, 1995, 2004). On a regional basis, Asia and Europe are the major potato producing regions, accounting for more than 80% of world production, while Africa produces the least, accounting for about 5% (FAO, 2008). China is currently the biggest potato producer, and almost a third of all potatoes are harvested in China and India (FAO, 2014). Within sub-Saharan Africa (SSA), the East and Central Africa region accounts for over 45% of potato production and 52% of area harvested. Kenya is the fifth biggest producer of potato in SSA after Malawi, Rwanda, Ethiopia and South Africa (FAO, 2014).

In Kenya, potato is an important food crop, second after maize in volumes produced (MoA, 1998; FAO, 2013,2014). It is grown mainly as a cash and food crop by small scale farmers, many of them women, although some large-scale growers specialize in commercial production (FAO, 2014). Potato therefore plays an important role in food security (MoA, 2005,2008; FAO, 2014). Potato is grown by about 800 000 farmers, on 158 000 ha per season, with an annual production of about 1 million tonnes in two growing seasons (Riungu, 2011; FAO, 2013, 2014; NPCK, 2014). The annual potato crop is valued at KES 13 billion (USD 150 million) at farm gate level, and KES 40 billion (USD 362 million) at the consumer level (FAO, 2013; ANN, 2009). Potato farming in Kenya employs 3.3 million people at all levels of the value chain. However, there has been a decline in potato

production in Kenya (Gregory et al., 2013) because of a number of production constraints. These include low soil fertility, an inadequate supply of certified seeds, the use of low yielding varieties, and diseases (FAO, 2013). The most common diseases include late blight, viral infections and bacterial wilt (Kaguongo et al., 2008; FAO, 2013).

Bacterial wilt, caused by *Ralstonia solanacearum* (Yabuuchi et al., 1995), is an important disease contributing to low potato yields globally (Kaguongo et al., 2008). The disease has been estimated to affect about 1.7 million ha in approximately 80 countries worldwide, with global damage estimates of over USD 950 million per annum (Champoiseau et al., 2009). In addition to potatoes, the disease also affects over 200 plant species from more than 50 families (Hayward, 1991). The disease is the second most important constraint on potato production in tropical and sub-tropical regions of the world after late blight (Priou et al., 1999c). Bacterial wilt is widely distributed in tropical, subtropical, and warm temperate climates of the world, and it occurs in about 45 countries in the southern hemisphere (Hayward, 1991). In Africa, it is found in Angola, Burkina Faso, Burundi, Cameroon, Congo, Ethiopia, Gabon, Gambia, Kenya, Madagascar, Malawi, Mauritius, Mozambique, Nigeria, Réunion, Rwanda, Senegal, Seychelles, Sierra Leone, Somalia, South Africa, Swaziland, Tanzania, Tunisia, Uganda, Zaire, Zambia, and Zimbabwe (EPPO, 2004).

Bacterial wilt of potato was first reported in Kenya in 1940 in the Embu district (currently Kirinyaga County), from where it spread to other parts of the country (Natrass, 1945). The disease is believed to have been introduced with tuber seeds imported from Europe (Todd, 1969). According to some studies, the disease is found in all the potato growing areas of the country affecting 77% of potato farms; it is followed by late blight (67%), and viral diseases (12%) (Kaguongo et al., 2010). Lately, the disease has been reported in all potato growing areas of the country (Muthoni et al., 2013; The Organic Farmer, June 2013).

Ralstonia solanacearum (the causal organism of bacterial wilt) has been classified into five races and five biovars on the basis of host range and carbon source, respectively (Hayward, 1991). Race 3 which correlates to biovar 2A (R3bv2A), causes bacterial wilt on potatoes in the cool climates worldwide, while race 1

causes the disease in the warm tropical lowlands (Hayward, 1983; French, 1994; EPPO, 2004). Because the bulk of the potato crop is grown in cool environments, bacterial wilt of potato is caused by race 3 in over 90% of the cases worldwide (EPPO, 2004) including Kenya. In Kenya, race 3, occurs in most potato growing highlands located in the former Central, Eastern, and Rift Valley provinces (Smith et al., 1995).

Rationale for the research

Protective measures have proven ineffective for the control of bacterial wilt because the bacterium resides in the host plant xylem, has a large host range and is soil-borne (Grimault et al., 1993). In addition, crop protection chemicals are ineffective and expensive (Champoiseau et al., 2010), and biological control agents are ineffective (Smith et al., 1998). Phytosanitary methods such as quarantine are either expensive or difficult to apply (Martin and French, 1985; Muthoni et al., 2010). Cultural methods such as crop rotation are largely impractical because the farms are too small to allow effective rotation, the pathogen has a wide host range, and it persists for a long time in the soil (Kaguongo et al., 2008; Muthoni et al., 2010). Methods such as positive and negative selection are only feasible on small farms (Gildemacher et al., 2007). Even in these cases, some farmers may not be able to identify the disease symptoms in the field and the likelihood of spreading the disease through latent infection is real. Development of resistant cultivars could therefore be the best option for managing the disease. However, the most productive and popular potato cultivars in Kenya are very susceptible to bacterial wilt. More resistant potato clones have recently been identified by CIP scientists, and this resistance needs to be incorporated into the popular but susceptible Kenyan potato cultivars to increase potato production in Kenya.

Research objectives

The overall objective of the study was to develop high yielding potato genotypes with resistance to bacterial wilt (*Ralstonia solanacearum)* for production in the Kenyan highlands.

The specific objectives of this study were as follows:

1) To document farmers' practices, key potato production and marketing constraints, and to determine farmers' potato cultivar preferences, the prevalence of bacterial wilt in the major potato growing areas and farmers' management practices of bacterial wilt.
2) To determine the response of the potato genotypes currently grown by farmers in Kenya as well as other clones from CIP to bacterial wilt.
3) To determine the genetic relationships among potato clones.
4) To determine the combining ability effects for yields, yield related traits and bacterial wilt resistance ofselected potato genotypes.
5) To estimate the magnitude of genotype x environment interaction (GEI) for potato tuber yield and bacterial wilt resistance.

Research hypotheses

The current study was based on the following test hypotheses:

1. Potato farmers in Kenya face various constraints in producing and marketing their produce with bacterial wilt being a major production constraint.
2. Considerable genetic variation for tuber yields and bacterial wilt resistance exist among potato varieties currently grown by farmers in Kenya and among the advanced clones from CIP.
3. Most of potato varieties grown by farmers in Kenya are closely related genetically.
4. The popular potato varieties grown in Kenya have good combining ability for tuber yields and bacterial wilt resistance.
5. Potato tuber yields and resistance to bacterial wilt are affected by changes in environment.

Thesis outline

This thesis consists of six distinct chapters (Table 1) reflecting a number of activities related to the above-mentioned objectives. Chapters 2 to 6 are written in the form of discrete research chapters, each following the format of a stand-alone research paper (whether or not the chapter has already been published). The referencing system used in the chapters of this thesis is based on the Journal of Crop Science system. This is the most recommended thesis format adopted by the University of KwaZulu-Natal. As such, there is some unavoidable repetition of

references and some introductory information between chapters. Chapter 2 has been published in the Journal of Agricultural Science; Chapter 3 has been published in the American Journal of Potato Research while Chapter 4 has been published in the Australian Journal of Crop Science.

Table 1. Thesis structure

Chapter	Title
-	Thesis introduction
1	Literature Review
2	Potato production in Kenya: Farming systems, production constraints and breeding priorities
3	Response of potato genotypes to bacterial wilt in the tropical highlands of Kenya
4	Genetic relationships among bacterial wilt resistant and susceptible potato genotypes revealed by SSR markers
5	Combining ability analysis of tuber yield and related traits and bacterial wilt resistance in potato
6	Genotype x environment interaction and stability of potato tuber yield and bacterial wilt resistance in Kenya
7	An overview of research findings

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Chapter One: A review of the literature

1.1 Introduction

This literature review covers topics relevant to the research focus and provides the theoretical basis for the research. It therefore seeks to give an insight into potato genetics as well as gene actions controlling various traits. In addition, it gives a summary of potato cultivars grown in Kenya and the major production constraints especially bacterial wilt. Distribution, symptoms and management of bacterial wilt are discussed in depth. Previous efforts in breeding for resistance are expounded, the difficulties are reviewed and new possibilities explored.

1.2 Origin and distribution of potato

The cultivated potato (*Solanum tuberosum* L) originated in the Andean mountains of Peru and Bolivia, where it has been cultivated for over 2 400 years (Acquaah, 2007). More than 200 potato varieties were developed by the Aymara Indians on the Titicaca plateau, in Peru about 3 000 meters above sea level (Sleper and Poehlman, 2006). These potatoes formed the main diet of the Aymara Indians and the Incas (Raker and Spooner, 2002).

The potato was introduced to Europe between 1565 and 1580 by the Spaniards. From here it was introduced into Germany in the 1620's where it became part of the Prussian diet by the time of the seven year war (1756-1763). After the war, it was introduced into France and thereafter, to the rest of Europe (Hijmans, 2001; Acquaah, 2007). It was introduced into Virginia, in the American colonies in 1621 (Sleper and Poehlman, 2006; Acquaah, 2007).

1.3 Genetics of Solanum tuberosum

Solanum tuberosum is an autotetraploid (2*n*=4*x*=48, 4EBN) and there can be four different alleles at a locus (Bradshaw and Mackay, 1994; Carputo et al., 2003). The tetraploid nature of cultivated potato can be exploited by the breeder to improve desirable characteristics. It is well known that asexually propagated species such as potatoes have evolved taking advantage of dominance or epistatic gene action (Sleper and Poehlman, 2006). Because of the autotetraploid nature of potato, intralocus interactions (dominance) and interlocus interactions (epistasis) occur, and are important when selecting breeding procedures to improve certain traits; it is assumed that increased heterozygosity leads to increased heterosis (Bradshaw and Mackay, 1994; Sleper and Poehlman, 2006). Heterosis in potato occurs when the

progeny surpasses the value of the best parent or the parental mean. The exploitation of heterosis is by far the most important goal in potato breeding. The inheritance of heterosis is by minor genes or by the side effects of the major genes. Their action can proceed in an additive (general combining ability [GCA]) or in a non-additive manner (specific combining ability [SCA]); in most cases both operate (Ross, 1986). Heterosis in potato is based mainly on non-addititve interactions of genes comprising intralocus (dominance) as well as interlocus (epistasis) interaction between genes and alleles (Ross, 1986). The level of heterozygosity is influenced by how different the four alleles are within a locus; the more diverse they are, the higher the heterozygosity and the greater the number of interlocus (epistatic) interactions and hence the greater the heterosis (Ross, 1986; Bradshaw and Mackay, 1994; Sleper and Poehlman, 2006). To establish how increased heterozygosity can lead to more epistatic interactions, it is necessary to identify the allelic conditions possible in an autotetraploid (Caligari, 1992; Sleper and Poehlman, 2006). Five tetrasomic conditions are possible at an individual locus in an autotetraploid (Table 1.1).

Table 1.1.The number of first-, second - and third-order interactions possible and their sums for the five different tetrasomic conditions in an autotetraploid

	Number of heteroallelic interactions				
Tetrasomic Condition	First-order	Second - order	Third-order	Total	Portion of haploids(2x) conserving one first-order Interaction
$a_1a_2a_3a_4$	6	4	1	11	All
$a_1a_1a_2a_3$	3	1	0	4	5/6
$a_1a_1a_2a_2$	1	0	0	1	2/3
$a_1a_1a_1a_2$	1	0	0	1	1/2
$a_1a_1a_1a_1$	0	0	0	0	none

Source: Sleper and Poehlman, 2006

$a_1a_1a_1a_1$ is a monoallelic locus where all alleles are identical.

$a_1a_1a_1a_2$ is an unbalanced diallelic locus where two different alleles are present in unequal frequency.

$a_1a_1a_2a_2$ is a balanced diallelic locus where two different alleles occur with equal frequency.

$a_1a_1a_2a_3$ is a triallelic locus where three different alleles are present.

$a_1a_2a_3a_4$ is a tetraallelic locus where four different alleles are present.

It is hypothesized that the tetraallelic condition provides the maximum heterosis because more interlocus interactions are possible for this tetrasomic condition than for the other configurations (Ross, 1986; Sleper and Poehlman, 2006). For example, in the tetrallelic condition, the six first-order interactions are: a_1a_2, a_1a_3, a_1a_4, a_2a_3, a_2a_4, a_3a_4. The four second-order interactions are $a_1a_2a_3$, $a_1a_2a_4$, $a_1a_3a_4$, $a_2a_3a_4$. The one third-order interaction is $a_1a_2a_3a_4$. There are a total of 11 different interactions possible for the tetraallelic condition. This is in contrast to the monoallelic condition, which has no interactions. The highest level of heterosis will occur as the frequency of tetraallelic loci increase. The greatest number of interlocus interactions will also occur as the frequency of tetraallelic loci increase. In breeding potatoes for higher tuber yields, inter- and intralocus interactions have been shown to be important. As such, procedures that maximize the frequency of tetraallelic loci should be considered in breeding potato for increased yields (Ross, 1986; Sleper and Poehlman, 2006). Therefore, the segregation of heterotic seedlings in a population is likely to be greatest when three conditions are fulfilled: 1) the parents possess as low a coefficient of inbreeding as possible, 2) as many loci as possible have different alleles and, 3) the parents belong to different genepools which improves the chances of allelic diversity, that is, wide hybridisation (parents should be as unrelated as possible) (Ross, 1986). In potatoes, heterosis is of direct relevance for improving traits under consideration as it gets fixed in the F_1 generation owing to the vegetative propagation of the crop. Because potato is a highly heterozygous crop, an increase in heterozygosity results in heterosis. Distantly related genotypes are more complementary and they produce heterotic progenies (Ross, 1986).

1.4 Combining ability studies in potato

According to Griffing (1956), the concepts of general combining ability (GCA) and specific combining ability (SCA) were introduced early in the 20th century (Sprague and Tatum, 1942; López and Biosca, 2004). GCA is the average performance of a parentin hybrid combinations and SCA is the contribution of a parent to hybrid performance in a cross with a specified genotype, in relation to its contributions in crosses with an array of specified genotypes (Sleper and Poehlman, 2006) i.e. the

departure of a progeny mean from that expected on the basis of the GCAs of its parents is called the SCA.

In potatoes both GCA and SCA are important in conditioning traits, and both are fixed in the F_1 generation. This is because with clonal propagation, there is no further segregation. GCA represents mainly the additive and additive x additive type of genetic variance (Gopal, 1998). In potatoes, the SCA was reported to be more important than GCA in the inheritance of tuber yields (Plaisted et al., 1962; Tai, 1976; Killick, 1977; Gopal, 1998), while the opposite was reported to be the case by Maris (1989), and Brown and Caligari (1989). Galarreta et al. (2006) and Gopal (1998) found that SCA was more important than GCA in determining yields, tuber number per plant and average tuber weight in the seedling and the first two clonal generations. In addition, Gopal (1998) found that GCA for various characters varied from generation to generation; correlation coefficients between generations for GCA ranged from r=0.5 to r=0.8. GCA seems to be significantly larger than SCA for tuber yield and quality traits in crosses between non-related parents while SCA appears to be more important among related parents (Ortiz and Golmirzaie, 2004). This is because in related material the number of different alleles is likely to be limited. Consequently, variation in additive gene action is limited as well while non-additive gene action, like episasis, can result in relatively large between progeny variation. In such experiments the SCA effects are likely to be prominent (Neele et al., 1991).

Plaisted et al. (1962) speculated that informal previous selection which narrowed the genetic base of the tested genotypes may be one of the possible causes for obtaining greater estimates of SCA variance for various characters. Killick and Malcolmson (1973), using a concept developed in evolutionary population genetics, suggested that traits subjected to directional selection would be expected to show little additive genetic variance, but a large degree of dominance and epistasis, whereas the reverse would be true for traits subjected to stabilising selection. GCA was found to be more important than SCA for maturity (Johansen et al., 1967; Killick, 1977; Maris, 1989), while SCA effects were found to predominate in determining resistance to late blight (Killick and Malcolmsom, 1973). In conditioning the after-cooking blackening in potatoes, it was reported that GCA was more important than SCA (Dalianis et al., 1966; Killick, 1977). Killick (1977) found SCA to

be most significant for many traits of agricultural importance in potato. Tai (1976) reported that variation between progenies for tuber yields and number of tubers per plant was dominated by SCA while for average tuber weight and specific gravity GCA was more important. Another study showed that GCA was more important in determining the inheritance of number of stems, stolon length, plant appearance, skin colour, tuber shape, tuber yield, eye depth, number of tubers per plant, average tuber weight, harvest index, foliage weight, and total biomass (Neele et al., 1991). In yet another study, it was found that GCA dominated in determining total tuber yield, number of tubers per plant and plant appearance while the mean tuber weight depended on both GCA and SCA (Brown and Caligari, 1989). Tung et al (1992) found that SCA was more important than GCA in conditioning resistance to bacterial wilt, and there was a strong genotype x environment interaction. From the foregoing, it appears the literature on combining ability in potatoes is conflicting.

1.5 Farmers' preferences and participatory variety development

Breeders have often been accused of failing to consider the special preferences of farmers especially those in marginal areas (Toomey, 1999; Banziger and Cooper, 2001), possibly because they are unaware of them. As a result, most of developed varieties remain in the shelves as farmers continue to grow their own varieties resulting in low yields and vicious cycle of poverty. In addition, most breeders focus on developing varieties that can yield high only under optimal, agronomically well-managed conditions without considering the plight of the farmers as well as the production environments. This leads to low adoption of improved varieties. Determination of the needs of various stakeholders and incorporation of these needs in the breeding programme will go a long way in enhancing adoption of the bred varieties.

1.6 Genotype X environment interaction

Although the phenotype of an individual is determined by both genotype and environment, these two effects are no always additive. Genotype x environment interaction (GEI) is the differential genotypic expression across environments. It results in inconsistent differences between genotypes across environments. Such inconsistency in performance is caused either by differential responses of the same set of genes to changes in the environment or by expression of different sets of genes in different environments. With GEI, the inconsistent differences between

genotypes are manifested either as rank order changes of the genotypes between environments (crossover GEI), or as alterations in the absolute differences between the genotypes without affecting the rank order (Crossa et al., 1995; Bernardo, 2002). The two forms of GEI are referred to as qualitative and quantitative respectively. These interactions are only important in selection when rank order changes occur. In such cases, genotypes must be bred for specific adaptation to certain environments. A cross-over interaction is a major problem in breeding (Cooper and Delacy, 1994; Crossa et al., 1995), because it can slow down selection progress as different cultivars are selected in different environments. There are different types of GEI which include genotype x location interaction (GLI), genotype x year interaction (GYI) and genotype x location x year interaction (GLYI) (Crossa, 1990). Breeders mostly desire genotypes that show little interaction with the environment as they are stable (Yan et al., 2007).

There are many methods of exploring GEI. The most commonly used are additive main effects and multiplicative interaction (AMMI) model which combines analysis of variance and PCA into a single analysis, with both additive and multiplicative components (Lin et al., 1986) and the GGE biplot analysis which is based on singular value decomposition (SVD) of environment-centred or within-environment genotype-by-environment data (GED) (Yan et al., 2000; Yan et al., 2007). These two methods are complementary.

1.7 Potato production in Kenya

In Kenya potato was introduced by British farmers in the 1880s (FAO, 2014). Over 60 potato varieties, both officially and non-officially released, are grown in Kenya (FAO, 2013). Currently, a farmer selection, Cangi, is the most popular with farmers (Muthoni et al., 2010; Muthoni et al., 2013; NPCK, 2014).

Despite potato being the second most important food crop in Kenya, its production is not achieving its potential because of a number of constraints. The main constraints are low soil fertility, inadequate supply of disease-free seeds, and diseases (Kaguongo et al., 2008; FAO, 2013). Inadequate supply of disease-free potato tuber seeds is a consequence of the potato seed systems currently operational in Kenya. Because the formal potato seed system can produce only 1.1% of the national certified seed requirement (The Organic Farmer, June 2013), farmers resort to

informal seed sources which include farm-saved (self supply), local markets or neighbours (Kaguongo et al., 2008; FAO, 2013). This informal seed system has been greatly responsible for the spread of tuber-borne diseases such as bacterial wilt.

Among potato diseases, the common ones are late blight, viral infections and bacterial wilt (Kinyae et al., 2004; Kaguongo et al., 2008; FAO, 2013). Late blight is highly destructive during the rainy season especially in the cool highlands(Sleper and Poehlman, 2006). However, the disease is effectively controlled using fungicides, although this raises production costs significantly thereby discouraging most small scale farmers (Kaguongo et al., 2008).

In addition to late blight, viral diseases are a serious problem hampering potato production in Kenya (Kaguongo et al., 2008). Most potatoes in Kenya are grown from seed tubers retained by farmers from previous harvests, acquired from local markets or from neighbours (Khurana and Garg, 2003; Kaguongo et al., 2008). Continuous recycling of own seeds leads to gradual debilitation of tubers through viral infections (Khurana and Garg, 2003). The most common viruses are Potato Virus A (PVA) Potato Virus X (PVX), Potato Virus Y (PVY), Potato Virus Z (PVZ), and Potato Leaf Roll Virus (PLRV)(KARI, 2000). Resistance to PVX, PVY, and PVA has already been incorporated into some potato varieties (Khurana and Garg, 2003). The PLRV, PVY and PVX are effectively controlled through apical meristem culture, in combination with thermotherapy at Kenya Agricultural Research Institute (KARI) potato programme (KARI, 2000).

Bacterial wilt, caused by *Ralstonia solanacearum* (Yabuuchi et al., 1995), is the second most important potato disease in Kenyaafter late blight (Kaguongo et al., 2008) and most of the local potato varieties are susceptible (Kaguongo et al., 2008).

1.8 Bacterial wilt

Globally, bacterial wilt has been estimated to affect 1.7 million ha of potatoes in approximately 80 countries, with global damage estimates of over USD 950 million per annum (Champoiseau et al., 2009). In addition to potatoes, the disease also affects over 200 plant species from more than 50 families (Hayward, 1991). Bacterial wilt is widely distributed in tropical, subtropical, and warm temperate climates of the world, and it occurs in about 45 countries in the southern hemisphere; the hardest hit

countries are Kenya, China, Uganda, Indonesia, Bangladesh, Bolivia, and Peru (Hayward, 1991; EPPO, 2004).

1.9 Bacterial wilt symptoms on potatoes

In the early stages of the disease, foliage symptoms include rapid wilting of the youngest leaves at the end of the branches during the hottest time of the day and plants may appear to recover at night when the temperatures are lower (Martin and French, 1985; Champoiseau et al., 2009). As the disease develops, all leaves may wilt quickly and desiccate, although they remain green (Champoiseau et al., 2009). This may be followed by yellowing of the foliage, and eventual plant death; other symptoms include epinasty, chlorosis, and stunting (Martin and French, 1985; Champoiseau et al., 2009). Wilting is possibly a result of restricted water movement due to the formation of slime that surrounds the bacterial mass in the stem vascular bundles (Martin and French, 1985). Infected stem vascular bundles may become visible as long, narrow, dark-brown streaks, and the stem may also collapse in young potato plants (Champoiseau, 2008). In well-established infections, cross-sections of stems may reveal brown discoloration of infected tissues (EPPO, 2004) and a white, slimy mass of bacteria may exude from the vascular bundles of the cross-sections (Martin and French, 1985; Hayward, 1991; EPPO, 2004). This slime also streams spontaneously, in form of threads, when the cut surface of a potato stem is suspended in water (Champoiseau, 2008). Such threads are not formed by other bacterial pathogens of potato (Champoiseau et al., 2009). The streaming test is of presumptive diagnostic value in the field (Martin and French, 1985; EPPO, 2004). Under cool growing conditions, wilting and other foliar symptoms may not occur (Hayward, 1991; EPPO, 2004).

On tubers, symptoms may be visible in the later stages of disease development (EPPO, 2004). The symptoms include bacterial ooze at the tuber eyes or at the point where the stolon attaches to the tuber; and soil may adhere to the tubers at the eyes (Martin and French, 1985; EPPO, 2004). Cutting the diseased tuber may reveal browning, and eventual necrosis of the vascular ring, and the immediate surrounding tissues (Martin and French, 1985). A milky-white sticky exudate usually appears spontaneously on the vascular ring a few minutes after cutting the tuber.

Plants with foliar symptoms may bear apparently healthy and diseased tubers, while plants that show no symptoms of the disease may sometimes produce diseased tubers (Martin and French, 1985; Hayward, 1991; EPPO, 2004). Because symptom expression is favoured by high temperatures, symptomless plants may remain latently infected for extended periods of time at low temperatures (French, 1994). In Kenya, certified and apparently healthy (but latently infected) potato seed tubers produced at altitudes of 1520-2120 meters above sea level showed infection when planted at lower altitudes (Nyangeri et al., 1984).

1.10 Causal organism of bacterial wilt

The causal organism of bacterial wilt is the bacterium *Ralstonia solanacearum* (formerly *Pseudomonas solanacearum* and *Burkholderia solanacearum*)(Yabuuchi et al., 1995), which was described for the first time as *Bacillus solanacearum* by Smith in 1896 (EPPO, 2004). *Ralstonia solanacearum* is a gram-negative, rod-shaped, chemoorganotroph and strictly aerobic bacterium that is 0.5–0.7 x 1.5–2.0 l in size (Smith, 1896). The bacterium is soil dwelling and enters the plants through the roots and colonizes in xylem tissues. The pathogen can be found in six of the seven continents (Fegan and Prior, 2005). Traditionally, the pathogen has been subdivided into races (based on host range under field conditions) and five biovars (based on carbon utilization patterns) (Buddenhagen et al., 1962; Hayward, 1964). Race 1 occurs in the lowland tropics and warm temperate lands (French, 1994). It attacks potato, tomato, brinjals, chilli, groundnuts, tobacco, diploid bananas, and many other *solanaceous* crops, as well as many hosts in other plant families (French, 1994). It has a high temperature optimum (35-37^{0}C), as do races 2, 4, and 5 (EPPO, 2004). Race 2 is indigenous to Central and South America, and attacks members of *Musaceae* family such as plantain, triploid bananas, and *Heliconia* (French, 1994). It causes moko disease on bananas and *Heliconia* in Central and South America, and bugtok disease on plantains in the Philippines (Martin and French, 1985; EPPO, 2004). Race 3 occurs at higher altitudes (in the tropics) and higher latitudes than race1 (EPPO, 2004). It mainly attacks potato, tomato (especially when planted after infected potato), geranium, occasionally *Pelargoniumzonale*, eggplants, capsicum, and some *solanaceous* weeds like *Solanum nigrum* and *Solanum dulcamara* (Martin and French, 1985; Janse, 1991; French, 1994). Race 3 also infects a number of non-*solanaceous* weeds asymptomatically (Wenneker et al., 1999; Pradhanang et al.,

2000). This race has a long association with potatoes and has an optimum temperature of 27 - 28^{0}C (French, 1994). Race 4 affects ginger in Asia and Hawaii, while race 5 affects mulberry in China (EPPO, 2004).

The bacterium has also been classified into five biovars. Biovars are based on their ability to utilize and oxidize several disaccharides and hexose alcohols (Buddenhagen, 1986; Seal et al., 1999; Table 1.2).

Table 1.2. Differentiation of *Ralstonia solanacearum* into biovars

Biochemical Test		Biovars				
		1	2A	3	4	5
	Mannitol	-	-	+	+	+
	Sorbitol	-	-	+	+	-
	Dulcitol	-	-	+	+	-
Oxidation of	Trehalose	+	-	+	+	+
	Lactose	-	+	+	-	+
	Maltose	-	+	+	-	+
Utilization of	Cellobiose	-	+	+	-	+

Source: Buddenhagen and Kelman, 1964

Biovars 3, 4, and 5 are the most versatile in terms of the range of carbon sources (Table 1.2). Later, a new group of *R. solanacearum* isolates from the Amazon basin was differentiated from the original biovar 2 using ribose and trehalose (Hayward, 1994). This group was named biovar 2-T or biovar N2 and the original biovar 2 strains are now referred to as biovar 2A. Generally, biovars do not correlate with the races and only race 3, the potato race, is equivalent to biovar 2A while race 5 is identical to biovar 5 (Hayward, 1983; Champoiseau et al., 2009; Table 1.3).

Table 1.3 Equivalence between biovars and races *of Ralstonia solanacearum*

Race	Biovars	Hosts	Location
1	1,3,4	All *Solanaceous* crops + many other hosts	Lowland tropics (Asia, Americas and Australia)
2	1,3	Bananas and other *Musa* species	American and Asian tropics (Caribbean, Brazil, Philippines)
3	2A	Potato and tomatoes	Cool climate worldwide
4	3,4	Ginger	Asia
5	5	Mulberry	China
Not known	2T	Numerous	Amazon basin

Source: EPPO, 2004

Biovar 2A has the least host range whereas biovar 3 has the widest (Table 1.3). Biovar 2A (race 3) is known as the potato or low temperature race and is found in

high latitudes, and high altitudes in the tropics (Seal et al., 1999; Hayward, 2000). Race 3/biovar 2A (R3bv2A) causes bacterial wilt of potato in over 90% of cases worldwide because potato is a cool season crop (French, 1994; EPPO, 2004). Potato is the common host for R3bv2A, but when there is high pathogen inoculum concentration in the soil, and high temperature, it can also infect tomatoes, or a few other crops, when they are grown in rotation (Buddenhagen, 1986; French, 1994; EPPO, 2004). The R3bv2A probably originated in the Andes and was apparently disseminated worldwide on potato tubers. It now occurs in tropical highlands and in subtropical and warm-temperate areas throughout the world, except in North America (Buddenhagen, 1986). It is also widespread in the higher latitudes as far as southern Sweden and southern Argentina (Champoiseau et al., 2009). The race R3bv2A is the main cause of bacterial wilt of potatoes in the Kenyan highlands (Smith et al., 1995). Although R3bv2A principally occurs in cool climates, it also occurs in potato plants grown in warmer locations from seed tubers harvested from cool climates (French, 1994). With the expansion of potatoes into warmer subtropical and tropical lands, in addition to global warming, cases of lowland bacterial wilt caused by race 1(biovars 1, 3 and 4) have occurred (French, 1994; EPPO, 2004).

A recent phylogenetic classification scheme based on DNA sequence analysis divided the species complex into four phylotypes that broadly reflect the ancestral relationships and geographical origins of the strains (Champoiseau et al., 2009). Phylotype I strains originated in Asia, phylotype II strains originated in the Americas, phylotype III strains in Africa, and phylotype IV strains in Indonesia. Phylotypes are further subdivided into sequevars based on the sequence of the endoglucanase (egl) gene (Prior and Fegan, 2005). Race 3 belong to phylotype II and sequevars 1 and 2 (Fegan and Prior, 2005).

1.11 Dissemination and survival of R3bv2A

In potatoes, R3bv2A is tuber borne, and is primarily disseminated through infected seed tubers (Champoiseau et al., 2009). Potato seed tubers carry the bacterium in the vascular tissue, lenticels, and on the surface (Kelman, 1953; Sunaina et al., 1989). The other source of inoculum is the infested soil; the bacteria is native in many tropical soils (Martin and French, 1985). Bacterial wilt is further spread through infected run-off water or soil adhering to tools and shoes (Martin and French, 1985; Pradhanang, 1999).

Under field conditions, plant infection usually occurs through the root system, especially through wounds (Kelman, 1953). The pathogen can also enter through stem wounds or stomata (EPPO, 2004). Wounds can occur due to cultivation activities, natural growth of secondary roots, attack by nematodes or other pests (Martin and French, 1985; Shekhawat and Chakrabarti, 1993).

Once introduced, the pathogen survives at soil depths of 1m or more, where microbial competition is low, or as slimy masses in the upper soil layers (Kinyua et al., 1998). The pathogen persists longer in wet but well-drained soil (Kinyua et al., 1998; Champoiseau et al., 2009). Survival of the pathogen in the soil is reduced by extreme cold, and the presence of antagonistic microorganisms, while volunteer host plants enable bacterial survival across seasons (Martin and French, 1985; Hayward, 1991; Milling et al., 2009). Survival depends also on the race involved; race1 usually persists for many years in the soil because of its numerous hosts, while R3bv2A tends to persist for a few years due to limited hosts (Martin and French, 1985; Champoiseau et al., 2009).

1.12 Management of bacterial wilt on potatoes

Control of bacterial wilt on potatoes is a problem because the physiologic race R3bv2A is the most virulent and no single control method has been found to be 100 % effective (Champoiseau et al., 2009). The common approach in the management of bacterial wilt in potatoes is an integrated combination of measures such as phytosanitation and cultural practices, chemical control, biological control, and host resistance (Champoiseau et al., 2010).

1.12.1 Phytosanitation and cultural practices

Phytosanitation and cultural practices are the most widely used practices for controlling bacterial wilt in the field (Martin and French, 1985; Champoiseau et al., 2010). These practices can be effective in regions where bacterial wilt is endemic, or in locations where it is present but not yet established (French, 1994; Champoiseau et al., 2010). Phytosanitation practices include planting disease-free tuber seeds, and quarantine measures, while cultural practices include crop rotation, intercropping, delayed planting, and soil amendments (Kinyua et al., 2001; EPPO, 2004; Champoiseau et al., 2010).

1.12.1.2 Use of disease-free tuber seeds

Although use of disease-free seed tubers is advocated in Kenya (Wakahiu et al., 2007), it is not effective because the quantities of disease-free certified tuber seeds produced by the formal seed system are insufficient to meet the farmers' requirements (Lung'aho et al., 1997; Ayieko and Tschirley, 2006; Kaguongo et al., 2008). Consequently, farmers use tuber seeds from informal sources, and the health status of such seeds cannot be guaranteed (Muthoni et al., 2010).

1.12.1.3 Quarantine

Quarantine measures on the other hand may prevent introduction of the pathogen into disease-free areas (Champoiseau et al., 2009). However, quarantine measures necessary to avoid spread of bacterial wilt to disease-free areas often restrict the production of tuber seeds; this may limits commercialization of ware potatoes thus affecting the local economy (Martin and French, 1985). Quarantine is not possible in Kenya because the movement of potato locally is uncontrolled and potato seed system is largely informal (Muthoni et al., 2010). Furthermore, international borders are porous leading to illegal importation of both ware and seed potatoes (Muthoni et al., 2010).

1.12.1.4 Crop rotation

Crop rotation of 5-7 years excluding host plants has been recommended to control R3bv2A in the soil (EPPO, 2004). Crop rotation as a control measure may not be effective in Kenya because the small farm sizes make proper crop rotations impossible to implement (Lemaga, 1997; Otipa et al., 2003; Kaguongo et al., 2008; Kaguongo et al., 2010). In addition, the small scale farmers do not have sufficient land to plant anything but essential food crops.

1.12.1.5 Intercropping

The importance of intercropping depends on the other crop used in the intercrop. In Burundi, intercropping of potatoes with beans resulted in less disease spread than intercropping potatoes with maize, while wide within-row spacing also reduced the incidence and spread of latent infection (French, 1994).

1.12.1.6 Delayed planting

Although delayed planting reduced bacterial wilt incidence in India and Japan, in Kenya, delay in planting time may not be the best option because the rainy seasons are short and erratic, and farmers may not be willing to risk losing a crop.

1.12.1.7Soil amendments

It has been reported that bacterial wilt incidence is increased by low soil pH, and low soil fertility (Lemaga et al., 2001; Lemaga et al., 2005; Messiha, 2006). However, soil amendments to raise pH or raise soil fertility may not be practical in Kenya because it is generally expensive to the small scale potato farmers.

1.12.2 Chemical control

The most commonly used chemical treatment has been fumigation of contaminated soil or portions of the farm with methyl bromide (Champoiseau et al., 2010). This is a very expensive and tedious exercise and cannot be used on large areas. In addition, methyl bromide has been banned in most parts in the world and is being phased out in Kenya. The other product commonly used at field level is sodium hypochlorite; it is appropriate for spot treatment of the holes left behind after rogueing of the wilting plants, and for general field sanitation (Kaguongo et al., 2008). However, use of sodium hypochlorite is expensive and tedious and therefore not practical in Kenya (Kaguongo et al., 2008; Kaguongo et al., 2010).

1.12.3 Biological control agents

Among biological control agents, a number of soil bacteria and plant growth promoting rhizobacteria (PGPR) are currently being investigated for their role in the control of R3bv2A (Champoiseau et al., 2010). However, none of them is currently available commercially, and their efficacy is yet to be determined on a commercial scale (Champoiseau et al., 2010). Search for a biological control agent for bacterial wilt from the local bacterial antagonists in Kenya was initiated in 1992; however, the biological control agents were largely ineffective (Smith et al., 1998).

1.12.4 Host resistance

Use of resistant potato varieties to control R3bv2A in Kenya is probably the cheapest and the most practical means because chemicals are generally ineffective, phytosanitation and cultural measures are difficult to apply, and biological control agents are not commercially available (Martin and French, 1985; Champoiseau et al., 2010).

1.12.4.1 Nature of resistance

The best that conventional breeding has achieved is moderate level of resistance to bacterial wilt on a regional level. When conditions are not excessively hot or wet,

some potato cultivars are less susceptible to bacterial wilt at least in some regions (Champoiseau et al., 2010). Resistance to R3bv2A available now in *Solanum tuberosum* originated mainly from the cultivated diploid, *Solanum phureja* (Martin and French, 1985). This resistance is seldom expressed as immunity because it is overcome by factors that favour the disease development i.e. high temperature, high soil moisture, low soil pH, low soil fertility, and damage to the plant root system (Martin and French, 1985; Low, 1997). The resistance, however, has been shown to be very unstable due to strong host-pathogen-environment interaction (French and Lindo, 1982; Tung et al., 1990; Tung, 1992; Tung et al., 1992b). Therefore, hosts resistant to the disease in one yearor location succumb to the disease in the other. Hosts are not resistant against all races of the pathogen (Grimsley and Hanson, 1998; López and Biosca, 2004) and a race at one location may overcome the resistance effective at another location (Grimsley and Hanson, 1998); more than one pathogen race may occur in a given field (Martin and French, 1985). Due to these host-pathogen-environment interactions, an essential step in the development of resistant varieties is local screening of the germplasm (Martin and French, 1985). Thus, use of potato germplasm that conforms to regional geographic boundaries is necessary for a successful local potato breeding programme. Because a race at one location may overcome the resistance effective at another location, an essential step in the development of resistant varieties is local screening (Martin and French, 1985).

Because high level of resistance has not been identified in potatoes, only moderately resistant cultivars are used such as ‘Cruza 148’ (unknown origin) in Africa, ‘Molinera’, ’Caxamaraca’, ‘Ampola’, and ‘Huanuquena’ in Peru, ‘Prisca’ and ‘Kinga’ in Madagascar, ‘Ndinamagara’ in Burundi, Rwanda, and Democratic Republic of Congo, and cultivar ‘Achat’ in Brazil (Hayward, 1991; French et al., 1997). In Uganda, clones 388575.5 and 388575.9 both from CIP are moderately resistant to bacterial wilt in the cool areas, while clones 390005.11, 388574.2B, and 388580.18A are moderately resistant to bacterial wilt in the warm areas (Kaguongo et al., 2008). In Kenya, varieties Kenya Dhamana (CIP-800228), Kenya Sifa, Kenya Karibu, Mauritius clone (89016), and Cruza-148 (CIP-720118) were rated as resistant to bacterial wilt, while varieties Asante (CIP-381381.20), Tigoni (CIP-381381.13), Nyayo, and Dutch Robyjin were highly susceptible (Ateka et al., 2001). To control

bacterial wilt of potatoes better, continuous development of resistant varieties is needed (Champoiseau et al., 2010).

1.12.4.2 Inheritance of resistance to bacterial wilt

It was reported that the resistance to bacterial wilt from *Solanum phureja* is controlled by a few genes (Martin and French, 1985); by three independent and dominant major genes (Buddenhagen, 1986), and that both additive and non-additive gene actions are important in the inheritance of the resistance (Rowe and Sequeira, 1970). Later, it was reported that this resistance is controlled by at least four major genes (French et al., 1997; Grimsley and Hanson, 1998). Recently, it was shown that there are around 70 genes and 15 inter-genes specific to the bacterial wilt pathogen by microarray technique (Guidot et al., 2009). Other studies reported that both major and minor genes are involved in the expression of resistance to bacterial wilt; and inheritance of this resistance involves both additive and non-additive gene actions (Tung et al., 1993; Tung and Schmiediche, 1995). Other reports showed significant general and specific combining abilities for bacterial wilt resistance indicating that both additive and non-additive gene actions are important in conditioning resistance expression (Chakrabarti et al., 1994). Other results indicated that resistance to bacterial wilt in potato is a partially dominant character (Tung et al., 1993), and in its inheritance, epistasis is important (Tung et al., 1992a; Tung et al., 1993). Other studies indicated that the resistance is polygenic and quantitative in nature, and involves genes with major and genes with minor effects (Tung et al., 1993; Cook and Sequeira, 1994). The major genes have been evolving independently from the pathogen interaction, whereas minor genes are thought to operate in a gene to gene way with the pathogen. There is also evidence that in the inheritance of resistance to wilt, non-additive gene action is important, and is largely of the epistatic type (Tung et al., 1992a; Tung et al., 1992b,1993). Some other reports (Tung, 1992) found that the non-additive variance component for disease severity was 4.5 times more than additive component and a large proportion of non-additive variance was due to epistasis. Therefore, breeding schemes designed to make use of both additive and non-additive gene actions seem most suitable in developing resistance. Moreover, the genetic background for adaptation is of crucial importance for expression of resistance (Tung, 1992; Tung et al., 1993). More evidence showed that the resistance to bacterial wilt in potatoes is very complex in

nature; it is probably a function of environmental adaptation with genes for adaptation being involved (Tung et al., 1990b; Tung et al., 1992a; Tung et al., 1992b). There is a large amount of interaction between genes for resistance and those for adaptation (Tung et al., 1992a; Tung et al., 1992b), and combining ability seems to be a considerable feature of the resistance (Tung et al., 1990b). Therefore, potato clones with a wide genetic background for both bacterial wilt resistance and adaptation tend to display a higher level of resistance, which is more stable over environments (Tung et al., 1993). Good adaptation of the potential host to a particular environment is likely to strengthen expression of the resistance to wilt (Tung et al., 1990; Tung et al., 1992b). In order to develop a stable resistance in potato populations, a wide genetic base for resistance and adaptation to the environment where the pathogen occurs would therefore be necessary (Tung et al., 1993). Hayward (1991) reported that resistance of different crop plants to *R. solanacearum* is a polygenic phenomenon and depends upon environmental conditions.

The strong interaction between genes for heat tolerance and those for resistance implies the presence of a large amount of favourable non-additive (epistatic) gene effects in expression of high resistance (Tung, 1992; Tung et al., 1993). Thus, breeding at the population level by incorporating multiple sources of resistance and heat tolerance should be effective in producing superior potato genotypes, suitable for production in the lowland tropics where high levels of bacterial wilt resistance and heat tolerance are much needed (Tung et al., 1993).

In tomatoes, it was found that both additive and non-additive gene action effects were significant for bacterial wilt resistance with additive gene action dominating (Osiru et al., 2001). They also found that this resistance is controlled by two genes. In groundnuts, it was reported that although both GCA and SCA were important for resistance to bacterial wilt, GCA was more important (Liao et al., 1990).

1.12.4.3Search for resistance

Other sources of resistance which have been evaluated, albeit at experimental level, are *S. stenotomum L* (cultivated), and *S. commersonii* Dun, which is wild (Laferriere et al., 1999; Fock et al., 2000; Fock et al., 2001; Carputo et al., 2009). They too show

moderate resistance and their hybrids harbour latent infection (Laferriere et al., 1999; Fock et al., 2000; Fock et al., 2001).

Recent developments in the search for resistance offer promise. Scientists from CIP have recently developed some improved potato clones that are moderately resistant to R3bv2A, although the clones have not been tested extensively (Bonierbale Merideth, personal communication, 2010)[1]. Therefore, introgression of resistance from the more resistant CIP germplasm into the more productive, more popular yet more susceptible Kenyan varieties may improve potato production in Kenya.

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Chapter Two: Potato production in Kenya: Farming systems, production constraints and breeding priorities

Abstract

Potato (*Solanum tuberosum* L.) is a major food and cash crop in the Kenyan highlands widely grown by small-scale farmers on mixed farms. Farmer practices and constraints in potato production differ from region to region. In view of this, a survey was conducted in three major potato producing districts, namely Bomet, Molo and Meru Central with the following objectives: 1) to document farmers' practices, key marketing and potato production constraints 2) to determine farmers' potato cultivar and trait preferences and 3) to assess the prevalence and farmers' management of bacterial wilt. The survey was carried out between November 2011 and March 2012. During the survey, a semi-structured questionnaire was administered to 253 individual farmers in three districts. The results show that the average household farm sizes are less than 2.4 ha in all the districts. The majority of farmers allocate more than 25% of their farms to potatoes; in Molo district, the allocation is more than 45%. Potato is produced both for food and cash by 90% of respondents in all the three districts. Farmers have varied cultivar and trait preferences; in Bomet district the red-skinned Dutch Robyjn is widely grown. In Molo district, the white- skinned Cangi is prominent while in Meru Central, the red-skinned Asante is predominantly grown by farmers. The cultivar preferences are mostly dictated by availability of markets, yield potential and taste. In addition to potatoes, other important crops in all the three districts include maize, dry beans and cabbage; these are rotated with potatoes. Over 75% of respondents indicated that the major production constraints are diseases with bacterial wilt being the most prominent. Farmers deploy different methods in managing the disease in the field such as spraying with fungicides, roguing and burning the wilting plants, and burying of the rotten tubers after harvest. However, these methods are tedious and expensive and at times impractical. Therefore integrated disease management with development of resistant varieties could be a cheap and environmentally friendly option in managing the disease. In addition to disease resistance, the cultivars should have a high market demand, be high yielding, early maturing and have a good taste.

Keywords: Bacterial wilt, Breeding priorities, Farming systems, Potato production.

2.1 Introduction

Potato (*Solanum tuberosum* L.) plays a major role in food security in Kenya and contributes to poverty alleviation through income generation and employment creation. Despite its importance, the potato sector is plagued by numerous problems such as lack of clean seeds, lack of proper pest and disease management, a disorganised marketing system and lack of clear policies on packaging (Riungu, 2011).

The shortage of clean (disease free) planting materials has led to low yields, poor quality produce, and spread of pests and diseases (GIZ-PSDA Kenya, 2011; Riungu, 2011). Kenya produces about 1.1% of the national certified seed demand. Because of shortage of clean planting materials, farmers are forced to plant seeds from informal sources such as farm-saved (self supply), local markets or neighbours. The informal system leads to the use of poor quality seeds and often accelerates the spread of seed-borne diseases such as bacterial wilt (Kinyua et al., 2001; Ng'ang'a et al., 2003). According to some studies, bacterial wilt has affected 77% of potato farms (Kaguongo et al., 2010). Because of the high prevalence of this disease, a strict rotation programme is required in the production of the crop; few farmers can rotate for the recommended one and a half years due to paucity of land (Riungu, 2011).

Control of bacterial wilt on potatoes is difficult and no single control method has been found to be totally effective (Champoiseau et al., 2009). The common approach in the management of the disease is a combination of measures such as phytosanitation (use of disease-free seeds and quarantine), cultural practices (crop rotation, intercropping and delayed planting), chemical control and biological control (Martin and French, 1985; Champoiseau et al., 2010). However, most of these measures have been found to be ineffective, impractical and/or expensive (Lemaga, 1997; Otipa et al., 2003; Kaguongo et al., 2008; Kaguongo et al., 2010; Riungu, 2011). Host resistance could therefore offer a more lasting solution.

Disease resistance, in addition to other good traits, may increase the chances of a cultivar being adopted by farmers as this may reduce production costs. The various end-uses of potatoes require specific tuber characteristics and cultivars. In a previous study, it was found that attributes considered in ranking a potato cultivar by

farmers are high yield potential, late blight resistance, taste, maturity period, market demand, bacterial wilt resistance, tuber size, and drought tolerance in that order (Kaguongo et al., 2008). In another study, it was found that farmers prefer cultivars for home consumption to be tasty, high yielding and resistant to late blight while the cultivars should have high market demand and be high yielding if they are destined for the market (McArthur, 1989). Tuber quality characteristics such as skin colour, tuber size, tuber shape and time to maturity are often key factors in cultivar acceptability based on local consumer preferences and criteria for potato processing (McArthur, 1989). Red-skinned cultivars, which are considered to boil quickly and mash easily are favoured for home consumption while white cultivars are preferred for making chips and french fries (McArthur, 1989). Different processing industries prefer different skin colour, tuber shape and sizes. For example, for making french fries, most processors in Kenya prefer the long and white-skinned cultivars while the round and red-skinned cultivars are preferred for making chips (Walingo et al., 1998). In addition, red-skinned cultivars have a greater demand in the fresh market probably because they do not turn green when exposed to the light as quickly white-skinned cultivars. In Kenya, red-skinned cultivars were found to be more popular than the white-skinned ones in Meru Central district while the opposite was found in Nyandarua district (Kaguongo et al., 2008). Early maturity is important for food security and enables households to generate income early to meet financial obligations. It is also an important trait in potato growing areas with high demand for land as early harvesting allows more crop cycles in a year. In addition, the short rainy season is often erratic and an early maturing cultivar stands a better chance of carrying the crop to full maturity.

Over time some potato cultivars have been rejected and replaced by others in Kenya; low yield and susceptibility to diseases were cited as the major weaknesses. For instance, Kerr's Pink was removed from its dominant position in Meru Central by Ngure; the latter has been replaced by Asante and Tigoni Red (Durr and Lorenzl, 1980; Crissman et al., 1993). Desiree has been largely abandoned due to low yields, poor market, poor taste and susceptibility to late blight (McArthur, 1989).

For a cultivar to be readily adopted, it must have farmers-preferred traits in addition to disease resistance. Without farmer participation either through participatory rural appraisal (PRA), participatory variety selection (PVS) or participatory plant breeding

(PPB), breeders often fail to target farmer-preferred traits (Witcombe et al., 1996) leading to low variety adoption rate (Fukuda and Saad, 2001). The initial stage of PPB involves identification of the end-users preferences and production environments. To achieve this, PRA can be employed (Witcombe et al., 2005). During the PRA, the breeder is able to identify and understand both the target environment and farmers. It creates a conducive environment where farmers and breeders exchange ideas and start working towards a common goal (Fukuda and Saad, 2001).

Against this background, a study was undertaken with the following objectives: to document farmers' practices, key marketing and potato production constraints and determine farmers' potato cultivar preferences, the prevalence of bacterial wilt in the major potato growing areas and establish farmers' management of bacterial wilt.

2.2 Materials and methods

2.2.1 Survey sites and descriptions

A survey was carried out in three major potato producing counties in Kenya namely, Meru, Bomet, and Nakuru between November 2011 and March 2012. These counties were selected because farmers ranked potatoes as their most important commercial crop (Kaguongo et al., 2010). In addition, Nakuru and Meru are among the five leading potato producing counties in Kenya (Ng'ang'a et al., 2003). Bomet County was chosen because its potatoes have a unique demand for processing into chips. Bomet and Nakuru are located northwest of Nairobi while Meru is northeast of Nairobi (Figure 2.1). In each county, sampling was done at several administrative levels: one district was selected per county, two divisions in each district were selected and all wards (in each division) where potato is a major crop were selected. In Meru County, Meru Central district was selected while in Bomet County, Bomet district was selected. Molo district was selected from Nakuru County.

Bomet district is located in the former Rift Valley Province. It has two divisions i.e. Bomet Central and Longisa. The district is home to the Kipsigis subgroup of the Kalenjin community. It is about 300km northwest of Nairobi and has intensively cultivated steep slopes. The area has a mean monthly temperature of 18ºC with an annual rainfall ranging between 1100mm and 1500mm (Jaetzold et al., 2006a).

Meru Central district is located in the former Eastern province and represents potato growing areas in the Mt Kenya region. The district is the ancestral home to the Meru community. The district lies to the east of Mt Kenya whose peak cuts through the southwest border of the district. In the district, potatoes are mainly produced in the Kibirichia and Abothuguchi West divisions located on the northern slopes of Mt Kenya. These divisions are characterized by annual precipitation ranging between 1400 and 2600mm and monthly temperature averaging 18ºC (Jaetzold et al., 2006b).

Molo district is located in the former Rift Valley province. It comprises two divisions; Molo and Elburgon. Molo is a cosmopolitan district with most of the inhabitants being immigrants from Central and Nyanza provinces. The main inhabitants are Kikuyu, Kalenjin and Kisii communities. The main economic activities are crop production, dairy and sheep keeping. The main cash crops are pyrethrum, potatoes, barley and maize (Jaetzold et al., 2006a).

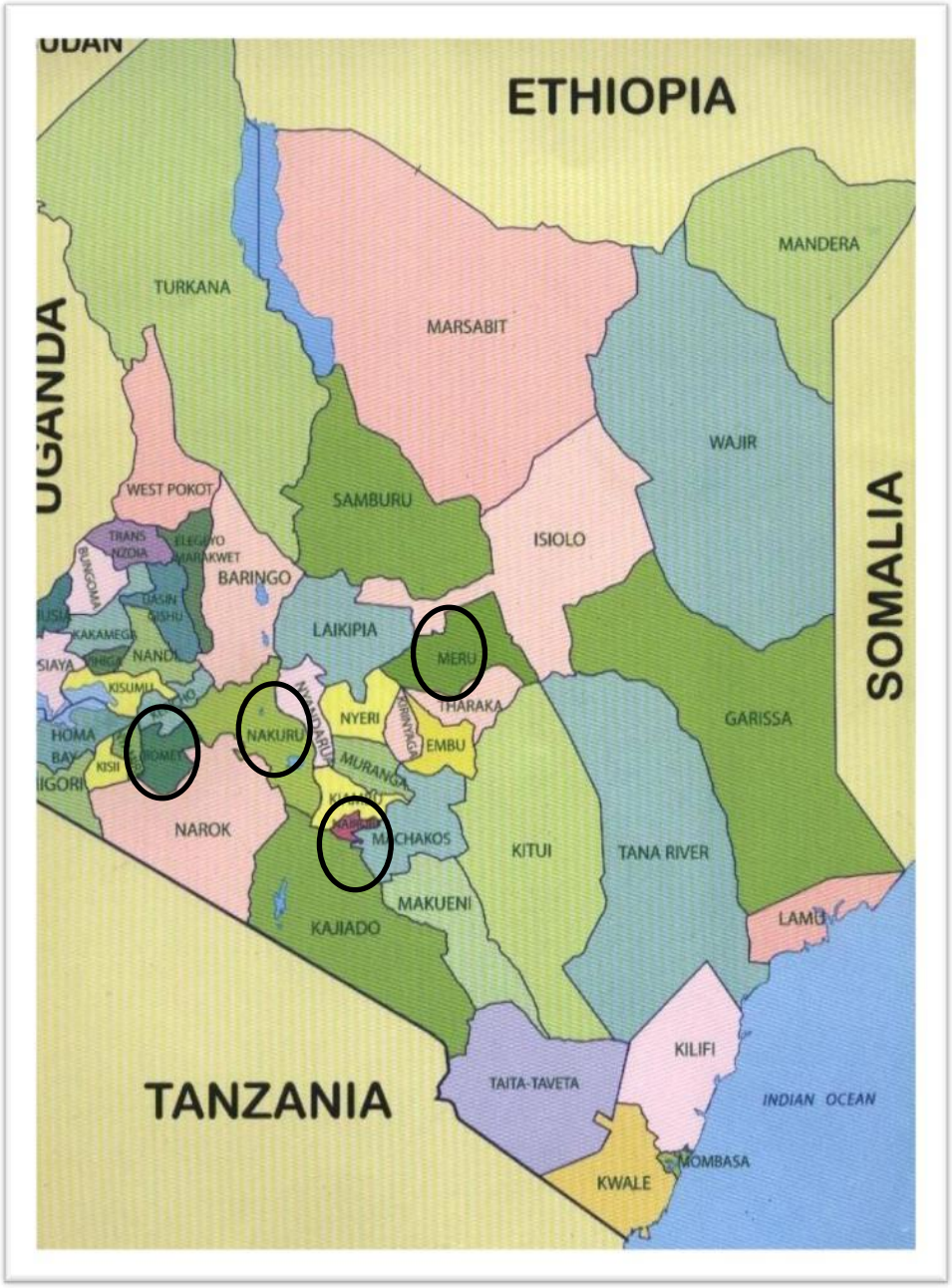

Figure 2.1. Administrative map of Kenya showing three potato producing counties which were surveyed

2.2.2 Sampling method, data collection and analysis

Primary data were collected by administering a semi-structured questionnaire to individual farmers. The questionnaire contained open-ended questions that allowed the respondents to express themselves fully in order to gain as much information as possible. After developing the questionnaire, planning meetings were held with the respective district agricultural officers to agree on the areas to be surveyed and the survey routes to be followed in each district. Following these discussions, the survey routes were mapped and the questionnaire pre-tested on ten households in each district. After pre-testing, changes were effected on the questionnaire and the formal survey commenced.

The survey team consisted of a breeder, a social scientist (both from KARI-Tigoni), an agricultural extension officer and three enumerators (selected from each district. Sampling of the households was both purposeful and systematic; one household (with a current potato crop in the field) within 3 km intervals along selected routes/paths was interviewed. If no household had a potato crop in the field within the 3 km interval, the next potato farm was sampled.

Interviews were carried out in the field using the questionnaire to capture data on farm size, area under potatoes, potato farming history, cropping system, bacterial wilt management and potato cultivar preferences. The interview was conducted in the local language whenever possible; otherwise it was conducted in Kiswahili, the national language.

The survey team visited the potato plot and scored for bacterial wilt incidence.The incidence was established by measuring the percentage of wilting plants. Prevalence of bacterial wilt was calculated as the number of farms affected by the disease expressed as a percentage of all farms visited in an area.

A global positioning system (GPS-Garmin Inc. Kansas, US) was used for geo-referencing purposes to supply coordinates (latitudes, longitudes, altitudes) for specific locations. The primary data was analysed using SPSS for Windows Release Version 18.0 (SPSS Inc., 2009). Data analysis was descriptive (means and percentages).

2.3 Results

2.3.1 Farmers and farm characteristics

A total of 253 farmers were interviewed. In each district, over 60% of the farmers interviewed were men (Table 2.1). The farms are located between 1933 and 2723 meters above sea level (masl).

Table 2.1.Descriptions of the three potato growing districts surveyed in Kenya

County	District	Divisions	Altitude (masl)	No of farmers interviewed	% Male farmers
Meru	Meru Central	Abothuguchi west	2126	52	61.8
		Kibirichia	2130	41	70.7
Bomet	Bomet	Longisa	1933	37	70.3
		Bomet Central	2279	42	88.1
Nakuru	Molo	Elburgon	2723	58	65.5
		Molo	2542	23	69.6
Total				253	

Masl= Meters above sea level

The area surveyed ranged from upper midlands (below 2000 masl) to upper highlands (over 2700masl) (Table 2.2). Molo and Elburgon represent the upper highlands while the other divisions represent the upper midlands and lower highlands.

Table 2.2. Agro-ecological zones of the six potato growing divisions in Kenya surveyed (% of respondents)

Agro-ecological zone	Altitude (masl)	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
Upper Midlands	2000 ≥	0.0	2.7	59.6	0.0	0.0	0.0
Lower Highlands	2001 -2400	100.0	97.3	40.4	95.1	0.0	0.0
Upper Highlands	2401-2700	0.0	0.0	0.0	4.9	50.0	100.0
Upper Highlands	2701 ≤	0.0	0.0	0.0	0.0	50.0	0.0

In all districts potatoes have been grown for more than nine years. In Meru Central district farmers have been growing potatoes for a longer period than in the other two districts (Table 2.3). The average farm sizes range from 0.9 to 2.1 ha (Table 2.3). This confirms the general observation that most potatoes in Kenya are predominantly grown by small-scale farmers; the mean farm size is about 2 ha while potato plots are about 0.5 ha (Kabira, 1983). These potato growing areas are densely populated and hence the small farm sizes.

A positive correlation (r=0.66) was observed between farm sizes and the area under potatoes. This means that farmers with bigger farms allocate bigger plots to potatoes. Wakahiu et al. (2007) found a correlation (r=0.26) between farm sizes and the area under potatoes in a previous study.

Table 2.3.Average farm size, area under potatoes, years of potato production in three districts in Kenya

District	Divisions	Av. Farm size (ha)	Av. area under potatoes ha (% of total farm size)	Av. years of potato growing
Bomet	Bomet Central	1.70	0.49 (28.8)	9.5
	Longisa	1.66	0.45 (25.5)	9.3
Meru Central	Abothuguchi West	0.97	0.28 (29.0)	16.0
	Kibirichia	1.17	0.49 (41.5)	23. 3
Molo	Elburgon	1.98	0.89 (45.7)	9.6
	Molo	2.10	1.13 (47.9)	9.2

Most potatoes are grown as pure stands in small scale intensive farming systems. Few farmers (5.5%) intercrop potatoes with crops such as maize or beans. The rest grow potatoes in pure stands and practice crop rotation (Table 2.4). Occasionally, in cases where farm size is very small, potatoes are grown without rotation. In Molo division, about 30% of the farmers surveyed do not practice crop rotation (Table 2.4).

Table 2.4. Common rotational sequences (% of respondents) of potato production in three districts in Kenya

Rotation sequence	District and divisions					
	Bomet		Meru Central		Molo	
	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
potato, maize, potato	14.3	2.7	1.9	12.2	34.5	8.6
potato, maize+beans, potato	50.0	37.8	25.0	0.0	19.6	34.8
potato, maize+bean/cabbage,potato	23.8	40.5	13.5	0.0	0.0	0.0
potato, maize/cabbage,potato	0.0	0.0	17.3	9.8	17.2	13.0
potato, cabbage, potato	0.0	0.0	5.8	9.8	5.2	4.3
potato, maize/wheat, potato	0.0	0.0	0.0	14.6	1.7	0.0
potato, maize+bean/wheat, potato	0.0	0.0	0.0	7.3	0.0	0.0
others	9.5	16.2	15.4	26.8	0.0	8.7
no rotation	2.4	2.7	20.2	19.3	21.5	30.4
Total	100	100	99.1	100	99.4	100

Maize+beans=maize intercropped with beans; maize+beans/cabbage= maize intercropped with beans or cabbage alone; potato, maize, potato=potatoes followed by maize then potatoes in that sequence; maize/cabbage= maize or cabbage

Other rotational sequences observed involve minor crops such as carrots, snow peas, millets. Over 99% of farmers plant a range of crops on their small farms mainly to cushion themselves against the risk of crop failure (Table 2.5). Wheat production is specific to Kibirichia while tea is specific to Bomet Central.

Table 2.5.Crops commonly grown by farmers (% of respondents) in three districts in Kenya

Crops	Districts and Divisions					
	Bomet		Meru Central		Molo	
	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
maize	97.6	89.2	92.3	75.6	91.4	82.6
beans	76.2	91.9	76.9	34.1	44.8	56.5
potatoes	100.0	100.0	100.0	100.0	100.0	100.0
cabbage	31.0	54.1	61.5	56.1	46.6	39.1
tea	47.6	0.0	15.4	0.0	0.0	0.0
coffee	0.0	0.0	9.6	0.0	0.0	0.0
wheat	0.0	0.0	0.0	41.5	3.4	0.0
bananas	0.0	0.0	11.5	0.0	0.0	0.0

2.3.2 Potato farming system

About 90% of all the farmers interviewed produce potatoes both for cash and food (Figure 2.2). This possibly explains the allocation of potatoes to large portions of their farms. Ng'ang'a et al. (2003) found that farmers in Nyandarua, Meru Central, Bomet, Nakuru, Nyeri and Keiyo districts grow potatoes mainly for cash, selling over 60% of their produce.

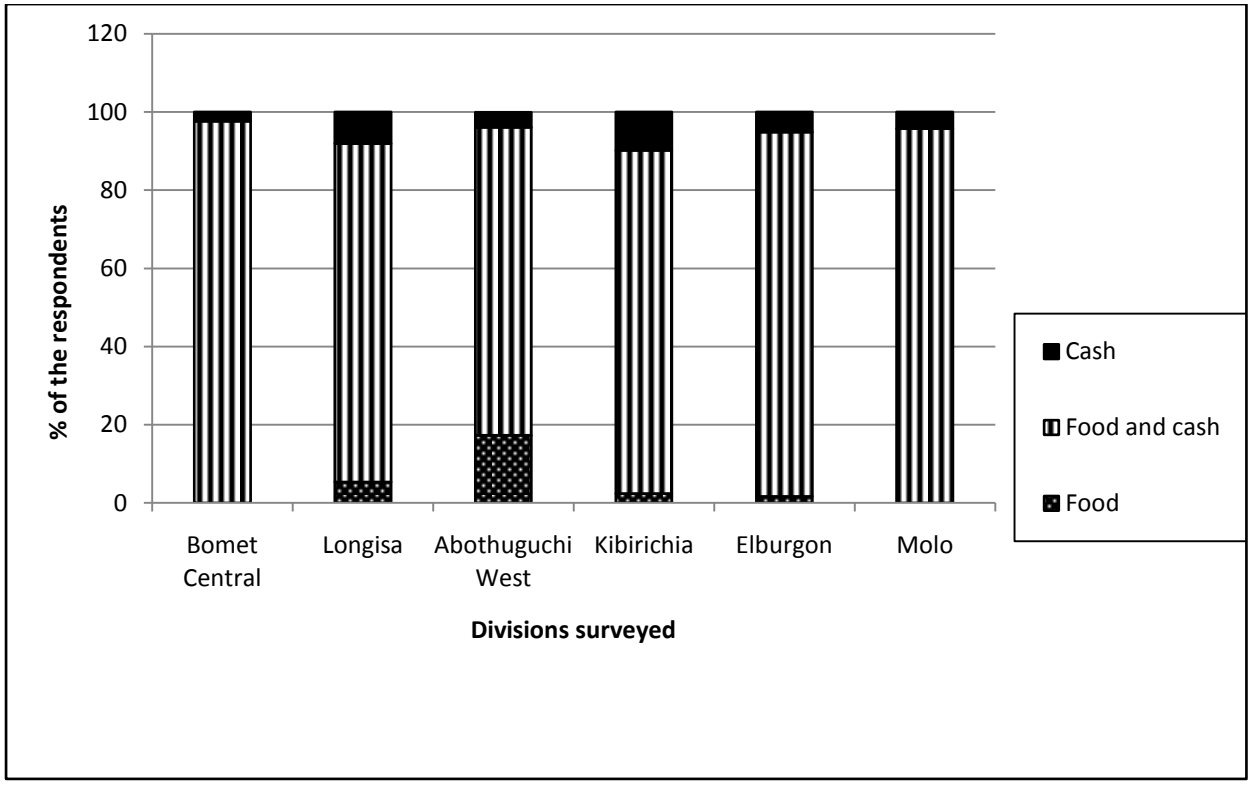

Figure 2.2. Proportion (%) of farmers that grow potatoes for cash, cash and food, or food in six divisions in Kenya

Farmers generally obtain seed tubers from informal sources (Table 2.6). The formal seed sources (ADC and KARI Tigoni) are utilized in Bomet district, and to a lesser extent, Elburgon division. The majority of farmers from Abothuguchi West obtain their seeds from the neighbouring Kibirichia division. They argue that potatoes from Kibirichia are rainfed and hence have a lower chance of having bacterial wilt. Farmers from Abothuguchi West believe that seed from their local area had bacterial wilt because it is mainly grown under irrigation.

Table 2.6. Percentage of farmers obtaining potato seeds from different sourcesin six divisions in Kenya

Seed source	Bomet Central	Longisa	Elburgon	Molo	Abothuguchu West	Kibirichia
ADC	4.8	2.7	0.0	0.0	0.0	0.0
ADC, neighbours	2.4	0.0	6.9	0.0	0.0	0.0
KARI Tigoni, own	2.4	8.1	0.0	0.0	0.0	0.0
neighbours	38.1	43.2	34.5	78.3	1.9	9.8
own (farm-saved)	33.3	21.6	50.0	17.4	3.8	90.2
own, neighbours	19.0	16.2	5.2	4.3	1.9	0.0
KARI Tigoni	0.0	8.1	0.0	0.0	1.9	0.0
local market	0.0	0.0	1.7	0.0	9.6	0.0
ADC,KARI Tigoni	0.0	0.0	1.7	0.0	0.0	0.0
farmers (Kibirichia)	0.0	0.0	0.0	0.0	76.9	0.0
market, own	0.0	0.0	0.0	0.0	3.8	0.0

ADC= Agricultural Development Corporation
KARI= Kenya Agricultural Research Institute

All farmers sampled from Bomet Central and Longisa divisions grow red-skinned potatoes (Figure 2.3). Farmers from Elburgon and Molo divisions grow mainly the white-skinned varieties. Most farmers in Kibirichia and Abothuguchi West grow the red-skinned varieties.

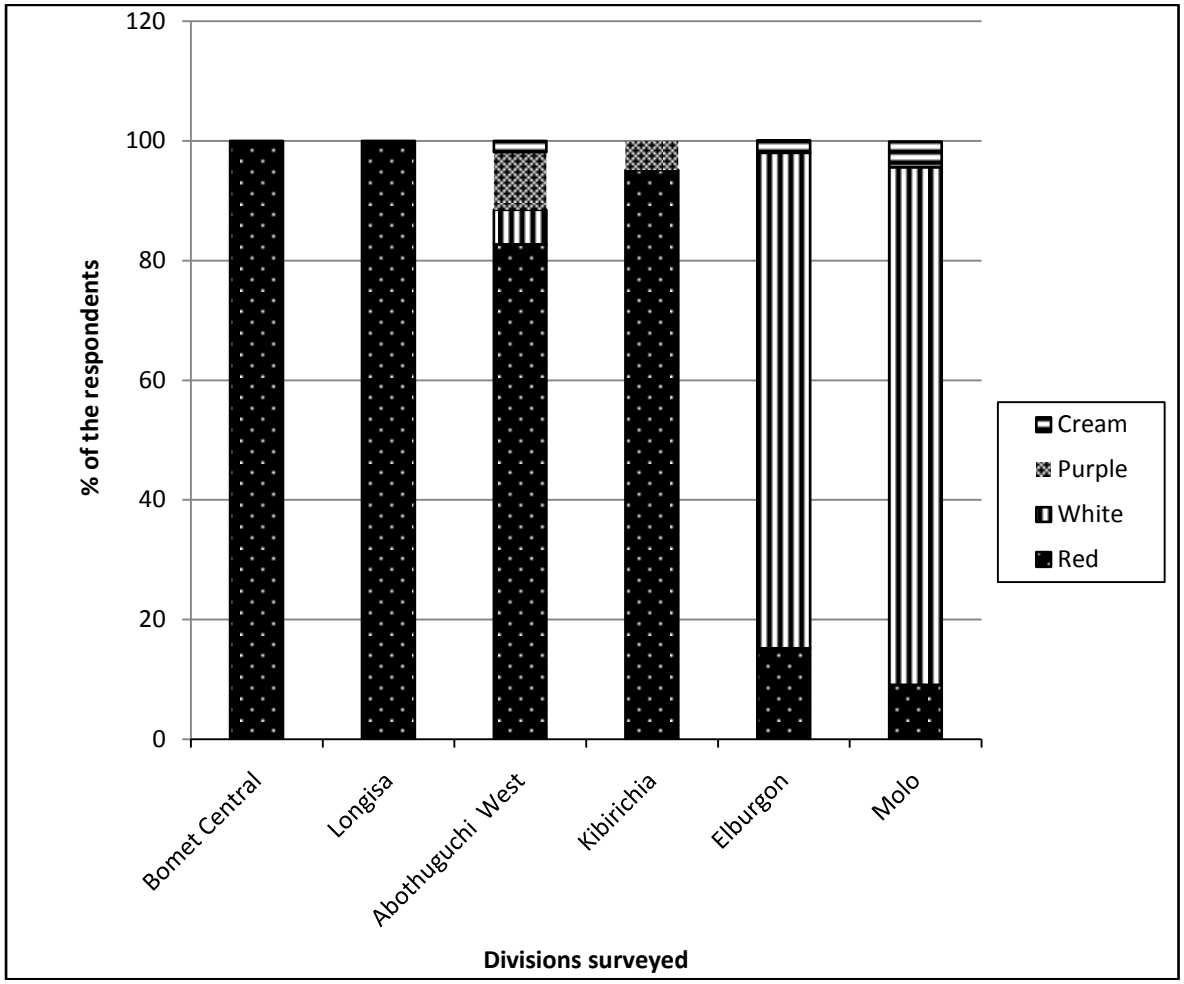

Figure 2.3. Skin colour of the potato cultivars grown by farmers in six divisions in Kenya

Most farmers across the districts grow white-fleshed potatoes (Figure 2.4).

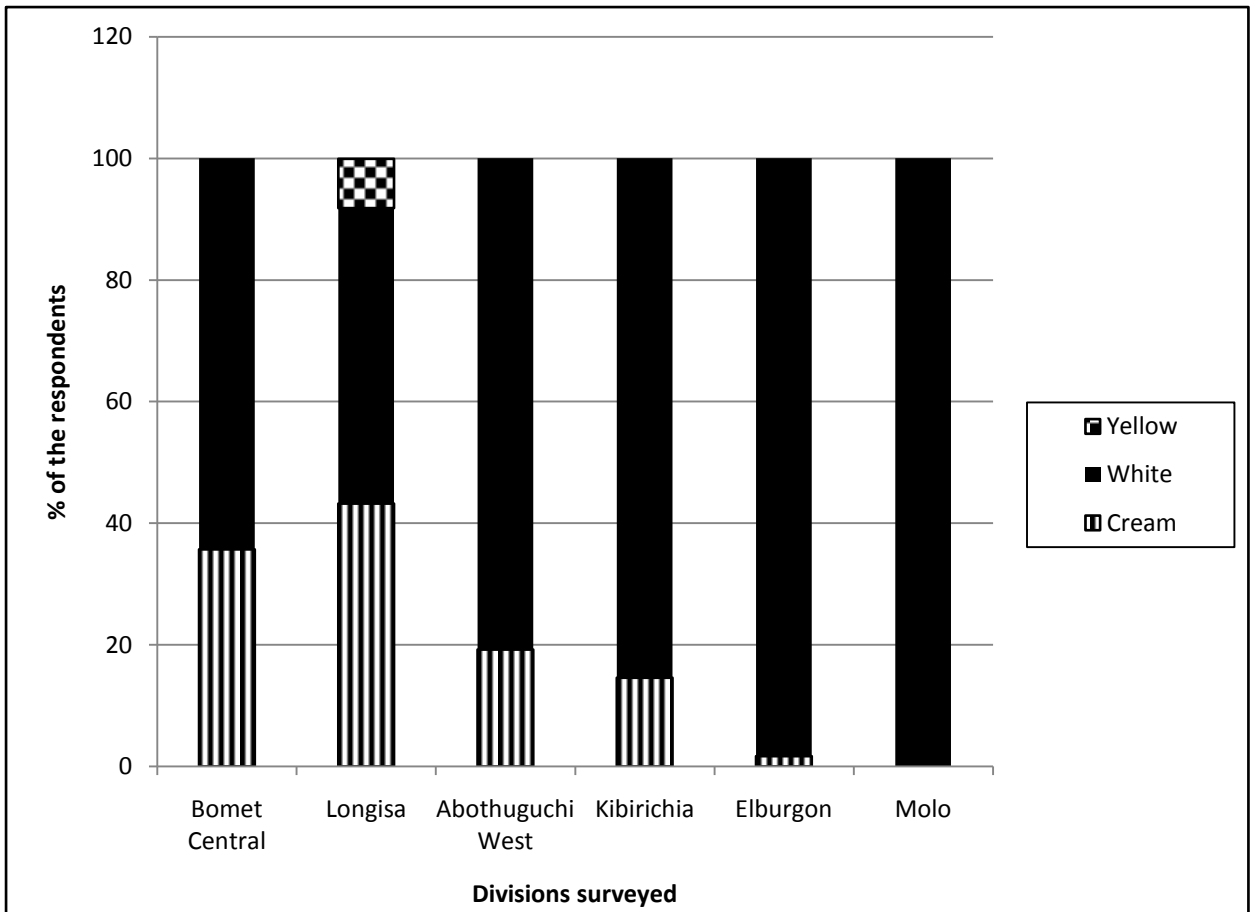

Figure 2.4. Flesh colour of the potato cultivars grown by farmers in six divisions in Kenya

All farmers in Bomet Central and almost all farmers in Longisa divisions grow the red-skinned Dutch Robyjn (Table 2.7). Their next popular variety is the red-skinned Desiree. In both Abothuguchi West and Kibirichia, the red-skinned Asante is grown by a majority of farmers followed by the white-skinned Tigoni. The white-skinned Cangi is the most popular in Elburgon and Molo divisions followed by the white-skinned Tigoni.

Table 2.7.Potato cultivars grown by farmers in six divisions in Kenya (% of the respondents)

Potato Cultivar	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
Dutch Robyjn	100.0	97.3	0.0	0.0	0.0	0.0
Desiree	26.2	16.2	0.0	0.0	1.7	0.0
Asante	2.4	2.7	88.5	82.9	10.3	0.0
Tigoni	9.5	5.4	59.6	41.5	44.8	26.1
Kenya Karibu	2.4	2.7	0.0	0.0	8.6	17.4
Cangi	0.0	2.7	0.0	2.4	96.6	100.0
Ngure	0.0	0.0	7.7	2.4	0.0	0.0
Kerr's pink	0.0	0.0	7.7	2.4	0.0	0.0
Kibururu	0.0	0.0	7.7	29.3	0.0	0.0
Kombiro	0.0	0.0	1.9	4.9	0.0	0.0
Arka	0.0	0.0	1.9	2.4	0.0	0.0
Komesha	0.0	0.0	0.0	2.4	10.3	17.4
Nyayo	0.0	0.0	0.0	0.0	13.8	8.7
Thimathuti	0.0	0.0	0.0	0.0	3.4	4.3

Market access is the most important factor considered by farmers in Bomet Central and Longisa divisions in deciding which potato cultivar to grow (Table 2.8). In all the other areas, high yield was the most important factor considered in the cultivar choice. Early maturity was considered an important factor by farmers from Elburgon and Molo divisions as it allows for more crop cycles per year. Early maturity and high yields are the main qualities that have made the variety Cangi very popular in these two divisions.

Table 2.8. Reasons given by potato farmers in deciding the potato cultivar to plant in six divisions in Kenya (% of respondents)

Reasons	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
drought tolerant	3.8	3.1	0.0	6.6	5.6	0.0
available market	44.3	54.7	1.4	8.2	27	32.7
high yielding	17.7	7.8	59.5	63.9	31	32.7
good taste	15.2	34.4	13.5	1.6	4.0	8.2
resists late blight	15.2	0.0	6.8	13.1	5.6	4.1
only available variety	3.8	0.0	0.0	3.3	0.8	2.0
matures early	0.0	0.0	14.9	3.3	25.4	20.4
long post-harvest storage	0.0	0.0	4.1	0.0	0.8	0.0

Generally, there was a high turnover of potato cultivars over the past five years (Table 2.9). About 34.5% farmers from Bomet Central and 32.0% from Longisa did

not change their favourite potato cultivar. Most farmers in Meru Central district abandoned the red-skinned Kerr's Pink and Ngure cultivars. Farmers from Molo district abandoned the white- skinned Nyayo cultivar.

Table 2.9. Potato cultivars abandoned by farmers over the past five years in six divisions in Kenya (% of respondents)

Abandoned varieties	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
Annett	6.9	0.0	0.0	0.0	0.0	0.0
Roslin Eburu	8.6	0.0	0.0	1.1	0.0	0.0
Tigoni	13.8	10.0	0.0	1.1	4.9	4.9
Desiree	8.6	20.0	5.3	3.4	13.2	14.6
Nyayo	3.4	4.0	6.3	2.3	24.3	22.0
Asante	1.7	2.0	1.1	1.1	2.1	0.0
Meru Mugaruro	10.3	0.0	0.0	3.4	3.5	4.9
K. Karibu	1.7	0.0	0.0	0.0	2.1	2.4
Arka	3.4	0.0	1.1	0.0	0.0	0.0
Kibururu	3.4	4.0	6.3	8.0	0.7	0.0
Cardinal	1.7	0.0	0.0	0.0	0.0	0.0
Kanongo	1.7	2.0	0.0	0.0	0.7	0.0
Othorongongo	0.0	4.0	0.0	0.0	0.0	0.0
Rangimbili	0.0	12.0	0.0	0.0	0.0	0.0
Kienyenji	0.0	8.0	0.0	0.0	0.0	0.0
Pimpernel	0.0	2.0	0.0	0.0	0.0	0.0
Kerr's pink	0.0	0.0	31.6	28.7	0.0	0.0
Roslin Tana	0.0	0.0	4.2	0.0	2.1	2.4
Ngure	0.0	0.0	24.2	33.3	0.0	0.0
Dutch	0.0	0.0	3.2	0.0	17.4	12.2
Munyiri	0.0	0.0	4.2	0.0	0.0	0.0
Komesha	0.0	0.0	1.1	2.3	0.7	2.4
Munyonge	0.0	0.0	1.1	0.0	0.0	0.0
Ntuka	0.0	0.0	1.1	0.0	0.0	0.0
Kombiro	0.0	0.0	0.0	3.4	0.0	0.0
Romano	0.0	0.0	0.0	3.4	0.0	0.0
Thimathuti	0.0	0.0	0.0	1.1	4.2	2.4
Karchi	0.0	0.0	0.0	2.3	0.0	0.0
Kiora	0.0	0.0	0.0	1.1	0.0	0.0
Ninty nine	0.0	0.0	0.0	1.1	0.0	0.0
Kihoro	0.0	0.0	0.0	0.0	10.4	14.6
Karoraiguru	0.0	0.0	0.0	0.0	1.4	0.0
Susana	0.0	0.0	0.0	0.0	1.4	0.0
Nderaciana	0.0	0.0	0.0	0.0	0.7	0.0
Mwezimoja	0.0	0.0	0.0	0.0	0.7	0.0
Baraka	0.0	0.0	0.0	0.0	0.0	2.4
None	34.5	32.0	9.5	2.3	9.7	14.6

Farmers who changed their popular potato cultivar in Bomet district mainly did so due to lack of market for the cultivars they had been growing (Table 2.10). Low yield was the main reason behind farmers in Meru Central and Molo districts rejecting some potato cultivars.

Table 2.10. Reasons given by farmers in six divisions in Kenya for rejecting some potato cultivars five years ago (% of respondents)

Reasons for rejection	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
lack of market	45.2	40.5	0.0	5.5	28.9	44.4
low yield	4.8	7.1	57.9	63.6	40.8	33.3
susceptibility to late blight	2.4	0.0	14.0	27.3	3.9	0.0
bad taste	0.0	9.5	0.0	0.0	0.0	0.0
late maturity	0.0	2.4	3.5	0.0	7.9	0.0
lack of seeds	0.0	2.4	7.0	0.0	0.0	0.0
poor post-harvest storage	0.0	0.0	1.8	0.0	0.0	0.0
none	47.6	38.1	15.8	3.6	18.4	22.2

2.3.3 Major potato marketing constraints

In all divisions surveyed, produce price fluctuation is the major marketing constraint (Table 2.11).

Table 2.11. Major marketing constraints encountered by potato farmers in six divisions in Kenya (% of respondents)

Marketing constraint	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
price fluctuations	45.2	40.5	19.2	17.1	41.4	17.4
poor roads	33.3	13.5	0.0	12.2	8.6	21.7
brokers	11.9	5.4	0.0	0.0	12.1	13.0
extended bag	2.4	21.6	7.7	9.8	36.2	26.1
lack of market	0.0	0.0	15.4	2.4	0.0	0.0
none	31.0	37.8	63.5	70.7	44.8	47.8

Farmers pack their potatoes in extended bags (Figure 2.5).

Figure 2.5. Extended potato bags used to pack potatoes in Kenya

2.3.4 Potato production constraints

Over 75% of the farmers in the surveyed divisions cited diseases as the main potato production constraint (Table 2.12). The high cost of fungicides and fertilizer was also mentioned as an important constraint. Lack of clean seeds and high seed costs were cited as production constraints by some farmers.

Table 2.12. Potato production constraints as cited by farmers in six divisions in Kenya (% of respondents)

Production Constraint	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
diseases	92.9	100	96.2	75.6	98.3	91.3
unpredictable rainfall	26.2	29.7	0.0	9.8	22.4	4.3
high fungicide costs	9.5	8.1	0.0	0.0	10.3	21.7
high fertilizer costs	16.7	21.6	34.6	51.2	27.6	21.7
lack of clean seeds	11.9	27	5.8	0.0	8.6	8.7
insect pests	2.4	2.7	7.7	24.4	5.2	4.3
high seed costs	0.0	2.7	19.2	12.2	1.7	0.0

Among the diseases, bacterial wilt is the most common in all divisions surveyed followed by late blight (Table 2.13). Despite 90% of farmers from Kibirichia using their own seed (Table 2.6), bacterial wilt prevalence is somehow lower than the other areas (Table 2.13).

Table 2.13. Major diseases affecting potato production in six divisions in Kenya (% of respondents)

Disease	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
*bacterial wilt	90.5	75.7	100.0	61.0	98.3	100.0
late blight	76.2	70.3	75.0	56.1	46.6	52.2
leaf rust	2.4	0.0	0.0	0.0	0.0	0.0
viruses	0.0	24.3	38.5	24.4	10.3	34.8
leafminer	0.0	0.0	5.8	0.0	0.0	0.0
nematodes	0.0	0.0	0.0	2.4	0.0	0.0
none	0.0	0.0	0.0	19.5	1.7	0.0

*= was directly observed in the fields. The other diseases were reported by the farmers during interview.

About 40% of all the farms visited in Kibirichia did not have bacterial wilt (Table 2.14). Most farms across all divisions had bacterial wilt incidence of 50% and below.

Table 2.14. Bacterial wilt incidence (%) in six divisions in Kenya(% of farms visited)

Bacterial wilt incidence (%)	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
0	9.5	24.3	0.0	39.0	1.7	0.0
1- 10	14.3	10.8	3.8	41.5	1.7	8.7
11-20	19.0	8.1	19.2	12.2	12	0.0
21-30	9.6	51.3	32.7	2.4	1.7	13
31-40	7.2	5.4	32.7	0.0	82.6	56.3
41-50	40.5	0.0	3.8	2.4	0.0	21.7
51-60	0.0	0.0	1.9	0.0	0.0	0.0
61-70	0.0	0.0	1.9	2.4	0.0	0.0
71-80	0.0	0.0	0.0	0.0	0.0	0.0
81-90	0.0	0.0	0.0	0.0	0.0	0.0
90-100	0.0	0.0	3.8	0.0	0.0	0.0

2.3.5 Management of bacterial wilt

In addition to crop rotation (Table 2.4), farmers use different methods in managing the disease in the field (Table 2.15). About 30% of the farmers surveyed in Molo division did nothing extra to control the disease (Table 2.15).

Table 2.15 Farmers' management of wilting plants in six divisions in Kenya (% of respondents)

Management of wilt	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
none	21.4	21.6	11.5	12.2	19	30.4
spray with fungicides	2.4	0.0	0.0	0.0	1.7	0.0
rogue and throw in a hole	14.3	2.7	7.7	4.9	0.0	0.0
rogue, throw in hole and bury	4.8	13.5	17.3	29.3	8.6	8.0
rogue and throw in a hole and burn, apply ash in the affected area	16.7	35.1	28.8	4.9	15.5	0.0
rogue and leave on the path	4.8	5.4	5.8	4.9	6.9	13.0
rogue and throw far away	16.7	5.4	7.7	0.0	41.4	43.5
rogue and feed cows	0.0	0.0	9.6	2.4	0.0	4.3
rogue and leave on the field	0.0	0.0	7.7	0.0	0.0	0.0
rogue, throw in a hole and burn	9.5	0.0	5.8	2.4	5.2	0.0
Not applicable	9.5	16.2	0.0	39	1.7	0.0

Over 15% of farmers in all divisions except Molo and Kibirichia manage the disease by uprooting and throwing the wilting plants and their tubers in a hole dug outside the field and burning them. They also remove the soil (from where the wilting plant has been uprooted) and throw it in the hole. Subsequently they apply two handfuls of ash in the place where the plant has been uprooted and mix it well with the soil (Table

2.15). After harvesting, the majority of the farmers in all the divisions surveyed (except Molo) throw the rotten tubers in a hole and bury (Table 2.16).

Table 2.16 Farmers' management of rotten tubers after harvesting potatoes in six divisions in Kenya (% of respondents)

Management of rotten tubers	Bomet Central	Longisa	Abothuguchi West	Kibirichia	Elburgon	Molo
throw in a hole	2.4	2.7	15.4	17.1	0	8.7
leave on the path	7.1	8.1	1.9	0	6.9	4.3
leave on the surface in the field	19	10.8	11.5	0	13.8	13
pile outside field and burn	7.1	2.7	11.5	7.3	1.7	0
throw in a hole and burn	2.4	2.7	0	2.4	0	0
throw in a hole and bury	52.4	45.9	44.2	29.3	29.3	8.7
feed cows	0	0	9.6	4.9	8.6	17.4
throw far away	0	10.8	7.7	2.4	13.8	26.1
pile outside farm and leave to rot	0	0	0	0	24.1	21.7
not applicable	9.5	16.2	0	39.0	1.7	0

A few farmers feed the rotten tubers to their animals. When the animals are given tubers, the uneaten infected tubers get mixed with manure; because most farmers use cattle manure in their fields, the disease is spread even further.

2.4 Discussion and conclusions

The study aimed at collecting information on potato production in Kenya, potato marketing and production constraints, cultivar preferences, and prevalence and management of bacterial wilt in Meru, Bomet, and Nakuru counties. Important information was gathered through individual interviews with farmers.

Molo district had the shortest history of potato production (Table 2.3). This could be attributed to the fact that most farmers are immigrants from other areas, mostly members of the Kikuyu community. The recent introduction of potato growing in Bomet district could be related to the establishment of a company which contracts farmers in this area to plant Dutch Robyjn for processing into chips and french fries. Bomet district is mainly a tea growing area where the good potato prices in recent years have lured farmers into potato farming.

There was a negative Spearman correlation (r=-0.295) between bacterial wilt incidence and altitude. This is to be expected because disease expression is favoured by high temperatures. However, this does not mean there are no bacterial

diseases; in the cold highlands the real danger is latent infection. A negative correlation (r=-0.354) between bacterial wilt incidence and altitude has previously been observed (Wakahiu et al., 2007). According to a previous study, the highest disease incidence was recorded in sites located 1800-2000 masl while the lowest incidence was observed in sites located over 2600 metres above sea level (Ateka et al., 2001).

There is a shortage of clean potato seed in Kenyaand farmers depend on informal seed sources which include farm-saved (self supply), local markets or neighbours. Due to limited supply, the certified potato seeds are highly priced (Ayieko and Tschirley, 2006). The informal system leads to use of poor quality seeds which often accelerates the spread of seed-borne diseases (Kinyua et al., 2001; Ng'ang'a et al., 2003).

Farmers allocate more than 25% of their farms to potatoes possibly due to its importance as cash and food crop. In Molo and Elburgon, the allocation is more than 45%. Wakahiu et al. (2007) found that farmers in Nyandarua County (another leading potato producer in Kenya) allocate about 50% of their farm to potato production. In Bomet district, farmers allocate less land to potatoes possibly because they grow tea; another lucrative cash crop. In addition, potatoes do not feature prominently in the diets of the local community. In contrast, potatoes are a major component of the diets of the local communities in Meru Central and Molo districts (McArthur, 1989).

Generally, farmers plant potatoes every second rainy season (Table 2.4). This is probably due to small farm parcels, limited choices of alternative crops as a result of unpredictable weather especially rainfall, and economic considerations due to a short potato growth period. However, this rotation is too short for proper management of soil fertility and plant diseases especially bacterial wilt. Wakahiu et al. (2007) found that 68.8% of farmers in Nyandarua practice a one season rotation. Furthermore, some farmers in the same county plant potatoes for 3-4 seasons consecutively.

In addition to potatoes, farmers grow other crops probably to meet various uses as well as hedge against the risk of crop failure. This was also reportedby McArthur (1989) and Kaguongo et al. (2008). Among the crops, maize is grown by a majority

of farmers in all divisions surveyed. In this study, it was found that taste, yields and availability of market are the major factors determining potato cultivars grown in an area. This is in agreement with previous studies by Wakahiu et al. (2007). In another study, farmers in the main potato growing counties in Kenya ranked high yields as the most important criterion for growing a specific cultivar (Ng'ang'a et al., 2003).

There are regional differences in potato cultivars grown (Table 2.7). All farmers in Bomet Central and almost all farmers in Longisa divisions grow the red-skinned Dutch Robyjn. Wakahiu et al. (2007), Kaguongo et al. (2008) and Kaguongo et al. (2010) also found that farmers in these divisions grew Dutch Robyjn. This could be due to the specific processing market that farmers in this area supply. Kaguongo et al. (2010) found that the most commonly grown potato cultivar in Kenya was Tigoni (cultivated by 25.7% of farmers) followed by Nyayo (cultivated by 24.8% of potato farmers) and then Thima thuti (22.7% of farmers). In addition, Tigoni was most popular in Nakuru County (grown by 61.9% of potato farmers and occupying 43.2% of potato area while Nyayo was grown by 37.1% of farmers on 16.3% of potato area in the same county. Tigoni and Nyayo are white-skinned and white-fleshed. The two have since been overtaken by Cangi (a white-skinned white-fleshed farmer selection) (Table 2.7). In Meru Central district, most farmers abandoned the red-skinned Ngure and Kerr's Pink (Table 2.9) for the equally red-skinned Asante (Table 2.7). It appears that despite changing the varieties, farmers did not change the skin colour. This indicates that market demand for a certain skin colour strongly affects variety choice.

Among the potato marketing constraints, price fluctuation is the most important (Table 2.11). Price fluctuations are due to seasonality in potato production leading to glut and lean times. Most farmers produce potatoes twice a year due to bimodal rainfall patterns in most potato growing areas (McArthur, 1989; Kinyae et al., 2004). The potato growers lack the ability to influence selling prices for their produce because of the poor keeping quality of potatoes and lack of adequate on-farm storage facilities. Over 80% of locally marketed potatoes go through brokers who shield the farmers from getting market information and in the process exploit them. In addition, potatoes are packed in extended bag (Figure2.5). Traders buy potatoes on per bag basis and not on weight basis thereby exploiting farmers even further. At the market, however, the traders sell the potatoes in smaller containers such as normal

sized bags or buckets. Therefore, an extended bag is advantageous to the trader but exploitative to the farmers.

In the potato producing districts most of the access roads are impassable during wet season. This results in high transportation costs of the produce and a lowering of farm-gate prices by the traders as soon as the rains begin.

Among the production constraints, diseases are the most important. Bacterial wilt is the most common disease in all divisions surveyed followed by late blight (Table 2.13). These findings are in agreement with previous studies by Kaguongo et al. (2010) who found that bacterial wilt is common in all potato growing areas of Kenya affecting 77% of potato farms followed by late blight (67%), and viral diseases (12%). The high prevalence of bacterial wilt in the potato growing areas can partly be due to planting of seeds from informal sources as well as inadequate rotation. Most farmers use seeds from informal sources (Table 2.6) partly due to high cost of certified seeds and/or lack of seeds (Ayieko and Tschirley, 2006). The informal system leads to use of poor quality seeds and often accelerates the spread of seed-borne diseases (Ng'ang'a et al., 2003). This, in addition to lack of effective control method makes bacterial wilt a major headache to small-scale potato farmers in Kenya.

Although most farmers practice some form of crop rotation (Table 2.4), the cycle is often too short to eliminate bacterial wilt inoculum in the soil. In addition, farmers leave volunteer potato plants thereby rendering rotation irrelevant. According to Gildemacher et al. (2007), a crop rotation sequence where potatoes are grown once in every four seasons is required so long as no other Solanaceous crop is grown. However, in most potato growing areas in Kenya there is not enough land for such a long rotation (Riungu, 2011). In addition to a suitable crop rotation scheme, removal of volunteers is extremely important (Gildemacher et al., 2007; The Organic Farmer, May 2012).

Some farmers manage bacterial wilt by uprooting and throwing the wilting plants and their tubers in a hole dug outside the field and burning them. They also remove the soil (from where the wilting plant has been uprooted) and throw it in the hole. Subsequently they apply two handfuls of ash in the place where the plant has been uprooted and mix it well with the soil (Table 2.15). This bacterial wilt management strategy is currently being promoted by KARI. Ashes and lime are known to suppress

the bacteria probably by raising the soil pH (Gildemacher et al., 2007). In addition, ashes have the added advantage of containing nutrients such as potassium and phosphorus. There is no rule on the exact amounts to be applied; one handful of lime or two handfuls of ashes can be used as a maximum dose per plant (Gildemacher et al., 2007).

The PRA study has provided an insight into potato production in the Kenyan highlands. Most of the farmers are small scale and grow other crops in addition to potatoes. Potatoes are grown for both cash and food. There are regional differences in cultivars planted by farmers; cultivar preferences are mostly dictated by availability of market, yields and taste. Bacterial wilt is a major production constraint; this is managed through many cultural methods including crop rotation. However, all these methods have not been effective; there is need to breed for host resistance.

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Chapter Three: Response of potato genotypes to bacterial wilt disease in the tropical highlands of Kenya

Abstract

The use of potato varieties resistant to bacterial wilt disease caused by *Ralstonia solanacearum* (Smith, 1896) (Yabuuchi et al., 1995) is probably the best management option of the disease. Because of strong host-pathogen-environment interaction, screening the potential parents for resistance under the target growing environmental conditions is the first important step for effective resistance breeding. The objective of this study was to determine the response to bacterial wilt of selected potato genotypes currently grown by farmers in the tropical highlands of Kenya and candidate clones from CIP. The study was carried out for three consecutive seasons between November 2011 and February 2013. Thirty six potato genotypes were established on an inoculated field at Kenya Agricultural Research Institute, National Agricultural Research Laboratories (KARI-NARL) using an alpha lattice experimental design with three replications. Data collected included, days from planting to onset of wilting (DTOW), area under the disease progress curve (AUDPC), total tuber weight (t ha^{-1}) (TTW), total tuber numbers/ha (TTN), proportion of ware sized tubers (PWTTW), proportion of symptomatic tubers based on weight (PSTTW), proportion of symptomatic tubers based on tuber numbers (PSTTN) and latent infection (LI) of the tubers. All the genotypes were generally susceptible; susceptibility ranged from moderate to high. The potato genotypes varied in their susceptibility to bacterial wilt and the most resistant genotypes were Kenya Karibu followed by Kenya Sifa. The study identified eight potato genotypes (Meru Mugaruro, Ingabire, Kenya Karibu, Sherekea, Kihoro, Tigoni, Bishop Gitonga and Cangi) to be used in a breeding programme to improve bacterial wilt resistance in Kenyan germplasm. The chosen genotypes are prolific in pollen production and are widely grown by potato farmers in Kenya.

Keywords: Bacterial wilt, Genotype, Potato, *Ralstonia solanacearum*, Resistance breeding

3.1 Introduction

Potato (*Solanum tuberosum* L., 2n=4x=28) production in Kenya has not achieved its full potential because of a number of production constraints. These include low soil

fertility, inadequate supply of certified seed, use of unimproved low yielding varieties, and diseases. The most common diseases in the country include late blight, viral infections and bacterial wilt (Kaguongo et al., 2008).

Bacterial wilt, caused by *Ralstonia solanacearum* (Smith 1896) (Yabuuchi et al., 1995), is the second most important potato disease after late blight locally and globally (Kaguongo et al., 2008). The disease has been estimated to affect about 1.7 million ha in approximately 80 countries worldwide, with global damage estimates of over USD 950 million per annum (Champoiseau et al., 2009). Reportedly, bacterial wilt caused yield losses between 50 and 100% in Kenya (Kaguongo et al., 2008). There are no suitable control measures of bacterial wilt as both crop protection chemicals (Champoiseau et al., 2010) and the biological control agents are ineffective (Smith et al., 1998). In addition, phytosanitary methods such as quarantine are either expensive or difficult to apply (Martin and French, 1985; Muthoni et al., 2010), and cultural methods such as crop rotations are largely impractical because the farms are too small to allow effective rotation, the pathogen has a wide host range, and it persists in the soil over a long period (Kaguongo et al., 2008).

Development of resistant cultivars could therefore be the best option for managing the disease. However, there are no known potato cultivars that are resistant to bacterial wilt. Cultivars such as Cruza 148 and Molinera have been found to have some degree of tolerance to bacterial wilt although they still transmit latent infection to their clonal progeny (French, 1994). In addition, the resistance has been shown to be very unstable due to strong host-pathogen-environment interaction (French and Lindo, 1982; Tung et al., 1990; Tung, 1992; Tung et al., 1992a). A pathogen race at one location may overcome the resistance effective at another location (Grimsley and Hanson, 1998) and more than one race may occur in a given field (Martin and French, 1985). It is therefore essential to screen the germplasm in the target production environment to identify well adapted, resistant clones which can be used as parents in a breeding programme (Martin and French, 1985).

Locally acceptable cultivars with good resistance to bacterial wilt are yet to be identified in Kenya (Ateka et al., 2001). Resistant potato clones have been identified by the International Potato Center (CIP) and this resistance could be incorporated into the popular but susceptible Kenyan potato cultivars. Screening the clones for

resistance under local environmental conditions is the first important step for effective resistance breeding. This study was therefore carried out to determine the response of the potato genotypes currently grown by farmers in Kenya as well as other clones from CIP to bacterial wilt in order to identify parents that can be used in a local breeding programme to develop resistant cultivars.

3.2 Materials and methods

3.2.1 Description of the study site

The experiment was carried out at the Kenya Agricultural Research Institute, National Agricultural Research Laboratories, (KARI-NARL). The station is located 7 km northwest of Nairobi at an altitude of 1795 meters above sea level, latitude of $1^0$15' 31.64" S and longitude 36^0 46' 17. 96" E (Jaetzold et al., 2006c). The average annual rainfall is 1295 mm with a bimodal distribution. A long rainy season occurs between March and May while a short rainy season is between October and December (Jaetzold et al., 2006c). The mean air temperature ranges from 13.3 to 22.9^0C. The soil type is humic-nitosol (alfisol) derived from quartz trachyte (UNESCO, 1977) and is locally referred to as the Kikuyu Red Clay. The experiment was carried out for three consecutive seasons i.e. 11th November 2011 to 24th February 2012 (first season), 7th April 2012 to 15th August 2012 (second season), and 16th October 2012 to 8th February 2013 (third season).

3.2.2 Field layout, bacterial wilt inoculation and crop management

Thirty six bacterial wilt free potato genotypes were obtained from the Kenya Agricultural Research Institute, National Potato Research Centre at Tigoni (KARI-Tigoni). The list and sources of the potato genotypes used in the study are described in Table 3.1.

Table 3.1 List and sources of potato genotypes used in the study

Genotype	Source/pedigree	Year of release
Desiree	The Netherlands	1972
Tigoni	CIP	1998
Kenya Sifa	CIP	2002
Kihoro	Farmers' variety	-
Meru Mugaruro	Farmers' variety	-
Nyayo	Farmers' variety	-
Ingabire	CIP	1998
Roslin Tana	Scotland	1974
Kenya Baraka	Scotland	1973
Kenya Furaha[1]	CIP	1998
393385.57	CIP	Not yet released
Tigoni Long[1]	Farmers' variety	-
Arka	The Netherlands	-
Kerr's Pink	Scotland	1927
Dutch Robyjn	The Netherlands	1945
Roslin Bvumbwe	Scotland	1974
Sterling		-
Bishop Gitonga	Farmers' variety	-
Annete	Germany	1972
Purple Gold	CIP	2010
Pimpernel	The Netherlands	-
Kenya Mpya	CIP	2010
B53	Scotland	1953
Sherekea	CIP	2010
Ngure[1]	Farmers' variety	-
Asante	CIP	1998
Kenya Mavuno	CIP	2002
Saturna[1]	Germany	-
396286.6	CIP	Not yet released
394906.6	CIP	Not yet released
387164.4	CIP	Not yet released
394903.3	CIP	Not yet released
394034.7	CIP	Not yet released
394905.8	CIP	Not yet released
394895.7	CIP	Not yet released
394904.17	CIP	Not yet released
Cangi[2]	Farmers' variety	-
Romano[2]	The Netherlands	-
Kenya Karibu[2]	CIP	2002
393382.44[2]	CIP	Not yet released

[2]= Not included in the first season. [1]= Not included in the second and third seasons. - Not available

The same genotypes were used in the second and third seasons; in the first season, four genotypes were different. The same field was used for three consecutive seasons; randomization was different for each season. The experimental design was an alpha lattice with four blocks each having nine plots with three replications. Each genotype was planted in four rows, and spacing was 75cm (inter-row) and 30cm

(intra-row). Di-ammonium phosphate (18% N: 46% P_2O_5) fertilizer was applied at the rate of 500 kg ha^{-1} in furrows before planting.

To ensure uniform inoculum distribution, a susceptible tomato cultivar, Moneymaker, was transplanted in the field at a spacing of 30cm x 60cm. Two weeks after transplanting, the tomato plants were inoculated by spraying a bacterial suspension (3.0 x 10^9cfuml^{-1}) at the base of each stem. About six weeks after inoculation, when at least 80% of the plants had wilted, the tomato plants were incorporated into the soil and the first evaluation trial planted. In the second and third seasons, a bacterial suspension concentrated at 3.0 x 10^9cfuml^{-1} was poured into the planting furrows (during planting of potato tubers but before covering them) at a rate of 400ml per plot to boost the inoculum concentration in the soil. The resident as well as inoculated bacteria were confirmed as biovar 2 by Plantovita, Lynn East, South Africa based on the ability of the bacteria to produce acid from several disaccharides and sugar alcohols (Buddenhagen and Kelman, 1964). Weeding and other cultural management were carried out according to recommendations for potato production in Kenya. For proper disease expression, supplemental watering using overhead irrigation was done during the dry times to avoid drought stress.

3.2.3 Data collection

The potato plants were first scored for wilt symptoms 30 days after planting and thereafter every 10 days. At each evaluation date, all the wilting plants on each plot were counted and expressed as a percentage of all the plants in the plot to give the bacterial wilt incidence (BWI). Final BWI score was taken at 120 days after planting. The BWI scores were used to calculate the area under the disease progress curve (AUDPC) (CIP, 2007) using the formula below:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{(S_i + S_{i+1})(t_{i+1} - t_i)}{2}$$

Where S_i is the BWI at days*i*, and *n* is the total number of sampling times, *t* is the number of days after planting.

Other data collected were days from planting to onset of wilting (DTOW). In each season, populations of *R. solanacearum* in the soil were determined three times using the modified semi-selective media from South Africa (SMSA) (Englebrecht, 1994). This was done before inoculating the field, at 60 days after planting and after

harvesting the potato crop. At each sampling time, eight soil samples were evenly collected from each replicate. From each soil sample, 10g were put in a sterile conical flask and 30ml of sterile distilled water added. This was mixed thoroughly for 30 minutes and then allowed to stand for 5 minutes. Thereafter, 1ml was drawn from the supernatant solution using a micro-pipette and put in a sterile Eppendorf tube to form the stock solution (10^0). From the stock solution, 0.1ml was drawn and put in sterile Eppendorf tube which already contained 0.9 ml of sterile distilled water. This formed the first dilution of the stock solution (10^{-1}). This serial dilution was continued upto 10^{-3}. From 10^{-3} dilution, 0.1ml of the suspension was drawn and plated on semi-selective media for *R. solanacearum*. The plates were incubated at 30^0C for 48 hours after which the bacterial colonies were counted. This was done in duplicate and the mean numbers of bacterial colonies were recorded.

Harvesting of potato tubers was done when the latest maturing genotype had reached 75% senescence. During harvesting, the six middle plants per plot were harvested, each plant separately. Total number of tubers was counted from each of the six plants. In addition, the number of symptomatic tubers (i.e. showing rotting or bacterial ooze in the tuber eyes or soil adhering to the eyes of the tubers) and healthy looking tubers (asymptomatic) were determined. The healthy looking tubers were then categorized based on size i.e. ware (>45mm diameter) and, seed and chatts (45>mm diameter). Their number and weights were recorded. The weights of symptomatic and ware tubers were expressed as percentage of the total yields. The percentage of symptomatic tubers was expressed both in weight, a value which is useful to determine yield losses (t ha^{-1}), and as a number of infected tubers, a value which is used for the calculation of infection tuber rates.

Only healthy-looking tubers selected above were analyzed for latent infection by *R. solanacearum.* For each plot, thirty healthy-looking tubers were placed in sugar paper and delivered to the laboratory for latent infection analysis. The tubers were washed and disinfected. They were then divided into five groups of six tubers each. Each group was extracted to constitute a composite sample which was then analyzed for latent infection using the post-enrichment enzyme-linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA) test as described by Priou et al. (1999a).

3.2.4 Data analysis

Data on soil bacterial count (SBC), days to onset of wilting (DTOW), AUDPC, total tuber numbers (TTN), total tuber weight in t ha^{-1}(TTW), percentage of symptomatic tubers based on total tuber numbers (PSTTN), percentage of symptomatic tubers based on total tuber weight (PSTTW), and percentage of ware sized tubers based on total tuber weight (PWTTW) values were subjected to analysis of variance using Genstat statistical package, 14th edition (Payne et al., 2011). Data on TTN, TTW, PWTTW, PSTTN and PSTTW were first averaged on a plot basis; the average value was then used to extrapolate values per ha. The total tuber weight (TTW) was given in t ha^{-1}. Where analysis of variance showed significant differences, mean separation was done using Fisher's protected LSD (Steel and Torrie, 1980). Data on latent infection (LI) level were subjected to the Kruskal-Wallis non-parametric test procedure using SPSS for Windows Release Version 18.0 (SPSS Inc., 2009). Data for different seasons were analyzed separately. Potato genotypes were also ranked based on % latent infection (% LI), final BWI, DTOW, TTN, TTW, PWTTW, PSTTW and PSTTN. Genotypes with low % LI, low final BWI, low PSTTW low PSTTN, more DTOW, high TTN, high TTW and high PWTTW were considered better and hence ranked high. Resistance of genotypes to bacterial wilt was determined using ranking based on % LI, AUDPC, DTOW, PSTTW and PSTTN. Genotypes with low % LI, low AUDPC, low PSTTW, low PSTTN and more DTOW were more resistant to bacterial wilt and hence ranked high. The percentage of total infected tubers (PTIT) was calculated taking into account the PSTTN and % LI. The PTIT was calculated as suggested by CIP (2007):

$$\text{PTIT} = \text{PSTTN} + \frac{(\%\ \text{healthy looking tubers x}\ \%\ \text{LI})}{100}$$

Where PTIT is the percentage of total infected tubers, PSTTN is the percentage of symptomatic tubers based on total tuber numbers and % LI is the % latent infection.

3.2.5 Selection of bacterial wilt resistant genotypes

The resistance of the potato genotypes to bacterial wilt was described using two criteria:

1) Using ranking based on % LI, AUDPC, DTOW, PSTTW and PSTTN. Small values of % LI, AUDPC, PSTTW and PSTTN as well as, high values DTOW indicate high resistance.

2) Using the percentage of total infected tubers (PTIT) (Table 3.2). Small values of PTIT indicates high reistance and hence high ranking.

Table 3.2 Reaction of potatoes to bacterial wilt based on PTIT

Resistance levels	Percentage of total infected tubers
Highly resistant	0
Resistant	1<15
Moderately resistant	15- <30
Moderately susceptible	30- <45
Susceptible	45- <60
Highly susceptible	≥60

Modified from CIP(2007)

3.3 Results

3.3.1 Weather data

The second season experienced much higher rainfall and slightly lower temperatures than the first season (Table 3.3). This was expected because the second season coincided with the long rains season (March-June) while the first season coincided with the short rains season (October-December). The third season experienced much higher temperatures than the first two seasons.

Table 3.3. Total rainfall (mm) and mean air temperatures (^{0}C) of the experimental site during the study period

	2011			2012											2013	
Month	Oct.	Nov.	Dec	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Total rainfall (mm	154.4	351	96.2	9.4	75.6	49.1	686.6	3746.6	456	26	96.8	33.5	416.1	252.1	289.4	89.2	6
No. of rainy days	16	12	4	1	3	3	21	23	11	3	2	2	12	15	12	7	2
Mean air temp (^{0}C).	16.8	17.8	18.4	19.4	16.9	19.7	18.0	16.2	14.9	14.8	16.24	23.1	24.6	23	22	23.1	25.2
Seasons	I			II								III					

3.3.2 Soil bacterial counts

There were significant differences (P≤0.01) in soil bacterial counts between seasons, among sampling times and in the seasons x sampling time interaction (Table 3.4). The third season had the highest number of soil bacteria counts followed by the second season while the first season had the least (Table 3.5).

Table 3.4. Analysis of variance on colony forming units (cfu) per gram of soil sampled during the three seasons at KARI-NARL

Source of variation	df	Fpr.
Block	2	
Season	2	<.001**
Sampling time	2	<.001**
Season * Sampling time	4	<.001**
Residual	205	
Total	215	

** = Significant at P≤0.01

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Table 3.5. Mean colony forming units (cfu) per gram of soil sampled during the three seasons at KARI -NARL

Sampling time	Season I	Season II	Season III	Mean
Before planting	855000 a	832500 a	1936250 a	1207917 a
60 days after planting	3352500 c	5361250 c	5556667 b	4756806 c
After harvesting	1373750 b	1490000 b	1568333 a	1477361 a
Mean	1860417 a	2561250 b	3020417 c	2480694
LSD(0.05) for Seasons = 294213.1				
LSD(0.05) for Sampling time= 294213.1				
LSD(0.05) for Seasons * Sampling time =509592.0				

Within each column, means followed by the same letter are not significantly different at P≤ 0.05

3.3.3 Bacterial wilt incidence and tuber traits

Genotypes exhibited significant differences (P≤0.05) in total tuber number per ha (TTN) and total tuber weight (TTW) (t ha^{-1}) in the first season (Table 3.6). There were no sisgnificant differences among potato genotypes in terms of percentage of

symptomatic tubers (PSTTN and PSTTW) as well as percentage of ware-sized tubers (PWTTW). In the second season, there were significant differences among the genotypes for all the five characters. In the third season, only TTN was not significant (Table 3.6). On average, the second season had the highest yields (TTW) (Table 3.8) followed by the third season (Table 3.9) while the first season had the least (Table 3.7). The PWTTW followed the same trend. There were significant differences ($P \leq 0.05$) among genotypes for latent infection (Chi-square=67.7; df=40). The mean % LI was higher in the first season than in the other two seasons (Tables 3.7, 3.8 and 3.9).

The AUDPC and DTOW were significantly different among potato genotypes in the first and third seasons (Table 3.6). For most genotypes, percentage wilting increased rapidly from 60 days after planting and levelled off at 90-100 days after planting (Figures 3.1, 3.2 and 3.3).

Table 3.6. Analysis of variance for some traits of 36 potato genotypes planted at KARI-NARL for three consecutive seasons

Source of variation	df	DTOW		AUDPC		TTN		PSTTN		TTW		PSTTW		PWTTW	
		MS	Fpr.	MS	Fpr.	MS	Fpr.	MS	Fpr.	MS	Fpr.	MS	Fpr.	MS	Fpr.
							Season I								
Block	2	295.85		2037682		2.633E+10		103.6		953.15		65.1		165.8	
Genotype	35	142.57	0.011*	956862	<.001**	7.526E+09	<0.001**	116.7	0.379	80.95	0.014*	225.1	0.046*	123.6	0.244
Residual	70	74.93		330975		2.348E+09		107.7		43.41		140.1		102.0	
							Season II								
Block	2	41.61		3767308		2.759E+09		15673.4		479.6		12010.7		5668.0	
Genotype	35	48.22	0.738	513482	0.057	8.704E+09	0.616	247.0	0.131	163.8	0.561	279.9	0.191	266.2	0.210
Residual	70	58.84		328677		9.589E+09		180.0		173.1		212.6		212.6	
							Season III								
Block	2	123.15		971864		4.401E+09		4564.0		487.4		3693.6		3227.5	
Genotype	35	248.78	<.001**	4197905	0.026*	9.076E+09	0.108	656.3	<.001**	182.2	0.017*	572.4	<0.001**	348.8	0.035*
Residual	70	66.96		2429912		6.407E+09		151.1		100.5		187.8		208.8	

df=Degrees of freedom; *= Significant at P≤0.05; **= Significant at P≤0.01; MS=Means squares; Fpr= F probability; DTOW= Days to onset of wilting; AUDPC= Area under the disease progress curve; TTN=Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); TTW= total tuber weight (tha^{-1}); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in $t\ ha^{-1}$); PWTTW= Percentage of ware sized tubers (% of total tuber weight in $t\ ha^{-1}$)

Table 3.7.Mean response and ranks among 36 potato genotypes for some agronomic and bacterial wilt resistance parameters ¨ during the first season

GENOTYPE	DTOW		PWTTW		TTW		PSTTW		TTN		PSTTN		AUDPC		LI	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	%	Rank
Kenya Baraka	60.0	1.0	30.0	7.0	39.7	12.5	29.6	4.0	330861	8.0	33.3	6.0	1355	2.0	50.0	15.5
Tigoni Long	56.7	4.0	22.7	20.5	37.0	24.0	37.8	21.5	31851	36.0	31.4	4.0	2250	25.0	33.3	3.5
Kenya Mavuno	56.7	4.0	19.8	31.0	41.3	9.5	33.6	12.0	335799	6.0	30.5	3.0	1845	14.0	53.3	20.5
Sterling	56.7	4.0	22.9	19.0	33.3	31.0	50.7	32.0	224689	34.0	46.9	32.0	2350	28.0	53.3	20.5
393385.57	56.7	4.0	21.5	25.0	38.7	17.0	38.3	23.0	325923	9.0	36.5	9.5	1405	3.0	60.0	28.5
Meru Mugaruro	56.7	4.0	34.5	2.0	41.3	9.5	31.7	8.0	308639	15.5	36.5	9.5	1770	7.0	66.7	34.5
394905.8	54.1	7.0	23.0	18.0	29.8	34.0	35.8	17.0	224694	33.0	40.0	19.5	2310	26.0	60.0	28.5
Kihoro	53.3	10.0	26.0	11.0	47.0	3.0	39.9	26.0	358021	3.0	43.8	29.0	2750	31.0	40.0	8.0
394903.3	53.3	10.0	28.7	9.0	52.3	1.0	30.1	5.0	437033	1.0	36.1	7.0	1860	15.0	60.0	28.5
394906.6	53.3	10.0	20.4	29.0	29.7	35.0	52.3	33.0	234566	31.5	50.8	33.0	1475	4.0	53.3	20.5
Nyayo	53.3	10.0	21.5	25.0	37.3	22.5	32.9	9.0	348144	4.0	36.9	13.0	2335	27.0	53.3	20.5
Asane	53.3	10.0	22.7	20.5	37.3	22.5	37.4	18.0	264195	24.0	52.8	34.0	2510	30.0	46.7	13.0
Desiree	50.0	14.5	33.0	5.0	33.3	31.0	30.5	6.5	259257	26.0	32.3	5.0	1785	9.0	40.0	8.0
Kenya Sifa	50.0	14.5	22.2	23.0	39.3	14.5	20.9	2.0	338268	5.0	29.7	2.0	1820	12.5	33.3	3.5
Purple Gold	50.0	14.5	23.4	16.0	38.3	19.5	37.8	21.5	298763	19.0	36.5	9.5	1995	28.0	53.3	20.5
Sherekea	50.0	14.5	23.1	17.0	31.7	33.0	30.5	6.5	256787	28.0	45.1	30.5	1820	12.5	60.0	28.5
394034.7	47.3	17.0	23.8	15.0	38.5	18.0	37.7	19.5	295677	20.0	40.0	19.5	1773	8.0	0.0	1.0
396286.6	46.7	19.0	20.0	30.0	28.4	36.0	54.5	34.0	178266	35.0	54.0	36.0	1245	1.0	40.0	8.0
B53	46.7	19.0	24.2	14.0	43.7	6.0	47.6	31.0	311108	13.5	41.0	26.0	2040	19.0	46.7	13.0
Kenya Furaha	46.7	19.0	30.7	6.0	43.3	7.0	35.1	15.0	316046	10.5	38.5	14.0	1895	27.0	66.7	34.5
Kenya Mpya	43.3	23.5	21.2	25.0	36.7	25.0	37.7	19.5	256788	27.0	40.2	21.5	1810	11.0	46.7	13.0
Saturna	43.3	23.5	20.6	27.5	33.7	28.5	34.1	13.0	274071	23.0	40.7	23.0	3445	35.0	60.0	28.5
Kerr's Pink	43.3	23.5	17.0	33.0	44.0	5.0	33.4	11.0	333330	7.0	43.1	27.5	2075	20.0	53.3	20.5
Bishop Gitonga	43.3	23.5	22.6	22.0	39.0	16.0	26.2	3.0	313577	12.0	40.9	24.5	2940	33.0	40.0	8.0
Arka	43.3	23.5	28.3	10.0	38.3	19.5	39.6	25.0	293824	21.0	43.1	27.5	1595	5.0	60.0	28.5
Roslin Tana	43.3	23.5	25.4	13.0	40.0	11.0	34.7	14.0	303701	18.0	40.9	24.5	1795	10.0	53.3	20.5

394904.17	40.0	30.0	29.6	8.0	33.3	31.0	43.2	29.0	254319	29.0	40.2	21.5	1870	26.0	40.0	8.0
Pimpernel	40.0	30.0	35.5	**1.0**	44.7	4.0	40.2	27.0	288886	22.0	39.7	18.0	3400	34.0	50.0	15.5
Annete	40.0	30.0	33.2	4.0	35.7	28.5	40.5	28.0	261726	25.0	53.2	35.0	2470	29.0	53.3	20.5
Ingabire	40.0	30.0	13.3	34.0	41.7	8.0	33.0	10.0	308639	15.5	36.5	9.5	2205	24.0	60.0	28.5
Dutch Robyjn	40.0	30.0	12.6	35.0	39.7	12.5	43.4	30.0	316046	10.5	39.3	17.0	2910	32.0	40.0	8.0
Tigoni	40.0	30.0	25.7	12.0	39.3	14.5	38.4	24.0	311108	13.5	39.0	16.0	3655	36.0	20.0	2.0
Ngure	40.0	30.0	18.2	32.0	36.3	26.0	57.9	36.0	306170	17.0	38.8	15.0	2185	23.0	66.7	34.5
387164.4	36.8	34.0	5.5	36.0	48.3	2.0	20.7	**1.0**	385243	2.0	29.4	**1.0**	2150	22.0	60.0	28.5
Roslin Bvumbwe	36.7	35.5	34.1	3.0	37.7	21.0	35.5	16.0	237035	30.0	36.8	12.0	2130	21.0	40.0	8.0
394895.7	36.7	35.5	20.6	27.5	36.0	27.0	54.7	35.0	234566	31.5	45.1	30.5	1665	6.0	66.7	34.5
Mean	47.3		23.8		38.5		37.7		295674		40.0		2135.78		49.4	
LSD(0.05)	14.1		16.4		10.7		19.3		78914.1		16.9		936.9			
SED	7.1		8.2		5.4		9.7		39567.1		8.5		469.7			
%CV	18.3		42.4		17.1		31.4		16.4		26.0		28.1			

DTOW= Days to onset of wilting; PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha^{-1});TTW= Total tuber weight (t ha^{-1}); PSTTW= Percentageof symptomatic tubers (% of total tuber weight in t ha^{-1}); TTN= Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); AUDPC= Area under the disease progress curve; % LI= % Latent infection.

Table 3. 8. Mean response and ranks among 36 potato genotypes for some agronomic and bacterial wilt resistance parameters ¨ during the second season

GENOTYPE	DTOW		PWTTW		TTW		PSTTW		TTN		PSTTN		AUDPC		LI	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	%	Rank
Sherekea	60	1.0	28.9	27	63.3	8.5	49.8	22.0	513575	1.0	56.2	23.0	1455	11	33.3	20.0
394905.8	59.6	2.5	14.1	36	51.5	29.0	70.0	36.0	440374	13.0	70.6	34.0	970	3	50.0	34.0
387164.4	59.6	2.5	25.6	29	**45.5**	36.0	59.7	32.0	351510	32.0	61.8	30.0	920	2	40.0	26.5
Roslin Tana	56.7	7.5	38.8	12	55.7	22.5	42.7	10.5	370367	26.0	49.5	15.0	2040	29	46.7	31.5
Pimpernel	56.7	7.5	21.1	34	47.7	35.0	60.4	34.0	286417	36.0	68.5	33.0	1430	10	40.0	26.5
Kerr's Pink	56.7	7.5	38.7	13	62.0	11.0	50.7	23.0	429625	17.0	57.5	25.5	2565	36	40.0	26.5
Tigoni	56.7	7.5	19.1	35	53.0	27.0	69.4	35.0	439502	14.0	71.6	35.0	1735	21	20.0	10.0
Bishop Gitonga	56.7	7.5	38.1	14	69.7	3.5	54.0	26.0	474069	5.0	54.0	20.0	1910	27	6.7	3.5
Annete	56.7	7.5	31.2	22	61.0	12.0	56.1	28.0	454316	8.5	55.9	22.0	1555	15	20.0	10.0
394034.7	56.7	7.5	22.0	32	60.7	13.0	54.3	27.0	459255	6.0	52.4	18.0	1315	6	26.7	14.5
393382.44	56.7	7.5	40.8	9	54.0	26.0	40.5	8.0	345676	34.0	47.6	10.0	1640	17	46.7	31.5
Sterling	53.3	16.0	40.0	11	51.3	30.0	40.3	7.0	353083	30.0	42.6	3.0	1880	26	20.0	10.0
Purple Gold	53.3	16.0	29.0	26	59.0	18.0	57.3	29.0	449378	11.0	60.5	29.0	1645	18	33.3	20.0
Nyayo	53.3	16.0	44.1	4	63.3	8.5	46.7	18.0	451847	10.0	50.3	16.0	1830	25	33.3	20.0
Kihoro	53.3	16.0	34.2	17	57.7	19.5	43.5	13.0	410490	19.0	46.2	9.0	1540	14	20.0	10.0
Kenya Sifa	53.3	16.0	60.8	1	69.3	5.0	33.6	2.0	385181	25.0	43.2	5.5	1300	5	40.0	26.5
Kenya Mya	53.3	16.0	31.4	21	65.7	6.0	47.2	19.0	488884	4.0	48.8	13.0	2130	30	33.3	20.0
Kenya Mavuno	53.3	16.0	28.0	28	63.0	10.0	49.2	21.0	454316	8.5	58.0	27.0	1480	12	20.0	10.0
Roslin Bvumbwe	53.3	16.0	47.0	2	78.0	1.0	34.6	4.0	490859	3.0	41.8	2.0	1815	24	46.7	31.5
394895.7	53.3	16.0	45.9	3	55.3	24.0	38.7	6.0	330861	35.0	44.3	7.0	1745	22	33.3	20.0
Kenya Karibu	50.0	26.5	35.8	15	57.7	19.5	42.7	10.5	429626	16.0	47.7	11.0	910	1	0.0	1.5
Romano	50.0	26.5	31.1	23	50.3	32.0	44.3	15.0	370366	27.0	53.5	19.0	1805	23	33.3	20.0
Kenya Baraka	50.0	26.5	33.9	19	71.3	2.0	57.5	30.0	498760	2.0	57.5	25.5	1265	4	60.0	35.5
Ingabire	50.0	26.5	43.7	5	59.7	16.0	31.7	1.0	385675	24.0	38.0	1.0	1670	19	20.0	10.0
Dutch Robyjn	50.0	26.5	41.5	8	69.7	3.5	35.3	5.0	351757	31.0	44.7	8.0	2525	35	0.0	1.5

Desiree	50.0	26.5	40.1	10	54.7	25.0	42.9	12.0	358021	28.5	48.6	12.0	2360	34	30.0	16.0
Cangi	50.0	26.5	25.2	30	55.7	22.5	58.5	31.0	404934	20.5	57.2	24.0	2265	33	13.3	5.0
B53	50.0	26.5	32.2	20	64.0	7.0	44.0	14.0	444440	12.0	42.9	4.0	2250	32	33.3	20.0
396286.6	50.0	26.5	23.9	31	48.7	34.0	60.1	33.0	404934	20.5	72.9	36.0	1375	9	40.0	26.5
394906.6	50.0	26.5	34.4	16	56.3	21.0	52.9	25.0	422218	18.0	65.8	32.0	2140	31	46.7	31.5
394903.3	50.0	26.5	30.6	24	50.7	31.0	52.8	24.0	392589	22.0	61.9	31.0	1335	7	26.7	14.5
393385.57	50.0	26.5	43.4	6	60.0	15.0	34.3	3.0	358021	28.5	43.2	5.5	1965	28	60.0	35.5
Meru Mugaruro	46.7	34.0	21.3	33	49.7	33.0	45.8	16.0	350614	33.0	52.2	17.0	1355	8	6.7	3.5
Asante	46.7	34.0	42.3	7	59.3	17.0	46.6	17.0	434564	15.0	55.0	21.0	1485	13	16.0	6.0
Arka	46.7	34.0	34.1	18	52.7	28.0	40.6	9.0	387650	23.0	49.3	14.0	1690	20	20.0	10.0
394904.17	43.3	36.0	29.9	25	60.3	14.0	48.5	20.0	456785	7.0	59.8	28.0	1570	16	4.00	26.5
Mean	52.7		34		58.5		48.5		411959		53.7		1690.7		30.4	
LSD (0.05)	12.5		23.7		21.4		23.7		159460.8		21.9		933.6			
SED	6.3		11.9		10.74		11.9		79952.8		11.0		468.1			
%CV	14.6		42.9		22.8		30.2		23.5		25		34.2			

DTOW= Days to onset of wilting; PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha^{-1});TTW= Total tuber weight (t ha^{-1}); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); TTN= Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); AUDPC= Area under the disease progress curve; % LI= % Latent infection.

Table 3.9. Mean response and ranks among 36 potato genotypes for some agronomic and bacterial wilt resistance parameters ¨ during the third season

GENOTYPE	DTOW		PWTTW		TTW		PSTTW		TTN		PSTTN		AUDPC		LI	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	%	Rank
Ingabire	60	2.5	62.2	1.0	73.0	1.0	25.9	5.0	404942	23	20.6	3	2925	9	13.7	3.0
Kenya Sifa	57	5.0	60.4	2.0	60.7	5.0	16.7	3.0	394646	26	22.3	4	1425	2	15.0	4.0
Kenya Baraka	60	2.5	55.6	3.0	66.3	2.0	11.6	2.0	496485	4	19.8	2	1730	4	6.7	2.0
394906.6	50	13.5	47.8	4.0	53.7	14.5	27.7	8.0	426898	19	31.7	11	4460	24	35.0	18.5
394034.7	53	9.0	46.3	5.0	54.3	12.0	29.2	9.0	452789	12	36.4	12	3550	14	25.0	8.5
393382.44	53	9.0	44.0	6.0	47.3	23.5	31.0	10.0	343090	35	38.3	13	2480	6	45.0	24.5
Kenya Karibu	67	1.0	43.7	7.0	53.0	17.0	11.4	1.0	465156	9	14.8	1	1465	3	5.0	1.0
393385.57	43	20.0	41.7	8.0	54.7	11.0	25.7	4.0	397541	24	27.9	7	2670	8	40.0	20.5
Roslin Bvumbwe	40	25.5	39.7	9.0	58.3	8.0	37.0	15.0	490088	5	41.2	15	1910	5	50.3	29.0
394895.7	43	20.0	38.4	10.0	49.3	21.0	35.2	14.0	365827	30	29.6	8	5140	33	26.7	14.0
394903.3	53	9.0	36.6	11.0	46.7	25.5	27.5	7.0	394521	27	46.3	20	3580	15	30.0	15.0
396286.6	47	16.5	33.7	12.5	46.3	27.5	26.0	6.0	413858	21	31.6	10	3910	19	26.3	13.0
Sterling	50	13.5	33.7	12.5	47.3	23.5	53.8	32.0	357842	32	45.8	19	3480	13	33.0	16.0
394905.8	50	13.5	33.3	14.0	46.7	25.5	32.7	11.0	427670	18	24.8	6	4525	25	25.0	8.5
Annete	37	30.0	33.1	15.0	53.7	14.5	35.0	13.0	473686	8	54.8	29	4220	22	45.0	24.5
Kenya Mavuno	43	20.0	31.8	16.0	59.3	6.0	47.8	20.0	456580	11	51.4	26	4840	29	23.3	6.0
Nyayo	40	25.5	31.4	17.0	41.3	34.0	49.9	24.0	440923	15	60.6	32	3430	12	53.3	31.0
394904.17	53	9.0	30.8	18.0	52.7	18.0	33.9	12.0	443887	14	24.4	5	1265	1	25.0	8.5
Romano	47	16.5	30.3	19.0	36.7	36.0	45.3	19.0	375242	29	52.8	27	3655	17	35.0	18.5
Asante	40	25.5	30.2	20.0	56.0	10.0	59.0	35.0	459900	10	44.0	16	3635	16	26.0	11.5
Kenya Mpya	43	20.0	29.3	21.0	62.0	3.0	49.5	23.0	511682	3	48.9	21	4425	23	33.3	17.0
Kerr's Pink	37	30.0	28.8	22.0	53.7	14.5	42.6	17.0	433481	17	49.3	23	4675	27	60.0	33.0
Kihoro	33	33.5	27.3	23.0	45.0	29.0	52.4	30.0	422287	20	69.0	35	5550	36	50.0	28.0
Sherekea	50	13.5	26.4	24.0	57.0	9.0	51.3	26.0	519960	1	40.8	14	4775	28	40.0	20.5
Tigoni	33	33.5	25.8	25.0	52.0	19.0	50.9	25.0	514773	2	71.8	36	3740	18	45.0	24.5

Arka	53	9.0	25.6	26.0	46.3	27.5	43.6	18.0	396743	25	49.7	24	3415	11	25.0	8.5
Desiree	43	20.0	25.4	27.0	41.0	35.0	48.3	21.0	363547	31	44.4	17	3995	20	26.0	11.5
B53	57	5.0	25.3	28.0	53.7	14.5	52.2	28.5	433996	16	45.7	18	4560	26	46.7	27.0
Meru Mugaruro	40	25.5	24.7	29.0	43.3	31.5	39.1	16.0	355682	33	53.3	28	4130	21	76.7	36.0
387164.4	57	5.0	24.1	30.0	44.0	30.0	49.0	22.0	344817	34	30.7	9	3120	10	20.3	5.0
Roslin Tana	33	33.5	24.0	31.0	42.7	33.0	57.0	34.0	379220	28	49.8	25	4985	31	53.3	31.0
Cangi	37	30.0	23.9	32.0	50.7	20.0	52.2	28.5	406765	22	49.2	22	4860	30	43.3	22.0
Pimpernel	40	25.5	23.6	33.0	43.3	31.5	51.7	27.0	288855	36	64.1	34	2630	7	45.0	24.5
Purple Gold	33	33.5	22.9	34.0	48.0	22.0	53.8	31.0	446832	13	62.8	33	5190	35	53.3	31.0
Bishop Gitonga	40	25.5	21.6	35.0	61.7	4.0	56.2	33.0	477053	7	60.1	31	5149	34	66.7	35.0
Dutch Robyjn	30	36.0	19.7	36.0	58.7	7.0	66.9	36.0	487847	6	58.9	30	5010	32	60.5	34.0
Mean	45.7		33.4		51.7		41.1		424031		43.5		3736.2		36.9	
LSD (0.05)	13.3		23.5		16.3		22.3		130343.6		20.0		2538.5			
SED	6.7		11.8		8.2		11.2		65353.6		10.0		1272.8			
% CV	17.9		43.2		19.4		33.4		18.9		28.2		41.8			

DTOW= Days to onset of wilting; PWTTW= Percentage of ware sized tubers (%of total tuber weight in t ha^{-1});TTW= Total tuber weight (t ha^{-1}); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); TTN= Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); AUDPC= Area under the disease progress curve; % LI= % Latent infection.

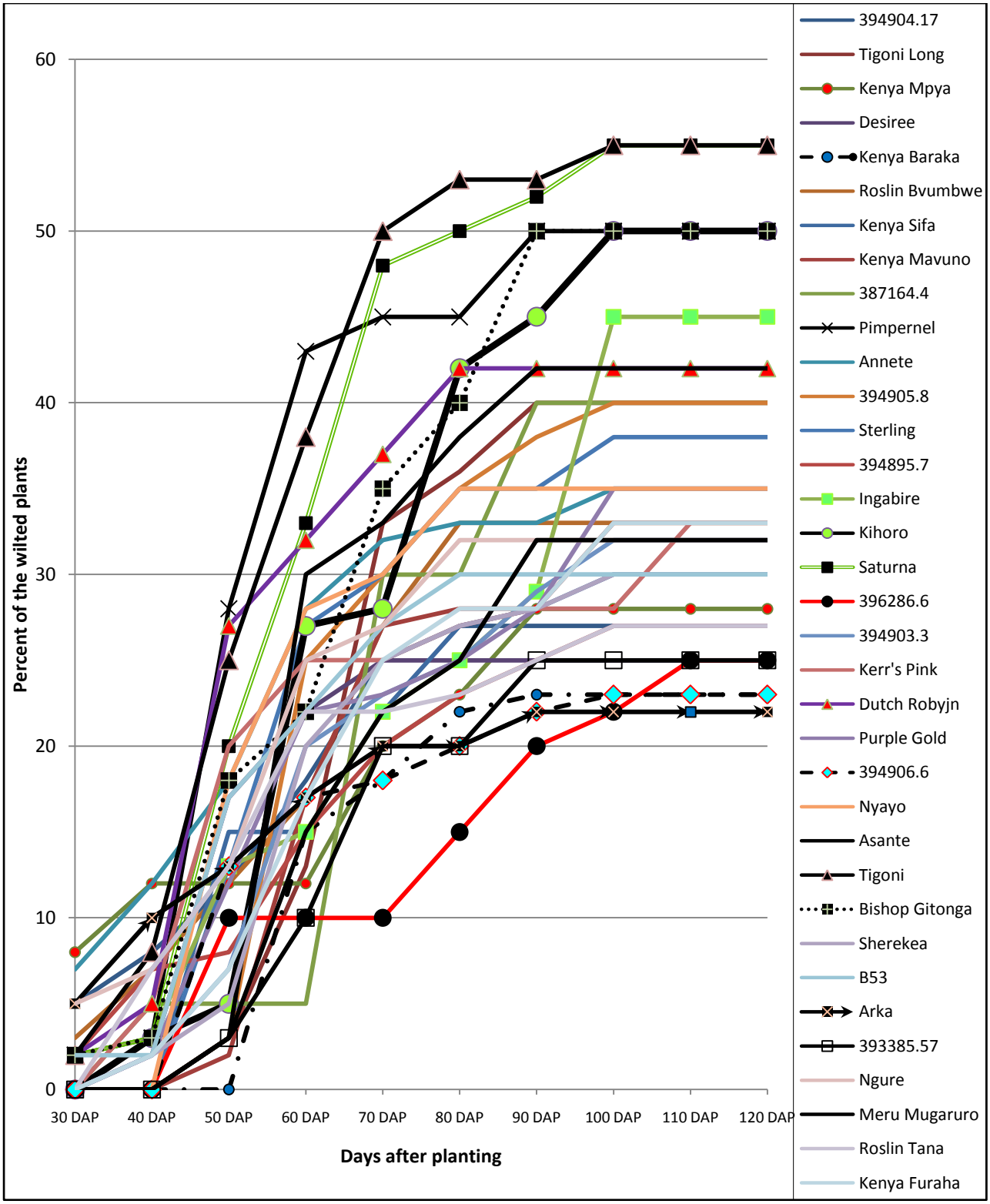

Figure 3.1. Bacterial wilt incidence (BWI) at 30 to 120 days after plating during the first season at KARINARL

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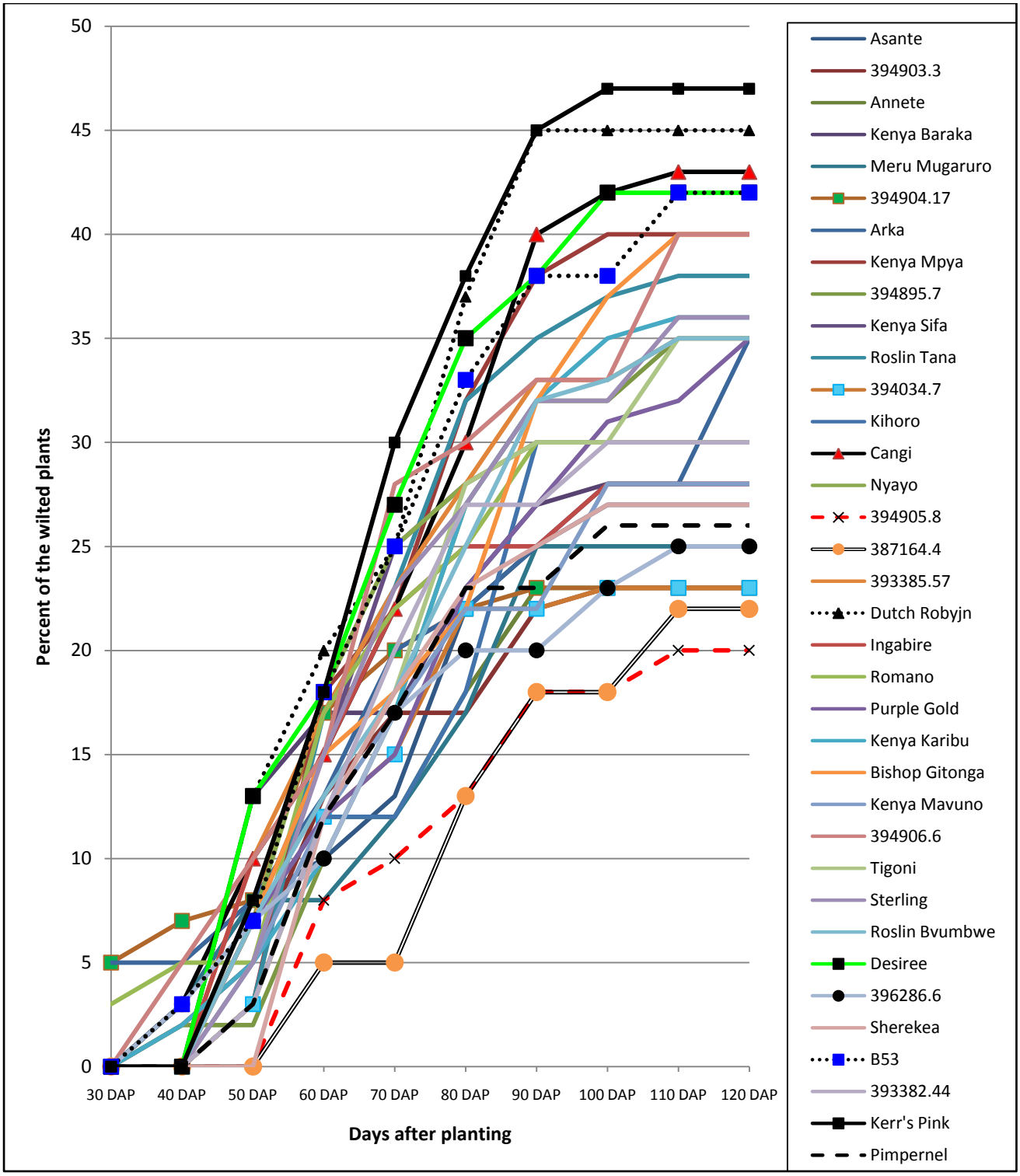

Figure 3.2. Bacterial wilt incidence (BWI)at 30 to 120 days after plating during the second season at KARINARL

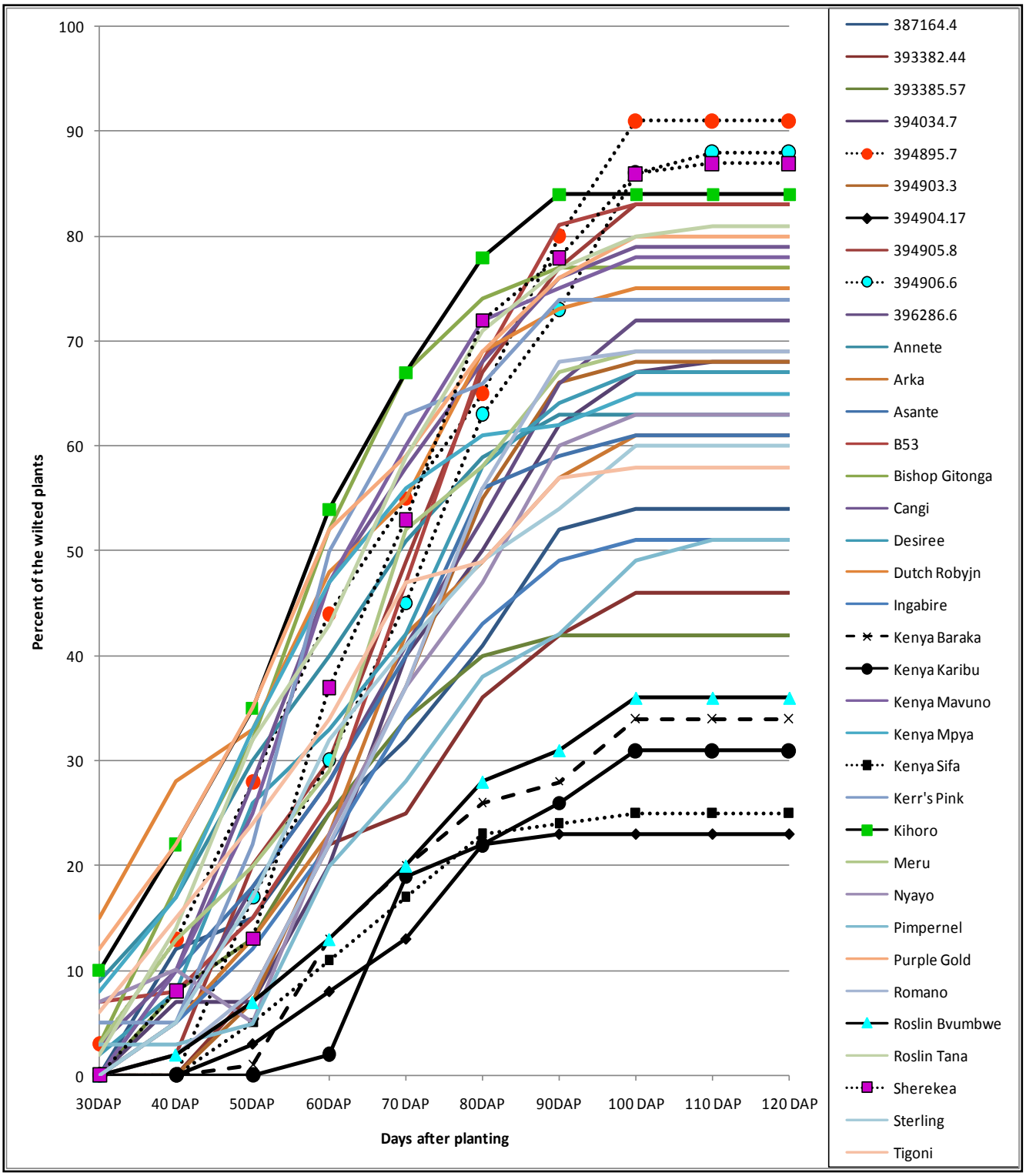

Figure 3.3. Bacterial wilt incidence (BWI) at 30 to 120 days after plating during the third season at KARI NARL

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3.3.4 Ranks of genotypes based on selected traits

When overall ranking was done based on % LI, AUDPC, DTOW, TTN, TTW, PWTTW, PSTTW, and PSTTN, the top ten genotypes were Kenya Baraka, clone 394903.3, Kenya Sifa, Meru Mugaruro, Kenya Mavuno, Desiree, clone 394034.7,

393385.57, Kihoro, and Kenya Furaha in that order during the first season (Table 3.10). In the second season, the top ten genotypes were Roslin Bvumbwe, Kenya Sifa, Kenya Karibu, Ingabire, Bishop Gitonga, Sherekea, Nyayo, Kihoro, Dutch Robyjn and clone 394034.7 in that order (Table 3.10).

3.3.5 Bacterial wilt resistance

Potato genotypes resistance to bacterial wilt as determined by ranks based on % LI, AUDPC, DTOW, PSTTW and PSTTN showed that the five most resistant genotypes were Kenya Baraka, Kenya Sifa, Desiree, Kenya Mavuno and Tigoni Long in that order in the first season (Table 3.11). In the second season, the most resistant genotypes were Kenya Karibu, Kenya Sifa, Ingabire, Sterling and Kihoro in that order (Table 3.12) while in the third season, the most resistant genotypes were Kenya Karibu, Kenya Baraka, Kenya Sifa, Ingabire and clone 394904.17 in that order (Table 3.13). When all the genotypes were ranked across the seasons, Kenya Karibu was the most resistant followed by Kenya Sifa while Kenya Baraka was third (Table 3.14). According to PTIT, all the genotypes had variable susceptibility ranging from moderate to high susceptibility (Table 3.15). The most resistant genotypes were Kenya Karibu followed by Kenya Sifa, Ingabire, clone 394034.7 while Kenya Baraka was the fifth (Table 3.15). The two ranking methods were in harmony because the five most resistant genotypes were the same in both cases.

Table 3.10. Seasonal ranking of the genotypes in the three seasons based on sum of ranks of DTOW, PWTTW, TTW, PSTTW, TTN, PSTTN, AUDPC and % LI in each season

	Season I		Season II		Season III	
GENOTYPE	Sum of ranks	*seasonalRank	Sum of ranks	SeasonalRank	Sum of ranks	*Seasonalrank
Kenya Baraka	**56.0**	**1.0**	144.5	19.0	21.5	**1.0**
Kenya Karibu			101.0	**3.0**	40.0	**2.0**
Ingabire	159.5	22.0	102.5	**4.0**	47.5	3.0
Kenya Sifa	77.0	**3.0**	86.0	**2.0**	51.0	**4.0**
394034.7	118.0	7.0	124.0	10.0	81.5	**5.0**
394904.17	172.5	27.0	172.5	26.0	85.5	6.0
393385.57	119.0	8.0	148.0	20.0	102.5	7.0
Roslin Bvumbwe	146.5	18.0	83.5	**1.0**	111.5	8.0
394906.6	196.0	30.0	201.0	34.0	112.5	9.0
394905.8	183.0	29.0	187.5	31.0	121.5	10.0
396286.6	198.0	31.0	216.5	36.0	125.5	11.0
393382.44			143.0	18.0	127.0	12.0
394903.3	76.5	**2.0**	180.0	28.0	129.5	13.0
Kenya Mpya	165.5	24.0	129.0	12.0	131.0	14.0
Kenya Mavuno	100.0	**5.0**	132.5	14.0	134.0	15.0
Sherekea	170.5	25.0	113.5	6.0	136.0	16.0
Asante	172.0	26.0	130.0	13.0	144.0	17.0
387164.4	126.5	11.0	190.0	32.0	145.0	18.0
Arka	160.0	23.0	156.0	22.0	149.0	19.0
394895.7	227.5	36.0	133.0	15.5	150.0	20.0
Annete	200.0	32.0	125.0	11.0	156.0	21.0
Sterling	200.5	33.0	133.0	15.5	161.5	22.0
B53	142.0	16.5	135.5	17.0	163.0	23.0
Romano			185.5	30.0	182.0	24.0
Desiree	105.0	6.0	164.0	24.0	182.5	25.0
Tigoni	148.0	20.0	184.5	29.0	183.0	26.0
Kerr's Pink	147.5	19.0	159.5	23.0	183.5	27.0
Nyayo	131.0	12.0	117.5	7.5	190.5	28.0
Bishop Gitonga	142.0	16.5	106.5	**5.0**	204.5	29.0
Cangi			192.5	33.0	206.5	30.0
Dutch Robyjn	175.0	28.0	118.5	9.0	217.0	31.0
Pimpernel	151.5	21.0	216.0	35.0	218.5	32.0
Meru Mugaruro	90.0	**4.0**	177.5	27.0	220.0	33.0
Purple gold	138.5	14.5	167.0	25.0	232.5	34.0
Kihoro	121.0	9.0	117.5	7.5	234.5	35.0
Roslin Tana	134.5	13.0	154.0	21.0	246.5	36.0
Kenya Furaha	123.5	10.0				
Tigoni Long	138.5	14.5				
Saturna	202.0	34.0				
Ngure	213.5	35.0				

Sum of ranks=sum of ranks due to DTOW,PWTTW,TTW, PSTTW, TTN, PSTTN,AUDPC and % LIin eaxh season. *Seasonalrank=ranking genotypes based on their sum of ranks in each season

Table 3.11. Average ranks of 36 potato genotypes for bacterial wilt resistance based on % LI, DTOW, PSTTW, PSTTN and AUDPC during the first season

GENOTYPE	Rank (% LI)	Rank (DTOW)	Rank (PSTTW)	Rank (PSTTN)	Rank (AUDPC)	Average rank	*Overall rank
Kenya Baraka	15.5	1.0	4.0	6.0	2.0	5.7	1.0
Tigoni Long	3.5	4.0	21.5	4.0	25.0	11.6	5.0
Kenya Mavuno	20.5	4.0	12.0	3.0	14.0	10.7	4.0
Sterling	20.5	4.0	32.0	32.0	28.0	23.3	30.0
393385.57	28.5	4.0	23.0	9.5	3.0	13.6	9.0
Meru Mugaruro	34.5	4.0	8.0	9.5	7.0	12.6	6.0
394905.8	28.5	7.0	17.0	19.5	26.0	19.6	19.0
Kihoro	8.0	10.0	26.0	29.0	31.0	20.8	24.0
394903.3	28.5	10.0	5.0	7.0	15.0	13.1	8.0
394906.6	20.5	10.0	33.0	33.0	4.0	20.1	21.0
Nyayo	20.5	10.0	9.0	13.0	27.0	15.9	10.0
Asante	13.0	10.0	18.0	34.0	30.0	21.0	26.0
Desiree	8.0	14.5	6.5	5.0	9.0	8.6	3.0
Kenya Sifa	3.5	14.5	2.0	2.0	12.5	6.9	2.0
Purple Gold	20.5	14.5	21.5	9.5	18.0	16.8	11.0
Sherekea	28.5	14.5	6.5	30.5	12.5	18.5	16.0
394034.7	1.0	17.0	19.5	19.5	8.0	13.0	7.0
396286.6	8.0	18.0	34.0	36.0	1.0	19.4	18.0
B53	13.0	19.5	31.0	26.0	19.0	21.7	28.0
Kenya Furaha	34.5	19.5	15.0	14.0	17.0	20.0	20.0
Kenya Mpya	13.0	23.5	19.5	21.5	11.0	17.7	13.0
Saturna	28.5	23.5	13.0	23.0	35.0	24.6	32.0
Kerr's Pink	20.5	23.5	11.0	27.5	20.0	20.5	23.0
Bishop Gitonga	8.0	23.5	3.0	24.5	33.0	18.4	14.0
Arka	28.5	23.5	25.0	27.5	5.0	21.9	29.0
Roslin Tana	20.5	23.5	14.0	24.5	10.0	18.5	16.0
394904.17	8.0	30.0	29.0	21.5	16.0	20.9	25.0
Pimpernel	15.5	30.0	27.0	18.0	34.0	24.9	33.0
Annete	20.5	30.0	28.0	35.0	29.0	28.5	36.0
Ingabire	28.5	30.0	10.0	9.5	24.0	20.4	22.0
Dutch Robyjn	8.0	30.0	30.0	17.0	32.0	23.4	31.0
Tigoni	2.0	30.0	24.0	16.0	36.0	21.6	27.0
Ngure	34.5	30.0	36.0	15.0	23.0	27.7	34.0
387164.4	28.5	34.0	**1.0**	**1.0**	22.0	17.3	12.0
Roslin Bvumbwe	8.0	35.5	16.0	12.0	21.0	18.5	16.0
394895.7	34.5	35.5	35.0	30.5	6.0	28.3	35.0

% LI= % Latent infection; DTOW= Days to onset of wilting; PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); AUDPC= Area under the disease progress curve. Average rank= average of rank due to % LI, rank DTOW, rank PSTTW, rank PSTTN and rank AUDPC. *Overall rank=ranking of genotypes based on their average rank

Table 3.12. Average ranks of 36 potato genotypes for bacterial wilt resistance based on % LI, DTOW, PSTTW, PSTTN and AUDPC during the second season

GENOTYPE	% LI	DTOW	PSTTW	PSTTN	AUDPC	Average Rank	*Overall Rank
Sherekea	20.0	1.0	22.0	23.0	11.0	15.4	10.0
394905.8	34.0	2.5	36.0	34.0	3.0	21.9	28.0
387164.4	26.5	2.5	32.0	30.0	2.0	18.6	18.0
Roslin Tana	31.5	7.5	10.5	15.0	29.0	18.7	19.0
Pimpernel	26.5	7.5	34.0	33.0	10.0	22.2	29.0
Kerr's Pink	26.5	7.5	23.0	25.5	36.0	23.7	31.0
Tigoni	10.0	7.5	35.0	35.0	21.0	21.7	27.0
Bishop Gitonga	3.5	7.5	26.0	20.0	27.0	16.8	14.0
Annete	10.0	7.5	28.0	22.0	15.0	16.5	13.0
394034.7	14.5	7.5	27.0	18.0	6.0	14.6	7.0
393382.44	31.5	7.5	8.0	10.0	17.0	14.8	8.0
Sterling	10.0	16.0	7.0	3.0	26.0	12.4	4.0
Purple Gold	20.0	16.0	29.0	29.0	18.0	22.4	30.0
Nyayo	20.0	16.0	18.0	16.0	25.0	19.0	20.0
Kihoro	10.0	16.0	13.0	9.0	14.0	12.4	5.0
Kenya Sifa	26.5	16.0	2.0	5.5	5.0	11.0	2.0
Kenya Mpya	20.0	16.0	19.0	13.0	30.0	19.6	22.0
Kenya Mavuno	10.0	16.0	21.0	27.0	12.0	17.2	15.0
Roslin Bvumbwe	31.5	16.0	4.0	2.0	24.0	15.5	11.0
394895.7	20.0	16.0	6.0	7.0	22.0	14.2	6.0
Kenya Karibu	1.5	26.5	10.5	11.0	1.0	10.1	1.0
Romano	20.0	26.5	15.0	19.0	23.0	20.7	26.0
Kenya Barka	35.5	26.5	30.0	25.5	4.0	24.3	33.0
Ingabire	10.0	26.5	1.0	1.0	19.0	11.5	3.0
Dutch Robyjn	1.5	26.5	5.0	8.0	35.0	15.2	9.0
Desiree	16.0	26.5	12.0	12.0	34.0	20.1	24.0
Cangi	5.0	26.5	31.0	24.0	33.0	23.9	32.0
B53	20.0	26.5	14.0	4.0	32.0	19.3	21.0
396286.6	26.5	26.5	33.0	36.0	9.0	26.2	35.0
394906.6	31.5	26.5	25.0	32.0	31.0	29.2	36.0
394903.3	14.5	26.5	24.0	31.0	7.0	20.6	25.0
393385.57	35.5	26.5	3.0	5.5	28.0	19.7	23.0
Meru Mugaruro	3.5	34.0	16.0	17.0	8.0	15.7	12.0
Asante	6.0	34.0	17.0	21.0	13.0	18.2	17.0
Arka	10.0	34.0	9.0	14.0	20.0	17.4	16.0
394904.17	26.5	36.0	20.0	28.0	16.0	25.3	34.0

% LI= % Latent infection; DTOW= Days to onset of wilting; PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); AUDPC= Area under the disease progress curve.Average rank= average of rank due to % LI, rank DTOW, rank PSTTW, rank PSTTN and rank AUDPC. *Overall rank=ranking of genotypes based on their average rank

Table 3.13. Average ranks of 36 potato genotypes for bacterial wilt resistance based on % LI, DTOW, PSTTW, PSTTN and AUDPC during the third season

GENOTYPE	Rank (% LI)	Rank (DTOW)	Rank (PSTTW)	Rank (PSTTN)	Rank (AUDPC)	Average Rank	*Overall Rank
Ingabire	3.0	2.5	5.0	3.0	9.0	4.5	4.0
Kenya Sifa	4.0	5.0	3.0	4.0	2.0	3.6	3.0
Kenya Baraka	2.0	2.5	2.0	2.0	4.0	2.5	2.0
394906.6	18.5	13.5	8.0	11.0	24.0	15.0	14.0
394034.7	8.5	9.0	9.0	12.0	14.0	10.5	7.0
393382.44	24.5	9.0	10.0	13.0	6.0	12.5	9.0
Kenya Karibu	1.0	1.0	1.0	1.0	3.0	1.4	1.0
393385.57	20.5	20.0	4.0	7.0	8.0	11.9	8.0
Roslin Bvumbwe	29.0	25.5	15.0	15.0	5.0	17.9	16.5
394895.7	14.0	20.0	14.0	8.0	33.0	17.8	15.0
394903.3	15.0	9.0	7.0	20.0	15.0	13.2	12.0
396286.6	13.0	16.5	6.0	10.0	19.0	12.9	11.0
Sterling	16.0	13.5	32.0	19.0	13.0	18.7	18.0
394905.8	8.5	13.5	11.0	6.0	25.0	12.8	10.0
Annete	24.5	30.0	13.0	29.0	22.0	23.7	26.0
Kenya Mavuno	6.0	20.0	20.0	26.0	29.0	20.2	20.0
Nyayo	31.0	25.5	24.0	32.0	12.0	24.9	27.0
394904.17	8.5	9.0	12.0	5.0	1.0	7.1	5.0
Romano	18.5	16.5	19.0	27.0	17.0	19.6	19.0
Asante	11.5	25.5	35.0	16.0	16.0	20.8	22.5
Kenya Mpya	17.0	20.0	23.0	21.0	23.0	20.8	22.5
Kerr's Pink	33.0	30.0	17.0	23.0	27.0	26.0	29.0
Kihoro	28.0	33.5	30.0	35.0	36.0	32.5	34.0
Sherekea	20.5	13.5	26.0	14.0	28.0	20.4	21.0
Tigoni	24.5	33.5	25.0	36.0	18.0	27.4	31.0
Arka	8.5	9.0	18.0	24.0	11.0	14.1	13.0
Desiree	11.5	20.0	21.0	17.0	20.0	17.9	16.5
B53	27.0	5.0	28.5	18.0	26.0	20.9	24.0
Meru Mugaruro	36.0	25.5	16.0	28.0	21.0	25.3	28.0
387164.4	5.0	5.0	22.0	9.0	10.0	10.2	6.0
Roslin Tana	31.0	33.5	34.0	25.0	31.0	30.9	32.0
Cangi	22.0	30.0	28.5	22.0	30.0	26.5	30.0
Pimpernel	24.5	25.5	27.0	34.0	7.0	23.6	25.0
Purple Gold	31.0	33.5	31.0	33.0	35.0	32.7	35.0
Bishop Gitonga	35.0	25.5	33.0	31.0	34.0	31.7	33.0
Dutch Robyjn	34.0	36.0	36.0	30.0	32.0	33.6	36.0

% LI= % Latent infection; DTOW= Days to onset of wilting; PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha).AUDPC= Area under the disease progress curve.Average rank= average of rank due to % LI, rank DTOW, rank PSTTW, rank PSTTN and rank AUDPC. *Overall rank=ranking based on average rank.

Table 3.14. Overall ranks of the most resistant potato genotypes across the three seasons at KARI-NARL based on average ranks of % LI, DTOW, PSTTW, PSTTN and AUDPC

	Average Ranks				
GENOTYPE	Season I	Season II	Season III	Average rank across the three seasons	*Overall Rank
Kenya Karibu		10.1	1.4	5.8	1.0
Kenya Sifa	6.9	11.0	3.6	7.2	2.0
Kenya Baraka	5.7	24.3	2.5	10.8	3.0
Ingabire	20.4	11.5	4.5	12.1	4.0
394034.7	13.0	14.6	10.5	12.7	5.0
393382.44		14.8	12.5	13.7	6.0
393385.57	13.6	19.7	11.9	15.1	7.0
387164.4	17.3	18.6	10.2	15.4	8.0
Desiree	8.6	20.1	17.9	15.5	9.0
394903.3	13.1	20.6	13.2	15.6	10.0

% LI= % Latent infection; DTOW= Days to onset of wilting; PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); AUDPC= Area under the disease progress curve;

*Overall rank=ranking of genotypes based on their average rank.

Table 3.15. The percentage of total infected tubers (PTIT) of potato genotypes across the three seasons

GENOTYPE	Season I	Season II	Season III	Average
Kenya Baraka	66.7	83.0	25.1	**58.3**
Kenya Mavuno	67.6	66.4	62.7	65.6
Sterling	75.2	54.1	63.7	64.3
393385.57	74.6	77.3	56.7	69.5
Meru Mugaruro	78.8	55.4	89.1	74.4
394905.8	76.0	85.3	43.6	68.3
Kihoro	66.3	57.0	84.5	69.3
394903.3	74.4	72.1	62.4	69.6
394906.6	77.0	81.8	55.6	71.5
Nyayo	70.6	66.9	81.6	73.0
Asante	74.8	62.2	58.6	65.2
Desiree	59.4	64.0	58.9	60.8
Kenya Sifa	53.1	65.9	34.0	**51.0**
Purple Gold	70.4	73.7	82.6	75.6
Sherekea	78.0	70.8	64.5	71.1
394034.7	40.0	65.1	52.3	**52.5**
396286.6	72.4	83.7	49.6	68.6
B53	68.5	61.9	71.0	67.1
Kenya Mpya	68.1	65.9	65.9	66.6
Kerr's Pink	73.4	74.5	79.7	75.9
Bishop Gitonga	64.5	57.1	86.7	69.4
Arka	77.2	59.4	62.3	66.3
Roslin Tana	72.4	73.1	76.6	74.0
394904.17	64.1	75.9	43.3	61.1
Pimpernel	69.9	81.1	80.3	77.1
Annete	78.2	64.7	75.1	72.7
Ingabire	74.6	50.4	31.5	**52.2**
Dutch Robyjn	63.6	44.7	83.8	64.0
Tigoni	51.2	77.3	84.5	71.0
387164.4	71.8	77.1	44.8	64.6
Roslin Bvumbwe	62.1	69.0	70.8	67.3
394895.7	81.7	62.9	48.4	64.3
393382.44		72.1	66.1	69.1
Kenya Karibu		47.7	19.1	**33.4**
Romano		69.0	69.3	69.2
Cangi		62.9	71.2	67.1

3.3.6 Correlations among traits

Correlations between DTOW and AUDPC were negative and significant in the first and third seasons (Table 3.16 and 3.17) and negative but non-significant in the second season (Table 3.16). Correlations between DTOW on one hand and PSTTW

and PSTTN on the other hand were negative and non-significant in the first season, positive and non-significant in the second season (Table 3.16) and, negative and significant (P≤0.01) in the third season (Table 3.17). Correlation between % LI and all the other traits were non-significant in the first two seasons. In the third season, correlation between % LI and DTOW was negative and significant (P≤0.01) while correlations between % LI and AUDPC, PSTTN and PSTTW were positive and significant (P≤0.01) (Table 3.17).

Table 3.16. Pearson correlation coefficients for various agronomic traits for 36 genotypes during season I (top diagonal) and season II (bottom diagonal)

Trait	%LI	DTOW	AUDPC	PSTTN	PSTTW	PWTTW	TTN	TTW
%LI	1	0.037 ns	-0.191 ns	0.048 ns	0.063 ns	-0.082 ns	0.181 ns	0.070 ns
DTOW	0.210 ns	1	-0.348**	-0.121 ns	-0.1531 ns	0.052 ns	0.015 ns	-0.220*
AUDPC	-0.050 ns	-0.183 ns	1	-0.023 ns	-0.049 ns	0.061 ns	0.104 ns	0.180 ns
PSTTN	0.175 ns	0.129 ns	0.242*	1	0.424**	0.004 ns	-0.393**	-0.296**
PSTTW	0.095 ns	0.187 ns	0.187 ns	0.939**	1	-0.175 ns	-0.357**	-0.130 ns
PWTTW	0.071 ns	-0.080 ns	-0.122 ns	-0.767**	-0.833**	1	0.066 ns	0.234*
TTN	0.041 ns	-0.080 ns	0.020 ns	-0.032 ns	0.006 ns	0.074 ns	1	0.743**
TTW	0.029 ns	-0.041 ns	0.125 ns	-0.359**	-0.372**	0.524**	0.708**	1

ns=Non-significant; *= Significant at P≤0.05; ** = Significant at P≤0.01; LI=% latent infection; DTOW= Days to onset of wilting; AUDPC= Area under the disease progress curve; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha^{-1});TTN=Total tuber number per ha;TTW= Total tuber weight (t ha^{-1}).

Table 3.17.Pearson correlation coefficients for various agronomic traits for 36 genotypes during the third season

Trait	% LI	DTOW	AUDPC	PSTTN	PSTTW	PWTTW	TTN	TTW
% LI	1							
DTOW	-0.740**	1						
AUDPC	0.512**	-0.636**	1					
PSTTN	0.725**	-0.487**	0.375**	1				
PSTTW	0.586**	-0.493**	0.300**	0.599**	1			
PWTTW	-0.599**	0.422**	-0.290**	-0.555**	-0.736**	1		
TTN	0.010ns	-0.078ns	-0.006ns	-0.079ns	0.031ns	0.052ns	1	
TTW	-0.250ns	0.079ns	-0.142ns	-0.387**	-0.193*	0.419**	0.631**	1

ns=Non-significant; *= Significant at P≤0.05; ** = Significant at P≤0.01; LI=% latent infection; DTOW= Days to onset of wilting; AUDPC= Area under the disease progress curve; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha^{-1});TTN=Total tuber number per ha;TTW= Total tuber weight (t ha^{-1}).

3.4 Discussion and conclusions

This study aimed at determining the reaction to bacterial wilt of the potato genotypes currently grown by farmers in Kenya as well as other advanced clones from CIP in order to identify parents that can be used in a local breeding programme to develop resistant cultivars.

The high soil bacterial count at 60 days after planting was probably due to the fact that this coincided with periods of high rainfall (Table 3.3). The aggressiveness of *R. solanacearum* is affected by temperature and moisture; high temperature and high soil moisture promote survival, reproduction, infectivity, and spread of the bacterium, and hence disease development (Harris, 1976; Martin and French, 1985). This high soil bacterial population combined with the vigorous vegetative plant growth probably led to the rapid increase in the disease incidence (number of wilting plants) in the field (Figures 3.1, 3.2 and 3.3). At around flowering time, the plants' water demand is very high and they wilt rapidly due to the blockage of the xylem tissue by the bacterial mass. In addition, due to high transpiration rates, the plants take up a lot of water (together with bacteria in the soil water) and hence wilt rapidly. The higher soil bacterial population in the third season compared to the other two seasons could be due to accumulation of bacterial population in the soil over time (the same experimental plot was used for three consecutive seasons), the high temperature and rainfall experienced in that period (Table 3.3) or a combination of these. This could also explain the higher AUDPC in the third season compared with the first two seasons (Tables 3.7, 3.8 and 3.9). The low AUDPC in the second season could be due to lower temperatures experienced during the second season compared to the other seasons (Table 3). The high total tuber weight (TTW) in the second season was likely due to the heavy rainfall and lower temperatures experienced in that season as well as lower BWI (low AUDPC). The heavy rains and cool conditions favoured crop growth because potato is a cool season crop. These conditions also led to the high PWTTW and TTN.

In terms of bacterial wilt resistance, the potato genotypes ranked differently across seasons (Table 3.11, 3.12 and 3.13). This could be due to differences in weather among the seasons especially with regards to temperature and rainfall. Resistance to R3bv2A available in *Solanum tuberosum* originated mainly from the cultivated

diploid, *Solanum phureja* (Martin and French, 1985). This resistance is very unstable due to strong host-pathogen-environment interaction; host resistant to the disease in one year/environment or location may succumb to the disease in the other year/environment or location (French and Lindo, 1982; Tung et al., 1990; Tung, 1992; Tung et al., 1992b; Tung et al.,1993). Previously, varieties Kenya Dhamana(CIP-800224), Kenya Sifa, Kenya Karibu, Mauritius (clone 89016), and Cruza-148 (CIP-720118) were rated as resistant to bacterial wilt, while varieties Asante (CIP-381381.20), Tigoni (CIP-381381.13), Nyayo, and Dutch Robyjin were highly susceptible (Ateka et al., 2001). In a later study it was found that Kenya Sifa and Kenya Karibu were the most resistant to bacterial wilt while Dutch Robyjin and Tigoni were the most susceptible (Felix et al., 2010). The present study found Kenya Karibu to be the most resistant followed by Kenya Sifa. The negative correlation between AUDPC and DTOW indicates that genotypes that took long before onset of wilting had low disease incidence. Correlation between latent infection and all the other traits was not consistent. According to some reports, *R. solanacearum* expresses different sets of genes during latent infection and during symptomatic disease development (Jill et al., 2004). Studies have shown that tuber latent infection and above ground plant susceptibility to bacterial wilt are not correlated; the clone's latent infection potential does not depend only on BWI but on other factors such as environment (Ciampi and Sequeira, 1980; Priou et al., 2001). Infection of tubers depends not only on above ground wilt severity but also on soil texture, humidity and temperatures (CIP, 2007).

This study has provided an insight into response to bacterial wilt of the potato genotypes currently grown by farmers in Kenya as well as other advanced clones from CIP. All the genotypes are generally susceptible; susceptibility ranged from moderate to high. From the evaluations, eight potato genotypes were selected to be used as pollen donors (males) in subsequent crossing. These are Meru Mugaruro, Ingabire, Kenya Karibu, Sherekea, Kihoro, Tigoni, Bishop Gitonga and Cangi. The choice of these genotypes was also determined by pollen production (a good paternal needs to produce a lot of pollen), and popularity of the genotype with the Kenyan farmers.

3.5 References

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Chapter Four: Genetic relationships among twenty potato genotypes as revealed by SSR markers

Abstract

The ability to quickly and accurately identify relationships among potato *(Solanum tuberosum* L.) clones is important to breeders, seed and commercial growers, and in marketing and utilization of cultivars. The DNA-based genotyping using simple sequence repeats (SSRs) or microsatellites has been shown to discriminate between tetraploid potato clones. The objective of this study was to determine the genetic relationships among potato clones so as to complement other bacterial wilt-resistance data in identifying parents for a breeding programme. Twenty potato clones were genotyped with twenty four SSR primer pairs. The twenty four SSR primer pairs identified 160 alleles among the 20 potato clones. The number of alleles per locus ranged from 2 to 14 with an average of 6.67. Seventeen SSR markers (71%) were highly informative and had polymorphic information content (PIC) values above 0.65; the PIC values ranged from 0.208 to 0.839. Three genetic clusters were identified; clone Meru Mugaruro formed its own cluster. The SSR markers generated useful information that will assist in identifying parents to include in the breeding programme.

Keywords: Bacterial wilt; Potato clones; SSR markers, Polymorphic information content.

4.1 Introduction

Information on the genetic interrelationships and diversity of crop plants allows systematic organization of the variability in the germplasm, creation of core collections in genebanks, and assists in selection of parents in a breeding programme hence paving the way to genetic gains (Powell et al., 1991; Sun et al., 2003). The characterization of genetic diversity is also important for cultivar identification, cultivar protection (e.g. potato tuber seed) as well as to ensure the trademark and intellectual property rights (Coombs et al., 2004). In a crop like potato, information on genetic diversity is used in co-ancestry/pedigree studies to avoid closely related parents and hence inbreeding depression (Tarn et al., 1992).

In determining genetic diversity, genetic markers representing genetic differences between genotypes or species are used. There are three major types of genetic markers: (1) morphological (also ‘classical’, ’phenotypic’ or ‘visible’) markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA (or molecular) markers, which reveal sites of variation in DNA sequence (Winter and Kahl, 1995; Jones et al., 1997). Molecular markers are the most widely used mainly because they are much more numerous than morphological markers, and they do not disturb the physiology of the organism. They reveal neutral sites of variation at the DNA sequence level. ‘Neutral' means that, unlike morphological markers, these variations do not show themselves in the phenotype, and each might be nothing more than a single nucleotide difference in a gene or a piece of repetitive DNA (Jones et al., 1997). Because polymorphisms are DNA sequence variations, these markers are applicable at any plant stage and tissue and are independent of growing conditions (Hahn and Grifo, 1996). They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Paterson, 1996). The most widely used molecular markers are restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSR) or microsatellites (Collard et al., 2005) and recently single nucleotide polymorphisms (SNPs) (Hamilton et al., 2011).

The SSRs or microsatellites (sometimes referred to as a variable number of tandem repeats or VNTRs) are short segments of DNA that have a repeated nucleotide sequences. These motifs exhibit extensive site-specific length polymorphism due to differing numbers of repeat units. Length polymorphisms at a particular SSR locus can be assayed on the basis of the differing electrophoretic mobilities of polymerase chain reaction (PCR) products amplified by primers flanking the motif (Rafalski et al., 1996). The nucleotide repeat motifs can be dinucleotide, trinucleotide or tetranucleotide repeats, and they tend to occur in non-coding DNA.

Simple sequence repeat (SSR) markers detect highly repetitive regions in the genome that can be derived from untranslated regions and introns (Ghislain et al., 2006). In solanaceous species the microsatellite frequency is greater in the intron untranslated regions 5' (upstream of the gene) and 3' (downstream of the gene) (Smulders et al., 1997). Moreover, although SSRs represent hypervariable areas of the genome, they are sufficiently conserved to be inherited for several generations in a Mendelian fashion (Morgnate and Olivieri, 1993). In this respect, the long-term stability of allele profiles in potato has been demonstrated (Love et al., 1992).

Unlike other DNA-based markers RFLP, RAPD, SNPs and AFLP, simple sequence repeats occur frequently in plants. Microsatellites are distributed throughout the genome of eukaryotes and prokaryotes. The frequency of SSRs varies between mammals and plants, being five times more frequent in the former (Lagercrantz et al., 1993). Within plants, the frequency is approximately one every 21.2 kb in dicots and every 64.6 kb in monocots (Wang et al., 1994a).

In potato, it was estimated that one SSR could be found in every 52 kb when screening for five different motifs (Ashkenazi et al., 2001). Microsatellites are ubiquitous, highly polymorphic and can be used to detect the heterozygosity at a locus due to their co-dominant behaviour. They also permit the analysis of multiple loci per individual (multiallelism) and can function with low quality DNA (Morgnate and Olivieri, 1993; Wang et al., 1994b). Microsatellites provide high genetic information, are highly reproducible, and simple to use. Additionally, the SSRs have the capacity to reflect ploidy status and the high heterozygosity of the tetraploid potatoes. Genetic fingerprinting using SSRs has been well established to effectively

discriminate between tetraploid potato clones (Kawchuk et al., 1996; Provan et al., 1996; Mc Gregor et al., 2000; Ashkenazi et al., 2001). Simple sequence repeats have been used to great advantage in potato for studies of diversity, genetic structure, and classification (Spooner et al., 2007); tracing germplasm migrations (Rios et al., 2007); fingerprinting (Provan et al., 1996; Moisan-Thiery et al., 2005); genetic linkage mapping (Feingold et al., 2005); establishment of core collections (Ghislain et al., 2006) and investigations of duplicate collections across genebanks (Del Rio et al., 2006).

Previous studies have resulted in selection of a new potato genetic identity (PGI) kit based on 24 SSR markers with two markers for each of the 12 linkage groups of potato and separated by at least 10 cM. The kit provides high locus-specific polymorphic information content and high quality of amplicons as determined by clarity and reproducibility (Ghislain et al., 2009). It thus seems that SSR markers are a powerful molecular approach for establishing genetic relationship, assessing genetic diversity and germplasm characterization in tetraploid potato.

Breeders commonly complement phenotypic information with a genotypic assessment of diversity and content using molecular markers to capture allelic diversity in a smaller core set of parents. They can also use genetic distance based on molecular markers to complement co-ancestry/pedigree analysis (Tarn et al., 1992; Gopal and Oyama, 2005) to avoid closely related parents and hence inbreeding depression and to ensure genetic variation for continued progress.

Against this background, the current study was undertaken to determine the genetic relationships among potato clones so as to complement other bacterial wilt-resistance data in identifying parents for a breeding programme.

4.2 Materials and methods

4.2.1 Plant materials

Twenty potato clones were used in the study (Table 4.1). The eight clones C1 to C8 are advanced clones from International Potato Center (CIP) and are reported to have high levels of resistance to bacterial wilt. The other clones are susceptible to bacterial wilt in varying degrees but are popular in Kenya because they are high

yielding, early maturing or have other preferred market qualities (Muthoni et al., 2014).

Table 4.1. List and sources of potato clones used in the study

Entry	Clone	Source	Year of release/status	Response to bacterial wilt
1	Tigoni	CIP	1998	Highly susceptible
2	Kihoro	Farmers' variety	-	Highly susceptible
3	Meru	Farmers' variety	-	Highly susceptible
4	Nyayo	Farmers' variety	-	Highly susceptible
5	Ingabire	CIP	1998	Susceptible
6	Kenya Furaha	CIP	1998	
7	Tigoni Long	Farmers' variety	-	
8	Bishop Gitonga	Farmers' variety	-	Highly susceptible
9	Kenya Mavuno	CIP	2002	Highly susceptible
10	Kenya Karibu	CIP	2002	Moderately susceptible
11	Kenya Faulu	CIP	2002	
12	Cangi	Farmers' variety		Highly susceptible
13	C1 (391919.3)	CIP	Advanced clone	
14	C2 (394904.9)	CIP	Advanced clone	
15	C3 (394905.8)	CIP	Advanced clone	
16	C4 (392278.19)	CIP	Advanced clone	
17	C5 (394895.7)	CIP	Advanced clone	
18	C6 (394903.5)	CIP	Advanced clone	
19	C7 (395438.1)	CIP	Advanced clone	
20	C8 (391930.1)	CIP	Advanced clone	

CIP=Centro Internacional de la Papa

4.2.2 DNA sampling

Fresh young leaves were picked from one month old plants in the field for DNA extraction. The DNA collection was done using Whatman FTA cards. The sampling protocol followed the modified protocols of FTA paper technology (Mbogori et al., 2006). The FTA classic card (Whatman Inc., Clifton, NJ) is a Whatman paper that is impregnated with a patented chemical formulation that lyses cells, then captures and immobilizes nucleic acids in the paper matrix. In addition, they contain compounds for denaturing, chelating and trapping free radicals which prevent damage of the nucleic acids (http://www.whatman.com). One FTA classic card measures 750 x 130mm and each was labeled prior to the day of sampling. Ten plants were sampled from each clone, one leaf per plant. Each sampled leaf was immediately placed on the FTA card and pressed using a pair of pliers until both sides of the FTA paper were soaked with the sap. Ethanol (70%) was used to clean the pliers between samples to prevent cross contamination. The FTA card was then hung on a drying line using a paper clip for air drying under room temperature for 2–5 hours. After

drying, the FTA cards were packed in an envelope and sent to the laboratory for analysis.

4.2.3 SSR analysis

In the laboratory, (INCOTEC, South Africa), samples on FTA cards from the twenty potato clones (10 samples per clone) were analysed. All the samples from each clone were bulked. A single punch of each card per submission was taken and homogenized in the Finnzymes dilution buffer (Kit). Then 2 uL of each of the bulked sample was used in the polymerase chain reaction (PCR). Twenty four SSR markers were used in this study. These were selected from previous studies based on their high polymorphic information content (PIC) (Ghislain et al., 2004; Feingold et al., 2005; Ghislain et al., 2009; Rocha, 2010). Twelve of them belong to the latest potato genetic identity (PGI) kit (Ghislain et al., 2009) while the others were identified from other studies and selected based on high PIC (Ghislain et al., 2004; Feingold et al., 2005; Ghislain et al., 2009; Rocha, 2010). The PCR products were fluorescently labeled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (AppliedBiosystems, Johannesburg, South Africa). Analysis was performed using GeneMapper 4.1. Euclidian distances were calculated between bulked samples, using the program GGT 2.0 (Van Berloo, 2007). Because potato is an autotetraploid, each individual could contain between one and four different alleles at any one locus. The SSR marker alleles were scored for presence or absence of the band for all the 20 potato clones and treated as dominant markers. Therefore, the bands generated by SSR markers were not considered allelic but evaluated as dominant markers, so the data were considered binary. Thus, to evaluate the results of SSR markers, each amplified fragment was considered as one locus. The genetic similarity matrix of the 20 potato clones was calculated using the Jaccard's coefficient (Anderberg, 1973).

The data matrices of the genetic distances were used to create the dendrogram using the unweighted pair group method with arithmetic mean allocated (UPGMA). The polymorphic information content (PIC), which is a measure of allelic diversity, was calculated, based on the equation: $PIC = 1 - \Sigma(pi^2)$, where pi is the frequency of i^{th} allele in the accessions (Nei, 1973; Rafalski et al., 1996).

4.3 Results

4.3.1 Genetic polymorphisms

The twenty four SSR primers identified 160 alleles among the 20 potato clones. The number of alleles scored across SSR loci ranged from 2 to 14, with an average of 6.67 alleles (Table 4. 2). The PIC estimated forall loci ranged from 0.839 to 0.208 with an average of 0.649. Expected heterozygosity (He) values, as a measure of allelic diversity at a locus varied from 0.856 to 0.236 with an average of 0.69 (Table 4. 2). Correlations were positive and strong between PIC and He (r= 0.986), PIC and number of alleles (r=0.772) and, He and number of alleles (r=0.715). Only seven SSR loci had PIC values less than 0.65 i.e. (STM1016=0.3750, STM0019a=0.5859, STPoAc58=0.2997, StI031=0.3750, STM1031=0.2078, STM2022=0.4482 and STM5121=0.3737). The remaining 17 SSR makers had potential to detect differences among the twenty potato clones.

Table 4.2.Description of repeat types, primer sequence, allelic information and PIC values of the 24 SSR loci used to genotype 20 potato clones

NO	Marker name (at SCRI)	Repeat	Primer sequences(5'-3') Forward-Reverse	No of alleles	Allele Size (bp)	PIC	He	PGI Kit
1	STM1052	(AT)14GT(AT)4(GT)6	CAATTTCGTTTTTTCATGTGACAC ATGGCGTAATTTGATTTAATACGTAA	7	224-248	0.7603	0.7846	Yes
2	STM2013	(TCTA)6	TTCGGAATTACCCTCTGCC AAAAAAAGAACGCGCACG	7	160-185	0.7594	0.7901	No
3	STM1104	(TCT)5	TGATTCTCTTGCCTACTGTAATCG CAAAGTGGTGTGAAGCTGTGA	10	182-200	0.7997	0.8233	Yes
4	STM1016	(TCT)9	TTCTGATTTCATGCATGTTTCC ATGCTTGCCATGTGATGTGT	2	163-175	0.3750	0.5000	No
5	STM1049	(ATA)6	CTACCAGTTTGTTGATTGTGGTG AGGGACTTTAATTTGTTGGACG	10	136-212	0.7838	0.8115	No
6	STM0019a	(AT)7(GT)10(AT)4 (GT)5(GC)4(GT)4	AATAGGTGTACTGACTCTCAATG TTGAAGTAAAAGTCCTAGTATGTG	8	195-256	0.5859	0.6089	Yes
7	STM1106	(ATT)13	TCCAGCTGATTGGTTAGGTTG ATGCGAATCTACTCGTCATGG	5	165-184	0.7127	0.7562	Yes
8	STM0037	(TC)5(AC)6AA(AC)7(AT)4	AATTTAACTTAGAAGATTAGTCTC ATTTGGTTGGGTATGATA	10	85-108	0.6834	0.728	Yes
9	STM0030	Compound(GT/GC)(GT)8	AGAGATCGATGTAAAACACGT GTGGCATTTTGATGGATT	9	152-186	0.8244	0.8432	No
10	STI0012	(ATT)n	GAAGCGACTTCCAAAATCAGA AAAGGGAGGAATAGAAACCAAAA	6	182-208	0.7167	0.7538	Yes
11	STI0023	(CAG)n	GCGAATGACAGGACAAGAGG TGCCACTGCTACCATAACCA	9	80-220	0.7949	0.8194	No
12	STI0030	(ATT)n	TTGACCCTCCAACTATAGATTCTTC TGACAACTTTAAAGCATATGTCAGC	6	73-122	0.6807	0.7284	Yes
13	STI0036	(AC)n(TC)imp	GGACTGGCTGACCATGAACT TTACAGGAAATGCAAACTTCG	9	131-163	0.8389	0.8555	No
14	STI0032	(GGA)n	TGGGAAGAATCCTGAAATGG TGCTCTACCAATTAACGGCA	6	124-150	0.6945	0.7323	Yes
15	STM5127	(TCT)n	TTCAAGAATAGGCAAAACCA CTTTTTCTGACTGAGTTGCCTC	7	254-295	0.8140	0.8357	Yes
16	STGBSS	(TCT)n	AATCGGTGATAAATGTGAATGC ATGCTTGCCATGTGATGTGT	4	161-177	0.7031	0.7500	No
17	STWAX-2	(ACTC)n	CCCATAATACTGTCGATGAGCA GAATGTAGGGAAACATGCATGA	6	232-259	0.7083	0.7519	No

18	StI046	(GAT)n	CAGAGGATGCTGATGGACCT GGAGCAGTTGAGGGCTTCTT	10	196-229	0.8362	0.8533	No
19	STPoAc58	(TA)13	TTGATGAAAGGAATGCAGCTTGTG ACGTTAAAGAAGTGAGAGTACGAC	5	246-254	0.2997	0.31	Yes
20	STM0031	(AC)5...(AC)3(GCAC)(AC)2(GCAC)2	CATACGCACGCACGTACAC TTCAACCTATCATTTTGTGAGTCG	14	110-210	0.7964	0.8207	Yes
21	StI031	(TCA)n	AGGCGCACTTTAACTTCCAC CGGAACAAATTGCTCTGATG	2	141-167	0.3750	0.5000	No
22	STM1031	(AT)13	TGTGTTTGTTTTTCTGTAT AATTCTATCCTCATCTCTA	2	276-290	0.2078	0.2355	No
23	STM2022	(CAA)3...(CAA)3	GCGTCAGCGATTTCAGTACTA TTCAGTAACTCCTGTTGCG	4	190-210	0.4482	0.5463	No
24	STM5121	(TGT)n	CACCGGAATAAGCGGATCT TCTTCCCTTCCATTTGTCA	2	300-310	0.3737	0.4974	Yes

SCRI= Scottish Crop Research Institute

PIC=Polymorphic information content; He= heterozygosity

4.3.2 Cluster analysis among potato clones

The dendrogram constructed using the UPGMA clustering algorithm based on SSR data matrices grouped the potato clones into three major clusters (Figure 4.1). The first cluster consisted of Meru Mugaruro alone while the third cluster consisted of Bishop Gitonga, C1, Kenya Furaha and Kenya Karibu. The shortest genetic distance was found between Tigoni Long and C4. With the exception of Meru Mugaruro, Bishop Gitonga, Cangi, Nyayo, Tigoni Long and Kihoro, the rest originated from CIP where they could have shared some parents and hence high level of similarity. In addition, Tigoni Long is suspected to have escaped from CIP germplasm during national performance trials (NPT) in Kenya (Kabira, Pers.Comm). Among the 20 clones, Meru Mugaruro was the least genetically related to the other clones (Figure 4.1). Meru Mugaruro is suspected to be a farmers' selection from Kerr's Pink. Kerr's Pink is an old Scottish variety released in Kenya in 1927 (ASARECA, 2004). This may explain the least genetic relationship between Meru Mugaruro and other potato clones. The results also show that the 24 microsatellite markers distinguished all the 20 potato clones.

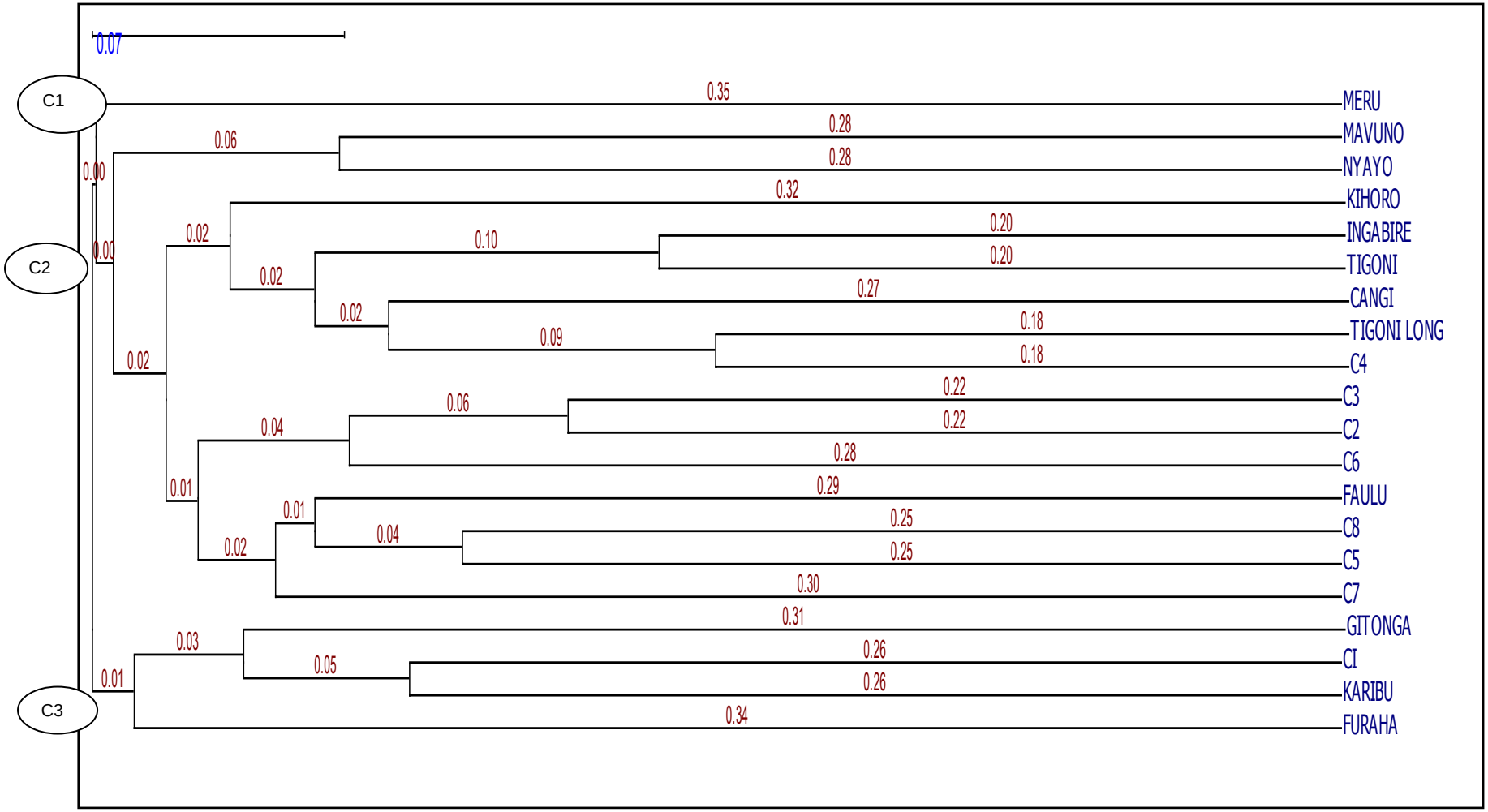

Figure 4.1.Dendrogram showing genetic relationship among 20 potato clones obtained using 24 SSR markers generated by UPGMA. The three clusters identified are C1, C2 and C3..
Furaha=Kenya Furaha; Karibu=Kenya Karibu; Gitonga=Bishop Gitonga; Faulu=Kenya Faulu; Mavuno=Kenya Mavuno; Meru- Meru Mugaruro

Table 4.3. .Jaccard's similarity matrix for 20 potato clones analyzed using 24 SSR markers

	MERU	GIT	FUR	CI	MAV	INGA	TIG	C3	CAN	C8	KIH	TIG. LONG	C7	C6	C4	C2	C5	NYAYO	FAU	KAR
MERU																				
BISHOP GITONGA	0.67																			
KENYA FURAHA	0.79	0.63																		
CI	0.74	0.58	0.67																	
KENYA MAVUNO	0.79	0.70	0.73	0.70																
INGABIRE	0.71	**0.83**	0.73	0.81	0.81															
TIGONI	0.65	0.67	0.73	0.63	0.65	**0.39**														
C3	0.70	0.67	0.68	0.60	0.62	0.76	0.65													
CANGI	0.65	0.75	0.65	0.65	0.70	0.56	0.63	0.61												
C8	0.74	0.75	0.67	0.68	0.58	0.72	0.65	0.67	0.63											
KIHORO	0.74	0.67	0.65	0.61	0.68	0.61	0.71	0.67	0.59	0.63										
TIGONI LONG	0.65	0.70	0.70	0.68	0.67	0.63	0.61	0.53	0.56	0.60	0.65									
C7	0.70	0.79	0.78	0.65	0.60	0.74	0.63	0.63	0.72	0.56	0.72	0.67								
C6	0.76	0.74	0.68	0.55	0.70	0.71	0.75	0.58	0.72	0.58	0.68	0.65	0.63							
C4	0.74	0.71	0.60	0.65	0.70	0.53	0.56	0.50	0.53	0.63	0.61	**0.36**	0.74	0.58						
C2	0.71	0.75	0.73	0.77	0.82	0.73	0.73	0.44	0.74	0.68	0.75	0.67	0.75	0.55	0.53					
C5	0.70	0.79	0.77	**0.85**	0.70	0.67	0.67	0.72	0.69	0.50	0.76	0.63	0.63	0.74	0.71	0.61				
NYAYO	0.74	0.79	0.78	0.82	0.57	0.81	0.70	0.70	0.63	0.65	0.75	0.72	0.74	0.75	0.74	0.70	0.78			
KENYA FAULU	0.65	0.78	0.75	0.71	0.67	0.65	0.68	0.63	0.65	0.61	0.74	0.56	0.63	0.58	0.65	0.60	0.56	0.58		
KENYA KARIBU	0.68	0.67	0.76	0.53	0.75	0.74	0.56	0.70	0.67	0.76	0.69	0.68	0.61	0.72	0.75	0.81	0.78	0.67	0.58	

GIT=Gitonga; FUR= Kenya Furaha; MAV= Kenya Mavuno; INGA=Ingabire; TIG. = Tigoni; CAN- Cangi; KIH=Kihoro; FAU=Kenya Faulu; KAR= Kenya Karibu

The genetic distance between clones ranged from 0.36 to 0.85 (Table 3). The short genetic distance between C4 and Tigoni Long (0.36) confirms the suspicion that Tigoni Long might have escaped from CIP germplasm. The short genetic distance between Tigoni and Ingabire (0.39) could be due the fact that both of them are selections from a single cross.

4.4 Discussion and conclusions

The current study was undertaken to determine the genetic relationships among potato clones so as to complement other bacterial wilt-resistance data in identifying parents for a breeding programme.

The SSR markers were chosen for potato genetic identification because of their high genetic information content, high reproducibility, and simplicity of use. They are appropriate, cost-effective and simple tools for laboratories in developing countries with financial constraints. The high PIC values in most of the SSR markers observed in this study could be due to the fact that most of the potato clones used in this study were from CIP and could be closely related. Some markers used in the present study had different PIC values in a previous study i.e. STM1016 had 0.84; STM1031 had 0.499; STM2022 had 0.621; STM5121 had 0.733 while STPoAc58 had 0.754 (Ghislain et al., 2009). In yet other studies, STM0019a had 0.8808; STM1031 had 0.6584; STM1016 had 0.7757; STM2022 had 0.7531 while STPoAc58 had 0.7033 (Ghislain et al., 2004); StI031 had 0.92 (Feingold et al., 2005) and StI046 had 0.97 (Rocha, 2010). This could be due to the fact that microsatellites are often useful for only closely related germplasm; amplification of moderately divergent cross species can lead to significant distortion in genetic similarity estimates (Peakall et al., 1998). In addition, differences in laboratory procedures may have also led to the discrepancies in PIC values. The SSR markers did not cluster the potato clones into different bacterial wilt resistance groups. This is probably because bacterial wilt resistance is very unstable due to strong host-pathogen-environment interaction; hosts resistant to the disease in one year/environment or location may succumb to the disease in the other year/environment or location (French and Lindo, 1982; Tung et al., 1990; Tung, 1992; Tung et al., 1992b; Tung et al., 1993). In addition, the pedigrees of some clones are unknown; some clones are farmer selections while for others, proper records may not have been kept during breeding. Despite the

discrepancies, the SSR markers generated useful information that will assist in identifying parents to include in the breeding programme.

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Chapter Five: Combining ability analysis of tuber yield and related traits and bacterial wilt resistance in potato

Abstract

Understanding the inheritance of any given trait helps in selecting suitable parents and crosses to use in a breeding programme and to determine the subsequent selection procedure to follow. In potatoes (*Solanum tuberosum* L. 2n=4x=48) both the general combining ability (GCA) effects of parents and specific combining ability (SCA) effects of crosses are important in conditioning economic traits. The objective of this study was to determine the combining abilities for tuber yield and related traits and bacterial wilt resistance in selected potato clones. Fourteen parents [eight males that are commonly grown in Kenya and six female clones with moderate resistance to bacterial wilt from the International Potato Center (CIP)] were crossed using the North Carolina II mating design. The resultant 48 families were evaluated for yield and yield components and bacterial wilt resistance in inoculated fields at Kenya Agricultural Research Institute, National Agricultural Research Laboratories (KARI-NARL) and at a farmer's field at Kinale using a 6 x 8 alpha lattice experimental design with three replications. Generally, crosses tested at Kinale took a longer time to start wilting (53 days), had lower values for the area under the disease progress curve (AUDPC) (1871.1), percentage of symptomatic tubers based on tuber numbers (PSTTN) (16.9) and percentage of symptomatic tubers based on weight (PSTTW) (18.0) than at KARI-NARL. Significant ($P<0.001$) GCA effects were observed for males for total tuber weight (TTW) and days to maturity (DTM) while the GCA effects for females were significant ($P\leq 0.001$) for TTW and ($P<0.01$) for total tuber numbers ha^{-1} (TTN) at KARI-NARL. The SCA effects were significant ($P\leq 0.05$) for TTN and ($P\leq 0.001$) for TTW, percentage of ware sized tubers (PWTTW) and DTM at KARI-NARL. At Kinale, significant ($P\leq 0.001$) differences were found among crosses for TTW and PWTTW. The current study also found that for all tuber yields related traits (TTN, TTW, PWTTW and DTM), SCA was greater than GCA. In addition, GCA was slightly more important than SCA in the expression of PSTTW and AUDPC (at KARI-NARL) and PSTTW and PSTTN at Kinale. For days to onset of wilting (DTOW), the GCA and SCA effects were almost equal.

Keywords: Bacterial wilt, Gene action, General combining ability, Potato, Specific combining ability

5.1 Introduction

Understanding the inheritance of any given trait helps in selecting suitable parents and their crosses to use in a breeding programme, to choose proper mating design or to identify the subsequent selection procedure to follow. Gene action reflects gene differences that provide the basis for the selection of desirable genotypes in plant breeding (Rasmusson and Gengenbach, 1983; Sleper and Poehlman, 2006). Gene inheritance is the transmission of genetic information to succeeding generations (Falconer, 1989). The efficient recovery and maintenance of desirable genes transmitted from crosses of selected parents to their progeny requires knowledge about the modes of gene action and its inheritance (Falconer and Mackay, 1996).

In potatoes (*Solanum tuberosum* L., 2n=4x=48) both the general combining ability (GCA) effects of parents and specific combining ability (SCA) effects of their crosses are important in conditioning economic traits. In this crop all genetic effects are fixed at the F_1 stage, as with clonal propagation, there is no further segregation. General combining ability is the average performance of a parental clone in hybrid combinations and SCA is the contribution of a clone to hybrid performance in across with a specified clone, in relation to its contributions in crosses with an array of specified clones (Sleper and Poehlman, 2006). General combining ability represents mainly the additive and additive x additive type of genetic variance (Gopal, 1998), while SCA is mainly due to genes with dominance and/or epistatic effects. Mating designs such as North Carolina II (NCII) and diallel, which partition the GCA and SCA are commonly used to determine combining abilities.

Resistance to bacterial wilt of potatoes [caused by *Ralstonia solanacearum* (Smith, 1896) Yabuuchi et al. 1995] was reported to be controlled by a few genes (Martin and French, 1985) and by three independent and dominant major genes (Buddenhagen, 1986). In addition, it was reported that both additive and non-additive gene actions are important in the inheritance of the resistance (Rowe and Sequeira, 1970). Later, it was reported that resistance is controlled by at least four major genes (French et al., 1997; Grimsley and Hanson, 1998). Other studies indicated that the resistance is polygenic and quantitative in nature, and involves genes with major and minor effects (Tung et al., 1993; Cook and Sequeira, 1994). Tung et al. (1992a)

found that the SCA effect was more important than the GCA effect in conditioning resistance to bacterial wilt, and there was a strong genotype x environment interaction.

There is also evidence that in the inheritance of resistance to bacterial wilt, non-additive gene action is important, and is largely of the epistatic type (Tung et al., 1992a; Tung et al., 1992b,1993). Therefore, breeding schemes designed to make use of both additive and non-additive gene actions seem most suitable in developing resistance. Moreover, the genetic background for adaptation is of crucial importance for expression of resistance (Tung, 1992; Tung et al., 1993). There is a large amount of interaction between genes for resistance and those for adaptation (Tung et al., 1992a; Tung et al., 1992b). Therefore, potato clones with a wide genetic background for both bacterial wilt resistance and adaptation tend to display a high level of resistance, which is stable over environments (Tung et al., 1993). In order to develop a stable resistance in potato populations, a wide genetic base for resistance and adaptation to the environment where the pathogen occurs would therefore be necessary (Tung et al., 1993).

In an attempt to develop improved potato clones with high yield, yield related traits and bacterial wilt resistance, the KARI-Tigoni potato research program in Kenya is constantly evaluating various locally grown varieties and clones from the International Potato Center (CIP) that are adapted to tropical highland environments. Information on combining ability for tuber yields and related traits as well as bacterial wilt resistance of potato clones that are commonly grown by farmers and clones from CIP is lacking. There is need to get this information since it is is essential for the success of the local breeding program. Therefore, the objective of this study was to determine the combining ability effects for yield and yield related traits and bacterial wilt resistance of selected potato clones and their crosses. Selected parental clones and promising families will be used for further breeding in Kenya and similar agro-ecologies.

5.2 Materials and methods

5.2.1 Study sites

The production of F_1 potato seeds and the seedling multiplication were done at the Kenya Agricultural Research Institute, National Potato Research Centre at Tigoni (KARI-Tigoni). The KARI-Tigoni station is located 40 km northwest of Nairobi at an altitude of 2051 meter above sea level (masl) latitude of $1^0$9'7.22" S and longitude $36^0$41'8.72" E (Jaetzold et al., 2006c). The average annual rainfall is 1096 mm with a bimodal distribution. The long rainy season occurs between March and May, while the short rainy season is between October and December (Jaetzold et al., 2006c). The mean annual air temperature is 18^0C and ranges between 12 and 24^0C. The soil type is humic-nitosol (alfisol) derived from quartz trachyte(Jaetzold et al., 2006c). The soil is very deep and well drained with a pH range of 5.5 to 6.5. The soil is of medium inherent fertility with organic carbon content of 1.65%. Exchangeable bases of potassium, calcium and magnesium are moderate to high with available potassium being about 21.2 ppm (Jaetzold et al., 2006c).

Determination of combining abilities for bacterial wilt resistance and tuber yield and its components was carried out at the Kenya Agricultural Research Institute, National Agricultural Research Laboratories (KARI-NARL) and at a farmer's field at Kinale. The KARI-NARL station has been described in section 3.3. The Kinale site is located 70 km northwest of Nairobi at an altitude of 2674 masl, latitude of $0^0$51' 30.43" S and longitude $36^0$36' 3.83" E (Jaetzold et al., 2006c). The average annual rainfall is 1276 mm with a bimodal distribution. A long rainy season occurs between March and May while the short rain season is between October and December (Jaetzold et al., 2006c). The mean air temperature ranges from 13.5 to 15.2^0C. The soil type is humic-andosol (Jaetzold et al., 2006c).

5.2.2 Plant materials and crosses

Eight potato clones selected previously in a bacterial wilt screening trial (Muthoni et al., 2014) were used as males for crossing using a North Carolina II mating design. The eight clones are high yielding and popular with Kenyan farmers, but highly susceptible to bacterial wilt (Muthoni et al., 2014). These clones were crossed to a set of six clones used as females, which were sourced from CIP. These six clones are reported to have moderate resistance to bacterial wilt (Priou, 2004). In the field,

all the 14 parents (Table 5.1) were planted out in a crossing block. Each parent was planted in three rows; each row had about 100 plants. Plants spacing was 75 x 30cm between and within rows respectively. During planting, (Diammonium phosphate (DAP) (18% N: 46% P_2O_5) was applied at the recommended rate of 500 kg ha^{-1}. Weeding, ridging and pests and late blight control were carried out as per recommendations for potato production in Kenya (KARI, 2008). Planting was done on 13th September 2012.

Table 5.1.Name, source, parentage, and reaction to bacterial wilt of the 14 potato parents

Parent	Germplasm maintainer	Male/Female	Response to bacterial wilt
Cangi	KARI- Tigoni	Male	Susceptible
Kenya Karibu	KARI- Tigoni	Male	Susceptible
Tigoni	KARI- Tigoni	Male	Susceptible
Sherekea	KARI- Tigoni	Male	Susceptible
MeruMugaruro	KARI- Tigoni	Male	Susceptible
Kihoro	KARI- Tigoni	Male	Susceptible
Ingabire	KARI- Tigoni	Male	Susceptible
Bishop Gitonga	KARI- Tigoni	Male	Susceptible
391919.3	CIP	Female	Resistant
394904.9	CIP	Female	Resistant
394905.8	CIP	Female	Resistant
392278.19	CIP	Female	Resistant
394895.7	CIP	Female	Resistant
394903.5	CIP	Female	Resistant

CIP= International Potato Center, KARI-Tigoni= Kenya Agricultural Research Institute, National Potato Research Centre, Tigoni

5.2.2 Generation of true potato seed and F_1seedlings

A few days after crossing, berries started forming on successful crosses and about 40 days later, they were harvested. The harvested berries were stored in khaki paper bags for three weeks to soften before processing. The ripened berries were processed by cutting them with a knife and emptying the seeds into a basin containing clean water. The seeds were washed and then spread on filter papers and placed on a table in the laboratory to air-dry overnight. The following day, all the seeds from each cross family were soaked in 1500 ppm GA_3 solution for 24 hours to break dormancy. Thereafter they were rinsed and immediately sown in plastic trays containing sterilized sand. Watering was done using a can and the seedlings were sprayed against pests and diseases as required. Four weeks later, all the seedlings were transplanted directly from the plastic trays into the field at KARI-Tigoni during the long rains season of 2013. Transplanting was done on 3rd April 2013.

5.2.3 Field management of F_1seedlings

The seedlings were transplanted in rows at a spacing of 75 x 30cm. At transplanting, DAP (18% N: 46% P_2O_5) was applied at the recommended rate of 500 kg ha^{-1}. Weeding, ridging and pests and late blight control were carried out as per recommendations for potato production in Kenya (KARI, 2008). When the crop was mature, it was harvested, each plant separately. From each cross family, 150 plants were randomly sampled and from each selected plant, two tubers were retained.

(The rest were later planted at KARI-Tigoni in the following season so as to generate more tubers for the second season bacterial wilt evaluation trial). One tuber from each of the 150 selected plants was picked and bulked together so as to come up with one bulked sample of 150 tubers. This was repeated again to generate a second bulked sample. Each of the two bulked samples consisted of 150 tubers. The two bulked samples were later planted out at KARI-NARL and Kinale respectively for determining the combining ability for bacterial wilt resistance and tuber yield and its components. To break tuber dormancy, the samples were treated by dipping them in a big container with GA_3 at 5ppm for ten minutes. Thereafter, they were air-dried and covered with a black polythene sheet for one week. They were then uncovered until sprouting.

5.2.4 Determination of combining abilities for bacterial wilt resistance and tuber yield and its components

Using the first clonal generation, combining ability effects for bacterial wilt resistance, yield and yield related traits were determined at the KARI-NARL and at a farmer's field at Kinale.

Once the two bulked tuber samples sprouted, they were planted out in the field at KARI-NARL and at Kinale during the 2013 short rains season so as to determine their reaction to bacterial wilt. Planting was done on 1st October 2013 at KARI-NARL and 2nd October at Kinale.

At each site, the experimental materials consisted of the 48 families. These were planted in a 6 x 8 alpha lattice design replicated three times. Each plot consisted of 50 plants i.e. 5 rows each consisting of 10 plants. The tubers were planted in furrows at a spacing of 75 x 30cm. During planting, DAP (18% N: 46% P_2O_5) was applied at the recommended rate of 500 kg ha^{-1}. Weeding, ridging and pests and late blight control were carried out as per recommendations for potato production in Kenya (KARI, 2008).

To ensure uniform distribution of bacterial wilt at KARI-NARL and Kinale, a bacterial suspension concentrated at 3.0 x 10^{9}cfuml^{-1} was poured into the furrows during planting at a rate of 1 litre per plot. The resident as well as inoculated bacteria were confirmed as bacterial wilt biovar 2 by Plantovita, South Africa based on the ability

of the bacteria to produce acid from several disaccharides and sugar alcohols (Buddenhagen and Kelman, 1964). For proper disease expression, supplemental watering using overhead irrigation was done during the dry times.

5.2.5 Data collection

Data collected included the number of days from planting to maturity (DTM), days to onset of wilting (DTOW) and bacterial wilt incidence (BWI). Time to maturity was counted as the number of days from planting to when 75% of the plants had senesced. These data were taken on a plot basis. The BWI scores were used to calculate area under the disease progress curve (AUDPC) (CIP, 2007) using the formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{(S_i + S_{i+1})(t_{i+1} - t_i)}{2}$$

Where S_i is the BWI at days *i*, and *n* is the total number of sampling times, *t* is the number of days after planting

During harvesting, the 24 middle plants per plot were harvested, each plant separately. Total number of tubers was counted from each of the 24 plants. In addition, the number of symptomatic tubers (i.e. showing rotting or bacterial ooze in the tuber eyes or soil adhering to the eyes of the tubers) and healthy looking tubers (asymptomatic) were determined. The healthy looking tubers were then categorized based on size i.e. ware (>45mm diameter) and seeds (<45mm diameter). Their number and weights were recorded. The weights of symptomatic and ware tubers were expressed as percentage of the total yields. The percentage of symptomatic tubers was expressed both in weight, a value which is useful to determine yield losses (t ha^{-1}), and as a number of infected tubers, a value which is used for the calculation of infection tuber rates.

Only healthy-looking tubers selected above were analyzed for latent infection by *R. solanacearum.* For each plot, 60 healthy-looking tubers were placed in sugar paper bags and delivered to the laboratory for latent infection analysis. The tubers were washed and disinfected. They were then divided into five groups of 12 tubers each. Sap from each group was extracted to constitute a sample which was then analyzed

for latent infection using the post-enrichment enzyme-linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA) test (Priou et al., 1999a).

5.2.6 Data analysis

5.2.6.1 Analysis of variance

Data on days to maturity (DTM), days to onset of wilting (DTOW), area under the disease progress curve (AUDPC), total tuber numbers (TTN), total tuber weight in t ha^{-1} (TTW), percentage of symptomatic tubers based on total tuber numbers (PSTTN), percentage of symptomatic tubers based on total tuber weight (PSTTW), and percentage of ware sized tubers based on total tuber weight (PWTTW) values were subjected to analysis of variance using the lattice procedure of Statistical Analysis Systems (SAS 9.1) statistical package (SAS, 2003).

Data on TTN, TTW, PWTTW, PSTTN and PSTTW were first averaged on a plot basis; the average value was then used to extrapolate values per ha. Data on latent infection (LI) level were subjected to the Kruskal-Wallis non-parametric test procedure using SPSS for Windows Release Version 18.0 (SPSS Inc., 2009). Data for different sites were analyzed separately. Resistance to bacterial wilt of the crosses was determined using ranking based on % LI, AUDPC, DTOW, PSTTW and PSTTN and the percentage of total infected tubers (PTIT). Crosses with low values of % LI, low AUDPC, low PSTTN, low PSTTW and high values of DTOW were considered more resistant to bacterial wilt and hence ranked high.The PTIT was calculated as suggested by CIP (2007):

$$\mathrm{PTIT} = \mathrm{PSTTN} + \frac{(\%\ \text{healthy looking tubers x}\ \%\ \mathrm{LI})}{100}$$

Where PTIT is the percentage of total infected tubers, PSTTN is the percentage of symptomatic tubers based on total tuber numbers and % LI is the % latent infection. Small values of PTIT indicates high resistance and hence high ranking. Based on PTIT, bacterial wilt resistance levels are categorized as indicated in Table 5.2 (CIP, 2007).

Table 5.2. Resistance levels of potatoes to bacterial wilt based on percentage of total infected tubers

Resistance levels	PTIT
Highly resistant	0
Resistant	1<15
Moderately resistant	15- <30
Moderately susceptible	30- <45
Susceptible	45- <60
Highly susceptible	≥60

Modified from CIP(2007)

5.2.6.2 Estimation of general and specific combining ability effects

Parents were considered as fixed effects in the test of significance.The GCA and SCA values for each trait were calculated following the NCII mating design across sites (Hallauer et al., 1988) as follows:

$$Yijk = \mu + gi + gj + sij + \varepsilon ijk$$

Where, Yijk = observed value of the ijth genotype in the kth environment

μ = overall mean;

gi = the GCA effects of the ith parent;

gj = the GCA effects of the jth parent;

Sij = the SCA effects for the cross between the ith parent and the jth parent

εijk = experimental error associated with ijth genotype in the kth environment.

As the parents were considered fixed, inferences drawn from this study cannot be generalised. The relative importance of GCA and SCA in influencing the performance of the crosses were estimated using the general predicted ratio (GPR) for all the traits (Baker, 1978);

$$\frac{GCA}{SCA} = \frac{\text{MSQ GCA (pooled)}}{\text{MSQ GCA (pooled)} + \text{MSQ SCA}}$$

$$\text{MSQGCA (pooled)} = \frac{(MSQ\ GCA\ male + MSQ\ GCA\ female)}{number\ of\ replications}$$

Where; MSQGCA and MSQSCA are the mean squares for GCA and SCA, respectively. When the ratio >0.5, GCA is more important than SCA in the inheritance of the character concerned, while the reverse is true when the ratio is <0.5 (Baker, 1978).

5.3 Results

5.3.1 Analysis of variance for crosses across sites

The combined analysis of variance showed significant differences among the crosses for TTW (P≤0.001), TTN (P≤0.05), PWTTW (P≤0.001), PSTTW (P≤0.05) and DTM (P≤0.01) (Table 5.3). The environmental (site) effect was significant (P≤ 0.001) for all the traits studied except PSTTN and AUDPC. The interaction between cross x site had significant (P≤ 0.05) effects for TTN and DTM.

There were significant differences (P≤0.001) among crosses for latent infection (Chi-square= 108.027; df =47) for Kinale and (Chi-square= 107.590; df =47) for the KARI-NARL site. In addition, % LI was higher at KARI-NARL (56.4) than at Kinale (53.8).

5.3.2 Ranking of crosses for bacterial wilt resistance across sites

Generally, the crosses planted at Kinale took a longer time to start wilting (53 days) and had lower values of AUDPC (1871.1), PSTTN (15.9) and PSTTW (18.0) than the crosses planted at KARI-NARL (Table 5.4). The resistance level of the potato crosses to bacterial wilt as determined by ranking based on the mean value across sites for % LI, AUDPC, DTOW, PSTTW and PSTTN showed that the five most resistant crosses were 392278.19 x Ingabire, 394903.5 x Meru Mugaruro, 394903.5 x Bishop Gitonga, 394903.5 x Cangi and 392278.19 x Meru Mugaruro in that order (Table 5.4). The resistance of the potato crosses to bacterial wilt as determined by ranking based on PTIT showed that the five most resistant crosses were 394903.5 x Ingabire, 394904.9 x Ingabire, 391919.3 x Ingabire, 394895.7 x Ingabire and 394905.8 x Ingabire in that order (Table 5.4). There was a significant (P≤ 0.05) and positive (r=0.318) correlation between the two ranking methods.

However, no cross was resistant to bacterial wilt [i.e PTIT, 1<15]; crosses 394903.5 x Ingabire and 394904.9 x Ingabire were moderately resistant while crosses 391919.3 x Ingabire and 394895.7 x Ingabire were moderately susceptible. The other crosses ranged from susceptible to highly susceptible.

Table 5.3.Combined analysis of variance for bacterial wilt resistance and, tuber yields and related traits at KARI-NARL and Kinale

Source of variation	df	Mean squares							
		TTW	TTN	PSTTN	PWTTW	PSTTW	AUDPC	DTOW	DTM
Sites	1	3541.17***	0.275422294E+16***	29639.23 ns	1950.47***	921.71***	1012.50 ns	975.35***	5210.50***
Rep(sites)	4	503.66***	508913253243.4***	21230.78 ns	45.49 ns	84.22 *	974801.00 ns	554.51***	39.93 ns
Crosses	47	1029.74***	1558531282495.7*	18950.26 ns	612.64***	41.33*	1462926.20 ns	98.22 ns	47.47*
Crosses x sites	47	65.75 ns	1525525137858.0*	18704.06 ns	16.67 ns	7.56 ns	936388.20 ns	42.01 ns	49.51*
Residual	188	124.71	1003526528920.5	18449.45	85.28	28.76	1143526.22	75.44	32.84

df=Degrees of freedom; *= Significant at P≤0.05; **= Significant at P≤0.01; ***= Significant at P≤0.001; ns=Non significant; TTW= Total tuber weight (tha^{-1}); TTN=Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha^{-1}); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); AUDPC= Area under the disease progress curve; DTOW= Days to onset of wilting; DTM= Days to maturity.

Table 5.4. Ranking of the potato crosses' resistance to bacterial wilt at Kinale and KARI-NARL

Cross	AUDPC		DTOW		PSTTW		PSTTN		% LI		Overall Rank (a)	PTIT	Overall Rank (b)
	Kinale	KARI-NARL	Kinale	KARI-NARL	Kinale	KARI-NARL	Kinale	KARI-NARL	Kinale	KARI-NARL			
391919.3 x Bishop Gitonga	1815	1928	57	57	17.1	20.1	18.1	22.3	53.3	60.0	19.0	65.4	26.5
391919.3 x Cangi	1413	1832	57	47	13.8	18.7	15.0	18.6	40.0	73.3	6.0	63.7	24.0
391919.3 x Kenya Karibu	1775	1847	57	50	18.0	20.9	15.1	18.5	53.3	53.3	8.0	61.2	18.0
391919.3 x Kihoro	1720	1870	60	57	17.8	19.7	18.6	22.0	53.3	53.3	14.0	62.8	23.0
391919.3 x Meru Mugaruro	1745	1790	50	50	16.4	19.1	17.4	18.2	80.0	66.7	25.0	78.1	43.0
391919.3 x Tigoni	1845	1908	57	47	17.8	21.5	16.8	18.7	53.3	53.3	16.0	61.6	20.0
391919.3 x Sherekea	1788	1882	57	50	16.3	19.0	18.1	22.2	60.0	80.0	22.0	75.8	40.0
391919.3 x Ingabire	2053	1747	53	53	19.7	19.2	13.3	26.0	13.3	20.0	15.0	32.8	**3.0**
394904.9 x Bishop Gitonga	2140	2183	50	47	18.9	22.2	18.9	23.0	60.0	60.0	43.0	68.4	32.0
394904.9 x Cangi	1837	1912	53	50	19.1	21.6	17.7	23.0	80.0	80.0	31.0	84.1	47.0
394904.9 x Kenya Karibu	2502	2650	53	43	20.0	31.0	18.8	29.7	66.7	73.3	48.0	77.1	41.0
394904.9 x Kihoro	1880	2267	47	43	21.6	23.0	17.1	25.0	73.3	80.0	47.0	81.5	45.0
394904.9 x Meru Mugaruro	2010	2097	53	47	18.6	24.6	17.0	17.8	60.0	26.7	26.0	53.3	6.5
394904.9 x Tigoni	1787	2400	50	53	18.1	28.6	16.6	26.3	53.3	40.0	32.5	58.4	13.0
394904.9 x Sherekea	2297	2453	53	50	20.6	25.0	19.4	22.4	66.7	66.7	44.0	73.6	38.0
394904.9 x Ingabire	1750	1817	53	50	16.0	19.3	17.2	21.9	13.3	13.3	10.0	30.3	**2.0**
394905.8 x Bishop Gitonga	2090	2133	47	43	21.3	25.0	16.4	22.2	60.0	46.7	39.0	62.5	22.0
394905.8 x Cangi	2213	1993	53	47	18.9	21.7	17.6	21.7	46.7	93.3	37.0	75.4	39.0
394905.8 x Kenya Karibu	1875	1945	53	53	19.2	20.8	17.0	21.9	66.7	46.7	23.0	65.4	26.5
394905.8 x Kihoro	1827	1970	50	47	20.0	22.2	16.5	20.4	86.7	80.0	34.0	86.5	48.0
394905.8 x Meru Mugaruro	1580	1715	50	50	15.7	18.7	14.2	19.8	46.7	60.0	12.0	61.1	17.0
394905.8 x Tigoni	1895	1933	57	47	17.7	22.7	16.1	18.4	60.0	46.7	21.0	61.5	19.0
394905.8 x Sherekea	2255	2198	53	43	19.2	24.5	20.5	23.1	53.3	26.7	40.0	53.26	6.5
394905.8 x Ingabire	1648	1667	53	47	17.9	16.9	19.7	24.0	20.0	40.0	20.0	45.1	**5.0**
392278.19 x Bishop Gitonga	1955	2077	50	47	21.1	23.0	14.4	22.1	66.7	80.0	38.0	78.0	42.0
392278.19 x Cangi	2073	2200	50	40	19.2	23.6	19.0	24.5	40.0	60.0	41.5	60.6	16.0
392278.19 x Kenya Karibu	2037	2190	53	50	19.2	23.2	14.1	18.8	66.7	66.7	29.0	72.2	36.0
392278.19 x Kihoro	2217	2397	57	47	22.7	27.5	18.5	24.9	66.7	26.7	36.0	58.9	14.0
392278.19 x Meru Mugaruro	1440	1588	57	57	14.9	16.2	14.2	16.2	80.0	80.7	**5.0**	83.0	46.0
392278.19 x Tigoni	2185	2218	47	40	20.3	26.0	16.9	24.4	60.0	26.7	41.5	55.7	10.0

392278.19 x Sherekea	1618	1735	53	60	17.4	19.5	18.5	21.4	60.0	73.3	18.0	73.2	37.0
392278.19 x Ingabire	1253	1362	50	60	12.2	17.1	13.8	15.2	46.7	46.7	**1.0**	54.4	9.0
394895.7 x Bishop Gitonga	1785	1860	50	60	15.2	20.2	15.4	18.0	53.3	66.7	9.0	66.6	29.0
394895.7 x Cangi	1815	2172	57	47	18.9	24.1	16.9	20.9	66.7	80.0	30.0	78.2	44.0
394895.7 x Kenya Karibu	1735	1853	57	47	15.7	18.5	14.1	16.0	73.3	60.0	11.0	71.8	35.0
394895.7 x Kihoro	1802	1878	57	53	17.1	20.6	17.4	20.9	86.7	26.7	17.0	65.5	28.0
394895.7 x Meru Mugaruro	1928	2028	50	47	18.7	21.5	17.8	23.2	53.3	73.3	35.0	70.6	34.0
394895.7 x Tigoni	1677	1812	60	60	16.4	17.7	16.8	19.2	73.3	46.7	7.0	67.4	30.0
394895.7 x Sherekea	1910	1887	53	47	18.7	20.4	17.2	20.8	46.7	66.7	27.0	64.7	25.0
394895.7 x Ingabire	1848	1913	53	47	15.3	20.0	18.6	17.1	13.3	46.7	13.0	42.6	**4.0**
394903.5 x Bishop Gitonga	1573	1632	53	63	16.1	17.5	17.4	23.3	20.0	66.7	**3.0**	54.2	8.0
394903.5 x Cangi	1293	1395	53	50	14.1	17.9	17.7	18.4	46.7	46.7	**4.0**	56.3	12.0
394903.5 x Kenya Karibu	1980	2133	53	50	19.6	25.0	16.8	19.1	20.0	73.3	28.0	55.9	11.0
394903.5 x Kihoro	7412	2130	47	40	19.9	24.3	19.2	26.2	66.7	33.3	45.0	61.9	21.0
394903.5 x Meru Mugaruro	1297	1452	53	53	15.5	16.1	13.6	14.9	33.3	73.3	**2.0**	59.9	15.0
394903.5 x Tigoni	2522	2690	43	37	21.4	28.4	16.6	23.4	53.3	73.3	46.0	70.3	33.0
394903.5 x Sherekea	1978	2110	50	47	18.0	22.4	18.0	22.4	53.3	66.67	32.5	67.9	31.0
394903.5 x Ingabire	2072	2117	57	47	18.6	20.9	14.0	15.0	13.3	6.7	24.0	23.1	**1.0**
Mean	1871	1978	53	49	18.0	21.6	16.9	21.1	53.8	56.4		63.6	
% CV	28.0	27.4	6.9	11.7	12.4	15.6	11.8	14.2					
SE	524.47	541.73	3.67	5.75	2.23	3.37	2.00	3.00					

% LI= % Latent infection; DTOW= Days to onset of wilting; PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); AUDPC= Area under the disease progress curve.PTIT= Percentage of total infected tubers.Average rank= average of rank due to % LI, rank DTOW, rank PSTTW, rank PSTTN and rank AUDPC.Overall rank (a) =Ranking of crosses based on theaverage rank.. Overall rank (b) =Ranking of crosses based on their mean PTIT.

5.3.3 General and specific combining ability estimates for selected tuber yield traits and bacterial wilt resistance at KARI-NARL

Significant differences were found among the crosses for TTW (P≤ 0.001), TTN (P≤ 0.05), PWTTW (P≤0.001) and (P≤0.001) DTM at KARI-NARL (Table 5.5). Significant (P≤0.001) GCA effects were observed for males for TTW and DTM while GCA for females was significant for TTW (P≤ 0.001) and TTN (P≤ 0.05). In addition, male parents had far much higher GCA effect for TTW (812.65) than the female parents (480.60) while the opposite was true for TTN where male parents had GCA of (316230799728.1) and the females (4597865057068.8) (Table 5.5). The SCA effects were significant (P≤0.05) for TTN and (P≤ 0.001) for TTW, PWTTW and DTM (Table 5.5). The SCA was more important than GCA in the expression of all traits except PSTTW and AUDPC (Table 5.5).

Among the male parents, Kihoro had the highest GCA effects for TTW (7.96) followed by Bishop Gitonga (6.75) while Meru Mugaruro had the lowest (-10.51) (Table 5.6). Ingabire had the lowest GCA effects for AUDPC (-208.16) and PSTTW (-2.73) followed by Meru Mugaruro (200.10) and (-2.26), respectively (Table 5.6). Among the female parents, 391919.3 had the highest GCA for TTW (5.24) followed by 394903.5 (3.16) while 392278.19 had the lowest (-7.17) (Table 5.6). In addition, 391919.3 had the lowest GCA effects for AUDPC (-128.02) and PSTTW (-1.84) followed by 394895.7 (-53.02) and (-1.25) respectively (Table 5.6).

Among the crosses, 394905.8 x Kihoro had the highest (31.94) SCA effect for TTW followed by 394903.5 x Kenya Karibu (31.46) (Table 5.7).

Table 5.5 Analysis of variance of general and specific combining abilities for selected traits at KARI-NARL

Source of variation	df	Mean squares							
		TTW	TTN	PSTTN	PWTTW	PSTTW	AUDPC	DTOW	DTM
Replications	2	0.05*	18265738589182**	42410.90 ns	3.637 ns	135.31 *	476441.15 ns	544.44 ***	9.90 ns
Crosses	47	645.64***	24765618979.7*	37644.73 ns	336.688 ***	34.03 ns	242465.39 ns	101.05 ns	56.56 ***
GCA Males	7	812.65 ***	316230799728.1 ns	38557.07 ns	88.891 ns	60.29 ns	389677.75 ns	74.50 ns	96.03 ***
GCA Females	5	480.60 ***	4597865057068.8*	36448.06 ns	130.849 ns	62.17 ns	385586.98 ns	69.03 ns	42.08 ns
SCA	35	635.81 ***	2941744091674.5*	37633.21 ns	415.653 ***	24.76 ns	192576.98 ns	110.93 ns	50.73 ***
GCA/SCA		0.40	0.36	0.40	0.15	0.62	0.57	0.30	0.46
Residual	94	132.81	1871410119854.1	36884.75	91.65	38.42	315083.70	95.51	23.37

df=Degrees of freedom; * = Significant at P≤ 0.05; **= Significant at P≤ 0.01; ***= Significant at P≤ 0.001; ns=Non significant; TTW= Total tuber weight (t ha^{-1});TTN=Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha^{-1}); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); AUDPC= Area under the disease progress curve; DTOW= Days to onset of wilting; DTM= Days to maturity.

Table 5.6.General combining ability effects of parents for different traits at KARI-NARL

Males	TTN	PSTTN	TTW	PWTTW	PSTTW	AUDPC	DTOW	DTM
Bishop Gitonga	486110.64	-15.36	6.75	2.49	0.28	-9.55	3.54	4.31
Cangi	202160.19	-15.99	-2.05	2.60	-0.35	-61.22	-2.57	0.42
Kenya Karibu	-81790.03	-16.50	-2.37	-0.45	1.63	124.62	-0.35	0.14
Kihoro	239197.31	114.50	7.96	0.68	1.27	106.84	-0.35	-0.14
Meru Mugaruro	-699073.36	-18.83	-10.51	-0.99	-2.26	-200.10	1.32	-3.47
Tigoni	66357.97	-15.44	5.58	1.36	2.53	181.84	-2.01	0.97
Sherekea	41666.64	-15.10	1.86	-1.87	0.19	65.73	0.21	-2.36
Ingabire	-254629.36	-17.30	-7.23	-3.82	-2.73	-208.16	0.21	0.14
SE(males' GCA)	322439.50	45.27	2.72	2.26	1.46	132.31	2.30	1.14
Females								
391919.3	54012.31	-16.35	5.24	-0.62	-1.84	-128.02	2.01	1.04
394904.9	-205246.69	-13.54	-0.90	-3.59	2.80	243.85	-1.32	1.67
394905.8	405863.81	-15.73	2.12	3.52	-0.07	-34.06	-2.15	-1.25
392278.19	-566357.53	-16.23	-7.17	0.64	0.39	-7.60	0.76	-1.67
394895.7	-279320.69	-17.66	-2.45	0.81	-1.25	-53.02	1.60	-0.42
394903.5	591048.81	79.50	3.16	-0.76	-0.04	-21.15	-0.90	0.63
SE (females' GCA)	279240.80	39.20	2.35	1.95	1.27	114.58	1.99	0.99

TTN=Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); TTW= Total tuber weight (tha^{-1}); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha^{-1}); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); AUDPC= Area under the disease progress curve; DTOW= Days to onset of wilting; DTM= Days to maturity.

Table 5.7. Specific combining ability effects of crosses for different traits at KARI-NARL

Cross	TTN	PSTTN	TTW	PWTTW	PSTTW	AUDPC	DTOW	DTM
391919.3 x Bishop Gitonga	-449073.64	16.87	-10.99	8.84	0.57	87.47	1.88	-6.60
394904.9 x Bishop Gitonga	-1374998.64	14.70	-18.63	-12.34	-1.94	-29.41	-4.79	1.11
394905.8 x Bishop Gitonga	384258.86	16.15	-0.62	0.15	3.73	198.51	-7.29	2.36
392278.19 x Bishop Gitonga	-273147.81	16.49	7.34	-18.20	1.23	115.38	-6.88	4.44
394895.7 x Bishop Gitonga	328703.36	13.80	15.37	13.13	0.16	-55.87	5.63	3.19
394903.5 x Bishop Gitonga	1384257.86	-78.01	7.53	8.44	-3.76	-316.08	11.46	-4.51
391919.3 x Cangi	427468.81	13.75	-3.23	-2.39	-0.77	42.47	-2.01	2.29
394904.9 x Cangi	94135.81	15.33	-16.07	-15.62	-2.47	-249.41	4.65	-5.00
394905.8 x Cangi	-516974.69	16.27	13.06	7.38	0.55	110.17	2.15	-2.08
392278.19 x Cangi	-211420.03	19.53	-5.65	5.64	1.97	290.38	-7.43	-5.00
394895.7 x Cangi	20061.81	17.37	8.75	-6.54	4.04	307.47	-1.60	5.42
394903.5 x Cangi	186728.31	-82.24	3.14	11.53	-3.31	-501.08	4.24	4.38
391919.3 x Kenya Karibu	-1510800.97	14.15	5.08	-22.13	-0.48	-128.37	-0.90	-0.76
394904.9 x Kenya Karibu	970678.03	22.59	2.03	2.51	4.97	303.09	-4.24	-1.39
394905.8 x Kenya Karibu	-1121912.47	16.97	-20.25	4.40	-2.42	-123.99	6.60	-1.81
392278.19 x Kenya Karibu	1628084.86	14.33	1.94	1.14	-0.41	94.55	0.35	1.94
394895.7 x Kenya Karibu	155864.03	13.03	-20.26	10.06	-3.48	-196.70	-3.82	-2.64
394903.5 x Kenya Karibu	-121913.47	-81.07	31.46	4.02	1.83	51.42	2.01	4.65
391919.3 x Kihoro	-794752.31	-113.30	3.35	11.99	-1.34	-87.26	6.88	-0.49
394904.9 x Kihoro	-979937.31	-113.18	-7.55	-5.24	-2.66	-62.47	-3.13	-4.44
394905.8 x Kihoro	1520060.19	-115.51	31.94	-0.19	-0.66	-81.22	1.04	3.47
392278.19 x Kihoro	-26234.47	-110.52	-14.13	2.88	4.20	318.99	-1.88	-1.11
394895.7 x Kihoro	-165123.31	-113.11	-12.51	5.56	-1.06	-153.92	3.96	2.64
394903.5 x Kihoro	445987.19	565.61	-1.09	-14.99	1.51	65.87	-6.88	-0.07
391919.3 x Meru Mugaruro	-152777.64	16.23	3.49	-5.62	1.58	139.69	-2.57	-3.82
394904.9 x Meru Mugaruro	995369.36	13.04	25.73	14.30	2.41	74.48	-2.57	2.22
394905.8 x Meru Mugaruro	976850.86	17.14	-13.29	-3.69	-0.62	-29.27	1.60	-1.53
392278.19 x Meru Mugaruro	319444.19	14.10	4.89	8.44	-3.56	-182.40	5.35	2.22
394895.7 x Meru Mugaruro	-412036.64	22.47	0.32	-15.46	3.36	303.02	-5.49	-2.36
394903.5 x Meru Mugaruro	-1726850.14	-82.98	-21.14	2.04	-3.16	-305.52	3.68	3.26
391919.3 x Tigoni	1896603.03	13.33	10.32	2.99	-0.77	-123.92	-2.57	3.40
394904.9 x Tigoni	-66357.97	18.10	-4.88	16.20	1.63	-4.13	7.43	1.11
394905.8 x Tigoni	-825616.47	12.37	-6.86	-14.51	-1.39	-192.88	1.60	-2.64
392278.19 x Tigoni	-1927467.14	18.89	-0.98	-9.79	1.49	65.66	-7.99	-2.22
394895.7 x Tigoni	-140431.97	15.10	12.23	11.43	-5.21	-295.59	11.18	-3.47
394903.5 x Tigoni	1063270.53	-77.79	-9.82	-6.31	4.25	550.87	-9.65	3.82
391919.3 x Sherekea	291666.36	16.46	-2.70	5.85	-0.94	-34.48	-1.46	-1.60
394904.9 x Sherekea	-782406.64	13.87	11.44	-4.92	0.44	165.31	1.88	1.11
394905.8 x Sherekea	-60185.14	16.77	-0.32	-8.68	2.77	188.23	-3.96	0.69
392278.19 x Sherekea	467592.19	15.61	-2.14	19.13	-2.73	-301.56	9.79	4.44
394895.7 x Sherekea	624999.36	16.43	10.92	-14.82	-0.18	-104.48	-4.38	-0.14
394903.5 x Sherekea	-541666.14	-79.13	-17.21	3.45	0.63	86.98	-1.88	-4.51
391919.3 x Ingabire	291666.36	22.51	-5.31	0.48	2.15	104.41	0.76	7.57
394904.9 x Ingabire	1143517.36	15.56	7.94	5.11	-2.37	-197.47	0.76	5.28
394905.8 x Ingabire	-356481.14	19.82	-3.67	15.16	-1.96	-69.55	-1.74	1.53
392278.19 x Ingabire	23148.19	11.57	8.72	-9.23	-2.19	-401.01	8.68	-4.72
394895.7 x Ingabire	-412036.64	14.92	-14.81	-3.35	2.36	196.08	-5.49	-2.64
394903.5 x Ingabire	-689814.14	-84.39	7.14	-8.16	2.01	367.53	-2.99	-7.01
SE (females x males)	789812.24	110.88	6.65	5.53	3.58	324.08	5.64	2.79

TTN=Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); TTW= Total tuber weight (tha^{-1}); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha^{-1}); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); AUDPC= Area under the disease progress curve; DTOW= Days to onset of wilting; DTM= Days to maturity.

5.3.4 General and specific combining ability estimates for selected tuber yield traitsand bacterial wilt resistance at Kinale

At Kinale site, significant (P≤ 0.001) differences were found among the crosses for TTW and PWTTW (Table 5.8). Significant GCA effects were observed for males for TTW (P≤ 0.001) while for females, the GCA effects were significant for TTW (P≤ 0.01). Male parents had higher GCA for TTW (552.97) than female parents (496.37); the opposite was the case for PWTTW where male parents had lower GCA (91.73) than the female parents (156.39) (Table 5.8). The SCA effects were significant (P≤ 0.001) for TTW and PWTTW (Table 5.8). The SCA was more important than GCA in the expression of all traits except PSTTW and PSTTN (Table 5.8). For these two traits, GCA was almost equal to SCA (Table 5.8).

Among the male parents, Meru Mugaruro had the lowest GCA for PSTTN (-1.18) and AUDPC (-315.52) (Table 5.9) followed by Ingabire PSTTN (-0.77) and AUDPC (-211.35). Kihoro had the highest GCA for TTW (8.34) followed by Bishop Gitonga (4.76). Among the female parents, 391919.3 had the lowest GCA for AUDPC (-212.81) followed by 394895.7 (-169.69).

Among the crosses, 391919.3 x Ingabire had the lowest SCA effects for PSTTN (-2.46) while 394903.5 x Cangi had the lowest SCA effects for AUDPC (-1014.48) (Table 5.10). Furthermore, 394905.8 x Kihoro had the highest SCA effects for TTW (27.13) followed by 394903.5 x Kenya Karibu (24.37) (Table 5.10).

Table 5.8.Analysis of variance of general and specific combining abilities for selected traits at Kinale

Source of variation	df	Mean squares							
		TTW	TTN	PSTTN	PWTTW	PSTTW	AUDPC	DTOW	DTM
Replications	2	581.79**	752087917305.3**	50.65*	87.34 ns	33.12 ns	1473160.9 ns	564.58 ***	69.97 ns
Crosses	47	449.85 ***	59290801374.0 ns	9.60 ns	292.62 ***	14.86 ns	2156849.0 ns	39.18 ns	40.42 ns
GCA Males	7	552.97***	71827207811.0 ns	18.11 ns	91.73 ns	21.85 ns	2262512.5 ns	20.24 ns	67.24 ns
GCA Females	5	496.37**	76811843318.7 ns	7.91 ns	156.39 ns	18.23 ns	1835623.6 ns	81.67 ns	21.42 ns
SCA	35	422.58***	54280514094.5 ns	8.13 ns	352.26 ***	12.98 ns	2181605.6 ns	36.91 ns	37.77 ns
GCA/SCA		0.45	0.48	0.52	0.19	0.51	0.39	0.48	0.44
Residual	94	116.61	35642937986.85	14.16	78.91	19.09	1971968.74	55.36	42.31

df=Degrees of freedom; * = Significant at P≤ 0.05; **= Significant at P≤ 0.01; ***= Significant at P≤ 0.001; ns=Non significant; TTW= Total tuber weight (t ha^{-1});TTN=Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha^{-1}); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); AUDPC= Area under the disease progress curve; DTOW= Days to onset of wilting; DTM= Days to maturity.

Table 5.9. General combining ability effects of parents for different traits at Kinale

GCA Males	TTN	PSTTN	TTW	PWTTW	PSTTW	AUDPC	DTOW	DTM
Bishop Gitonga	-126758.15	-0.10	4.76	4.02	0.24	-89.13	-1.81	1.91
Cangi	73486.96	0.43	-1.98	0.05	-0.69	-208.02	0.97	1.35
Kenya Karibu	9290.24	-0.87	-1.41	-0.19	0.60	1.70	1.53	-0.31
Kihoro	48795.85	1.00	8.34	0.78	1.78	827.26	-0.14	0.52
Meru Mugaruro	-22808.65	-1.18	-9.01	-2.39	-1.41	-315.52	-0.69	2.47
Tigoni	43857.63	-0.24	4.33	0.65	0.58	2.81	-0.69	-3.09
Sherekea	11759.18	1.74	-1.07	0.60	0.33	-7.74	0.42	-1.98
Ingabire	-37623.04	-0.77	-3.97	-3.52	-1.41	-211.35	0.42	-0.87
SE(males' GCA)	86808.52	0.89	2.55	2.09	1.03	330.99	1.75	1.53
GCA Females								
391919.3	16697.53	-0.32	7.40	-0.95	-0.94	-212.81	2.92	-0.38
394904.9	-96449.76	0.95	-2.12	-4.50	1.08	43.02	-1.25	0.03
394905.8	-16635.60	0.35	-0.27	3.08	0.70	-59.27	-0.83	-1.42
392278.19	64845.07	-0.69	-6.04	0.80	0.35	-134.90	-0.83	1.49
394895.7	-11080.10	-0.09	-1.35	0.57	-1.06	-169.69	1.67	0.03
394903.5	42622.86	-0.21	2.38	1.01	-0.13	533.65	-1.67	0.24
SE (females' GCA)	75178.38	0.77	2.20	1.81	0.89	286.65	1.52	1.33

TTN=Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); TTW= Total tuber weight (tha^{-1}); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha^{-1}); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); AUDPC= Area under the disease progress curve; DTOW= Days to onset of wilting; DTM= Days to maturity.

Table 5.10. Specific combining ability effects of crosses for different traits at Kinale

Cross	TTN	PSTTN	TTW	PWTTW	PSTTW	AUDPC	DTOW	DTM
391919.3 x Bishop Gitonga	15648.03	1.67	-13.09	7.44	-0.23	134.76	2.64	-1.28
394904.9 x Bishop Gitonga	-6019.35	1.18	-14.76	-11.94	-0.50	203.92	0.14	-0.03
394905.8 x Bishop Gitonga	48981.15	-0.78	5.46	0.67	2.32	256.22	-3.61	3.09
392278.19 x Bishop Gitonga	-151016.85	-1.65	3.81	-16.10	2.50	196.84	-0.28	3.51
394895.7 x Bishop Gitonga	13796.32	-1.29	15.35	12.22	-2.06	61.63	-2.78	-1.70
394903.5 x Bishop Gitonga	78610.69	0.87	3.23	7.70	-2.04	-853.37	3.89	-3.58
391919.3 x Cangi	22808.25	-1.96	7.64	2.29	-2.62	-148.02	-0.14	-2.40
394904.9 x Cangi	91511.54	-0.58	-9.25	-14.99	0.69	19.48	0.69	2.19
394905.8 x Cangi	56141.38	-0.07	-5.09	5.64	0.90	498.44	0.28	-1.35
392278.19 x Cangi	4290.04	2.36	-7.20	6.80	1.52	434.06	-3.06	-0.94
394895.7 x Cangi	-23487.46	-0.31	15.69	-11.95	2.60	210.52	1.11	-1.15
394903.5 x Cangi	-151263.75	0.56	-1.79	12.22	-3.10	-1014.48	1.11	3.65
391919.3 x Kenya Karibu	12932.64	-0.58	1.44	-19.31	0.30	3.92	-0.69	0.94
394904.9 x Kenya Karibu	-155399.74	1.88	3.74	3.89	0.30	474.76	0.14	-1.15
394905.8 x Kenya Karibu	-101881.90	0.62	-13.29	2.68	-0.13	-49.62	-0.28	0.31
392278.19 x Kenya Karibu	127745.43	-1.18	1.48	-1.17	0.25	187.67	-0.28	-5.94
394895.7 x Kenya Karibu	55523.93	-1.77	-17.74	7.24	-1.84	-79.20	0.56	3.85
394903.5 x Kenya Karibu	61079.64	1.03	24.37	6.67	1.13	-537.53	0.56	1.98
391919.3 x Kihoro	225275.36	0.99	1.08	11.16	-1.11	-876.63	4.31	3.44
394904.9 x Kihoro	-209720.01	-1.74	-1.08	-6.49	0.66	-972.47	-4.86	1.35
394905.8 x Kihoro	6759.15	-1.74	27.13	0.30	-0.55	-923.51	-1.94	-0.52
392278.19 x Kihoro	162313.15	1.34	-14.09	1.70	2.50	-457.88	4.72	-0.10
394895.7 x Kihoro	16018.32	-0.36	-10.85	4.76	-1.68	-838.09	2.22	1.35
394903.5 x Kihoro	-200645.97	1.52	-2.19	-11.43	0.19	4068.58	-4.44	-5.52
391919.3 x Meru Mugaruro	-73486.81	2.03	-9.06	-9.94	0.68	291.15	-5.14	3.16
394904.9 x Meru Mugaruro	54475.15	0.33	19.59	17.44	0.91	300.31	2.36	2.74
394905.8 x Meru Mugaruro	211695.65	-1.84	-5.88	-4.23	-1.63	-27.40	-1.39	-0.80
392278.19 x Meru Mugaruro	-240151.68	-0.78	9.10	10.67	-2.10	-91.77	5.28	-5.38
394895.7 x Meru Mugaruro	-30894.51	2.19	-0.79	-13.86	3.09	431.35	-3.89	2.74
394903.5 x Meru Mugaruro	78362.19	-1.93	-12.95	-0.08	-0.96	-903.65	2.78	-2.47
391919.3 x Tigoni	-80894.42	0.51	6.40	2.09	0.09	72.81	1.53	-1.28
394904.9 x Tigoni	-86264.46	-1.03	-10.57	15.39	-1.56	-241.35	-0.97	-3.37
394905.8 x Tigoni	-210522.63	-0.89	0.18	-16.25	-1.65	-30.73	5.28	-0.24
392278.19 x Tigoni	152436.71	0.99	1.25	-6.19	1.36	334.90	-4.72	5.17
394895.7 x Tigoni	80215.21	0.24	11.93	10.23	-1.19	-138.65	6.11	-3.37
394903.5 x Tigoni	145029.58	0.19	-9.20	-5.27	2.95	3.02	-7.22	3.09
391919.3 x Sherekea	-33981.31	-0.20	-2.04	2.37	-1.14	26.70	0.42	0.94
394904.9 x Sherekea	153239.32	-0.13	9.36	-5.37	1.15	279.20	1.25	-4.48
394905.8 x Sherekea	43795.82	1.48	-1.41	-0.82	0.17	339.83	0.83	0.31
392278.19 x Sherekea	-82128.85	0.53	1.39	13.70	-1.28	-221.22	0.83	4.06
394895.7 x Sherekea	38240.32	-1.30	-0.38	-8.61	1.35	105.24	-1.67	-6.15
394903.5 x Sherekea	-119165.31	-0.38	-6.92	-1.28	-0.25	-529.76	-1.67	5.31
391919.3 x Ingabire	-88301.75	-2.46	7.63	3.90	4.03	495.31	-2.92	-3.51
394904.9 x Ingabire	158177.54	0.10	2.97	2.07	-1.66	-63.85	1.25	2.74
394905.8 x Ingabire	-54968.63	3.22	-7.10	12.02	0.57	-63.23	0.83	-0.80
392278.19 x Ingabire	26512.04	-1.61	4.26	-9.41	-4.75	-382.60	-2.50	-0.38
394895.7 x Ingabire	-149412.13	2.61	-13.22	-0.04	-0.27	247.19	-1.67	4.41
394903.5 x Ingabire	107992.92	-1.86	5.46	-8.54	2.08	-232.81	5.00	-2.47
SE (females x males)	212636.57	2.17	6.23	5.13	2.52	810.75	4.3	3.76

TTN=Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha); TTW= Total tuber weight (tha^{-1}); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha^{-1}); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); AUDPC= Area under the disease progress curve; DTOW= Days to onset of wilting; DTM= Days to maturity.

5.4 Discussion and conclusions

This study aimed at determining the combining abilities for bacterial wilt resistance as well as tuber yield and related traits in selected potato cultivars currently grown by farmers in Kenya as well as other advanced clones from the International Potato Center.

Potato crosses planted at Kinale took longer time to start wilting and had lower values of % LI, AUDPC, PSTTN and PSTTW than the crosses planted at KARI-NARL possibly due to the low temperatures experienced at Kinale compared to KARI NARL. Kinale site is in Upper Highland (UH_1) agro-ecological zone as opposed to KARI-NARL which is at Lower Midland (LM_3)(Jaetzold et al., 2006c). It has previously been reported that high temperature promote survival, reproduction, infectivity, and spread of *R.solanacearum* and hence disease development (Harris, 1976; Martin and French, 1985).

The current study found that for all tuber yield related traits (TTN, TTW, PWTTW and DTM), SCA was greater than GCA. This is due to the fact that most of the parents used in this study were bred at CIP (except Kihoro, Bishop Gtonga, Cangi and Meru Mugaruro) and are closely related.

It was previously reported that GCA is significantly larger than SCA for tuber yield and quality traits in crosses between non-related parents while SCA appears to be more important among related parents (Neele et al., 1991; Ortiz and Golmirzaie, 2004). This is because in closely related breeding material, the number of different alleles at a locus is likely to be limited. Consequently, variation in additive gene action is limited while non-additive gene action, like dominance or epistasis, can result in a relatively large variation between progenies. Plaisted et al. (1962) speculated that informal previous selection which narrowed the genetic base of the tested genotypes may be one of the possible causes for obtaining greater estimates of SCA variance for various characters. Killick and Malcolmson (1973), using a concept developed in evolutionary population genetics suggested that traits subjected to directional selection would be expected to show little additive genetic variance, but a large degree of dominance and epistasis, whereas the reverse would be true for traits subjected to stabilising selection.

Previous studies (Johansen et al., 1967; Killick, 1977; Maris, 1989) found the GCA to be more important than SCA for maturity; this is in agreement with the findings of the current study. Tai (1976) reported that variation between progenies for tuber yields and number of tubers per plant was dominated by SCA effect while for average tuber weight and specific gravity the GCA effect was more important.

The current study also found that GCA was more important than SCA in the expression of PSTTW and AUDPC (at KARI-NARL) and PSTTW and PSTTN at Kinale. For DTOW, the GCA and SCA effects were almost equal. This is in agreement with previous studies which reported that both major and minor genes are involved in the expression of resistance to bacterial wilt; and inheritance of this resistance involves both additive and non-additive gene actions (Tung et al., 1993; Tung and Schmiediche, 1995). Furthermore, epistasis was found to be important in the inheritance of this resistance (Tung et al., 1992a; Tung et al., 1993). Other reports showed significant GCA and SCA effects for bacterial wilt resistance indicating that both additive and non-additive gene actions are important in conditioning resistance expression (Chakrabarti et al., 1994). Additionally, it was found that the non-additive variance component for disease severity was 4.5 times more than additive component and a large proportion of non-additive variance was due to dominance or epistatic genetic effects (Tung, 1992). Given these contradictory results, selection of a resistant parent or cross should be done cautiously. This could be due to the strong host-pathogen-environment interaction that affects the expression of resistance (French and Lindo, 1982; Tung et al., 1990; Tung, 1992; Tung et al., 1992b).

Among the crosses, the nine crosses with the highest SCA effects for TTW at KARI-NARL were 394905.8 x Kihoro (31.94), 394903.5 x Kenya Karibu (31.46), 394904.9 x Meru Mugaruro (25.73), 394895.7 x Bishop Gitonga (15.37), 394905.8 x Cangi (13.06), 394895.7 x Tigoni (12.23), 394904.9 x Sherekea (11.44), 394895.7 x Sherekea (10.92) and 391919.3 x Tigoni (10.32) in that order. At Kinale, the nine crosses with the highest SCA effects for TTW were 394905.8 x Kihoro (27.13), 394903.5 x Kenya Karibu (24.37), 394904.9 x Meru Mugaruro (19.59), 394895.7 x Cangi (15.69), 3948957 x Bishop Gitonga (15.35), 394895.7 x Tigoni (11.93), 394904.9 x Sherekea (9.36), 392278.19 x Meru Mugaruro (9.10) and 391919.3 x

Cangi (7.64) in that order. These crosses were selected for high tuber yield and will be evaluated in future. For bacterial wilt resistance, the best general combiners were Ingabire, Meru Mugaruro, 391919.3, 394895.7 and 394903.5. These parents were selected for future crosses.

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Chapter Six: Genotype x environment interaction and stability of potato tuber yield and bacterial wilt resistance in Kenya

Abstract

Breeders mostly desire high and stable yielding genotypes that show minimal interaction with the environment. The additive main effects and multiplicative interaction (AMMI) analysis and genotype main effect and genotype x environment interaction (GGE) biplot analysis are widely used to measure stability of yield and its components. The objective of this study was to estimate the magnitude of GEI for potato tuber yield and bacterial wilt resistance and to identify the most discriminating and representative environments for potato testing in Kenya. The study was conducted in four environments. Forty eight potato families were evaluated using a 6 x 8 alpha lattice design replicated three times. Data on days from planting to onset of wilting (DTOW), area under the disease progress curve (AUDPC), total tuber weight (t ha^{-1}) (TTW), total tuber numbers ha^{-1} (TTN), proportion of ware sized tubers (PWTTW), proportion of symptomatic tubers based on weight (PSTTW), proportion of symptomatic tubers based on tuber numbers (PSTTN) and latent infection (LI) of the tubers were subjected to combined analysis of variance to identify crosses that were resistant to bacterial wilt. Data on tuber yields (TTW) were analysed using AMMI and GGE biplot methods in order to identify the highest yielding and most stable family as well as the most discriminating and yet representative test environment. The potato families were ranked differently in terms of resistance against bacterial wilt across the four environments. Family 20 (394905.8 x Kihoro) was closest to the ideal genotype; it was the highest yielding (104.7 t ha^{-1}) and most stable; it was closely followed by family 43 (394903.5 x Kenya Karibu) which yielded 98.3 t ha^{-1}. The environment ENVI 1(short rains of 2013 at Kinale) was the closest to ideal environment and therefore the most desirable of the four test environments.

Keywords: Additive main effects and multiplicative interaction, Bacterial wilt, Genotype x environment interactions. GGE biplot, Potatoes, Yield stability

6.1 Introduction

Genotype x environment interaction (GEI) is the differential genotypic expression across environments (Fox et al., 1997). It results in inconsistent ranks between

genotypes across environments. Such inconsistency in performance is caused either by differential responses of the same set of genes to changes in the environment or by expression of different sets of genes in different environments. With GEI, the inconsistent differences between genotypes are manifested either as rank order changes of the genotypes between environments (crossover GEI), or as alterations in the absolute differences between the genotypes without affecting the rank order (Crossa et al., 1995; Bernardo, 2002). The two forms of GEI are referred to as qualitative and quantitative, respectively. These interactions are only important in selection when rank order changes occur. In such cases, genotypes must be bred for specific adaptation to certain environments. A crossover interaction is a major problem in breeding (Cooper and Delacy, 1994; Crossa et al., 1995), because it can slow down selection progress as different genotypes are selected in different environments. The GEI tends to have a greater effect on quantitative than qualitative traits (Mather and Jinks, 1982; Dabholkar, 1992; Falconer and Mackay, 1996). Breeders mostly desire high and stable yielding genotypes that show minimal interaction with the environment (Yan et al., 2007).

Stability analysis is an important tool for plant breeders to identify and recommend widely or specifically adapted genotypes for a target set of environments. Several statistical techniques to measure stability have been developed for studying GEI effects, and to facilitate variety recommendations in multiple environments. The widely used and powerful tools are additive main effects and multiplicative interaction (AMMI) analysis (Gauch and Zobel, 1997) and genotype main effect and genotype x environment interaction (GGE) biplot analysis (Yan et al., 2000). In the AMMI model, the main effects are retained as additive effects, while the GEI is treated as a multiplicative effect (Gauch and Zobel, 1988). The AMMI procedure utilizes an analysis of variance (ANOVA) for the effects due to genotypes and environments, and principal component analysis (PCA) for the GEI (Bernardo, 2002). The AMMI generates a family of models; AMMI 0 uses the additive genotypic and environmental means to describe the data matrix and thus ranks genotypes identically at each environment, ignoring the GEI. The second model, AMMI1 considers the main effects as well as one interaction principal component axis (IPCA1) to interpret the residual matrix. The third model, AMMI2, considers the main

effects and two axes, IPCA1 and IPCA2 for the non-additive variation. The higher order multiplicative components that are not significant can be ignored. When one IPCA accounts for most GEI, a feature of AMMI is the biplot procedure. In the AMMI 1, genotypes and environments are plotted on the same diagram facilitating inference about specific interactions of individual genotypes and environments by using the sign and magnitude of IPCA 1 values.

The genotype and genotype x environment (GGE) biplots display both genotype (G) and genotype x environment interactions (GEI), which are the two sources of variation that are relevant for genotype evaluation (Kang, 1993; Yan et al., 2007). The GGE biplot is constructed by plotting the first two principal component axis (PCA1 and PCA 2) derived from singular value decomposition (SVD) of the environment-centred data. Models that decompose the environment-centred data are commonly referred to as sites regression models or SREG, and SREG with two PCAs, such as GGE biplot, is referred to as $SREG_2$ (Yan et al., 2001). The GGE biplot is useful in two major aspects: first is to display the "which- won – where" pattern of the data that may help in identifying high- yielding and stable cultivars and, second, in determining the discriminating ability and representativeness of the test environments (Yan et al., 2001). It provides useful information regarding genotype yield and stability performance. Furthermore, it has the ability to identify environments with power to discriminate between genotypes, and to measure the representativeness or stability of the target environments (Yan and Tinker, 2006; Yan et al., 2007).

Breeding work has been going on at the Kenya Agricultural Research Institute, National potato Research Centre at Tigoni (KARI-Tigoni) to develop potato cultivars that are high yielding and at the same time resistant to bacterial wilt (Muthoni et al., 2014). So far, the potato families have been propagated up to the second clonal generation. There is need to identify and select promising families at this stage so as to reduce them to manageable levels. This study was therefore set up to estimate the magnitude of GEI for potato tuber yield and bacterial wilt resistance and to identify the most discriminating and representative environments for potato testing in Kenya. The experimental materials used in this study were in the early stages of

breeding; they were families in second clonal generation. Therefore, this study was not meant for cultivar recommendation *per se* but to undertake early family selection.

6.2 Materials and methods

6.2.1 Study sites

The study was carried out at the Kenya Agricultural Research Institute, National Research Laboratories (KARI-NARL) and at a farmer's field at Kinale. The two sites have been described in section 5.3.1. The study was carried out for two consecutive seasons; between 1st October 2013 and 11th February 2014 for the first season and 28th March 2014 to 15th August 2014 for the second season. The two seasons and two sites constituted the four environments (Table 6.1).

Table 6.1. Four environments under which the study was carried out

Season	Site	Environment[a]
Short rains (2013)	Kinale	ENVI 1
Short rains (2013)	KARI-NARL	ENVI 2
Long rains (2014)	Kinale	ENVI 3
Long rains (2014)	KARI-NARL	ENVI 4

[a]ENVI=Environment

6.2.2 Plant materials, families and agronomic management

The study used 48 potato families developed as follows: eight potato clones selected previously in a bacterial wilt screening trial (Muthoni et al., 2014) were selected as males. The eight clones are high yielding and popularly grown by Kenyan farmers but are highly susceptible to bacterial wilt (Muthoni et al., 2014). These males were crossed to a set of six female clones sourced from CIP using a North Carolina mating design II that yielded 48 families. The seedlings were first sowed in trays and transplanted to the field one month later. During harvesting, 150 plants were randomly sampled from each family and from each selected plant, two tubers were retained. (The rest were later planted at KARI-Tigoni in the following season so as to generate more tubers for the long rains of 2014 season evaluation trial). One tuber from each of the 150 selected plants was picked and bulked together so as to come up with one bulked sample of 150 tubers. This was repeated again to generate a

second bulked sample. Each of the two bulked samples consisted of 150 tubers. The two bulked samples were planted out at Kinale and KARI-NARL during the short rains season of 2013. (From the tubers planted at KARI-Tigoni, 150 plants were randomly sampled from each family and from each selected plant, two tubers were retained. One tuber from each of the 150 selected plants was picked and bulked together so as to come up with one bulked sample of 150 tubers. This was repeated again to generate a second bulked sample. Each of the two bulked samples consisted of 150 tubers. The two bulked samples were planted out at Kinale and KARI-NARL during the long rains season of 2014). For both seasons and both sites, the families were planted in a 6 x 8 alpha lattice design replicated three times. Each plot consisted of 50 plants i.e. 5 rows each consisting of 10 plants. The tubers were planted in furrows at a spacing of 75 x 30cm. During planting, DAP (18% N: 46% P_2O_5) was applied at the recommended rate of 500 kg ha^{-1}. Planting was done on 1st October 2013 at ENVI 2 and 2nd October at ENVI 1. At ENVI 3 and 4, planting was done on 28th March 2014 and 31st March 2014, respectively. Weeding, earthing-up and spraying against pests and late blight were carried out as per recommendations for potato production in Kenya (KARI, 2008).

6.2.3 Inoculation of bacterial wilt

To ensure uniform distribution of bacterial wilt at KARI-NARL and Kinale, a bacterial suspension concentrated at 3.0 x 10^9cfuml^{-1} was poured into the planting furrows (during planting of potato tubers but before covering them) at a rate of 1 litre per plot to boost the inoculum concentration in the soil. The resident as well as inoculated bacteria were confirmed as biovar 2 by Plantovita, Lynn East, South Africa based on the ability of the bacteria to produce acid from several disaccharides and sugar alcohols (Buddenhagen and Kelman, 1964). For proper disease expression, supplemental watering using overhead irrigation was done during the dry times.

6.2.4 Data collection

Weather data (mean monthly temperature and rainfall) were recorded from the nearest meteorological station less than 300 meters from the experimental field. Plant data collected included the number of days from planting to maturity (DTM), days to onset of wilting (DTOW) and bacterial wilt incidence (BWI). Time to maturity was counted as the number of days from planting to when 75% of the plants had

senesced. These data were taken on a plot basis. The BWI scores were used to calculate area under the disease progress curve (AUDPC) (CIP, 2007) using the formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{(S_i + S_{i+1})(t_{i+1} - t_i)}{2}$$

Where S_i is the BWI at days *i*, and *n* is the total number of sampling times, *t* is the number of days after planting

During harvesting, the 24 middle plants per plot were harvested, each plant separately. Total number of tubers was counted from each of the 24 plants. In addition, the number of symptomatic tubers (i.e. showing rotting or bacterial ooze in the tuber eyes or soil adhering to the eyes of the tubers) and healthy looking tubers (asymptomatic) were determined. The healthy looking tubers were then categorized based on size i.e. ware (>45mm diameter) and, seeds (<45mm diameter). Their number and weights were recorded. The weights of symptomatic and ware tubers were expressed as percentage of the total yields. The percentage of symptomatic tubers was expressed both in weight, a value which is useful to determine yield loses (t ha^{-1}), and as a number of infected tubers, a value which is used for the calculation of infection tuber rates.

Only healthy-looking tubers selected above were analyzed for latent infection by *R. solanacearum.* For each plot, 60 healthy-looking tubers were placed in sugar paper and delivered to the laboratory for latent infection analysis. The tubers were washed and disinfected. They were then divided into five groups of 12 tubers each. Each group was extracted to constitute a composite sample which was then analyzed for latent infection using the post-enrichment enzyme-linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA) test (Priou et al., 1999a).

6.2.5 Data analysis

6.2.5.1 Analysis of variance

A combined analysis of variance (ANOVA) across the environments (seasons and sites) was performed to determine the effects of environment, genotype and GEI on potato tuber yields and bacterial wilt resistance. Data for each environment was also analysed separately. Data on days to maturity (DTM), days to onset of wilting

(DTOW), area under the disease progress curve (AUDPC), total tuber numbers (TTN), total tuber weight in t ha^{-1} (TTW), percentage of symptomatic tubers based on total tuber numbers (PSTTN), percentage of symptomatic tubers based on total tuber weight (PSTTW), and percentage of ware sized tubers based on total tuber weight (PWTTW) values were subjected to analysis of variance using the lattice procedure of Statistical Analysis Systems (SAS 9.1) statistical package (SAS, 2003). Data on TTN, TTW, PWTTW, PSTTN and PSTTW were first averaged on a plot basis; the average value was then used to extrapolate values per ha.

Data on latent infection (LI) level were subjected to the Kruskal-Wallis non-parametric test procedure using SPSS for Windows Release Version 18.0 (SPSS Inc., 2009). The resistance to bacterial wilt was determined using ranking based on % LI, AUDPC, DTOW, PSTTW and PSTTN and the percentage of total infected tubers (PTIT). Small values of % LI, AUDPC, PSTTW and PSTTN as well as high values of DTOW indicates high resistance. The PTIT was calculated as suggested by CIP (2007):

$$\text{PTIT} = \text{PSTTN} + \frac{(\%\ \text{healthy looking tubers x}\ \%\ \text{LI})}{100}$$

Where PTIT is the percentage of total infected tubers, PSTTN is the percentage of symptomatic tubers based on total tuber numbers and % LI is the % latent infection. Small values of PTIT indicates high resistance. Based on PTIT, bacterial wilt resistance levels are categorized as indicated in Table 6.2 (CIP, 2007).

Table 6.2. Resistance levels of potatoes to bacterial wilt based on percentage of total infected tubers

Resistance levels	PTIT
Highly resistant	0
Resistant	1<15
Moderately resistant	15- <30
Moderately susceptible	30- <45
Susceptible	45- <60
Highly susceptible	≥60

Modified from CIP(2007)

6.2.5.2 AMMI model

Tuber yield data (TTW) was analysed using the AMMI model that combines into a single model analysis of variance (Aksenova et al., 2013) for genotype and environment main effects with principal component analysis (PCA) for the GEI. The complete AMMI model is shown below (Crossa, 1990).

$$Yij = \mu + gi + ej + \sum_{k=1}^{t} \lambda k\, \alpha ik \gamma jk + \varepsilon ij$$

Where, Y_{ij} = is the mean yield (t ha^{-1}) of the i^{th} genotype in the j^{th}environment, μ is the overall mean, g_i and e_j are the main effects of the genotype and environment respectively, *t* is the number of PCA axes considered, λ_k is the singular value of k^{th}PCA axis, α_{ik} and $\gamma_{jk}$are scores for the i^{th} genotype and j^{th} environment on the k^{th}PCA axis, and εij is the residual term which includes experimental error.

In this model, AMMI analysis of variance and ranking of potato families per environment were presented to interpret the results. The AMMI biplot showing the main effects (genotype and environment) and the first interaction principal components axis (IPCA 1) was also presented to assess the relationships among potato families, test environments and GEI for potato tuber yield.

6.2.5.3 GGE biplot

Variation in tuber yield (TTW) due to genotype (G) and genotype x environment interaction (GEI) was explained using GGE biplot based on the principal component analysis (PCA) of environment-centred data (Yan et al., 2000; Yan, 2002). The GGE biplot was analysed using Genstat statistical package (14th Edition) (Payne et al., 2011). The GGE mathematical model based on PCA of environment-centred data (which contains G and GE as the main sources of variation) subjected to singular value decomposition (SVD) was used to visualize the relationship among potato families and the environments. The basic model for a GGE biplot as described by Yan (2002) is:

$$\mathrm{Yij} - \mu - \beta \mathrm{j} = \sum_{l=1}^{k} \lambda l \gamma \mathrm{i} l \eta l \mathrm{j} + \varepsilon \mathrm{ij}$$

Where Yij = Mean tuber yield (t ha^{-1}) of the i^{th} genotype in the j^{th} environment;

μ = Overall mean

βj = Main effect of the environment;

λ_l = Eigen value associated with IPCA l

$\gamma i l$ = The eigenvector of genotype *i*for PC l

$\eta l j$= The eigenvector of environment *j* for PC l

εij= Error term associated with potato genotype i in environment j.

Interrelationships among the test environments (Cooper et al., 1997) and potato families (Yan et al., 2001) were visualised using various GGE biplot graphs. A GGE polygon was used to identify high yielding families in specific environments through analysis of the "which-won-where-pattern" (Yan et al., 2000; Yan, 2002). The GGE biplots based on average environment coordination (AEC) and drawn on the genotype-focused biplot (Yan and Kang, 2003) was used to determine yield performance and stability of the 48 potato families. Environment-focused scaling was used totest the relationship of the test environments.

6.3 Results

6.3.1 Weather conditions in the test environments

The four environments differed in terms of rainfall and temperature (Table 6.3). Generally, KARI-NARL was warmer than Kinale.

Table 6.3. Weather conditions in the test environments during the study period

	Oct. 2013	Nov. 2013	Dec. 2013	Jan. 2014	Feb. 2014	March 2014	April 2014	May 2014	June 2014	July 2014	August 2014
Seasons	Short rains season					Long rains season					
	Kinale										
Total rainfall (mm)	0	89.1	162.6	71.1	238.8	130.4	166.5	36.9	40.9	95.6	51.3
No. rainy days	0	9	8	3	9	5	8	3	4	4	4
Mean air temp. (^{0}C)	20.3	20.6	21.3	20.5	15.2	14.9	13.3	11.4	10.3	10.6	9.1
	KARI-NARL										
Total rainfall (mm)	33.4	91.0	192.2	36.4	23.6	119.1	256.6	165.6	56.9	86.3	12.6
No. rainy days	4	5	9	2	2	8	10	6	3	4	3
Mean air temp. (^{0}C)	23.3	24.2	23.9	25.2	24.2	19.7	17.8	16.2	15.9	14.2	13.6

6.3.2 Analysis of variance across environments

The combined analysis of variance showed significant family x site x season effect for PSTTN, TTW and PSTTW (Table 6.4). In addition, site had significant effect for TTN, PSTTN, TTW, PSTTW, PWTTW, DTM (P≤0.001) and AUDPC (P≤0.01) (Table 6.4). The season effect was significant for TTN, PSTTN, TTW, PSTTW, PWTTW, DTOW, DTM (P≤0.001), and for AUDPC (P≤0.01) (Table 6.4). Signficant families x sites interation was found for TTN and TTW (P≤0.05) and, PSTTN and PSTTW (P ≤0.001). There were significant differences (P≤0.001) among families for latent infection across environments (Table 6.5). In addition, % LI was higher in environments 2 and 3 than in the other two (Table 6.5).

Table 6.4. Combined ANOVA for tuber yields and other traits of potato families evaluated across four environments

		Mean squares							
Source of variation	df	TTN	PSTTN	TTW	PSTTW	PWTTW	AUDPC	DTOW	DTM
Families	47	2.33E+12 ns	180.027***	807.219***	178.903***	565.228***	478264.3*	130.437***	55.570**
Seasons	1	2.49E+14***	15396.880***	8702.558***	9840.392***	1522.333***	26666465.7**	21756.250***	2889.063***
Sites	1	7.31E+15***	15378.687***	16801.128***	10858.595***	14107.204***	2134642.8**	136.111 ns	12844.444***
Rep	2	3.28E+12 ns	996.779***	605.519ns	400.851***	271.805 ns	1409658.7**	1054.861***	49.783 ns
Iblock in Rep	15	1.23E+12 ns	77.693**	547.694**	93.374**	236.143*	283622.9 ns	65.833 ns	25.052 ns
Season x Site	1	1.27E+14***	5354.825***	2066.839**	3753.962***	3143.424***	29828.2 ns	1056.250***	126.563 ns
Family x Season	47	1.78E+12 ns	145.504***	726.062***	149.601***	425.957***	489033.0**	53.413 ns	35.428 ns
Family x Site	47	2.59E+12*	135.025***	299.024*	135.050***	157.158 ns	306581.9 ns	53.842 ns	46.483 ns
Family x Site x Season	47	1.91E+12 ns	132.604***	329.434*	126.323***	157.205 ns	308697.3 ns	49.867 ns	37.821 ns
Residual	367	1.74E+12	31.329	211.957	37.054	121.707	300612.866	69.853	34.173

df=Degrees of freedom; *= Significant at P≤0.05; **= Significant at P≤0.01; ***= Significant at P≤0.001; ns=Non significant; TTN=Total tuber number per ha; PSTTN= Percentage of symptomatic tubers (% of total tuber number per ha);TTW= Total tuber weight (t ha^{-1}); PSTTW= Percentage of symptomatic tubers (% of total tuber weight in t ha^{-1}); PWTTW= Percentage of ware sized tubers (% of total tuber weight in t ha^{-1}); AUDPC= Area under the disease progress curve; DTOW= Days to onset of wilting; DTM= Days to maturity.

Table 6.5 Chi square test statistics for latent infection of families across four environments

	Environments			
Statistics	ENVI 1	ENVI 2	ENVI 3	ENVI 4
Chi square	118.149	121.258	128.751	108.027
Asymp. Sig.	<0.001	<0.001	<0.001	<0.001

6.3.3 Ranking for bacterial wilt resistance across environments

The potato families were ranked differently in terms of resistance against bacterial wilt across the four environments and between the two ranking methods (Table 6.6).

Table 6.6 .Resistance of potato families against bacterial wilt for the four environments

		ENVI1		ENVI2		ENVI3		ENVI4	
Family Code	Family (genotype)	Overall Rank (a)	Overall Rank (b)	Overall Rank (a)	Overall Rank (b)	Overall Rank (a)	Overall Rank (b)	Overall Rank (a)	Overall Rank (b)
1	391919.3 x Bishop Gitonga	14.0	23.0	21.0	24.0	24.0	32.0	1.0	11.0
2	391919.3 x Cangi	**1.0**	9.0	22.0	35.0	42.5	15.0	2.0	37.0
3	391919.3 x Kenya Karibu	6.0	16.0	16.0	19.0	31.0	26.0	3.0	15.5
4	391919.3 x Kihoro	15.0	24.0	15.0	21.0	20.0	25.0	4.0	5.0
5	391919.3 x Meru Mugaruro	19.0	45.0	11.0	28.0	48.0	48.0	5.0	47.0
6	391919.3 x Tigoni	13.0	20.0	23.0	20.0	11.0	11.0	6.0	15.5
7	391919.3 x Sherekea	16.0	30.0	26.0	45.0	30.0	28.0	7.0	46.0
8	391919.3 x Ingabire	8.5	1.0	12.0	4.0	2.0	2.0	8.0	13.0
9	394904.9 x Bishop Gitonga	38.5	32.0	33.0	25.0	16.0	33.0	9.0	31.0
10	394904.9 x Cangi	29.5	46.0	29.0	46.0	47.0	44.0	10.0	3.0
11	394904.9 x Kenya Karibu	44.0	38.0	48.0	40.0	22.0	34.0	11.5	21.0
12	394904.9 x Kihoro	37.0	43.0	46.0	47.0	37.0	41.0	11.5	30.0
13	394904.9 x Meru Mugaruro	24.0	29.0	25.0	3.0	45.0	22.0	13.0	9.0
14	394904.9 x Tigoni	8.5	18.0	35.5	12.0	12.0	14.0	14.0	45.0
15	394904.9 x Sherekea	47.0	40.0	40.0	32.0	46.0	39.0	15.0	14.0
16	394904.9 x Ingabire	5.0	3.0	6.0	2.0	5.0	4.0	16.0	28.0
17	394905.8 x Bishop Gitonga	40.0	27.0	38.0	18.0	17.0	31.0	17.0	6.0
18	394905.8 x Cangi	29.5	14.0	34.0	48.0	41.0	17.0	18.0	38.0
19	394905.8 x Kenya Karibu	31.0	36.0	20.0	17.0	23.0	27.0	19.0	22.0
20	394905.8 x Kihoro	34.0	47.0	31.0	42.0	25.0	46.0	20.0	40.0
21	394905.8 x Meru Mugaruro	3.0	12.0	8.0	23.0	42.5	37.0	21.0	8.0
22	394905.8 x Tigoni	20.0	26.0	24.0	14.5	15.0	12.0	22.0	23.0
23	394905.8 x Sherekea	43.0	25.0	37.0	6.0	44.0	21.0	23.0	1.0
24	394905.8 x Ingabire	17.5	7.0	17.5	10.0	9.0	5.0	24.0	7.0
25	392278.19 x Bishop Gitonga	32.0	34.0	39.0	44.0	38.0	43.0	25.0	2.0
26	392278.19 x Cangi	36.0	10.0	45.0	26.0	32.0	16.0	26.0	10.0
27	392278.19 x Kenya Karibu	28.0	33.0	28.0	29.0	40.0	29.0	27.0	36.0
28	392278.19 x Kihoro	46.0	37.0	41.0	8.0	28.5	36.0	29.0	43.0
29	392278.19 x Meru Mugaruro	7.0	44.0	5.0	41.0	18.5	47.0	29.0	34.0
30	392278.19 x Tigoni	42.0	28.0	44.0	7.0	9.0	8.0	29.0	29.0
31	392278.19 x Sherekea	22.0	31.0	14.0	37.0	36.0	24.0	31.0	39.0
32	392278.19 x Ingabire	**2.0**	11.0	1.0	11.0	1.0	13.0	32.5	25.0
33	394895.7 x Bishop Gitonga	12.0	17.0	7.0	27.0	13.0	23.0	32.5	24.0

34	394895.7 x Cangi	33.0	35.0	42.0	43.0	33.0	38.0	34	18.0
35	394895.7 x Kenya Karibu	17.5	41.0	13.0	22.0	39.0	42.0	35.0	41.0
36	394895.7 x Kihoro	35.0	48.0	9.0	5.0	14.0	45.0	36.5	26.0
37	394895.7 x Meru Mugaruro	38.5	21.0	35.5	38.0	26.0	35.0	36.5	42.0
38	394895.7 x Tigoni	25.0	42.0	2.0	16.0	8.0	7.0	38.0	33.0
39	394895.7 x Sherekea	27.0	13.0	27.0	30.0	35.0	30.0	39.0	20.0
40	394895.7 x Ingabire	21.0	4.0	19.0	13.0	4.0	3.0	40.0	17.0
41	394903.5 x Bishop Gitonga	11.0	6.0	10.0	33.0	6.0	9.0	41.0	44.0
42	394903.5 x Cangi	10.0	15.0	3.5	14.5	28.5	18.0	42.0	32.0
43	394903.5 x Kenya Karibu	26.0	5.0	30.0	36.0	18.5	10.0	43.0	19.0
44	394903.5 x Kihoro	48.0	39.0	43.0	9.0	21.0	40.0	44.0	35.0
45	394903.5 x Meru Mugaruro	4.0	8.0	3.5	34.0	34.0	19.0	45.0	12.0
46	394903.5 x Tigoni	45.0	19.0	47.0	39.0	3.0	6.0	46.0	4.0
47	394903.5 x Sherekea	41.0	22.0	32.0	31.0	27.0	20.0	47.0	48.0
48	394903.5 x Ingabire	23.0	**2.0**	17.5	1.0	10.0	1.0	48.0	27.0

Overall rank (a) =ranking of families based on the means of AUDPC, DTOW, PSTTW, PSTTN and % LI.
Overall rank (b) =ranking of families based on their mean PTIT.

6.3.4 AMMI analysis of variance

The AMMI analysis of variance showed significant (P≤0.001) effects of the families, environments and the G x E interaction (Table 6.7). The AMMI model (families, environments and G x E interaction) captured 62.17% of the total sum of squares. Of the AMMI model (treatment) sum of squares, the families contributed 33.15%, the environments 23.43% and the G x E interaction 43.42%. The IPCA 1 was significant (P ≤0.001) and it explained 34.68% of the treatment sum of squares which is 79.88% of the G x E interaction sum of squares. The IPCA 2 was nonsignificant and it explained 7.8% of the treatment sum of squares which is 17.96% of the G x E interaction sum of squares. Combined, the IPCA 1 and IPCA 2 explained 97.84% of the total G x E interaction. Therefore, AMMI 1, in which the families and environment main effects are plotted againist IPCA 1 was used to describe the G x E interaction. The AMMI 1 biplot explained 91.26% of the treatment variation.

Table 6.7. Analysis of variance for potato tuber yields (t ha^{-1}) for 48 families grown in four test environments

Source	df	SS	MS	% Total SS explained	% Treatment SS explained	% G X E interaction SS explained
Treatments	191	80134	419.5 ***	62.17		
Families (G)	47	26566	565.2 ***		33.15	
Environments (E)	3	18773	6257.7 ***		23.43	
Block	8	999	124.9 ns			
Interaction (G X E)	141	34795	246.8 ***		43.42	
IPCA 1	49	27794	567.2 ***		(34.68)	79.88
IPCA 2	47	6250	133 ns		(7.80)	17.96
Interaction residuals	45	752	16.7 ns		(0.94)	2.16
Error	376	47753	127	37.05		
Total	575	128886	224.1			

df=Degrees of freedom; *= Significant at P≤ 0.05; **= Significant at P≤ 0.01; ***= Significant at P≤ 0.001; ns = Non significant;; SS = sum of squares, MS = mean squares.

6.3.5 Ranking of the best four AMMI selections per environment

There were variations in the ranking of potato families for tuber yields across the four test environments (Table 6.8). In environments ENVI 1 and ENVI 2, the families were ranked similarly; in both environments, the four best families were 394895.7 x Bishop Gitonga, 392278.19 x Sherekea, 394903.5 x Cangi and 394905.8 x Ingabire (Table 6.8).

Table 6.8. The best four potato families from AMMI per environment

Environment	Mean yields (t ha^{-1})	PCA Score	Rank 1	2	3	4
ENVI 4	48.41	8.086	394895.7 x Sherekea	391919.3 x Cangi	391919.3 x Kihoro	392278.19 x Kenya Karibu
ENVI 3	62.98	-0.239	392278.19 x Tigoni	394895.7 x Tigoni	391919.3 x Ingabire	394904.9 x Kenya Karibu
ENVI 1	55.05	-3.815	394895.7 x Bishop Gitonga	392278.19 x Sherekea	394903.5 x Cangi	394905.8 x Ingabire
ENVI 2	49.83	-4.032	394895.7 x Bishop Gitonga	392278.19 x Sherekea	394903.5 x Cangi	394905.8 x Ingabire

6.3.6 AMMI biplots: classification of families and environments

Environments ENVI 1, ENVI 2 and ENVI 4 had positive IPCA1 values (Figure 6.1). Families that had the same sign and IPCA values close to these environments were G39, G27, G45 and G47; these families were specifically adapted to these three environments. Environment ENVI 3 had a large negative IPCA 1 value; thus it strongly interacted with the potato families that had the same IPCA sign. ENVI 3 also had the highest mean yields. Most families had IPCA 1 values between +1.0 and -1.0 indicating general adaptation to the test environments. Family G15 (394904.9 x Sherekea) was the lowest yielding followed by G16 (394904.9 x Ingabire) (Figure 6.1).

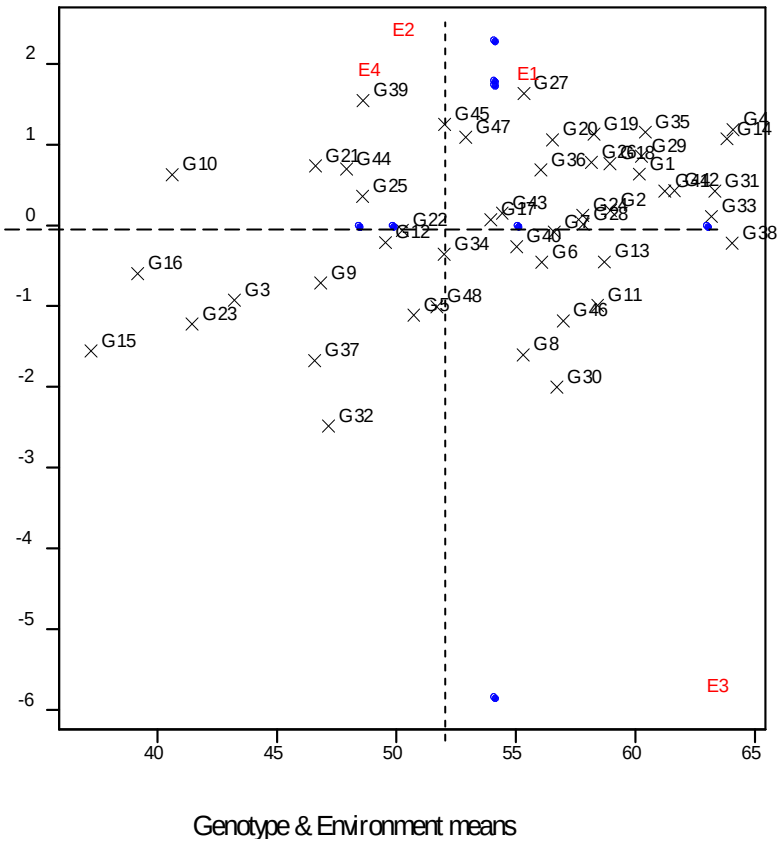

Figure 6.1.AMMI 1 biplot of TTW of 48 potato families (G1-G48) across the four environments (ENVI 1-ENVI 4).See Table 6.1 for environments and Table 6.6 for family codes

6.3.7 GGE biplot analysis

In the GGE analysis, IPCA 1 contributed 48.23% while IPCA 2 accounted for 26.65% of the total variation. The GGE biplot therefore explained 74.88% of the G and GxE interaction variation (Figure 6.2). The GGE biplot for potato tuber yield showed the most discriminating environment as ENVI 4 followed by ENVI 2 while ENVI 1 was third (Figure 6.2). Environments ENVI 1 and ENVI 2 were very similar (a small angle between their vectors); in case of limited resources, ENVI 1 can be dropped. Because ENVI 3 had the shortest vector, it had the least discriminating power and was therefore the least informative; genotypic differences in ENVI 3 may not be reliable for selection purposes.

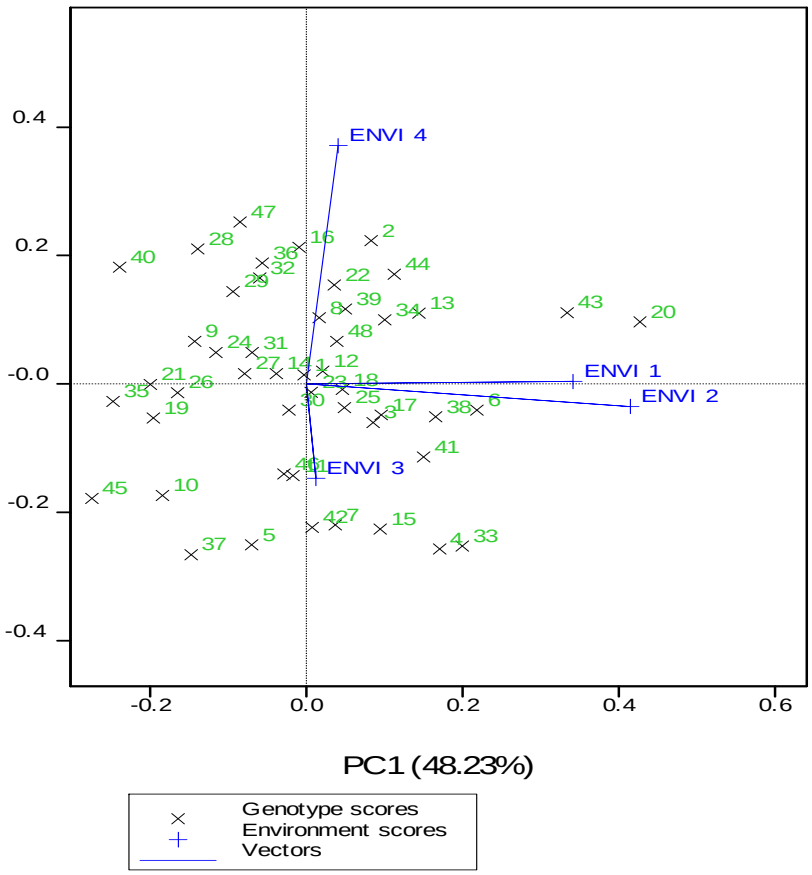

Figure 6.2 Vector view of the GGEbiplot showing the discriminating power and representativeness of the test environments. See Table 6.1 for environments and Table 6.6 for family codes

The highest yielding family in environments ENVI 1 and ENVI 2 was 20 followed by 43 (Figure 6.3). This indicates that family 20 (394905.8 x Kihoro) is the most

specifically suited to these two environments. In ENVI 4, the highest yielding family was 47 followed by 40 while in ENVI 3, the highest yielding family were 45, 37 and 33. There were three mega- environments; the first one consisted of ENVI 1 and ENVI 2, the second one was ENVI 4 while the third one was ENVI 3 (Figure 6.3).

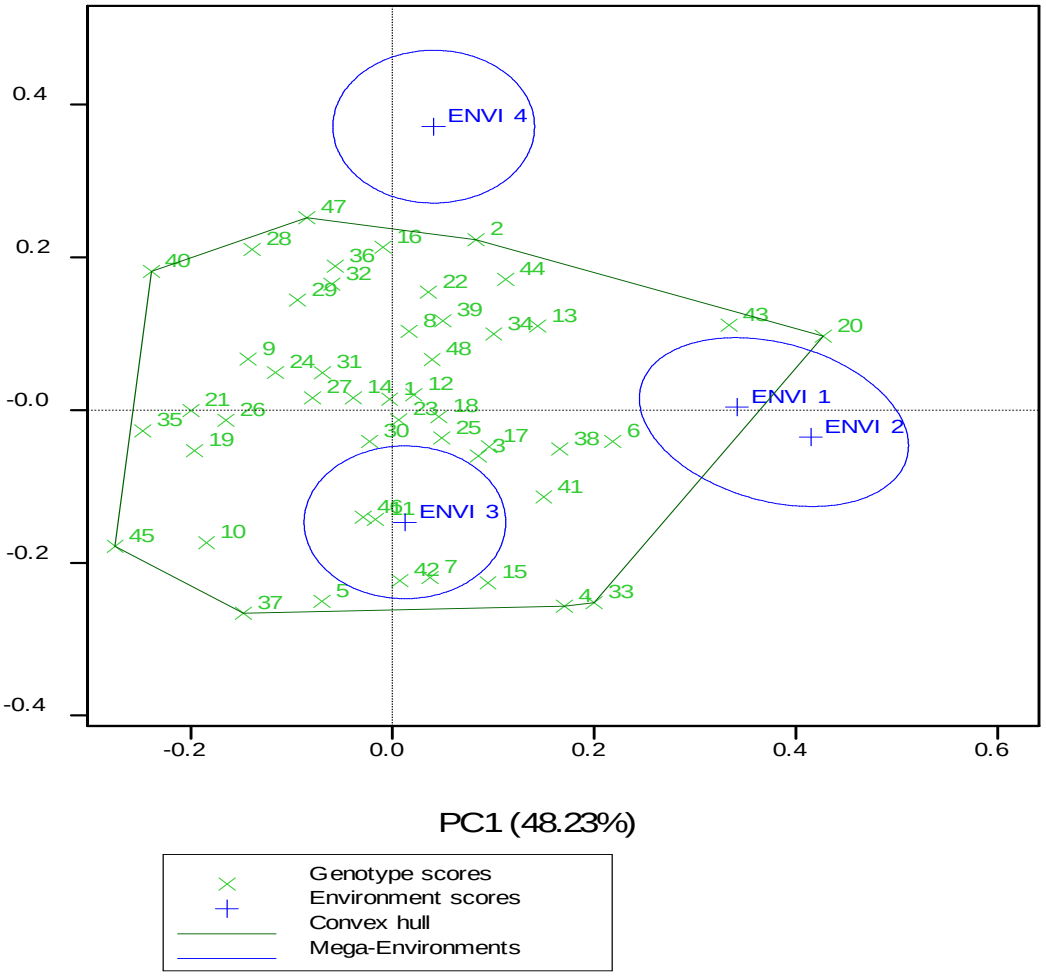

Figure 6.3. The “which-won-where” view of the GGEbiplot under each mega-environment constructed based on environment-centred and symmetrical singular-value partitioning. See Table 6.1 for environments and Table 6.6 for family codes.

The family 20 (394905.8 x Kihoro) was closest to the ideal genotype and was also the highest yielding; it was closely followed by family 43 (394903.5 x Kenya Karibu) (Figure 6.4). In addition, family 20 was the most stable.

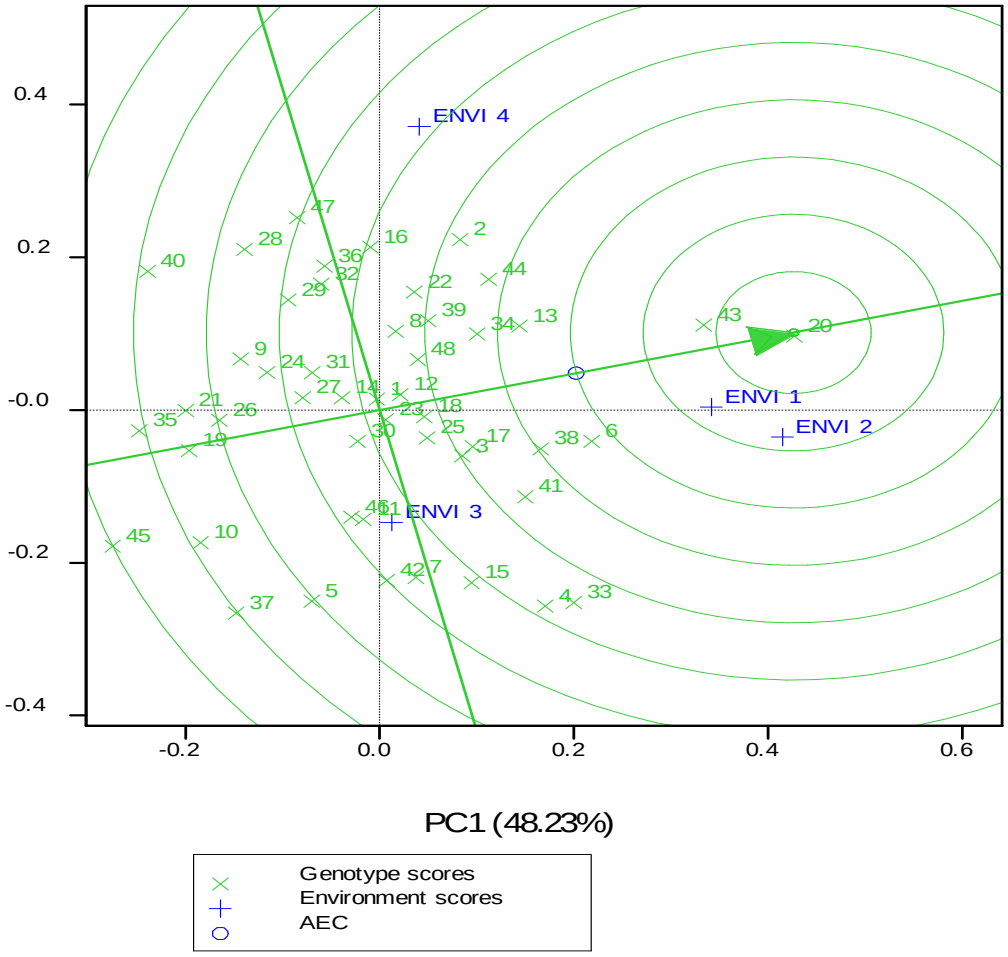

Figure 6.4. Biplot showing comparison of all genotypes with ideal genotype constructed based on environment-centred and genotype-focused singular-value partitioning. See Table 6.1 for environments and Table 6.6 forfamily codes

The ENVI 1 (short rains during 2013 at Kinale, Table 6.1) was the closest to ideal environment and therefore the most desirable of the four environments (Figure 6.5). It had great discriminating power and was representative of the test environments. The ENVI 4 did not appear representative of other environments. However, since it had the longest vector, it had the most discriminating power; it was also a unique environment.

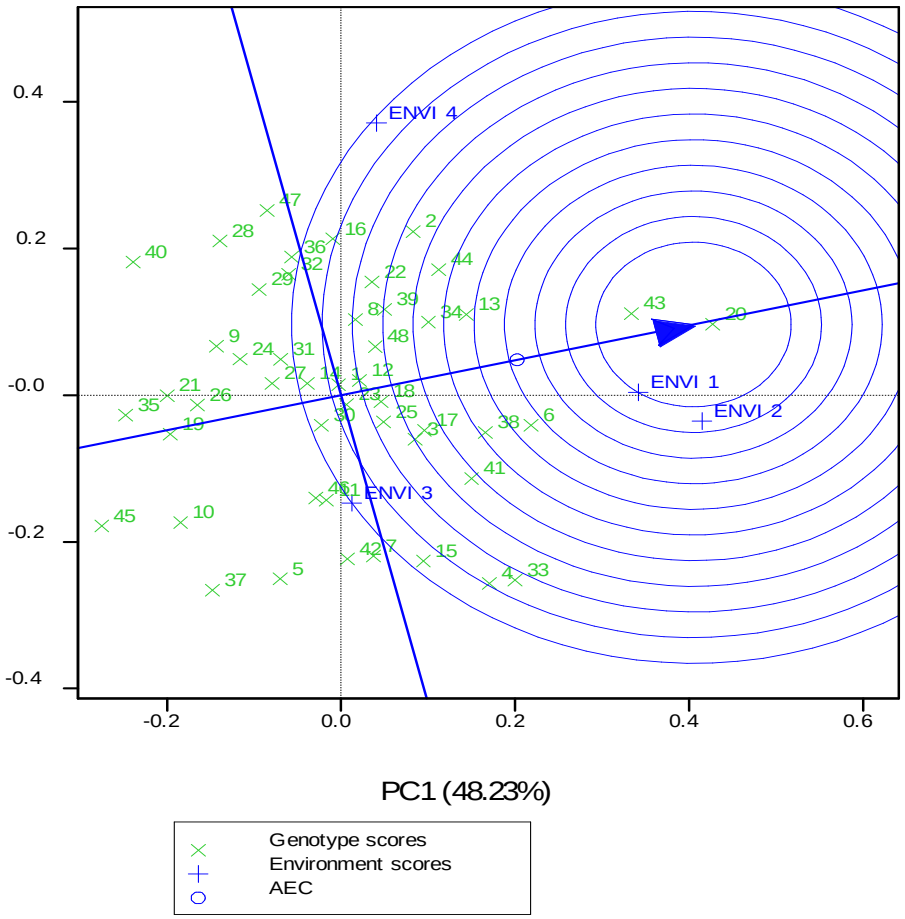

Figure 6.5 .Biplot for comparison of all environments with the ideal environment constructed based on environment-centred and environment-focused singular-value partitioning.See Table 6.1 for environments and Table 6.6 for family codes.

6.4 Discussion and conclusions

This study was set up to estimate the magnitude of GEI for potato tuber yield and bacterial wilt resistance in Kenya and to identify the most discriminating and representative environments for potato testing in Kenya. The experimental materials used in this study were in the early stages of breeding; they were families in second clonal generation. Therefore, this study was not meant for cultivar recommendation *per se* but to undertake early family selection.

The potato families were ranked differently in terms of resistance against bacterial wilt across the four environments and between the two ranking methods (Table 6.6). This is a case of crossover GEI. This inconsistency in ranking could partly be explained by the cross x site, cross x season and cross x site x season interactions displayed in Table 6.4. The genotype x environment (G x E) interactions could be due to differences in temperature and rainfall across the four environments (Table 6.3). Soil moisture and temperature are known to greatly influence survival and infectivity of *R. solanacearum* (Harris, 1976; Martin and French, 1985). It has previously been reported that resistance to bacterial wilt is very unstable due to strong host-pathogen-environment interaction; hosts resistant to the disease in one year/environment or location may succumb to the disease in the other year/environment or location (French and Lindo, 1982; Tung et al., 1990; Tung, 1992; Tung et al., 1992a; Tung et al., 1992b; Tung et al., 1993). The G x E interactions could have been complicated by the fact that the test materials were heterogeneous since they were early families and not of advanced clones.

From the AMMI analysis (Table 6.7), the first two IPCA's accounted for 97.84% of the G x E interaction. This corroborates with previous findings that G x E data sets are best described by AMMI models with one or two multiplicative terms (Gauch and Zobel, 1988). The high yields in ENVI 3 (Figure 6.1) could be due to cool temperatures experienced there (Table 6.3). Potato is a cool season (C_3) crop and cool conditions lead to high tuber yields (Haverkort et al., 1990). The ENVI 1 and ENVI 2 were similar (Figure 6.2) and ranked their first four families similarly (Table 6.8). This similarity could have been due to the fact that both of them experienced relatively high temperatures (Table 6.3).

This study has provided an insight into magnitude of GEI for potato tuber yield and bacterial wilt resistance in Kenya. The potato families were ranked differently in terms of resistance against bacterial wilt across the four environments; this was an indication of crossover GEI. Family 20 (394905.8 x Kihoro) was closest to the ideal genotype; it was the highest yielding and most stable; it was closely followed by family 43 (394903.5 x Kenya Karibu). The environment ENVI 1(short rains during 2013 at Kinale) was the closest to ideal environment and therefore the most desirable test site of the four environments.

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Chapter Seven: General overview of the thesis

7.1 Introduction and research objectives

In Kenya, potato is an important food crop, second after maize in volumes produced. It is grown mainly as a cash and food crop by small scale farmers, many of them women, although some large-scale growers specialize in commercial production. Potato therefore plays an important role in food security. However, there has been a decline in potato production in Kenya because of a number of production constraints. Bacterial wilt is the second most important biotic factor limiting potato production in Kenya; it has no known effective chemical treatment and biological and cultural control methods are ineffective. This chapter summarises the research objectives and highlights the core findings of the study.

The objectives of this study were:

1) To document farmers' practices, key potato production and marketing constraints, and to determine farmers' potato cultivar preferences, the prevalence of bacterial wilt in the major potato growing areas and establish farmers' management of bacterial wilt.
2) To determine the response of the potato genotypes currently grown by farmers in Kenya as well as other clones from CIP to bacterial wilt.
3) To determine the genetic relationships among potato clones
4) To determine the combining ability effects for yields, yield related traits and bacterial wilt resistance of selected potato genotypes.
5) To estimate the magnitude of genotype x environment interaction (GEI) for potato tuber yield and bacterial wilt resistance.

7.2 Research summary

To document farmers' practices, key potato production and marketing constraints, and to determine farmers' potato cultivar preferences, the prevalence of bacterial wilt in the major potato growing areas and establish farmers' management of bacterial wilt, a participatory rural appraisal (PRA) was conducted in three major potato growing counties involving 253 potato growers. The main outcomes were as follows:

- Farmers varied in cultivar and trait preferences; in Bomet district the red-skinned Dutch Robyjn is widely grown. In Molo district, the white- skinned Cangi is prominent while in Meru Central, the red-skinned Asante is predominantly grown by farmers.
- The cultivar preferences are mostly dictated by availability of markets, yield potential and taste.
- Over 75% of respondents indicated that the major production constraints are diseases with bacterial wilt being the most prominent.
- Farmers employ different methods in managing the disease in the field such as spraying with fungicides, roguing and burning the wilting plants, and burying of the rotten tubers after harvest.

To determine the respone of the potato genotypes currently grown by farmers in Kenya as well as other clones from CIP to bacterial wilt and to identify parents that can be used in a local breeding programme to develop resistant cultivars, field experiments were conducted in order to evaluate 36 potato genotypes for their response to bacterial wilt for three consecutive seasons between November 2011 and February 2013. The main outcomes were as follows:

- All the genotypes are generally susceptible to bacterial wilt; susceptibility ranged from moderate to high.
- The potato genotypes varied in their susceptibility to bacterial wilt and the most resistant genotypes were Kenya Karibu followed by Kenya Sifa.
- The study identified eight potato genotypes (Meru Mugaruro, Ingabire, Kenya Karibu, Sherekea, Kihoro, Tigoni, Bishop Gitonga and Cangi) to be used in a breeding programme to improve bacterial wilt resistance in Kenyan germplasm.

To determine the genetic relationships among potato clones so as to complement other bacterial wilt resistance data in identifying parents for a breeding programme, 20 selected potato genotypes were evaluated for genetic variability using 24 SSR primer pairs selected based on high polymorphism. The main outcomes were as follows:

- The twenty four SSR primer pairs identified 160 alleles among the 20 potato clones.
- The clones were grouped into three clusters; cluster I ahd Meru Mugaruro, cluster II had CIP clones while cluster II had the local varieties.
- Therefore, the SSR markers generated useful information that will assist in identifying parents to include in the breeding programme.

To determine the combining ability effects for yield and yield related traits and bacterial wilt resistance of selected potato varieties and clones and their crosses, 14 potato genotypes were identified as promising parents for further breeding programme based on their resistance to bacterial wilt. These parents were crossed in a North Carolina II mating design to generate 48 families for determining combining ability. The main outcomes were as follows:

- Parents with highest general combining ability were Ingabire, Meru Mugaruro, 391919.3, 394895.7 and 394903.5.
- These parents were selected for future crosses.
- In addition, nine crosses with the highest SCA effects for total tuber yield (TTW) at KARI-NARL were 394905.8 x Kihoro (31.94), 394903.5 x Kenya Karibu (31.46), 394904.9 x Meru Mugaruro (25.73), 394895.7 x Bishop Gitonga (15.37), 394905.8 x Cangi (13.06), 394895.7 x Tigoni (12.23), 394904.9 x Sherekea (11.44), 394895.7 x Sherekea (10.92) and 391919.3 x Tigoni (10.32) in that order.
- At Kinale, the nine crosses with the highest SCA effects for TTW were 394905.8 x Kihoro (27.13), 394903.5 x Kenya Karibu (24.37), 394904.9 Meru Mugaruro (19.59), 394895.7 x Cangi (15.69), 3948957 x Bishop Gitonga (15.35), 394895.7 x Tigoni (11.93), 394904.9 x Sherekea (9.36), 392278.19 x Meru Mugaruro (9.10) and 391919.3 x Cangi (7.64) in that order.
- These crosses were selected for high tuber yield and will be evaluated in future.

To estimate the magnitude of GEI for potato tuber yield and bacterial wilt resistance and to identify the most discriminating and representative environments

for potato test in Kenya, the GEI effect of 48 potato families were evaluated at two sites for two consecutive seasons (making a total of four environments). The main outcomes were as follows:

- The potato families were ranked differently in terms of resistance against bacterial wilt across the four environments.
- In terms of yield stability, family 20 (394905.8 x Kihoro) was closest to the ideal genotype; it was the highest yielding (104.7 t ha^{-1}) and most stable; it was closely followed by family 43 (394903.5 x Kenya Karibu) which yielded 98.3 t ha^{-1}.
- The environment ENVI 1(short rains of 2013 at Kinale) was the closest to ideal environment and therefore the most desirable of the four test environments.

7.3 Implications of the research findings to breeding potato for higher yield and resistance to bacterial wilt

The following implications for breeding were noted:

- Farmers' participation in potato varietal selection and identification of breeding priorities is important for better and faster adoption of improved varieties. Their views and priorities will be considered in the potato breeding programme in Kenya.
- There is considerable genetic variability for potato tuber yield and bacterial wilt resistance among the potato varieties currently grown by farmers in Kenya.
- The SSR genetic markers were useful and provided three distinct genetic groups enabling breeders to design targeted crosses for hybrid development to exploit heterosis, and maintain diversity among the clusters.
- The importance of both additive and non-additive effects in controlling potato tuber yields, bacterial wilt resistance and other agronomic traits suggest that breeding gain can be realized through hybridization and selection strategies in the program.

- In general, the study identified valuable potato families with high combining ability for tuber yields and bacterial wilt resistance from which new high yielding, bacterial resistant clones can be selected for future release as cultivars.