

**Genetic analyses for resistance to soybean rust (*Phakopsora pachyrhizi*) and yield stability among soybean genotypes in Kenya**

**By**

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**A thesis submitted in partial fulfillment of the requirements for the  
degree of Doctor of Philosophy (PhD) in Plant Breeding**

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December, 2012

## Thesis abstract

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Soybean (*Glycine max* (L.) Merr.) occupies an important position in the world economy of the feedstock of high quality protein and vegetable oils. However, its production is threatened by, Asian soybean rust (ASR), caused by the rust fungus *Phakopsora pachyrhizi* Syd. & P. Syd. This fungus is highly dependent on environmental conditions, has a wide range of hosts, and evolves rapidly into novel races, making it difficult to control. In addition, most commercial varieties are susceptible to rust, the rust has already developed resistance to triazole fungicides, and most small-scale farmers cannot afford expensive systemic fungicides to control the disease. The use of resistant varieties is the most viable, long-term option to manage ASR, especially in the small-holder soybean farming sector. This study was therefore designed to undertake the following goals: (i) to identify farmers' preferred varieties and desired traits, their knowledge of ASR, and other key constraints affecting soybean production in Kenya; (ii) to evaluate soybean accessions for rust resistance, and to determine the correlation of rust resistance with other agronomic traits; (iii) to determine the mode of inheritance for ASR resistance and selected agronomic traits; and (iv) to determine yield stability of soybean advanced lines at multiple sites in Central and Eastern Kenya.

To understand farmers' preferred varietal characteristics, knowledge of ASR and other key constraints to soybean production, a survey was conducted using a structured questionnaire in the major soybean growing areas of Kenya. The farmers preferred local varieties because of their desirable characteristics, which included high yields, early maturity, drought tolerance and seed availability. Although the majority of the participating farmers expressed a willingness to grow improved varieties, financial limitations, seed unavailability and lack of information were the major barriers to their use of improved varieties. High yield, early maturity, adaptability and grain quality were the traits that most farmers sought in an ideal soybean variety. Knowledge of the cause of ASR was limited, and its occurrence was largely attributed to environmental factors, poor soil fertility conditions, poor agronomic practices, physiological maturity and specific species of weeds. Their investments in control methods were minimal due to a lack of technical knowledge, poor access to fungicides, and limited resources. Other constraints faced by soybean farmers included: lack of access to grain markets; lack of knowledge in processing and utilization of soybean grain; the unavailability of seeds; losses to pests and diseases; the lack of inputs such as fertilizers; frequent dry spells; and low yielding varieties.

A total of 110 soybean accessions were evaluated for their rust reactions and correlations with selected agronomic traits. These included plant introductions possessing single rust resistant genes (*Rpp1-4*), tolerant lines, gene bank accessions, commercial varieties and advanced lines. Soybean genotypes varied significantly in their reactions to rust severity, sporulation, lesion type and area under disease progress curve (AUDPC) values. Genotypes possessing *Rpp4* (G10428) and *Rpp2* (G8586) resistant genes, and non-characterized genotypes MAK BLD 11.3, GC 00138-29 and Namsoy 4M, were the most resistant accessions, as indicated by low rust severity scores, low AUDPC values, red brown lesions and low sporulation scores. Other genotypes with known resistant genes including G7955 (*Rpp3*), G58 and Tainung 4 (*Rpp1*), a few tolerant lines, and one advanced line (BRS Sambaiba) were moderately resistant. All the other advanced lines, commercial varieties, gene bank accessions and collections from the farmers' fields were highly susceptible to rust. Rust severity was positively correlated with rust sporulation, indicating that reduction of sporulation made a significant contribution towards rust resistance.

An  $F_2$  population was generated from a half diallel mating design, involving 4 resistant, 2 moderately resistant and 2 susceptible genotypes selected as parents. The  $F_2$  populations along with their parents were evaluated in two environments to determine the type of gene action for rust resistance and other quantitative traits in soybeans. The results revealed that both general combining ability (GCA) and specific combining ability (SCA) were significant for most of the traits studied, indicating that both additive gene action and non-additive gene action played a major role in the inheritance of rust resistance and selected agronomic traits. The GCA/SCA ratio was close to unity for rust severity, rust sporulation, days to flowering, days to maturity and plant height. This indicated that additive gene action played a more significant role in the inheritance of these traits than non-additive gene action. Non-additive gene action was only predominant for soybean grain yield. Parental lines G10428, G8586 and Namsoy 4M were the best general combiners for improving rust resistance across the environments. The most promising parents for early flowering were G7955, G8586 and G58. Parent Maksoy 1N was the best general combiner for early maturity while parents Maksoy 1N, G58, G7955 and Nyala contributed effectively towards reduced plant height.

Yield stability analysis was conducted for 30 genotypes in 6 environments, using additive main effects and multiplicative interaction (AMMI), genotype main effect and genotype x environment interaction (GGE) biplot analyses. Genotypes 916/5/19 and G7955 were identified as the high

yielding and most stable across the environments. On the other hand, genotypes BRS MG46 and Sable were high yielding but unstable and specifically suitable for the environments EM2 and MW2, respectively (both environments have long rainy seasons). Environment EM2 was identified as the most discriminating and representative among the six environments. Environments IG1 and MW1 (short rainy seasons) were less informative on genotypes tested, as confirmed by short environment vectors. Environment EM1 was better for discriminating genotypes but was a poor representative of the test environments, hence it should only be utilized for developing specifically adapted genotypes. Further analysis using GGE biplot approach grouped the environments into three putative mega-environments in Central and Eastern Kenya.

Overall, this study established the need to educate farmers on the cause of ASR, to develop ASR resistant varieties, and to incorporate farmers' desired traits in the breeding programme, especially by the use of participatory breeding approaches. The resistant and moderately resistant genotypes identified in this study could be used as sources of resistant genes to develop ASR resistant varieties in Kenya. This study also established that genetic improvement for ASR resistance and selected agronomic traits in soybeans is possible based on the use of recurrent selection breeding procedures that result in the accumulation of additive gene effects. Selection of late segregating generations would be effective for soybean grain yield improvement. This study identified potential parents for ASR resistance and selected agronomic traits, but they require further breeding to improve on farmers' desired traits.

## Declaration

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I, **Susan Wothaya Wanderi**, 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
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Signed.....Date.....

**Susan Wothaya Wanderi**

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

Signed.....Date.....

**Prof. Githiri Mwangi (Principal supervisor)**

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**Prof. Mark Laing (Co-supervisor)**

Signed.....Date.....

**Dr. Julia Sibiya (Co-supervisor)**

## Acknowledgements

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I would like to express my sincere appreciation to my supervisors; Professor Githiri Mwangi of the Jomo Kenyatta University of Agriculture and Technology (formerly of the University of KwaZulu Natal), Professor Mark Laing and Dr. Julia Sibiya of the African Center for Crop Improvement, University of KwaZulu-Natal, for their support and guidance throughout this study and thesis compilation. My appreciation is extended to my in-country supervisor, Dr. James Muthomi, of the University of Nairobi, for his valuable input during the study.

I am deeply indebted to the Alliance for a Green Revolution in Africa (AGRA) for the financial support through the African Center for Crop Improvement (ACCI), without which this study could not have been possible. I am grateful to the ACCI Director, Prof. Mark Laing, and the entire staff for their facilitation and logistic support that has made it possible for me to accomplish this study.

My sincere appreciation goes to Kenya Agricultural Research Institute (KARI) Director, Dr. Mukisira, for granting me study leave and institutional support. I also acknowledge the support of the KARI-Embu Center Director, Dr. Njoka, and members of staff for the provision of laboratory equipment to carry out the research work, technical and logistic support. Specifically, I thank Mr. Mohasen Mwangi, Mugo and Lucy for their technical assistance in the field research. My appreciation also goes to KARI-Mwea Centre Director, Dr. Gitonga and Dr. Kimani, for allowing me to conduct field experiments in their station. My sincere appreciation to Christine, Susan and Nancy for the assistance they offered to me during the research period.

Special thanks to Dr. Tukamuhabwa of Makerere University, Dr. Mahasi and Dr. Gethi of Kenya Agricultural Research Institute (KARI-Njoro), and Mr. Muthamia of the National Genebank of Kenya (NGBK) for the provision of soybean germplasm for this study.

Last but not least, I acknowledge the love and support of my parents, Wanderi Mukira and Agatha Wangechi, my son Daniel, my brothers Simon, John, Peter and Paul, and my sisters, Alice and Teresah, throughout the study period. Special thanks to my sister Alice for her commitment to take care of my son while I was away from home. I would also like to thank my friends Peter Gakuyia, Njeru and Mirriam for their assistance in various ways during the period of this study. May God bless you all and reward you abundantly. Amen.

## **Dedication**

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This thesis is dedicated to the entire Wanderi's family; my son Daniel Wanderi, my parents Wanderi Mukira and Agatha Wangechi, my brothers Simon Gitonga, John Warugu, Peter Wachira and Paul Maina and my sisters Alice Muthoni and Teresah Wanjugu.

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## Introduction to Thesis

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### 1. Soybean production and uses

Soybean (*Glycine max* (L.)) is a major source of vegetable oil and high quality protein in the world (Tukamuhabwa et al., 2012). It is ranked first in the international world market (55%) among other oil seed crops such as cotton seed, peanuts (groundnuts), sunflower seed, rape seed, palm and coconut (Chung and Singh, 2008). Currently, United States of America is the largest world producer of soybeans accounting for about 38% of the global production followed by Brazil (26%), Argentina (21%), China (7%) and India (4.3%) (FAOSTAT, 2012). Africa accounts for approximately 1% of the world soybean production. However, the potential to increase soybean production in Africa exists, if the major constraints are properly addressed.

In Kenya, soybean is mainly produced by small-scale farmers in Western, Nyanza, Eastern and Central regions and a few large-scale farmers in the Rift Valley region (Chianu et al., 2008). Over the last few years, soybean demand both locally and at industrial level has increased considerably in the country (Nassiuma and Wasike, 2002). This is probably due to its potential as a source of dietary quality foods for the rapid increasing human population, livestock feed, source of income and soil fertility improvement. The soybean seed is highly valued for its high protein content (40%) that is used either for fresh green vegetables or processed products such as soyflour, soymilk, roasted soy beverage, fried soynuts and soymeat for human consumption (Hartman et al., 2011). In addition, more than 90% of the soybean cake is used for livestock, poultry and aquaculture feeds due to its high protein content. Its high oil content (20%) is used for making processed food products (margarine and cooking oil) and industrial products such as cosmetics, plastics and paint removers, among others. Soybean is also an important source of isoflavines, which are used for reducing health risks associated with blood cholesterol and other diseases in human beings. Furthermore, soybean improves soil fertility through biological nitrogen fixation, thereby alleviating soil fertility problems, and when grown in rotation with maize, it reduces *Striga* infestations of maize (De Groote et al., 2010).

### 2. Soybean production constraints

Soybean is an important crop in Kenya as highlighted by its multiple uses and wide adaptation. However, the area under soybean production, yields and production quantities have been constant during the period of 2006 to 2010 (Table 1) (FAOSTAT, 2012). The current production level in the country is below 10,000 tonnes (Wasike et al., 2009) and cannot meet the increasing

demand for food and feed processing industries, as well as other local buyers. The deficit has to be acquired through importation from the neighbouring countries (Uganda and Tanzania). This demand is projected to increase to more than 150,000 tonnes per year, in the next 10 years indicating the need to increase the local production. Soybean production in Kenya is low because of a number of challenges, including low yielding varieties, lack of markets, poor agronomic practices, lack of awareness for its potential, competition with other legumes, drought, water logging, and pest and disease attacks (Hartman et al., 2011). Among the diseases, ASR caused by *Phakopsora pachyrhizi* Syd. & P. Syd. (1914) is by far the most devastating in soybean production (Calvo et al., 2008; Li et al., 2012).

**Table 1: Soybean area, yield and production in Kenya from 2006 to 2010**

Year	Yield (tonnes/ha)	Area (ha)	Production quantity (tonnes)
2006	0.827	2513	2077
2007	0.840	2500	2100
2008	0.791	2615	2042
2009	0.693	2965	2054
2010	0.917	2400	2200

Source: UN Food and Agriculture (FAOSTAT, 2012).

### **3. Soybean rust**

Asian soybean rust (ASR) was first reported in Japan in 1902, and the pathogen subsequently spread from Asia to Africa, South America, and United States of America (Schneider et al., 2005). Since its discovery in Kenya, in 1996 (Kawuki et al., 2003b), ASR has continued to have negative impacts on farmers' livelihood, especially in the main growing areas, and in soybean-dependent food and feed industries. Currently, there is no formal documentation on ASR distribution and prevalence in Kenya, though heavy infestations have been reported in Busia, Kakamega and Nakuru counties (Wasike and Janey Leaky, *personal communication*<sup>1</sup>). Severe damage caused by ASR has also been observed in farmers' fields in the Mwea and Embu

<sup>1</sup> Wasike, V.W. (Soybean rust in Western Kenya): Kenya Agricultural Research Institute, Hqs, Nairobi, Kenya.

Janey Leaky (Soybean rust in Nakuru County): Director, Leldet Seed Company, Rwajera Farm, Nakuru, Kenya.

counties (personal observation). The increased incidence of ASR in different regions of the country is raising great concern for plant breeders and pathologists.

Yield losses due to ASR have not been quantified in Kenya. However, significant yield losses of between 10 and 80% have been reported in unprotected fields in different parts of the world including some African countries (Yorinori et al., 2005). For example, Caldwell and Laing (2001) reported yield reduction ranging from 10 to 80% in South Africa, and 60 to 80% in Zimbabwe. In Uganda, yield losses up to 40% were reported by Kawuki et al. (2003a). Therefore, without adequate control measures, ASR is likely to cause serious yield losses and economic damage to soybean production in Kenya, similar to those observed in other countries. This situation is complicated by the fact that the disease is highly dependent on environmental conditions for its development and infection (Kawuki et al., 2004), making its outbreaks unpredictable and difficult to control, especially in the diverse agro-ecological zones of Kenya where soybeans are grown. The local farming systems almost certainly contribute to the development of ASR because soybean is mainly grown as an intercrop with important leguminous food crops including beans (*Phaseolus vulgaris* L.), pigeonpea (*Cajanus cajan* (L.) Millsp.) and cowpeas (*Vigna unguiculata* (L.) Walp) that acts as inoculum reservoirs for *P. pachyrhizi*, further exacerbating the ASR problem (Maphosa et al., 2012). In addition, most of the commercial varieties (e.g. Nyala, Gazelle and EAI 3600) grown in Kenya are highly susceptible to ASR (Mahasi et al., 2009). Furthermore, the majority of soybean farmers are resource-poor and they have limited access or funds to buy fungicides to control ASR (Li et al., 2012). Therefore, breeding for rust resistant varieties would be the most economically feasible and environmentally friendly approach for reducing yield losses in the soybean farming sector in Kenya (Twizeyimana et al., 2008; Pham et al., 2010).

#### **4. Breeding for ASR resistance**

Breeding for ASR resistance has been in progress for many years in Asia (Hartman et al., 1992) and more recently in USA (Miles et al., 2006) and Africa (Kawuki et al., 2004; Twizeyimana et al., 2007). As a result specific resistance, partial resistance and tolerance against ASR have been identified (Hartman et al., 2005). For example, eight specific rust resistant dominant genes in six loci: *Rpp1*, *Rpp1b*, *Rpp2*, *Rpp3*, *Rpp4*, *Rpp5*, *Rpp Hyuuga?* and *Rpp6* have been identified in different germplasm collections (Hartman et al., 2005; Calvo et al., 2008; Li, 2009; Li et al., 2012). In addition, three single recessive genes (*rpp2*, *rpp3* and *rpp5*) controlling ASR were recently identified by Calvo et al. (2008) and Ray et al. (2011). Despite the discovery of

these resistance mechanisms, there are no commercial varieties with universally acceptable levels of resistance to rust that have been released. This is because specific genes are resistant to some *P. pachyrhizi* isolates but ineffective against other isolates. This is due to the presence of races of *P. pachyrhizi* with virulence against the genes involved in monogenic resistance (Hartman et al., 2005). Therefore it is necessary to verify the effectiveness of these genes against local isolates before they are utilized in breeding programmes. In addition, local varieties and advanced lines may possess resistance to some isolates that need to be verified for proper utilization in the breeding programmes.

Another problem that has made breeding for rust resistance slow is lack of information regarding the genetic mechanisms controlling the inheritance of ASR resistance. Previous genetic studies on the inheritance of ASR resistance have reported variable findings on the type of gene action and mode of inheritance among different sources (Garcia et al., 2008). Some studies reported that resistance to ASR is predominantly controlled by additive gene action (Maphosa et al., 2012; Ribeiro et al., 2007), while others reported partial and complete dominant gene action (Laperuta et al., 2008), and more recently epistatic gene action was detected (Garcia et al., 2008; Laperuta et al., 2008). These studies have provided useful genetic information to the plant breeders but it is applicable to specific germplasm and range of tested environments. Therefore, further genetic studies may be useful to identify sources of resistance that are applicable to Kenyan environments.

In addition, genotype x environment interaction (GEI) has continued to slow the selection progress and development of stable soybean cultivars (Ahmadi et al., 2012), as well as the identification of suitable testing environments (Badu-Apraku et al., 2012), given that in the many agro-ecological zones in Kenya where soybeans are grown GEI is unavoidable. Therefore, understanding the patterns and nature of GEI would play an important role in developing a successful breeding programme for soybean in Kenya. Additive main effects and multiplicative interaction (AMMI) model, and genotype main effect and genotype x environment interaction (GGE) biplot analyses are powerful statistical tools that have provided detailed information on GEI in several crops. Their application in soybeans may provide useful information on stable or specific genotypes that could be used in the breeding programmes or recommended for commercial production in Kenya.

The International Institute of Tropical Agriculture (IITA) has developed high yielding and rust resistant soybean varieties with the objective of increasing soybean production in Africa (Vandeplas et al., 2010). However, most of these “improved” soybean varieties have not been adopted by farmers, who continue to grow their local varieties (Mahasi et al., 2009). This is probably because the traits that are valued by farmers were not incorporated during the development of these varieties. It is therefore important that the researchers work in collaboration with farmers to identify their varietal preferences and desired traits, and identify the constraints that farmers face during soybean production, marketing and consumption. The involvement of farmers through participatory approaches from the beginning of a breeding programme is likely to enhance the adoption rate of the improved varieties.

## **5. Problem statement**

In Kenya, soybean is a relatively new crop; hence its improvement through the breeding programme is still in early stages. As a result, there have been no studies on ASR, despite to the threat it poses to soybean production in Kenya. There is therefore an urgent need to establish a breeding programme that will develop rust resistant varieties that will meet the increasing demand for soy products, and consequently reduce the current levels of soybean importation. Important research conducted elsewhere has identified several rust resistant and tolerant varieties that could be used as source of resistance genes. However, their effectiveness against local ASR isolates may differ due to diverse environmental conditions, the rapid evolution of races of *P. pachyrhizi* with novel virulence genes, the wide range of hosts for ASR providing for year-round sites for infection and spore generation, and the diverse farming systems present in Kenya. Therefore further testing of these plant introductions is needed to identify new sources of resistance that could be used to develop resistant varieties in the country. There is also need to understand the genetic mechanisms governing ASR and other agronomic traits for effective selection and breeding procedures.

In addition, GEI analysis for soybean cultivation has not received adequate attention in Kenya. It would therefore be important to determine the magnitude of GEI and yield stability of soybean advanced lines, and to identify the most suitable testing environments for evaluating soybeans in Central and Eastern Kenya. Information on farmers' knowledge of ASR and other constraints affecting soybean production, as well as a clear understanding of the agronomic and quality traits that farmers demand in the soybean cultivars that they grow is still lacking in Kenya. For an effective soybean breeding programme, it is necessary to clearly understand the key



soybean production and marketing constraints, and farmers' preferences for the purpose of developing soybean varieties that will meet farmers' requirements and flourish in their particular agro-ecological zone.

## **6. Objective of the study**

The overall objective of this study was to contribute to improved food security through increased soybean productivity by developing stable, high yielding varieties with rust resistance and farmers preferred traits. The specific objectives were to:

- (i) Identify farmers' preferred varieties, desired traits, knowledge of ASR, and identification of other constraints facing soybean production in Kenya;
- (ii) Evaluate soybean genotypes for ASR resistance, and determine its correlation with selected agronomic traits in Kenya;
- (iii) Determine the combining ability for resistance to soybean rust and selected agronomic traits in soybeans;
- (iv) Analyse genotype x environment interaction and stability for grain yield of advanced soybean lines in Kenya.

## **7. Research hypotheses**

The following research hypotheses were tested:

- (i) Farmers in Kenya are aware of ASR and they prefer specific traits that meet their selection criteria.
- (ii) High levels of resistance/tolerance to ASR are available in local soybean accessions and plant introductions and this resistance can be identified and exploited in breeding programmes.
- (iii) The inheritance of genes for resistance to ASR and other agronomic traits are controlled by both additive and non-additive gene action.
- (iv) Soybean grain yield performance and stability are affected by genotype x environment interaction.

## **8. Thesis outline**

The thesis outline is presented in such a way that each specific objective represents a chapter intended for journal publication. The chapters are divided as follows;

Introduction to the thesis

Chapter 1: Review of literature;

- Chapter 2: Identification of farmers' preferred varieties, desired traits, perceptions on ASR and other constraints facing soybean production in Kenya;
- Chapter 3: Evaluation of soybean genotypes for ASR resistance and its correlation with selected agronomic traits in Kenya;
- Chapter 4: Combining ability for resistance to soybean rust and selected agronomic traits in soybeans;
- Chapter 5: Genotype x environment interaction and stability for soybean grain yield in Kenya;
- Chapter 6: Overview and conclusion of the research.

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## 1 Literature review

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### 1.1 Introduction

This chapter provides a brief overview necessary to guide research into breeding soybean for rust resistance and other agronomic traits in Kenya. Soybean taxonomy, origin, distribution, its economic importance and production constraints are described. Asian soybean rust (ASR), which is caused by *Phakopsora pachyrhizi* Sydow and is the most economically important disease affecting soybean in Kenya, is discussed. Its geographical distribution, symptoms, epidemiology, factors affecting its development and its negative effects on soybean growth, yield and seed quality are reviewed. Also discussed are the efforts made to control ASR, with an emphasis on host resistance; identification of rust resistance mechanisms and their mode of inheritance, and suggested breeding approaches. Finally, this review highlights the importance of assessing genotype x environment interaction in soybean breeding programmes.

### 1.2 Soybean taxonomy, cytology, floral and pollination system

Soybean belongs to the genus *Glycine* of Fabaceae (Leguminosae) family. Genus *Glycine* is further grouped into three subgenera: *Glycine* subgenus *Leptocyamus* (Benth.) F.J. Herm., *Glycine* (perennials), and *Soja* (Moench) annuals (Chung and Singh, 2008). *Glycine* subgenus *Leptocyamus* comprises of 6 species, including the most important species, *G. tabacina* (Labill.) Benth., *G. tomentella* Hayata and *G. canescens* F.J.Herm. The subgenus *Glycine* (perennials), on the other hand comprises of 2 species *G. petitiiana* (A.Rich.) Schweinf. and *G. javanica* L. with several subspecies while the cultivated soybean (*Glycine max* (L.) Merr) and the wild soybean (*Glycine soja* Siebold and Zucc.) belong to subgenus *Soja*. Both the cultivated and the wild soybean are paleoploid with  $2n=40$  chromosomes and they are cross compatible (Singh and Hymowitz, 1999).

Soybean is a self pollinated crop with a typical papillionaceous flower. The flower is complete consisting of the calyx, corolla, pistil, and stamen. The calyx has five unequal sepals that surround the corolla, pistil and the stamen. The corolla consists of a standard (posterior and bannel petal) with two wings and two keel petals that are not fused. The pistil has a single ovary with one to five ovules and a club shaped stigma. The flower has ten stamens that form a ring a round the pistil. During anthesis, the filaments elongate pushing the anthers above the stigma thus ensuring self pollination (Singh, 2007). The receptivity of the stigma to pollen occurs one day before the pollen is shed.

### **1.3 Soybean origin and distribution**

Soybean (*G. max.*) originated from China and was domesticated from the wild soybean (*G. soja*) between 1500-1100 BC (Pathan and Sleper, 2008). It was introduced to European countries between the 16<sup>th</sup> and 17<sup>th</sup> centuries from China, Korea and Japan. In North America it was introduced in 1765 and was only introduced to Central and South America in the mid-1900s. In Africa, soybean was introduced by Chinese traders along the East coast in the nineteenth century (Giller and Dashiell, 2006). Today, soybean is grown throughout the world, in diverse climates ranging from temperate to tropical and subtropical regions (Tukamuhabwa et al., 2002). The main producers of soybeans are United States of America, Brazil, Argentina, China and India (Pathan and Sleper, 2008). In Africa, countries with the greatest soybean production are Nigeria, South Africa and Uganda (FAOSTAT, 2012). In Kenya, soybean production is currently gaining prominence due to the increasing demand for its products. It is mainly grown by small-holder farmers in high potential areas of Western, Nyanza, Eastern and Central regions, and by a few large scale farmers in Laikipia, Uasin Gishu, Trans-Nzoia and Nakuru counties (Wasike et al., 2009).

### **1.4 Importance of soybeans**

Soybean is an economically important leguminous crop that is grown for its oil and protein products (Tefera et al., 2009). The soybean seed contains an average of 40% protein and 20% oil that is used for making nutritious food products such as soymilk, miso, soyflour, soysauce and tofu (Fabiya, 2006). It is also an important source of proteins in feed supplements for livestock. Besides its nutritive value, soybean has medicinal properties due to isoflavones content that reduces blood cancer, osteoporosis, blood cholesterol and heart diseases in human beings (Pathan and Sleper, 2008). In addition, it improves soil fertility through biological nitrogen fixation, thus reducing the cost of purchasing inorganic fertilizers by resource constrained farmers (Misiko et al., 2008). In rotational system with cereals, soybean dual purpose varieties have shown its potential in reducing the levels of Striga (*Striga hermonthica* (Delile) Benth.) infestations (Chianu et al., 2006b). Furthermore, soybean is used as a raw material in industries for production of biodiesel, cosmetics, pesticides, hydraulic fluids, lubricants, paint removers and plastics; hence it can be utilized as a beneficial crop by small-holder farmers for income generation (Pathan and Sleper, 2008).

## **1.5 Soybean production constraints in Kenya**

Soybean production in Kenya is characterized by low yields. The actual yield in the farmers' fields is 0.6 t ha<sup>-1</sup> which is far below the potential yield of 2.5 t ha<sup>-1</sup> in research managed trials (Chianu et al., 2006b). The low yields are as a result of several challenges which include abiotic, socioeconomic and abiotic constraints (Kawuki et al., 2003b; Mohammad et al., 2003). The major abiotic constraints in soybean production are weather-related factors (e.g. extreme temperature, drought, waterlogging and frost), soil nutrient availability, salinity and photoperiodism (Sleper and Poehlman, 2006; Hartman et al., 2011). Apart from environmental factors, soybean production is constrained by several socio-economic factors including lack of high yielding varieties, poor agronomic practices, lack of awareness of soybean processing and utilization, lack of markets, lack of inputs, and lack of supportive policies by the government, among others. Biotic constraints such as pests (aphids and thrips), diseases (ASR, brown spot, soybean mosaic, downy mildew, frogeye leaf spot and bacterial blight), and weeds are also harmful to soybean production, reducing soybean yields. Among the biotic constraints, ASR is the major cause of low yields in many areas of the world (Hartman et al., 2011).

## **1.6 Asian soybean rust**

Asian soybean rust (ASR) is the most devastating foliar disease in soybean growing areas (Garcia et al., 2008; Li et al., 2012). It is caused by two obligate fungal species, *P. pachyrhizi* and *Phakopsora meibomia* (Arthur) Arthur (Bonde et al., 2006). The species *P. meibomia* is mainly found in the western hemisphere, is less aggressive and does not cause substantial yield losses in soybeans. On the other hand, *P. pachyrhizi* is more aggressive, and is responsible for significant yield reduction worldwide (Calvo et al., 2008). This review focuses on *P. pachyrhizi*.

### **1.6.1 Geographical distribution of ASR**

ASR occurrence was first observed in Japan in 1902, and later spread throughout the main soybean growing areas of Asia, Australia and India in 1951 (Miles et al., 2003). The disease later spread to Africa probably through airborne urediniospores movements; but the date of first appearance on the continent is not well documented (Levy, 2005). In Africa, ASR was first reported in East African countries i.e. Uganda, Kenya and Rwanda in 1996 and thereafter it spread southwards to Zambia and Zimbabwe in 1998, and by 2001 it had reached other Southern African countries i.e. Mozambique and South Africa (Levy, 2003). The disease also spread westwards into Nigeria in 1999 and Ghana in 2007 (Akinsanmi et al., 2001; Bandyopadhyay, 2007). ASR later moved from Africa to South America, where it was reported

first in Paraguay in 2001, Brazil in 2002 and Argentina in 2003 (Bonde et al., 2006). More recently, it was reported in the United States of America in 2004 (Garcia et al., 2008; Sconyers et al., 2006). The wide distribution of ASR and severe yield losses it causes, makes it the most harmful disease in soybeans worldwide (Li, 2009a).

### **1.6.2 Symptoms of ASR**

The initial symptoms of ASR are small water-soaked lesions that develop either into grey, tan or reddish brown lesions mainly on the lower side of the leaves; but sometimes they may appear on the petioles, pods, cotyledons, and stems (Li, 2009b) (Fig 1.1). In most cases, the lesion colour varies depending on the lesion age, pathogen aggressiveness, host plant, and the interaction between the pathogen and the host (Li, 2009a). There are three major types of lesions and are described as tan, red brown and immune (Bromfield and Hartwig 1980; Bromfield, 1984). Tan-coloured lesions (TAN) indicate a highly susceptible reaction with many urediniospores and high sporulation levels. Reddish brown lesion (RB) on the other hand, is a form of resistance that is characterized by small, irregular lesions without urediniospores, while an immune reaction is a complete resistance without visible symptoms. In some cases, an intermediate response with both TAN and RB lesions has been reported (Bonde et al., 2006). This reaction is described as mix (MIX) and is attributed to mixtures of *P. pachyrhizi* races in the inoculum.



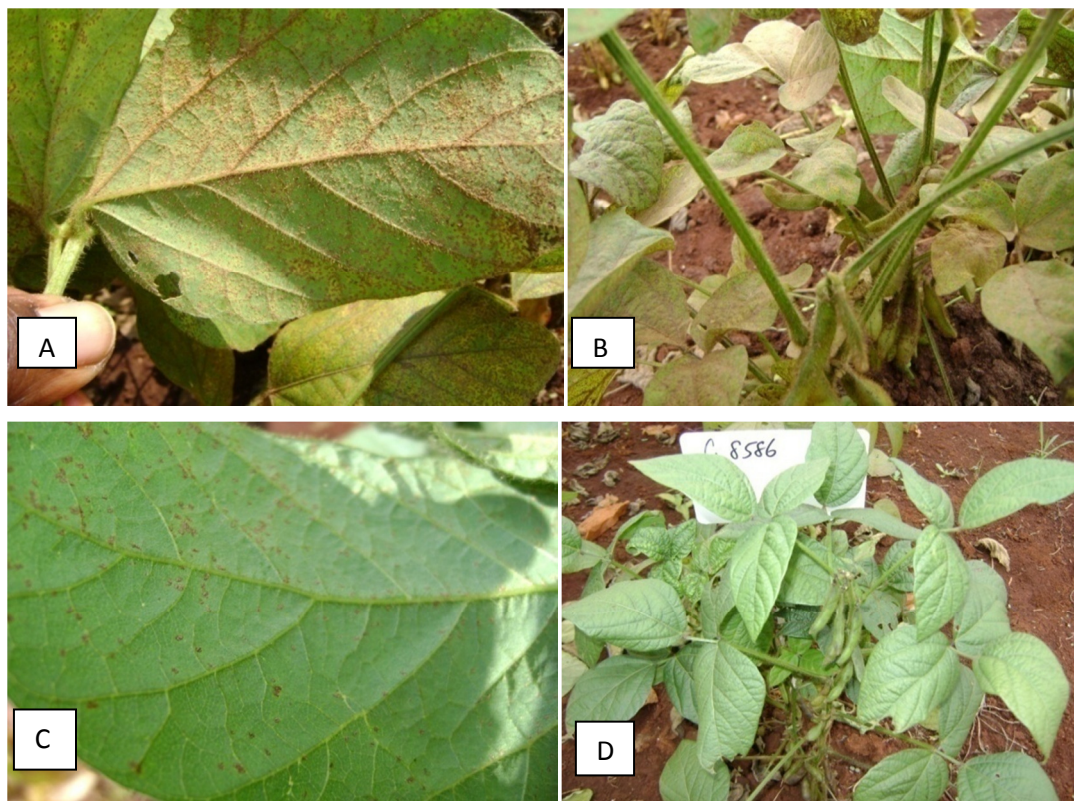


Fig 1.1: (A) *P. pachyrhizi*'s prolific sporulation on the lower side of the leaf; (B) pustules on the upperside of the leaf and pods; (C) Red brown lesions and (D) Immune response

### 1.6.3 Diagnosis of ASR

ASR is mainly diagnosed visually using a hand lens or a dissecting microscope. The key characteristic of ASR is the occurrence of uredinia that have urediniospores. In the field at early stages of ASR development, non-sporulating symptoms are easily confused with other fungal and bacterial diseases such as bacterial blight (*Pseudomonas syringae* Van Hall pathovar *glycinea*), brown spot (*Septoria glycines* Hemmi), frog-eye leaf spot (*Cercospora sojina* K.Hara), and downy mildew (*Peronospora manshurica* Naumov) Syd.) (Sinclair, 1982). It is, therefore, recommended that diseased leaf samples be incubated in a moist chamber and left overnight to enhance rust development and sporulation for accurate diagnosis. PCR tools (polymerase chain reaction) are also useful for ASR diagnosis when sporulating pustules are not visible (Frederick et al., 2002).

### 1.6.4 Epidemiology of ASR

ASR infection process involves spore germination, formation of appressorium, penetration, development of urediniospores and finally sporulation (Li, 2009b). According to Bromfield (1984)

urediniospores are the key primary source of inoculum of *P. pachyrhizi* that initiates ASR epidemics. After the initial infection, a single germ tube germinates from each urediniospore, and subsequently forming an appressorium that has a hyphal tube on its bottom part, enabling direct penetration of the epidermal cells through the leaf cuticle. This explains why *P. pachyrhizi* has a wider range of hosts than most other rust pathogens, which penetrate the leaf via the stomata or wounds (Miles et al., 2003). Under favourable environmental conditions, new urediniospores are developed in pustules and the first urediniospores are released about 9 days after the initial infection (Kawuki et al., 2003b). Urediniospores may be released continuously for several weeks, depending on the initial inoculum, and the volume of spores produced within the first three weeks. This infection cycle is repeated on the same plant, neighbouring plants or distant plants, of the same or many other legume species, as long as the environmental conditions are suitable and susceptible host plants are available.

Telia, basidiospores and teliospores have also been described in the life cycle of *P. pachyrhizi* but they are not the primary source of inoculum. The telial stage (sexual stage) is not very common but it has been induced under laboratory conditions to produce basidiospores. In the field, telia are sometimes observed towards the end of the growing cycle of soybean plants (Bromfield, 1984). However, importance of telia, teliospores and the basidial stage in the life cycle of *P. pachyrhizi*, and in epidemics of ASR, is not well understood. If there is an alternate host for the aecial stage of *P. pachyrhizi*, then it remains to be discovered.

## **1.7 Factors affecting ASR development**

ASR infection process is affected by biotic factors of the host plant and the pathogen, as well as abiotic factors of the environment (Li, 2009b).

### **1.7.1 Host range of ASR fungus**

ASR pathogen has a wide range of hosts, unlike other rust pathogens that are limited to a few host species (Posada and Frederick, 2005). According to Li (2009b), *P. pachyrhizi* infects more than 150 species from 53 genera of the family Leguminosae. The most susceptible host of *P. pachyrhizi* is kudzu (*Pueraria lobata* (Willd.) Ohwi), a weed species that is commonly found in the United States of America. Other common hosts are medic (*Medicago arborea* L.), lupine (*Lupinus hirsutus* L.), sweet clover (*Melilotus officinalis* (L.) Lam), vetch (*Vicia dasycarpa* Ten), common beans (*Phaseolus vulgaris* L.), lima and butter beans (*Phaseolus lunatus* L.), pigeonpea (*Cajanus cajan* (L.) Millsp), garden peas (*Pisum sativum* L.) and cowpeas (*Vigna*

*unguiculata* L.) Walp.) (Bromfield, 1984). Most of these legume hosts like beans, cowpeas, pigeonpea and peas are commonly grown as food crops in Kenya. Farmers plant them either in an intercrop system, or in the small pure portions in the same fields. Both systems result in the creation of a local source of inoculum, making ASR control a big challenge (Maphosa et al., 2012b).

### **1.7.2 Environmental factors affecting ASR development**

One factor that contributes greatly to the survival of ASR is its ability to spread over long distances. This is because it is characterized by airborne urediniospores that are easily dispersed by wind or rain (Garcia et al., 2008). The optimal temperature for urediniospore germination ranges between 18 and 25°C (Del Ponte et al., 2006; Sinclair, 1982). According to Caldwell et al. (2005), temperatures less than 15°C or greater than 30°C do not support spore viability and infection. Urediniospore germination also requires 6-16 hours of wetness and it is faster in regions with an even rainfall distribution compared to regions with uneven rainfall distribution (Tschanz, 1984). Li et al. (2009) also reported that precipitation not only provides moisture needed for germination and infection of urediniospores, but it also facilitates deposition of the spore, especially for long-distance dissemination. Relative humidity of 75-80% is also required for urediniospores germination and infection (Park et al., 2008). Sunlight in the wavelengths of 0.285-2.800 µm also promotes spore germination, as does UV light (0.295-0.385 µm) (Isard et al., 2006).

The environment affects both host genotype and *P. pachyrhizi* fungus either directly or indirectly, resulting into a complex interaction (Li, 2009a). With the current climate change, ASR is becoming a major concern in Kenya. This is because climate change is likely to transform the host physiology, resistance and the rate at which the pathogen develops (Tukamuhabwa and Maphosa 2011). This calls for more studies on the influence of climatic factors, alternative hosts and cropping systems on ASR epidemiology.

### **1.7.3 Effect of ASR on soybean development stages and maturity**

Soybean phenological stages and maturity duration play an important role in the development of ASR (Tschanz et al., 1985). According to Kawuki et al. (2004), soybean plants are susceptible to rust at all developmental stages. However, infections tend to be more severe during the reproductive stages (flowering and pod-filling stages) (Maria et al., 2007). With regard to maturity duration, early maturing varieties are heavily infected with higher rates of ASR

incidences than late maturing varieties as reported by Tschanz et al. (1985). In contrast, a recent study conducted by Oloka et al. (2008) reported that late maturing varieties are heavily infested by ASR that result into substantial yield losses than early maturing varieties. These findings imply that breeders' choice of soybean maturity group, and the use of a consistent maturity stage is crucial for the accurate evaluation of rust resistance.

#### **1.7.4 Effect of ASR on yield and seed quality**

ASR spreads fast, causing severe crop damage, which leads to significant yield and quality losses. Soybean grain losses of 10-80% have been reported (Yorinori et al., 2005). The level of loss experienced depends upon prevailing weather patterns, the genotype and the maturity stage at the time of infection (Wang and Hartman, 1992). Kumudini et al. (2008) reported that yield losses are mainly attributed to premature leaf fall, reduced green leaf area in the canopy, reduced dry matter accumulation and reduced harvest index. Bennett (2005) also reported that heavily infected plants had significantly reduced pods/plant, seeds/pod, number of filled pods/plant, 1000 seed weight, seed germination and oil content. In Kenya, however, the magnitude of yield and quality losses associated to ASR has not been quantified. This information would be crucial for guiding proper planning of suitable control measures against ASR (Kawuki et al., 2003a).

### **1.8 Control strategies for ASR**

Since ASR affects vegetative growth, reproductive stage, yield and seed quality, effective control strategies are needed to enable soybean crop to withstand the devastating effects of the disease. Various control strategies have been suggested, including cultural practices, nutrition management, bio-control, biological, fungicide application and host resistance but they are all limited options in one way or another.

#### **1.8.1 Cultural practices and nutrient management strategies**

Cultural practices, such as controlling wild weed hosts, adjusting planting dates, planting early maturing varieties and site selection have been proposed to reduce ASR incidences and severity (Bromfield, 1984). The main challenge with cultural practices is that some of the alternative hosts are perennials, resulting into year-round spore production, thus making cultural practices unsuitable for ASR control. Fertilization with potassium, chloride, manganese, and phosphorous may affect the development of fungal diseases (Ebelhar et al., 2008). However, their effects on ASR have not been fully explored.

### **1.8.2 Biopesticidal and biological control**

Borges (2007) examined application of oils and plant extracts to control ASR infections. He reported that *Pelargonium* spp. and *Lavandula officinalis* Mill. extracts had similar effects as fungicide applications in controlling ASR. These extracts also reduce environmental hazards, but they are expensive and may not be available in large quantities.

Biological control of ASR using fungi has been reported to be an effective method of controlling the disease by various authors. For example, *Tricothecium roseum* (Pers.) Link was reported to reduce ASR infections (More and Kamble, 2009). Kumar and Jha (2002) reported that *T. roseum* caused 90% inhibition in germination of the infected urediniospores of *P. pachyrhizi*. Ward et al. (2012) also observed that *Simplicillium lanosoniveum* (J.F.H. Beyma) Zare and W. Gams significantly lowered the amount of DNA in *P. pachyrhizi* and reduced disease severity. Therefore, some fungal species might have the potential to slow rust development.

### **1.8.3 Chemical control**

Several systemic and protectant fungicides are used as the primary control measure for managing ASR globally (Levy, 2005). However, the appropriate application of fungicides is locally specific due to differences in the environmental factors (weather patterns), cropping systems and socio-economic factors (availability, equipment, labour, cost, etc.). In addition, the fungicide efficacy and timing of application is complicated by the maturity stage of the host and the disease progress. This makes fungicide control measures less applicable in most of the developing countries, including Kenya (Oloka et al., 2008).

### **1.8.4 Host plant resistance**

Host plant resistance is a more affordable method for managing ASR in agricultural systems of resource poor farmers (Twizeyimana et al., 2008). For this reason, considerable efforts have been directed towards screening soybean germplasm for resistance to *P. pachyrhizi* for many years. For example, more than 16,000 soybean accessions have been screened in the USA (Miles et al., 2006), 9,000 accessions at AVRDC (Tschanz et al., 1985) and 2,700 in China (Li, 2009b). In addition, about 1,000 wild *Glycine soja* and perennial *Glycine* species have been evaluated for rust resistance (Hartman, 1995). In Nigeria, 178 breeding lines from IITA and 101 accessions from USDA-ARS and Uganda have also been screened for rust resistance

(Twizeyimana et al., 2008). From these evaluations, three types of resistance mechanisms against *P. pachyrhizi* were identified. These were specific resistance, partial resistance and tolerance. All three forms of ASR resistance can be considered for incorporation into soybean breeding programmes.

#### **1.8.4.1 Specific rust resistance and its application in breeding**

Plant introductions with specific rust resistance conditioned by four independent dominant genes have been identified (Table 1.1). These are PI 200492 (*Rpp1*), PI 230970 (*Rpp2*), PI 462312 (*Rpp3*), and PI 459025 (*Rpp4*) (Bromfield and Hartwig, 1980; McLean and Byth, 1980; Hartwig and Bromfield, 1983; Hartwig, 1986). Generally, specific rust resistance is associated with immune lesions, formation of reddish-brown (RB) lesions, reduced disease incidence and severity, as well as reduced sporulation levels (Miles et al., 2006). For example, *Rpp1* gene confers an immune (complete resistance) type of reactions without visible ASR infections. On the other hand, *Rpp2*, *Rpp3* and *Rpp4* genes confer reddish-brown lesions; a resistant reaction without or sparsely urediniospores that restrict fungal development and sporulation (Bonde et al., 2006). The reduction in size and number of urediniospores is also a desirable indicator of resistance when assessing single ASR resistance genes (Bonde et al., 2006).

Generally, specific rust resistance is not long-lasting and is easily matched by virulent races of *P. pachyrhizi*. These virulent races evolve rapidly, which is to be expected, given the prolific production of urediniospores from infections of the fungus's many hosts (Table 1.1). McLean and Byth (1980) studied the resistance of PI 200492 and Tainung 3 that possess *Rpp1* gene. They reported that PI 200492 was susceptible to an Australian isolate of *P. pachyrhizi*, while Tainung 4 was resistant. Recent studies in different regions have shown that PI 200492 was resistant to Mississippi isolates but susceptible to isolates from Brazil, Paraguay, Thailand and Zimbabwe (Li, 2009a; Miles et al., 2011). Another study conducted by Bromfield and Hartwig (1980) evaluated the *Rpp2* dominant resistance gene in PI 230970. They reported that this genotype remained resistant to India-73-1, Phillipines-77-1 and Taiwan-72-1 isolates but it was susceptible to a Taiwan 80-2 isolate. The same genotype was susceptible to a Brazilian isolate (Yamanaka et al., 2011). Genotype PI 462312, on the other hand, was susceptible when challenged with Ugandan isolate (Oloka et al., 2008) but resistant to Brazilian isolates (Yamanaka et al., 2011). The variable performance of single resistant genes suggests that there is much variation in the virulence profiles of *P. pachyrhizi* races in different geographical regions. This makes it essential to test the reactions of genotypes with single gene resistance

against the local population of ASR races before incorporating them in the breeding programmes.

Table 1.1: Single resistant genes in different soybean accessions and their reactions to *P.pachyrhizi* isolates

Name of the single gene	Accession number	Resistant reaction to <i>P.p</i> isolate	Susceptible reaction to <i>P.p</i> isolate	References
<i>Rpp1</i>	PI 200492	IN 73-1, MS06-1, MS07-1, MS07-2	TW 72-1, TW 80-2, BZ01-1, PG01-2, TH01-1, ZM01-1	(McLean and Byth, 1980; Li, 2009a; Miles et al., 2011)
<i>Rpp2</i>	PI 230970	AU 72-1, IN 73-1, PH 77-1, TW 72-1	TW 80-2, BRP-2	(Bromfield and Hartwig, 1980; Yamanaka et al., 2011)
<i>Rpp3</i>	PI 462312	IN 73-1, BRP-2	TW 72-1, TW 80-2	(Hartwig and Bromfield, 1983; Yamanaka et al., 2011)
<i>Rpp4</i>	PI 459025	IN 73-1, TW 72-1, TW 80-2, BRP-2	Uganda isolate, North America isolate	(Hartwig, 1986; Oloka et al., 2008; Yamanaka et al., 2011; Walker et al., 2011)
<i>rpp5</i> <i>rpp2</i> <i>rpp3</i>	PI 200456 PI 224270 PI 567099A	Brazil isolate		(Calvo et al., 2008; Ray et al., 2011)
<i>Rpp5</i>	PI 200526, PI 471904	Brazil isolate		(Garcia et al., 2008)
<i>Rpp5</i>	PI 200487	Brazil isolate	Southeastern United state isolate	(Garcia et al., 2008; Walker et al., 2011)
<i>Rpp?</i> (Huyuga)	PI 506764	Georgia isolate		(Monteros et al., 2007)
<i>Rpp1b</i>	PI594538A			(Chakraborty et al., 2009)
<i>Rpp6</i>	PI 567102B	MS06-1, LA04-1		(Li et al., 2012)

*P. p* isolate is the *P. pachyrhizi* isolates. AU is an isolate from Australia; Brazil/BZ is an isolate from Brazil; Georgia is an isolate from Georgia; IN is an isolate from India; PH is an isolate from the Philippines; TW is an isolate from Taiwan; MS is an isolate from Mississippi; LA is an isolate from Louisiana; PG is an isolate from Paraguay; TH is an isolate from Thailand; and ZM is isolate from Zimbabwe.

Several studies have established the occurrence of different *P. pachyrhizi* races either on soybeans or on other leguminous crops in the same field, or in different geographical areas (Hartman et al., 2004). For example, Yamaoka et al. (2002) identified eighteen pathogenic races in samples collected from soybean plants and wild hosts in Japan. Recently, Twizeyimana et al. (2009) reported seven physiological races in Nigeria while Oloka et al. (2008) reported three physiological races in Uganda, thereby confirming the occurrence of diverse *P. pachyrhizi* races in Africa. Because studies on physiological race variability are limited in Kenya, it is not known how many races could be present in their soybean fields. In addition, pathogenic variability studies are conducted using a few set of differential hosts that succumb to certain ASR isolates. For instance, using South African *P. pachyrhizi* isolates, all AVRDC differentials recommended

for identification of ASR pathogenic races were susceptible (Caldwell et al., 2003). Therefore, increasing the number of differential hosts or using other methodologies like molecular tools will be useful in identifying and characterizing ASR races.

Due to multiple virulence genes of *P. pachyrhizi*, no varieties have been bred with rust resistance genes. This is because specific rust resistance is not stable when introgressed into the commercial varieties and its value in the breeding programmes is limited (Miles et al., 2008). However, in some areas of Asia, Africa and South America, specific resistance has been the major control strategy for ASR. This is because some specific resistance genes have remained effective against the ASR races in these regions. For instance, in Brazil, among the four sources of resistance *Rpp1*, *Rpp2*, *Rpp3* and *Rpp4*, only those with genes *Rpp2* and *Rpp4* remained resistant to rust, while in Uganda only *Rpp2* was effective (Oloka et al., 2008; Silva et al., 2008). In Nigeria, *Rpp1* and *Rpp4* genes were effective (Twizeyimana et al., 2009). In addition, this type of resistance is preferred by breeders because it is simply inherited and simple to work with, especially in a backcrossing programme, where the resistance can be introgressed into elite lines within seven generations. Furthermore, single resistant genes may offer an opportunity of pyramiding genes into various combinations for more resistant genes (Miles et al., 2011).

Efforts to search for additional resistance genes have led to the discovery of new loci that may play an important role in soybean breeding programmes. These include *Rpp5* in PI 200526, PI 471904 and PI 200497 (Garcia et al., 2008) and *Rpp?* (*Hyuuga*) in PI 506764 (Monteros et al., 2007) (Table 1.1). Recently, two new soybean resistant genes were identified, *Rpp6* in PI 567102B (Li et al., 2012), and a second rust resistant gene in *Hyuuga* (Kendrick et al., 2011). Another study conducted by Calvo et al. (2008) and Ray et al. (2011) identified three single recessive genes (*rpp*) controlling ASR. Chakraborty et al. (2009) also discovered a new allele (*Rpp1b*) at *Rpp1* from soybean PI 504538A that conferred an RB lesion type resistance to ASR. These genes are more likely to contribute to the development of soybean varieties with stable ASR resistance because no races of *P. pachyrhizi* with virulence to these resistance genes have developed yet.

#### **1.8.4.2 Partial rust resistance and its application in breeding**

Partial resistance that involves reduction in the rate of disease progress has also been reported in soybean (Hartman et al., 2005). Unlike specific resistance, partial resistance is quantitatively



inherited and it has the advantage of being effective against all the races of the pathogen. This type of resistance might be very useful for *P. pachyrhizi* because it has multiple virulence genes (Wang and Hartman, 1992). In the field, soybean genotypes with partial resistance are mainly rated as moderately resistant because they are characterized by few or sparsely lesions that develop on the plants throughout the growing season. In the greenhouse, partially resistant soybean genotypes show red-brown infection type with longer latent periods, reduced numbers of pustules over time and smaller lesions when compared to genotypes with tan infection type (Hartman, et al., 2005). Bonde et al. (2006) also proposed that a reduction in the size and number of urediniospores should be evaluated as a useful index for detecting partial resistance to ASR. However, accurate identification and assessment of partial resistance in breeding programmes is challenging and time consuming (Hartman et al., 2005). In particular, it is often affected by interplot interference, where a susceptible neighbouring plot makes a variety with partial resistance appear equally susceptible (Parlevliet and Danial, 1992). Consequently, its assessment in the field is difficult especially when screening a segregating population or genotypes with different maturities (Hartman, 1995). This is further complicated by environmental influences, resulting in plants maturing at different time periods. To correct this variations in plant maturity, relative soybean life time (RLT) regressed on logit transformation of rust severity was suggested by Tschanz and Shanmugasundaram (1985). A further challenge is that partial resistance in most crops is governed by additive genes (Ribeiro et al., 2007). Hence, there is a need to start with susceptible parents (avoiding any specific rust resistance), and then use recurrent selection to accumulate additive genes for resistance to ASR, together with other traits. The challenge with soybean is to make enough crosses to make this approach viable, given the difficulty of hand-pollinating soybeans successfully. As a result, despite the importance of this form of resistance, no soybean cultivars with partial resistance have been released for commercial purposes. The difficulties associated with breeding for partial resistance, and the lack of durability of specific rust resistance, have forced AVRDC to look for other forms of resistance, such as rust tolerance for improving yield of rust affected cultivars (Tschanz et al., 1985, Hartman et al., 2005).

#### **1.8.4.3 Tolerance to rust and its application in breeding**

Tolerance to rust, defined as yielding potential of soybean genotypes under rust stress, has been used to minimize yield losses associated with ASR (Hartman et al., 2005). Tolerance assessment is mainly based on genotypic adaptation, and the evaluation is for genotypes that yields highly in a target environment, and also maintains these high yields under high levels of

rust infection (Jarvie, 2009). This type of assessment is complicated because selection of yield stability is highly influenced by genotype x environment interactions. Hence, selection of tolerant lines is often based on comparisons of yield in plots with and without fungicide application. Breeders also use percentage yield loss, rust tolerance index or stress tolerance index to assess soybean genotypes for rust tolerance (Kawuki et al., 2003a). Preliminary evaluations conducted in Uganda established significant variation in tolerance among soybean genotypes that could be exploited by breeders in soybean breeding programmes (Kawuki et al., 2004).

## **1.9 Other breeding and selection procedures for ASR**

This section discusses some of the breeding and selection methods used or recommended by breeders for development of more ASR resistance genotypes.

### **1.9.1 Introgression of rust resistance from perennial *Glycine* spp. to cultivated **soybeans****

Sources of resistance to ASR have been identified in the wild perennial *Glycine* spp. including *G. tomentella*, *Glycine argyrea* Tindale, *Glycine canescens* F.J. Herm *Glycine clandestina* J.C.Wendl, *Glycine latifolia* (Benth.) C.A. Newell & Hymowitz and *Glycine microphylla* (Benth.) Tindale (Hartman et al., 1992). However, this source of resistance has not been fully exploited in breeding programmes due to sterile hybrids among these species. For instance, the relationship between *G. max* and *G. canescens* species is not well understood because hybrids produced by these two species are unfertile. However, all loci in the two species have dominant alleles conferring ASR resistance (Calvo et al., 2008). For this reason, only a limited number of sterile F1 hybrids have been produced through hybridization.

However, in some studies, fertile soybean lines have been developed. Singh et al. (1990) developed fertile hybrids by crossing *G. max* (2n=40) x *G. tomentella* (2n=78) and consequently obtained backcross BC<sub>2</sub>, BC<sub>3</sub> and BC<sub>4</sub> progenies. More recently, studies on inter-subgeneric hybrids were developed between *G. max* (cv. *Altona*) and *G. tomentella*. As a result of this hybridization, amphiploid hybrid lines (2n =118) were developed and when they were further backcrossed to *G. max* (cv. Clark 63), they generated fertile lines (2n = 40) (Patzoldt et al., 2007). Amphiploid hybrids maintained rust resistance that was derived from the *G. tomentella* parent but the fertile lines were susceptible to ASR. Therefore, using backcross procedure, there is a possibility of transferring rust resistant gene(s) from *G. tomentella* species to cultivated soybean.

### 1.9.2 Mutation breeding

Mutation breeding in soybeans was initiated in 1974 in Kasetsart University with the objective of breeding high yielding soybean varieties with resistance to rust (Smutkupt et al., 1986). Preliminary studies on ASR resistance showed that some mutant lines (81-1-038 and 81-1-113) were resistant while other lines had moderate resistance. Through mutation breeding, identified mutant lines could be used as additional sources of rust resistance or for development of new resistant lines.

### 1.9.3 Use of molecular markers and marker assisted selection

Molecular markers have an important role in plant breeding as a tool for gene identification, and potentially, they could be used to introgress ASR resistance genes into the elite soybean cultivars. Using SSR markers, Hyten et al. (2007) mapped *Rpp1* locus to molecular linkage group MLG G. Silva et al. (2008) mapped *Rpp2* to molecular linkage group MLG J and *Rpp4* to MLG G using SSR markers. Hyten et al. (2009) mapped the *Rpp3* gene to MLG C2 using single nucleotide polymorphism (SNP) in bulk segregant analysis. *Rpp?* (*Hyyuga*) from the Japanese cultivar was also mapped to the same region MLG C2 as *Rpp3* using SSR markers (Monteros et al., 2007). The resistance gene *Rpp5* was mapped to MLG N using SSR markers (Calvo et al., 2008; Garcia et al., 2008). Recently, Chakraborty et al. (2009) mapped a new *Rpp1* allele known as *Rpp-1b*. Identification of molecular markers related to ASR resistance genes could be useful in implementation of marker-assisted selection (MAS) in soybean breeding programmes. It will also help breeders to select ASR resistant plants in the early stages of soybean development, even when the pathogen is absent (Garcia et al., 2008).

### 1.9.4 Pyramiding ASR resistant genes

With the aid of molecular markers, ASR resistance loci have been mapped, offering an opportunity of pyramiding resistance genes into a single genotype that could result in durable ASR resistance (Garcia et al., 2008). Maphosa et al. (2012a) investigated the effectiveness of gene combination for three single resistance genes *Rpp2*, *Rpp3* and *Rpp4* using SSR markers and proposed the use of marker gene pyramiding to increase numbers of rust resistance genes. Another case of gene pyramiding was reported in *Hyyuga*, because of the natural occurrence of gene pyramiding for ASR resistance in this cultivar (Ray et al., 2011). However, successful gene pyramiding will depend on a number of factors. These include; the number of genes, the distance between genes and closest markers, selected genotypes in each generation and the

kind of germplasm used. Use of modern tools like DNA markers, micro arrays, and SNPs could speed up the progress in pyramiding (Joshi and Nayak, 2010).

### **1.10 Participatory breeding approaches**

Plant breeders mainly use conventional plant breeding to select superior genotypes that fit into high potential environments. This methodology is powerful but its objectives are not always realized because this approach may fail to consider the farmers' and consumers' expectations, their perceptions and their knowledge on the crops and diverse environmental conditions (Ceccarelli et al., 2001). As a result, farmers frequently reject new, "improved" cultivars. In recent years, farmers' participatory research approaches such as participatory plant breeding (PPB), participatory rural appraisal (PRA), participatory variety selection (PVS) and other approaches are being used for variety/technology development and dissemination, with the overt goal of ensuring that farmers adopt the new cultivars. For instance, participatory plant breeding actively involves farmers during the selection of the segregating materials in the early stages of the breeding process (Doward et al., 2007). Participatory variety selection, on the other hand, has been used to assist breeders in identifying farmer-preferred varieties that match with their environmental conditions, available resources, quality traits, and consumers' needs (Pandit et al., 2007). Another important aspect of the participatory variety selection is that it reduces the cost of research, new varieties are released sooner, and the level of adoption of new varieties is much higher (Doward et al., 2007). Participatory variety selection also enhances the mechanisms of dissemination (Doward et al., 2007) and it increases cultivar diversity (Abebe et al., 2005).

Participatory approaches also help researchers to understand farmers' knowledge and management of pest and diseases, thereby providing the basis for further development of integrated pest and disease management strategies that the farmers are more likely to adopt (Hoffmann et al., 2007). However, participatory research tools have not been used in soybean farming in Kenya to understand farmers' perceptions and knowledge on ASR and its management in different regions. This information may be useful in identifying resistant landraces, and to develop an integrated disease management programme for ASR. In addition, use of farmers' participatory approaches will be useful to researchers for identifying soybean production constraints, selection criteria for new soybean varieties, and understanding the traits in soybean that the farmers prefer (Chianu et al., 2006a). Therefore, participatory approaches will allow better incorporation of end users perspectives, reach the resource poor farmers,

develop varieties that are well adapted to high-stress environments and diverse conditions, and incorporate several traits that the farmers prefer in the breeding programmes.

### **1.11 Genetic analysis for inheritance of ASR resistance**

A few genetic studies have been conducted with the goal of understanding the genetics of ASR resistance. Some studies have shown that rust resistance is qualitatively inherited and largely controlled by single dominant genes. For instance, Bromfield and Hartwig (1980) determined the inheritance of ASR resistance in two  $F_2$  populations with PI 230970 and PI 230971 as the resistant parents. Their analysis of these  $F_2$ s showed that their rust resistance was dominant and qualitatively (simply) inherited. Other studies have reported partial to complete dominance action in the inheritance of ASR resistance (Garcia et al., 2008; Ray et al., 2009).

Quantitative inheritance has also been reported to control inheritance to ASR resistance. Ribeiro et al. (2007) used a 6x6 full diallel mating design and reported that ASR resistance was quantitatively inherited, which was predominantly controlled by additive gene action. These findings were supported by Maphosa et al. (2012b) who found that ASR resistance was predominantly controlled by additive gene action. Given the predominantly additive effects, recurrent selection was recommended as the most efficient selection procedure for developing rust resistant varieties with stable resistance. Recurrent selection would be possible if male sterility was developed for soybean breeding programme (Acquaah, 2007). The use of male gametocides provides an alternative route to enhanced cross pollination in soybean. Using male gametocides, successful crosses have been achieved in several crops including soybeans (Lai et al., 2004), coriander (*Coriandrum sativum* L.) (Kalidasu et al., 2009) and pigeonpeas (*Cajanus cajan*) (Singh et al., 2012).

In contrast, epistatic gene action has also been reported to be important in some cases of ASR resistance. Garcia et al. (2008), using both genetic and molecular analysis, detected multiple alleles or closely linked genes governing ASR resistance. Laperuta et al. (2008) also detected epistatic gene action in the inheritance of rust resistance. Contrary to these findings, Ribeiro et al. (2007) detected non-allelic interaction that did not play an important role in controlling rust resistance. This raises questions on the significance of epistatic gene action in rust resistance, which needs further investigation.

### **1.12 Diallel mating design**

In order to understand the type of gene action controlling ASR, an appropriate mating design is needed. A diallel mating design is one of the tools that is commonly used for genetic analysis (Gravina et al., 2003). This method makes useful predictions on early generations, thereby increasing the effectiveness of the plant breeding programme. It provides estimates of additive, dominance, maternal effects, environmental effects and non-allelic interactions. Several procedures for analysis and understanding diallel mating design have been suggested by Gardner and Eberhart (1966), Griffing (1956) and Hayman (1954). In this study, Griffing's (1956) method was used to provide information on the general combining abilities of superior parents, which is associated with additive gene action. It was also used to estimate the performance of hybrids or crosses that would be expected from the average performance of the parents, which is associated with non-additive gene action (specific combining ability). These estimates provided useful information for the selection of the best parent combinations and an indication of the most productive breeding procedures to be used in future soybean breeding for resistance to ASR.

### **1.13 Genotype x environment interactions (GEI)**

Soybean performance for different traits such as yield, oil content, protein content and rust disease varies from one environment to the other, due to the influence of GEI (Twizeyimana et al., 2008; Fekadu et al., 2009; Tukamuhabwa et al., 2012). GEI is a major concern for plant breeders because it reduces the heritability of a trait, thus decreasing its genetic gains (Balestre et al., 2009; Ahmadi et al., 2012). Consequently, it complicates direct selection of superior cultivars and their release (Ahmadi et al., 2012). GEI also demands the identification of optimum test environments to reflect the likely environments the cultivars will be exposed to if they are released (Badu-Apraku et al., 2012). Therefore, evaluation of GEI is important when breeding soybean varieties for wide adaptation.

There are several stability models that have been adopted by many researchers for assessing, studying and interpreting GEI. The most commonly used techniques are linear regression analysis (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966) and multivariate analysis. The multivariate analytical techniques have become increasingly important tools for analyzing GEI, with the additive main effects and multiplicative interaction (AMMI) model (Gauch and Zobel, 1997), and the genotype main effect and genotype x environment interaction (GGE) biplot (Yan et al., 2000) being the most powerful tools in common use (Asrat et al., 2009).

The AMMI model joins together both the main effects of the genotype (G) and environment (E) as additive effects, and the G x E interaction as a multiplicative component of principal component analysis (Asrat et al., 2009). This technique is often preferred because it removes the largest portion of G x E 'noise', which then allows for the estimation of genotype performance across the environments, resulting into more successful predictive selection of stable genotypes (Crossa, 1990). It is a useful tool in visualizing multi-environment data in regard to adaptability and stability; hence its suitability in identifying superior genotypes and the best test environment.

For further interpretation of multi-locational trials, Yan et al. (2000) developed the technique of biplot analysis, with the genotype as the main effect, and the genotype x environment interaction (GGE) as the interaction effect. This technique considers the genotype and G x E effects, but it does not consider the main effect of the environment, even though it has the highest contribution of the total yield variation (Kaya et al., 2006). GGE biplot explains most of the data using the graphic axes to represent the first two principal components, which are derived from environment centered data ("*yield variation due to GGE*") (Yan et al., 2000). GGE is effective in evaluating test environments, i.e., it has the power to discriminate among genotypes (informative) in target environments and the representativeness (stable) of the test environments, which is not possible with AMMI analysis. In addition, it is effective in identifying superior cultivars ("which won where") and possible mega-environments (Kaya et al., 2006).

Many researchers are currently using both AMMI and GGE biplot analyses for interpreting GEI in several crops such as maize (Badu-Apraku et al., 2012), wheat (Ahmadi et al., 2012) and soybeans (Asrat et al., 2009). However, there have been no attempts to apply these models to provide information on GEI and soybean phenotypic stability in Kenya. It is hoped that, this study will provide useful information on stable genotypes that could be used in the breeding programmes or recommended to farmers. It will also give an insight on the most ideal testing environments and possible mega environments for testing new soybean varieties.

#### **1.14 Summary**

Currently, the major constraint affecting soybean production worldwide is ASR caused by *P. pachyrhizi*. The disease is already established in Kenya and it will cause substantial soybean yield losses and economic damages if control measures are not taken. However, the regional distribution, yield losses and economic impact of ASR in Kenya remains undocumented. In

addition, ASR pathogen is highly variable and it is not known how many pathogenic races could be present in Kenya. It is, therefore, important that more studies on ASR be explored for proper planning of suitable management strategies.

Efforts to control ASR have not been fruitful, though cultural practices, nutrient management, bio-control, biological and fungicide control measures have been proposed, and some adopted to manage ASR and therefore to enhance soybean production. Breeding for rust resistance in soybean varieties offers the best long term solution to meet the increasing demand for the crop. Several soybean accessions have been evaluated in various parts of the world in search of resistant/tolerant varieties but there have been no studies on ASR resistance in Kenya. Specific resistance to ASR has been identified but it is ineffective to some *P. pachyrhizi* isolates, while partial resistance and tolerance to ASR are not well defined in soybean. This is mainly attributed to the wide range of ASR hosts, high variability and complex virulence of *P. pachyrhizi* races, susceptibility of different maturity groups, environmental factors and the interaction among the pathogen, host and the environment. Furthermore, the genetic control of ASR resistance is complicated by the type of gene action controlling its inheritance. These difficulties warrant more studies on the ASR pathogen and a continuous programme to develop resistant soybean genotypes in different geographical regions using multiple approaches. These include screening previous identified resistant plant introductions and local germplasm for rust resistance using local isolates, and developing ASR resistant cultivars using conventional and participatory breeding approaches, integrated with molecular breeding techniques. Further studies on GEI are recommended for development of stable soybean cultivars, and to identify suitable test environments to screen new soybeans varieties in Kenya.

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## **2 Identification of farmers' preferred varieties, perceptions on Asian soybean rust (ASR) and other constraints facing soybean production in Kenya**

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### **Abstract**

In recent years, soybean farming has been gaining popularity in Kenya because of the increasing demand for its products. However, information on the soybean sub-sector in the country is limited. A survey was conducted in the major soybean growing areas of Kenya between December 2009 and February 2010 using a structured questionnaire to determine farmers' preferred traits, variety selection criteria, perceptions on Asian soybean rust (ASR) and other constraints facing soybean production. The results revealed that 77% of the farmer's preferred local varieties which were characterised by moderate yields, early maturity, drought tolerance and seed availability. However, the majority of farmers (61%) showed a willingness to grow improved varieties, but financial limitations, seed unavailability and lack of information were the major drawbacks. Farmers had several selection criteria across the surveyed regions for soybean varieties they grew in their fields. High yielding, early maturing, readily cooked soybeans, drought tolerance, low shattering ability, and pest and disease resistance were the main characteristics used by most farmers in variety selection. Other traits considered important included high protein and oil content and stay-green characteristics. Results showed low (39%) awareness of ASR among the participating farmers. Farmers attributed ASR occurrence to environmental factors, poor soil fertility, use of susceptible varieties, physiological maturity and weeds. Only 24% of the farmers applied rust control methods due to lack of technical know-how and resources. Soybean farmers also faced other challenges which included lack of markets, lack of knowledge in processing and utilization, the unavailability of commercial seed, pests and diseases, lack of farm inputs, frequent dry spells and dependence upon low yielding varieties. Therefore, there is a need to improve farmers' knowledge on ASR, breed for ASR resistant varieties and to address the other constraints facing soybean production in Kenya. In addition, incorporating the most important farmers' desired traits in the breeding programme is likely to increase the adoption of the improved soybean varieties.

## 2.1 Introduction

Soybean farming in Kenya is increasingly becoming important among farmers (Nassiuma and Wasike, 2002) due to the high demand for it as a human food product, as a livestock feed, for income generation, for soil fertility improvement, as a raw material for industrial and pharmaceutical purposes, and as a source of bio-diesel (Chianu et al., 2009). According to Mahasi et al. (2009), upto 80% of soybean produced in the country is consumed in the animal feed industries while 20-30% is used for human consumption. However, soybean production in Kenya contributes only a fraction of what is demanded by the food and feed industries. It is, therefore, important that local soybean production be increased to meet the required quantities. The most strategic option to increase soybean production is to develop high yielding varieties that incorporate farmers' desired attributes through a participatory breeding approach.

To address this problem, the Crop Improvement Program of the International Institute of Tropical Agriculture (IITA) has developed and released varieties that are high yielding, resistant to pests and diseases, with promiscuous nodulation, seed longevity, low pod shattering, and appealing seed colour (Vandeplas et al., 2010). Since 2005, the Tropical Soil Biology and Fertility–Institute of the International Centre for Tropical Agriculture (TSBF-CIAT), in collaboration with IITA, has contributed significantly in promoting improved soybean varieties through field evaluations especially in Western Kenya (Chianu et al., 2006). Despite these efforts, the improved soybean varieties have not been adopted by the Kenyan farmers and the majority of them still grow local varieties such as Bossier, EAI 3600 and Hill and other varieties such as Nyala, Gazelle, Sable, SCS-1 and Duicker that were released in Zimbabwe (Nassiuma and Wasike, 2002) and introduced in Kenya in 1990s (hereafter referred to as local varieties) that are highly susceptible to ASR (Mahasi et al., 2009).

Development of varieties using a top-down approach (Chianu et al., 2006), without collaboration between farmers and researchers, is probably the major reason for the low adoption of improved varieties. It is also possible that improved varieties do not match with small-scale farmers' resources such as land, labour, capital and management (Chianu et al., 2006). In addition, farmers' interests and preferences are not taken into account during the development of varieties, yet they are capable of selecting varieties that are suitable for their environments (Abebe et al., 2005). In order to enhance the adoption rate, it is important that researchers work in collaboration with farmers through participatory approaches (Kiros-Meles and Abang, 2008).

Participatory approaches help researchers to understand farmers' awareness, knowledge and management of diseases, thereby providing the basis for further development of integrated disease management strategies (Hoffmann et al., 2007). Involving farmers is also important in understanding the occurrence of pests and diseases in a particular environment and how their risks can be managed (Misiko et al., 2008). Researchers also use participatory approaches to identify production constraints of several crops, to reveal selection criteria for varieties, and understand desirable traits that farmers prefer while selecting varieties (Chianu et al., 2006). However, participatory research tools have not been used extensively to evaluate soybean farming in Kenya, in order to understand the traits that farmers desire, their perception of ASR and its management in different regions. This information may be useful in the development of an integrated ASR management programme, as well as identifying and incorporating farmers' desired traits in the breeding programme with the aim of enhancing farmers' adoption rates of new varieties. This study was therefore designed to; (i) identify soybean variety preferences and selection criteria; (ii) understand farmers' perceptions, knowledge, and management of ASR; and (iii) identify key soybean production and marketing constraints in Kenya.

## **2.2 Materials and methods**

### **2.2.1 Study area and sampling**

This study was conducted in selected counties located in Western, Nyanza, Rift Valley, Central and Eastern regions of Kenya. The geographical location of the surveyed regions is shown in Figure 2.1. The counties were selected because they represent major soybean growing areas and diverse agro-ecological zones in Kenya. In addition, these counties differ with respect to socio-economic activities, soybean cultivars grown, their utilization, and their contribution to the community's livelihood and hence, various soybean production systems and ASR management strategies exist.

With the assistance of extension officers from the Ministry of Agriculture, one county was purposively selected in each region except for Western and Eastern region where two counties were selected (Table 2.1). The selection was based on the intensity of soybean production, spatial and agro-ecological location. For instance, in Western region, Busia and Kakamega counties were selected. In Nyanza and Rift valley regions, Kisii and Nakuru counties were selected, respectively. Kirinyaga county was selected in central region. In Eastern region, two counties were selected, namely Embu and Meru. Geographical information (latitude, altitude and longitude) was recorded in all the counties using a global positioning system (GPS). In

addition, environmental data (rainfall distribution and pattern, temperatures) and soil type was obtained from the Ministry of Agriculture office (Table 2.1).



Fig 2.1: Map of Kenya showing counties (with stars) where the survey was conducted. Source: <http://softkenya.com/county>.

Table 2.1: Climatic characteristics and soil types of the study sites

Region	County	Latitude	Longitude	A(m)	AEZ	T (°C)	R(mm/yr)	R/pt	Soil types
Western	Busia	0° 27' 16N	34° 4' 33E	1179-1485	UM3	14-30	760-1800	Bi modal	Ferralsol orthic Acrisol
	Kakamega	0° 16' N	34° 45'E	1585	UM3	14-32	1700	Bi modal	Dystro- mollic Nitisol
Nyanza	Kisii	0° 44' 50.7S	34° 41' 7.7E	1800-2100	UM1&UM2	30-32	2100-2500	Bi modal	Acrisols
Rift valley	Nakuru	0° 19' 24.3N	34° 31' 7E	1500	LM2	27-30	1000	Bi modal	Acrisols
Central	Kirinyaga	0° 34' 5.8"S	37° 19' 52.6"E	1159	UM3	22.8	769	Bi modal	Vertisols
Eastern	Embu	0° 33' 0.35S	37° 35' 37.1E	300-600	UM3	28-30	1495	Bi modal	Cambisols
	Meru	0° 7' 33.4S	37° 43' 27.2E	1160-1350	LM3	20.9-22	800-900	Bi modal	Eutric nitisols

AEZ is the Agroecological zones, A is Altitude, T is the annual mean temperatures, R is the annual average rainfall, and R/pt is the rainfall pattern. UM1, UM2 and UM3 represent the Upper Midland zone 1, 2 and 3 respectively; LM2 and LM3 is the Lower Midland zone 2 and 3 respectively.

## 2.2.2 Survey methodology, data collection and analysis

Information about soybean farming was gathered using a structured questionnaire (Appendix 1). A total of 105 farmers (both male and female farmers) were interviewed between December 2009 and February 2010 by a team comprising of the principal investigator, two enumerators and local extension staff. Information on farmers' demographic characteristics, general production of soybeans, land size, land allocated to soybean production, soybean varieties grown, farmers preferences for different varieties; comparison of the local and improved varieties and their desired attributes were collected. Data on the farmers' perception and knowledge on ASR, source of planting materials and soybean rust control methods was also collected. Other key constraints facing soybean production and marketing were also recorded. This data was analysed using SPSS (Statistical Package for Social Science) Version 15 (SPSS, 2005).

## 2.3 Results

### 2.3.1 Respondents characteristics

Across all the surveyed regions, a total of 105 respondents (men and women) participated in the survey. These comprised 66% male farmers and 34% female farmers. Most of the households interviewed were headed by males (88%) while only a few (12%) were headed by females. The age of household heads ranged between 30 and 75 years. Ninety percent of the household heads were married while 6 and 4% were single or widowed, respectively. Sixty four percent of the respondents had attained secondary school education, 27% had primary school education and only 9% had no formal education.

### 2.3.2 Soybean production in the different counties

About 61% of the farm holdings owned by the participating farmers was less than 2.0 hectares while 22% of the farmers owned between 2.0 and 4.0 hectares (Table 2.2). This was mainly observed in Busia, Embu, Kakamega, Kirinyaga, and Kisii counties where the average land holdings ranged between 0.4 and 2.0 hectares. Only a small portion of each farm, less than 0.2 hectares, was allocated to soybean production by 86% of the farmers, while the rest of the land was allocated to other food crops (Table 2.2). However, in Nakuru and Meru counties, some farmers owned more than 8 hectares (5.7%) and soybean farming was allocated about 0.4 hectares in Meru (9.5%) and more than 4 hectares of land in Nakuru county (<1%).

Table 2.2: Farm sizes in hectares and land allocated to soybean production by the participating farmers (%) in different counties

		Counties							Total
Description		Busia	Embu	Kakamega	Kirinyaga	Kisii	Meru	Nakuru	
Land size	0.4-2 ha	100	66.7	71.4	69.6	77.8	55.6	19	61.9
	2.4-4 ha	0.0	33.3	14.3	26.1	16.7	22.2	33.3	22.9
	4.4-8 ha	0.0	0.0	14.3	4.3	5.6	11.1	28.6	9.5
	> 8 ha	0.0	0.0	0.0	0.0	0.0	11.1	19.0	5.7
Land allocation for soybean	0.2 ha	100	100	100	100	100	86.7	38.1	86.7
	0.4 ha	0.0	0.0	0.0	0.0	0.0	11.1	47.6	9.5
	0.8 ha	0.0	0.0	0.0	0.0	0.0	0.0	9.5	2.8
	> 4 ha	0.0	0.0	0.0	0.0	0.0	0.0	4.7	0.95



### 2.3.3 Purpose of soybean cultivation

Soybean was mainly grown for income (37%) and as a source of food and beverage (36%) in all the counties (Fig 2.2). A few farmers (7.6%) processed soybeans into various products including soyflour, soymilk, soymeat and fried soynuts. After processing, the left overs were dried and mixed with other products to make poultry or livestock feeds (10%). Stems and leaves were used as fodder for the animals. Soybeans were either intercropped or grown in rotation with other crops to improve soil fertility (4%). In Kirinyaga county, farmers sold soybeans as a green vegetable (3%) for the export market.

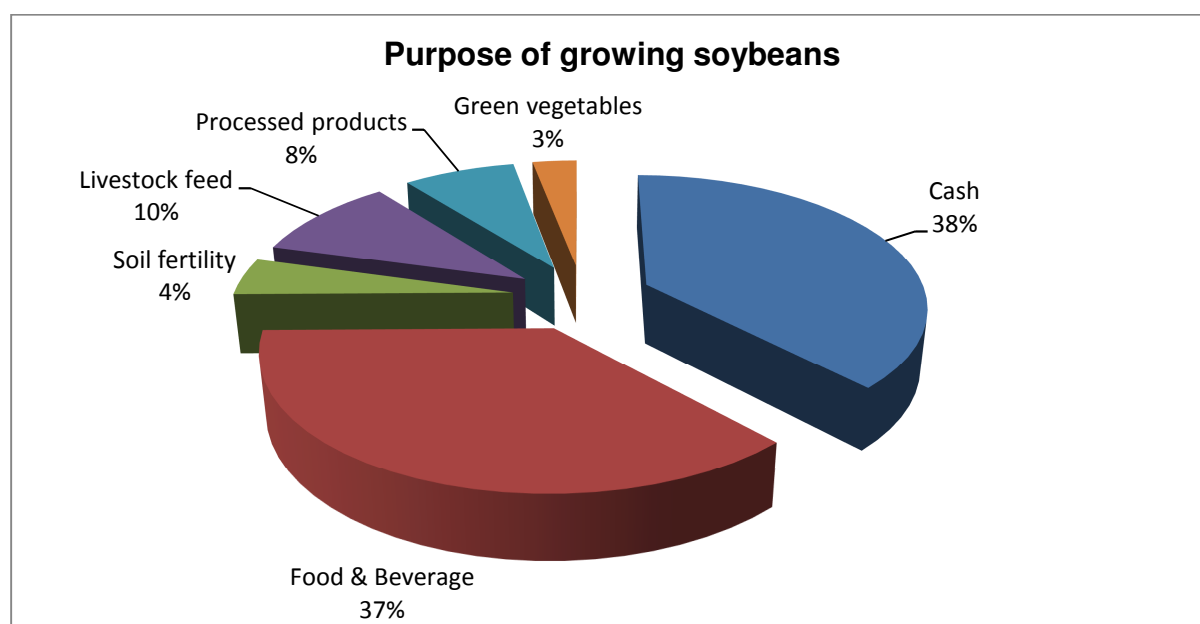


Fig 2.2. Purpose of growing soybeans by the participating farmers

### 2.3.4 Soybean cropping systems

Soybean was either grown as sole crop or intercropped with other crops (Fig 2.3a). About 62% of the farmers in this study grew soybean as an intercrop with cereals (maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), upland rice (*Oryza sativa* L.), and finger millet (*Eleusine corocana* Gaertn.), legumes (beans (*Phaseolus vulgaris* L.), cowpeas (*Vigna unguiculata* (L.) Walp.), and pigeonpea (*Cajanus cajan* (L.) Millsp.), perennial cash and fruit crops (tea (*Camellia sinensis* L.) Kuntze), mangoes (*Mangifera indica* L.), sugarcane (*Saccharum* L.) and coffee (*Coffea arabica* L.) and oil crops (sunflower (*Helianthus annuus* L.). Farmers practising intercropping were mainly located in Busia (100%), Kisii (94%), Kakamega (85%), Kirinyaga (78%) and Embu (54%) counties. The most common reasons for intercropping as mentioned by the farmers were food security against poor harvest or drought, soil fertility improvement,

limiting land size and control of pests and diseases (Fig 2.3b). In Nakuru county, soybean was mainly grown as a sole crop (95%) in large parcels of land (over 4 ha), while in Meru county, the crop was grown in pure stands in small portions (72%).

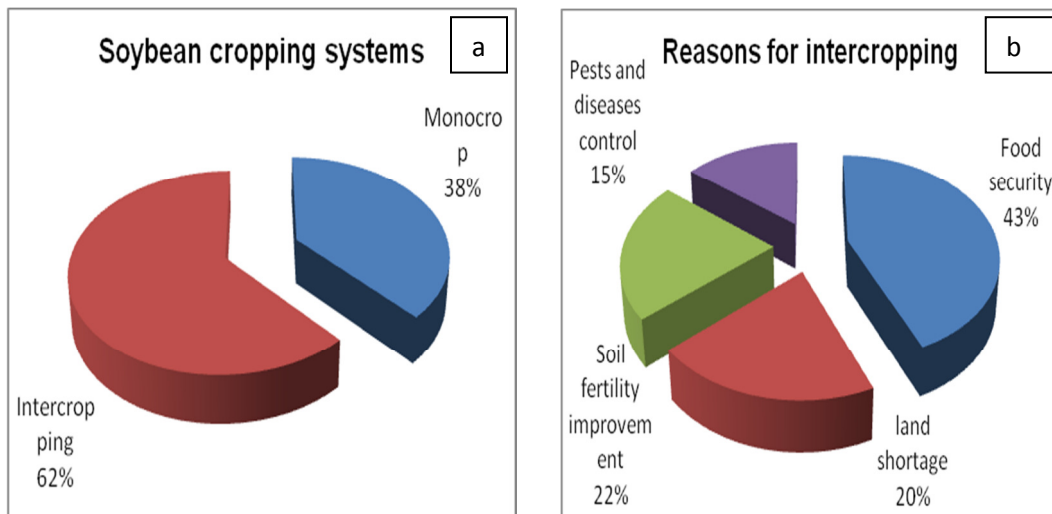


Fig 2.3: (a) Soybean cropping systems and (b) reasons for intercropping soybeans with other crops.

### 2.3.5 Soybean varieties commonly grown by farmers in Kenya

Farmers listed the soybean varieties that they grew in their fields. A total of 13 named varieties, both local and improved were being grown in Kenya as presented in Table 2.3. Farmers also grew other varieties (10%) that could not be identified by names. Farmers in Busia, Kakamega and Kisii counties grew both local and improved varieties, while in Embu, Kirinyaga and Nakuru counties the farmers mainly grew local varieties. Nyala and Gazelle were the most preferred varieties in all the counties. These varieties had a number of positive traits: moderate yields, early maturity, drought tolerance, high oil content, readily cooked seeds and a good physical appearance, which were appealing to most farmers. However, all the local varieties were highly susceptible to pests and diseases, prone to shattering, had difficulties in harvesting and poor nodulation. Variety Duicker was preferred for its stay green characteristics, especially in Kirinyaga county where green pods are sold for the fresh market. Among the improved varieties Namsoy 4m was rated high for its N fixing capacity, high quality grain and high sale price. However, it had negative attributes that included late maturity, low yields, poor seed viability and their unsuitability for intercropping. Farmers had similar perceptions of several other varieties, namely SB-15, SB-22, SB-20, Maksoy-1N, and FH-1.

Table 2.3: Soybean varieties grown by farmers in different counties of Kenya and the percentages of farmers growing a particular variety.

Soybean varieties	Local/ improved	Counties							Total
		Busia	Embu	Kakamega	Kirinyaga	Kisii	Meru	Nakuru	
Nyala	Local*	33.3	37.0	43.5	44.7	27.6	48.6	56.4	42.9
Gazelle	Local*	16.7	44.4	19.6	23.7	31.0	45.7	10.3	26.5
SCS-1	Local*	8.3	3.7	0.0	2.6	6.9	2.9	2.6	3.4
Sable	Local*	0.0	0.0	0.0	0.0	6.9	0.0	10.3	2.5
Duicker	Local*	0.0	3.7	0.0	5.3	0.0	0.0	0.0	1.3
EAI 3600	Local	0.0	3.7	0.0	0.0	0.0	0.0	0.0	0.4
Bossier	Local	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.4
Unknown	(Others)	0.0	7.4	0.0	21.1	20.7	2.9	20.5	10.5
Namsoy 4M	Improved	33.3	0.0	4.3	0.0	6.9	0.0	0.0	5.0
SB-15	Improved	0.0	0.0	10.9	0.0	0.0	0.0	0.0	2.1
SB-20	Improved	0.0	0.0	8.7	0.0	0.0	0.0	0.0	1.7
SB-22	Improved	0.0	0.0	13.0	0.0	0.0	0.0	0.0	2.5
Maksoy 1N	Improved	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.4
FH-1	Improved	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.4
<b>Total</b>		<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>

\*Soybean varieties released in Zimbabwe and introduced in Kenya in 1990's. (Nassiuma and Wasike, 2002).

### 2.3.6 Adoption of improved soybean varieties

The proportion of farmers growing local varieties (77%) was higher than those growing improved varieties (12%) across the counties. The local varieties were mainly grown because they had preferred traits that were absent in the improved varieties. The improved varieties were mainly grown in Busia (41%) and Kakamega (36%) counties and to a less extent in Kisii (6.9%) county (Table 2.3). In Nakuru, Kirinyaga, Embu and Meru counties farmers mainly grew the local varieties. The majority of participating farmers (61%) indicated a willingness to grow improved varieties (Fig 2.4). However, lack of information (37%), the unavailability of improved soybean seeds (37%), farmers' preference of the local varieties compared to the improved varieties (7.1%), and financial limitations (18.9%) were the main reasons as to why farmers did not plant improved varieties. About 38% of the farmers from Kirinyaga, Embu and Meru counties were content with the local varieties and they were not aware of the improved varieties.

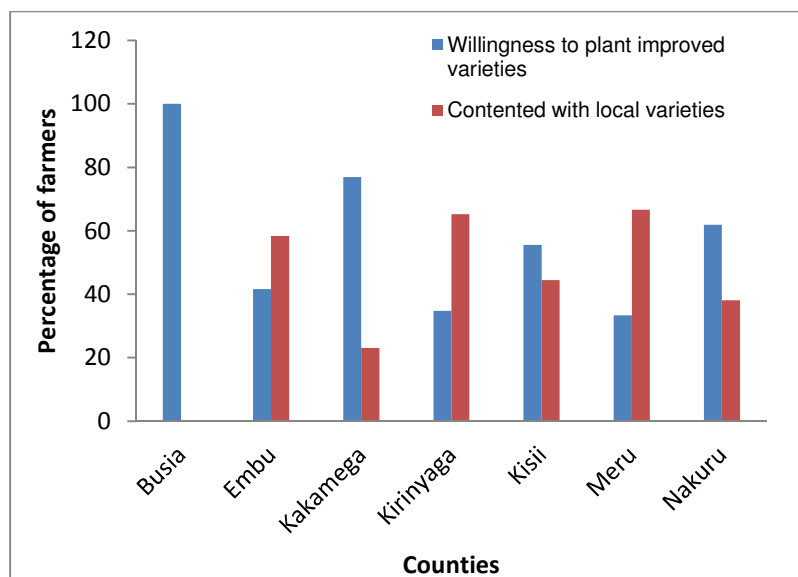


Fig 2.4: Farmers' (%) willingness to grow improved soybean varieties and those contented with the local varieties.

### 2.3.7 Farmers preferences for soybean varieties

Farmers' choices of either local and improved varieties were based on a number of key traits, which included yield potential, maturity, pest and disease resistance, drought tolerance, soil fertility improvement, seed availability, seed colour, and quality traits (Table 2.4). Improved varieties scored high in terms of soil fertility improvement, resistance to pests and diseases, high biomass production and high quality traits, but they were regarded as poor with regard to time-to-maturity, seed availability, low yield and susceptibility to lodging. On the other hand, local varieties were rated highly on yield, early maturity, drought tolerance and seed availability. The local varieties also scored high for lodging resistance, good viability and for being easier to cook. The local varieties were rated poorly for their pest and diseases susceptibility, harvesting difficulties, poor nodulation and farm input requirements. It was also observed that early maturing local varieties were prone to pod shattering but this was not the case for the improved varieties. Appealing seed colour, easy processing and adaptability to the local environment were rated as high in local varieties but low for improved varieties.

Table 2.4: Farmers' preferences for soybean varieties based on the desirable and undesirable attributes

	Local varieties (n=101)	%	Improved varieties (n=88)	%
<b>Desirable attributes</b>				
	High yielding	24	Soil fertility improvement	31
	Drought tolerant	16	High biomass production	11
	Good viability	7	Highly viable	6
	Seed availability	11	Easily cooked	3
	Lodging Resistant	8	Resistant to pests and diseases	22
	Early maturity	18	High quality trait	10
	Appealing seed color	5	Does not require input application	7
	Quality processed products	4	shattering resistant	1
	Well adapted to environment	1	High yielding	6
	Easily cooked	7	Early maturing	3
<b>Undesirable attributes</b>				
	<b>Attributes (n=52)</b>	<b>%</b>	<b>Attributes (n=97)</b>	<b>%</b>
	Pests / diseases susceptibility	31	Low yields	13
	Hard to uproot	15	Late maturity	28
	High shattering ability	17	Seeds unavailability	15
	Requires manure application	10	Hard to uproot	6
	Low yields	10	Lodging susceptible	13
	Poor viability	6	Intercropping incompatibility	4
	Poor nodulation	12	Poor appearance	5
			Uncooked grains	6
			Unappealing seed colour, poor adaptation, uneven maturity, poor storage, beany flavour	6

### 2.3.8 Sources of seed

The majority of farmers (41.1%) obtained their planting seed from the nearby open markets, while others (24.3%) sourced their seeds from their neighbours (Fig 2.5). Only a few farmers obtained seed from government and non-government organisations such as TSBF-CIAT, Kenya Agricultural Research Institute (KARI), Mwea Irrigation Development (MIAD) Centre, Ministry of Agriculture (MoA), German Technical Corporation (GTZ) and Agrovets.

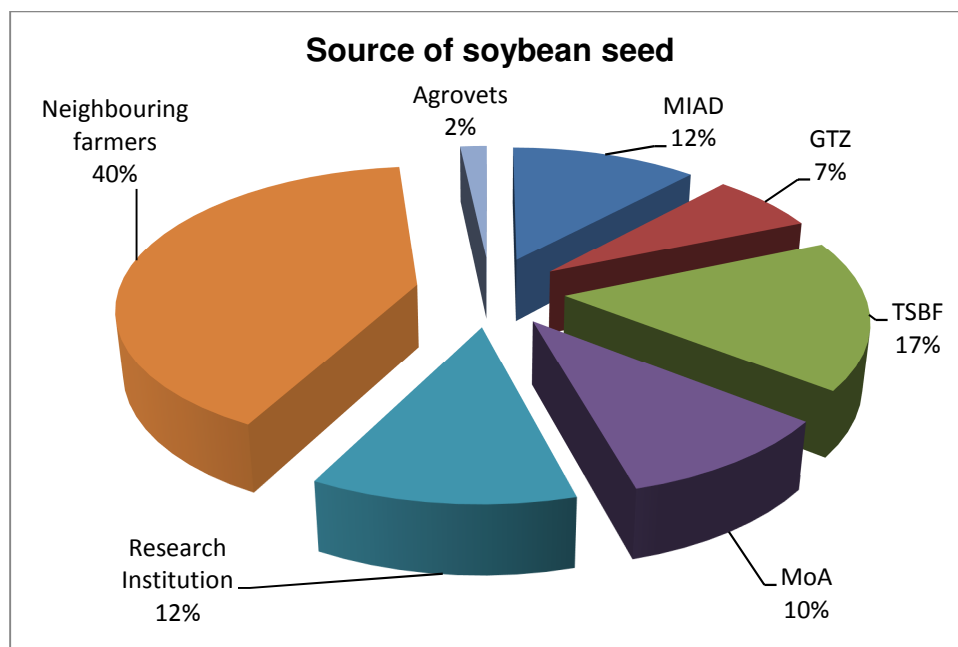


Fig 2.5: Sources of soybean seeds

### 2.3.9 Farmers' desired traits in an ideal soybean variety

Farmers used a number of criteria for selecting soybean varieties that they would like to grow in their fields (Table 2.5). Their main criterion was high yielding ability (16.5%) in terms of large grain size, number of pods per plant, number of seeds per pod and the number of branches per plant. The farmers also indicated that they desired early maturing varieties because these varieties could escape drought, pests and diseases, and could be harvested earlier, giving them an opportunity to prepare the land for the following crop. About 7.8% of the farmers considered ease of cooking to be a key trait that they would want in soybean varieties. They also preferred varieties with excellent agronomic traits such as drought tolerance, low shattering ability, pest and disease resistant and lodging resistance. Farmers also wished to have varieties with longer seed viability, an enhanced ability to fix nitrogen, ease of harvesting (threshing and uprooting) and intercrop compatibility characteristics. They also considered quality traits such as high oil and protein content, appealing seed colour, easy utilization and processing, marketability and the stay-green characteristic as important traits.

Farmers desired traits in soybean varieties differed across the regions (Table 2.5). In Busia, Kakamega, Kisii and Nakuru counties, farmers expressed a strong desire for high yielding varieties, followed by early maturing varieties and readily cooked grain. In Embu and Kirinyaga

counties, farmers desired early maturity, high yielding, drought tolerant and shattering-resistant varieties, in order of importance. High yielding varieties, early maturing varieties, drought tolerant, shattering-resistant and marketable varieties were the most desired traits in Meru County. High nitrogen fixing and lodging resistant varieties were also highly valued in Busia and Kakamega counties compared to other regions.

Desirable post-harvest traits also varied from one region to the other. In Kisii county farmers preferred varieties with appealing seed colour, that were easily cooked and processed into a high quality beverage. Farmers in this county consume soybean beverage as an alternative to tea and coffee because of their religious beliefs. On the other hand, Busia farmer's valued varieties that could easily be processed into various products while Nakuru farmers preferred varieties with high protein contents for their livestock feeds. The stay-green characteristic was highly valued in Kirinyaga county for a fresh pod market.

#### **2.3.10 Farmers desired traits based on gender**

Men and women rated high yielding and early maturing varieties as the most important criteria they would like in a soybean variety. However, other desired traits differed based on gender. For instance, men desired varieties that were drought tolerant, pest and disease resistant and the ability to fix nitrogen as the most important traits. On the other hand, women preferred varieties with desirable post-harvest characteristics, that is, varieties that were readily cooked, easy to harvest, with a low shattering frequency and easily processed seeds. They also favoured varieties with good crop vigour/appearance, high seed viability, appealing seed colour as well as high protein and oil content (Table 2.5).

Table 2.5: Farmers' desired traits (%) in soybeans based on counties and gender

Farmers desired traits	Counties							Gender		Total
	Busia	Embu	Kaka mega	Kirin yaga	Kisii	Meru	Nakuru	Men	Women	
Yield	18.6	13.4	12.5	16.7	20.9	19.0	16.5	17.1	15.6	16.5
Maturity	14.0	17.9	6.9	17.6	10.4	19.0	13.0	15.6	11.1	14.0
Drought tolerant	0.0	13.4	5.6	8.3	1.5	11.9	7.8	8.4	5.0	7.2
High Nitrogen fixation	9.3	6.0	8.3	5.6	4.5	2.4	3.5	6.6	3.3	5.4
Resistant to lodging	4.7	0.0	4.2	0.9	0.0	0.0	1.7	1.2	2.2	1.6
Resistant to shattering	0.0	10.4	6.9	7.4	3.0	9.5	9.6	6.8	7.4	7.2
Pests and disease resistance	0.0	9.0	6.9	7.4	3.0	7.1	7.8	6.9	5.6	6.4
High and protein content	4.7	0.0	4.2	1.9	4.5	2.4	9.6	4.2	4.4	4.3
Seed colour	0.0	0.0	1.4	0.0	4.5	2.4	0.9	1.5	1.6	1.6
Crop vigour/ appearance	4.7	0.0	5.6	2.8	6.0	2.4	2.6	1.2	7.2	3.3
Good seed viability	4.7	4.5	6.9	4.6	9.0	2.4	7.0	5.7	6.1	5.8
Intercropping compatibility	2.3	1.5	1.4	3.7	4.5	0.0	2.6	2.7	2.2	2.5
Readily cooked	9.3	4.5	6.9	7.4	10.4	2.4	10.4	5.7	11.7	7.8
Easy to harvest	2.3	7.5	8.3	2.8	7.5	2.4	5.2	2.7	10.0	5.3
Marketable varieties	4.7	7.5	0.0	3.7	1.5	9.5	1.7	4.2	2.2	3.5
Easy to process	11.6	3.0	13.9	4.6	9.0	2.4	0.0	4.8	7.2	5.6
Quality by-products	9.3	1.5	0.0	2.8	0.0	4.8	0.0	2.4	1.1	1.9
Stay green characteristics	0.0	0.0	0.0	1.9	0.0	0.0	0.0	0.3	1.3	1.3

### 2.3.10 Common pests and diseases in soybeans

Participating farmers listed soybean pests and diseases that are commonly found in their regions. Aphids and thrips were the most prevalent pests in all the surveyed regions. Whiteflies were also widespread, but they were not mentioned in Busia, Kisii and Meru counties (Table 2.6). Other pests that were mentioned included birds, caterpillars, bean fly, monkeys and termites. The most common diseases mentioned in some regions were ASR (39%), powdery mildew, Phytophthora root rot, soybean bacterial blight and soybean mosaic virus.

### 2.3.11 Farmers' perceptions of ASR

Among the diseases, ASR was considered the most common, by 39% of the farmers mainly from Busia, Kakamega and Nakuru counties (Table 2.6). However, 61% of the respondents were not aware of its existence. This was mainly observed in Kirinyaga, Embu, Kisii and Meru counties, where the disease was not perceived as an important production constraint and the farmers had no idea of what causes ASR, its symptoms and how it attacked the crop.



Table 2.6: Common pests and diseases of soybeans and farmers perception (%) on soybean rust in Kenya

Soybean pests	Counties (%)							Total
	Busia	Embu	Kakamega	Kirinyaga	Kisii	Meru	Nakuru	
Aphids	25	38.5	87.5	44.4	50.0	71.4	40.0	49.3
Thrips	75	30.8	0.0	22.2	33.3	28.6	26.7	26.8
Birds	0	0.0	0.0	0.0	16.7	0.0	13.3	4.2
Monkeys	0	0.0	0.0	0.0	0.0	0.0	6.7	1.4
Whiteflies	0	15.4	12.5	11.1	0.0	0.0	13.3	9.9
Termites	0	7.7	0.0	0.0	0.0	0.0	0.0	1.4
Catepillars	0	7.7	0.0	11.1	0.0	0.0	0.0	4.2
Beanfly	0	0.0	0.0	11.1	0.0	0.0	0.0	2.8
<b>Soybean diseases</b>								
Soybean rust	55.6	15.4	64.3	26.1	27.8	33.3	52.0	38.7
Soybean blight	0.0	0.0	0.0	4.3	0.0	0.0	4.0	1.8
Powdery mildew	11.1	15.4	0.0	0.0	0.0	0.0	4.0	3.6
Root rot	0.0	0.0	0.0	0.0	0.0	11.1	8.0	2.7
Soybean mosaic virus	0.0	0.0	7.1	0.0	0.0	0.0	0.0	0.9
None	33.3	69.2	28.6	69.6	72.2	55.6	32.0	52.3
<b>Soybean rust knowledge</b>								
Awareness of soybean rust	55.6	18.2	64.3	26.1	27.8	22.2	61.9	40.0
Lack of awareness	44.4	81.8	35.7	73.9	72.2	77.8	38.1	60.0

### 2.3.12 Farmers' perception on ASR - predisposing factors and symptoms

Farmers mainly associated the occurrence of ASR to excessive soil moisture, poor soil fertility conditions, high planting density, use of susceptible varieties and physiological maturity (Table 2.7). Other ASR predisposing factors were weeds (*Oxalis* spp. L.), frost and drought. None of the farmers associated the disease with fungal infections. The indicators of ASR as perceived by the farmers were leaf fall, yellowing of leaves, appearance of brown patches and dust on soybean leaves.

Table 2.7: Farmers perception on soybean rust description and predisposing factors

Soybean rust description	%	Soybean rust causes	%
Brown patches on the leaves	23	Excessive moisture / heavy rainfall	31.6
Yellowing of leaves	27	Low soil fertility	17.5
Brown dust on the leaves	19	High planting density	10.5
Dropping of leaves	31	Specific types of weeds like Oxalis	7.0
Total	100	Drought	5.3
		Frost	7.0
		Physiological maturity	10.5
		Total	100

### 2.3.13 Management of ASR

The majority of farmers applied minimal measures to control ASR. About 24% of the farmers used fungicides while a limited number practiced traditional methods such as seasonal planting, uprooting diseased plants, weeding, wide spacing, crop rotation and planting early maturing varieties (Fig 2.6). Other control methods like use of resistant varieties, soil fertility improvement and growing soybeans in the intercrop system were mentioned but not necessarily applied by the farmers. More than 30% of the farmers did not attempt to control the disease.

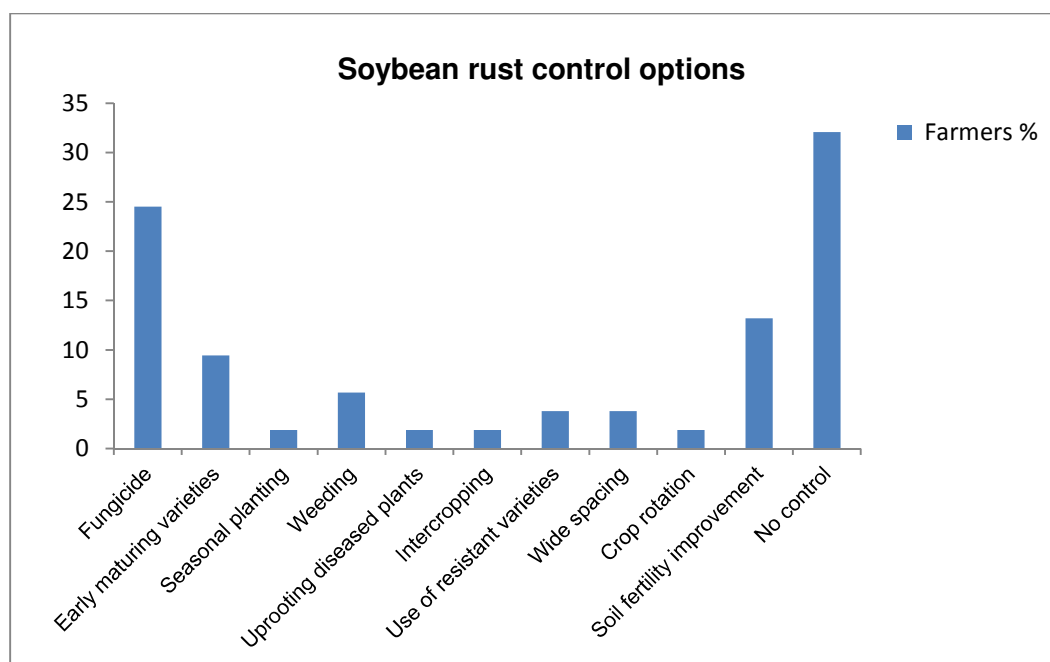


Fig 2.6: Soybean rust control options mentioned by the farmers.

#### **2.3.14 Other soybean production and marketing constraints**

Soybean farmers in this study reported a wide range of challenges (Table 2.8). Lack of market was mentioned by 16.5% of the farmers as a major constraint across all the regions. Low prices, lack of competition between the few buyers, lack of access to processing equipment, long distances to the market, and exploitation by middle men were the major challenges the farmers faced in marketing. Lack of appropriate processing and utilization methods (11%), unavailability of improved seeds (10%), pest and disease susceptibility (9%) were ranked second, third and fourth, respectively. Other production constraints were: limited access to farm inputs, low yielding varieties, frequent drought, uncooked soybean seeds, poor seed viability, uprooting and threshing difficulties, low demand for soybeans and high shattering incidence, among others.

Farmers' responses on the important constraints differed across the counties. For instance, marketing and lack of processing techniques were considered as the major constraints in Embu, Kirinyaga and Kisii counties. Lack of markets followed by pests and disease susceptibility, lack of processing techniques and seed unavailability were considered to be the major drawbacks in Nakuru county. In Busia county, pests and diseases were rated as the top constraint followed by poor nodulation, lack of farm inputs and lack of readily cooked soybeans. In Meru county; lack of markets, seed unavailability, frequent dry spells, and pest and disease susceptibility were the major constraints. High pod shattering was a major problem in Embu, Meru and Nakuru counties (Table 2.8). Low demand for soybeans compared to other legumes like beans, cowpeas and pigeonpea was cited in Meru, Embu, Kirinyaga and Busia counties. In Kisii county, lack of technical know-how, credit facilities and weak policy support were major constraints compared to the other counties. Intercropping incompatibility was also a major concern in Kisii county. In Busia county, poor nodulation of the local varieties was a major problem. Limited access to farm inputs, poor seed viability, highly lodging varieties, labour shortages and difficulties in harvesting were also a major concern in the area. In Kirinyaga county, water logging was a major constraint as black cotton soils in the area has a very low water absorption capacity.

Table 2.8: Soybean production and marketing constraints (%) in Kenya

Soybean constraints	Counties							Total
	Busia	Embu	Kakamega	Kirinyaga	Kisii	Meru	Nakuru	
Lack of markets and low prices	5.7	20.3	9.1	19.4	20.3	17.1	16.5	16.5
Lack of awareness on processing and utilization of soybeans	2.9	11.9	3.6	16.5	17.2	8.6	9.7	11.2
Low yielding soybean varieties	2.9	3.4	5.5	5.8	6.3	5.7	6.8	5.5
Seed unavailability	2.9	13.6	12.7	10.7	6.3	14.3	9.7	10.1
Frequent dry spells	0.0	6.8	0.0	6.8	3.1	11.4	7.8	5.5
Pest and disease susceptibility	17.1	3.4	18.2	4.9	4.7	11.4	10.7	9.0
Poor seed viability	2.9	1.7	9.1	4.9	3.1	8.6	2.9	4.4
Lack of farm inputs	11.4	6.8	5.5	5.8	3.1	2.9	6.8	5.9
Lack of credit facilities	0.0	0.0	1.8	0.0	3.1	0.0	1.0	0.9
Poor cookability	11.4	5.1	7.3	3.9	3.1	2.9	3.9	4.8
Poor nodulation	14.3	0.0	3.6	1.9	3.1	0.0	1.9	2.9
High pod shattering ability	0.0	5.1	1.8	1.9	1.6	8.6	6.8	3.7
Uprooting and threshing difficulties	2.9	6.8	3.6	1.0	4.7	0.0	8.7	4.4
Weak policy support	0.0	0.0	0.0	0.0	1.6	0.0	0.0	0.2
Highly lodging	5.7	0.0	3.6	0.0	0.0	0.0	1.9	1.3
Low demand of soybeans compared to other legumes	8.6	5.1	1.8	4.9	3.1	8.6	2.9	4.4
Lack of technical know-how	2.9	3.4	1.8	2.9	7.8	0.0	1.9	3.1
Intercropping incompatibility	2.9	0.0	0.0	1.9	3.1	0.0	0.0	1.1
Land size	0.0	0.0	1.8	2.9	3.1	0.0	0.0	1.3
Non uniform maturity	2.9	0.0	1.8	0.0	1.6	0.0	0.0	0.7
Labour shortages	2.9	6.8	3.6	2.9	0.0	0.0	0.0	2.2
Beany flavour	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.2
Late maturing compared to other legumes	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.2
High cost of production	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.2
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>

## 2.4 Discussion

### 2.4.1 Soybean production, its importance and cropping systems

This study revealed soybean to be a potential crop in Kenya for cash, food and beverage, processed products, soil fertility improvement, livestock fodder/feeds and green vegetables. However, the crop was cultivated only in small plots compared to other food crops. This is because soybean is a relatively new crop in Kenya (Chianu et al., 2009), and thus, has received little attention from the farmers. The limited immediate utilization of soybean at the household level compared to other legumes also limits the expansion of its production area (Tinsley, 2009). However, the potential to increase production exists, if the crop could be processed at

household level, and at the same time incorporated into the farming system as a cash crop that would compete better with already established food crops. This means more emphasis should be placed on its promotion, research and development while considering farmers needs and preferences.

Soybean was mainly cultivated by small-holder farmers as an intercrop with cereals, perennial cash crops, fruit trees, oil crops and other legumes depending on the region. These results are in agreement with those reported by Misiko et al. (2008). Intercropping was highly valued for food security and a coping strategy for land shortages. It also improves nutrition status, soil fertility and reduces pest and disease infestations (Yu et al., 2009). The high proportion of farmers growing the crop as an intercrop suggests the need for breeding suitable soybean varieties that could fit within this type of cropping system.

#### **2.4.2 Soybean varieties grown by the farmers**

The majority of farmers preferred local varieties over improved varieties because they had more desirable traits that satisfied their needs (socially, culturally and economically) and they were suitable for their environments. Similar findings were reported by Mulatu and Zelleke (2002) in maize. Local varieties ranked high in terms of yields (though potential yield has not been achieved) along with early maturity, drought tolerant and seed availability. Local varieties are also well adapted to local environments and their reduced vegetative growth contributes to their suitability in the intercrop, fitting well in small-holder farming systems. These traits may be used for selecting suitable parental materials for future breeding to develop soybean varieties that are attractive to farmers.

Only a few farmers in this study, mainly from Busia and Kakamega counties, grew improved soybean varieties. This is because TSBF-CIAT in conjunction with KARI, MoA and other non-governmental organizations have been promoting utilization and consumption of improved soybean varieties in these regions (Misiko et al., 2008). The majority of farmers also expressed their interest in growing improved varieties but they cited lack of information, limited availability of improved seeds, lack of resources and farmers preferences of the local varieties over the improved varieties as the major draw backs. Nevertheless, use of improved varieties could be sustainable if information on agronomic practices was provided to the farmers, effective seed production to ensure seed availability, and farmers were involved during variety development.

Improved varieties were rated high with regard to soil fertility improvement and biomass production. These are promiscuous varieties that nodulate freely without artificial rhizobium inoculation, thus fixing N that is beneficial to subsequent crops, while the substantial amount of biomass improves soil fertility through litter fall (Sanginga, 2003). Improved varieties were also highly ranked in terms of pest and disease resistance, high quality traits, better performance without application of manure and highly viable seeds. Based on these characteristics, improved varieties could be used as parents for introgressive breeding to improve deficit traits in the local varieties.

Farmers also mentioned negative aspects of improved and local varieties that needed improvements. For instance, improved varieties were disliked because of their late maturity, low yields (Mahasi et al., 2009), seed unavailability, highly lodging and poor crop appearance. Farmers also indicated that improved varieties were not acceptable because of their uneven maturity, unappealing seed colour, poor adaptation to the environment and their tall high branching ability and longer maturity durations that made them unsuitable for intercropping. Farmers were aware that local varieties were highly susceptible to pest and diseases, prone to shattering, had poor nodulation, high input requirements, and poor viability, but they were associated with other good qualities. Therefore, opportunities exist for either developing the local varieties for deficit traits and retain their desired traits or introduce improved varieties that will satisfy farmers' needs.

#### **2.4.3 Farmers' desired traits in an ideal soybean variety**

Farmers expressed differences in their desired traits across the study regions. In Kirinyaga county, farmers valued stay green characteristics for fresh soybean market while those in Kisii county preferred easily cooked processed seeds for beverage because of their religious beliefs. Farmers in Busia county preferred easily processed soybean seed for various products while Nakuru county valued high protein seeds for livestock feed. This variation may be attributed to the differences in cultural practices, availability of resources, constraints faced during production, environmental conditions and the purpose of growing soybeans. These results are in agreement with those reported by Mulatu and Zelleke (2002). Since soybean desired traits depended on the regions, it is important that the soybean breeding programme focuses on specific regions.

In general, farmers preferred varieties based on yield and maturity as the most important criteria for selection. Similar findings were reported by Mahasi et al. (2009) in Kenya and Idrisa et al. (2010) in Nigeria. In Kenya, soybean production is low compared to other countries like Brazil and USA thus expressing the need for developing high yielding varieties (Chianu et al., 2009). The desire for early maturing varieties was an important criterion, particularly in Embu, Kirinyaga and Meru counties where drought stress is common. This is because early maturity allow crops to escape dry spells, pod shattering and to some extent pest and disease infestations (Idrisa et al., 2010; Mahasi et al., 2009). Furthermore, early maturing varieties are fast income-generating activities for farmers thus reducing food insecurity.

Farmers also desired adaptation traits such as drought tolerance, and pest and disease resistance. Pests were more prevalent in Embu county while diseases were mostly mentioned in Nakuru and Kakamega counties. Given that farmers had minimal control measures and fungicides/pesticides are expensive, they indicated the need for resistant varieties. Drought tolerance was also ranked among the top soybean desired traits especially in Embu, Kirinyaga and Meru counties, possibly because of frequent drought stress. Farmers also pointed out that high yielding ability and early maturing varieties may not perform well without the ability to escape drought, pests and diseases. This suggests that breeding for pest and disease resistant and drought tolerant varieties must be a priority in soybean improvement.

Varieties that possess a high shattering resistance were also a priority to the farmers as pod shattering leads to seed losses of between 50-100% (Tukamuhabwa et al., 2002). Breeding for pod shattering resistant varieties would give farmers an opportunity to prepare for harvesting and reduce seed losses. Lodging was also an important criterion which made harvesting difficult and reduced yields. Although lodging can be reduced through adjustments of irrigation and soil fertility practices, as well as low planting densities, breeding for short determinate varieties or varieties that will maintain erect position throughout the growing season is necessary for maximum yields. Farmers in Busia county also recognized benefits from soybean including soil fertility improvement, preservation of soil moisture and weed suppression thereby reducing labour (Misiko et al., 2008) and other farm input costs they incurred during production. This led their desire for high nitrogen fixing varieties.

Farmers' desire for quality traits like high oil and protein content, easy utilization and processing, stay green characteristic and marketable varieties with appealing seed colour was also evident.

Since soybean is mainly processed into different products, industries are interested in soybean seeds with quality oil and protein to produce nutritious oil for human consumption, livestock feed, biofuel and other industrial products. However, the quality properties of the current soybean varieties limit its use for many industrial applications (Cahoon, 2003). Therefore, breeding efforts need to focus on the development of high quality varieties to improve soybean value for processors and acceptability among consumers.

#### **2.4.4 Gender participation in soybean production**

Both men and women rated high yielding and early maturing varieties as the key desired traits. However, they scored other traits differently indicating that men and women have specific preferences for certain traits that reflect different roles and responsibilities they perform along soybean value chain. Women preferences for high yielding and early maturing varieties reveal their responsibility in ensuring daily food availability while consideration of readily cooked varieties, with ease harvesting and easy processing reflects their roles in food preparation, harvesting and processing, respectively. Doward et al. (2007) had similar observations for rice in Ghana. For men, they concentrated more on yielding potential and adaptability traits because they are concerned with management of food availability for a longer time. This suggests that preferences by both men and women need to be integrated in breeding programmes to increase adoption rate of improved varieties and enhance breeders' knowledge during selection.

#### **2.4.5 Farmers' perceptions of ASR and its control**

Farmers' awareness on the occurrence and destructive nature of ASR was quite low, particularly in Embu, Kirinyaga, Kisii and Meru counties. Although the disease was present, it was probably not severe for the farmers to recognize it implying the need to train farmers on disease identification. The disease is also rare in semi-arid areas, characterized by low rainfall and high temperatures, especially during the short rain season (October- December), conditions not conducive for ASR development resulting in low inoculum to cause severe damages in the field. This confirms that environmental factors have an enormous impact on ASR development depending on location, season and cultivar as reported by Kawuki et al. (2003). ASR urediniospores require relative humidity of 75-80% and high rainfall with even distribution throughout the season for germination and rapid establishment (Hartman et al., 2005). The humid conditions in Western Kenya along with even rainfall distribution could be the reason why ASR is prevalent in the region. This is probably why farmers were aware of ASR in the region.



Farmers' cropping systems and lack of technical know-how also influence ASR spread. Preferences of the local rust susceptible varieties (Nyala and Gazelle) because of their desired attributes has significantly contributed to the wide spread of ASR (Mahasi et al., 2009). High planting density and poor spacing also facilitate rust development and they are clear indications that farmers lack technical know-how required for soybean production. Since ASR pathogen (*P. pachyrhizi*) has a wide range of hosts that can infect many other legumes (Hartman et al., 2005), there is continuous production of inoculum when soybeans are intercropped with other legumes including pigeonpea, cowpeas and beans. This calls for efforts towards increasing farmers' technical know-how to reduce disease incidences.

Farmers' identification of ASR was mainly based on visual observations. Brown patches and dust on leaves, yellowing leaves and defoliation of infected plants were the major indicators of ASR as perceived by the farmers. This concurs with the common symptoms of ASR because as the disease progresses, more lesions (either brown or tan in colour) are formed on the leaves and subsequently develop into chlorosis that eventually results into premature defoliation causing significant yield reductions (Kawuki et al., 2003). Farmers mainly attributed ASR occurrence to environmental factors. Excessive moisture was the most predisposing factor that farmers thought facilitated rapid development of ASR. These findings are supported by Li et al. (2009) who reported that moisture is needed for the germination, infection of urediniospores and deposition of the spore, especially for long-distance dissemination. Farmers also attributed ASR to frost and drought, but they have not been reported to have a significant effect on rust development (Delaney et al., 2007).

ASR was also related to physiological maturity, weeds, susceptible varieties, high planting density and poor soil fertility conditions. Farmers indicated that ASR was affected by plant age. This observation is in agreement with that reported by Srivastava et al. (2009) because as soybean crop approaches physiological maturity, ASR severity, lesion density, sporulation, and number of pustule per lesion increases. Farmers thought that weeds played a major role in the development of ASR possibly because some weeds have been reported to be alternative hosts of rust pathogen serving as sources of inoculum (Hartman et al., 2005). High ASR incidences and severity were mainly observed in more densely planted soybean fields compared to low plant densities simply because rust is more severe in shaded areas (Dias et al., 2011). Some farmers also felt that ASR was caused by susceptible varieties which acted as sources of inoculum. This is supported by Silva et al. (2008) that most of the current commercial varieties

grown in different regions are susceptible to ASR infection, though at varying degrees. Other farmers associated ASR with poor soil fertility conditions, probably due to the yellow leaves of the infected plants. Lack of major soil nutrients such as nitrogen, phosphorous and potassium has been reported to affect crop health making it susceptible to several diseases. However, the effect of soil nutrients on ASR is not known thus further investigations are needed.

ASR control measures observed in the farmers' fields were limited. This means that the urgent development of affordable control methods is needed, including breeding for ASR resistance and development of integrated disease management strategies. In most cases fungicide are used for controlling rust, but only a few of the interviewed farmers were using them. It is possible that farmers lack knowledge, have poor access to fungicides and resources are not readily available making it difficult to control rust using chemicals. This concurs with Danial et al. (2007) findings. Due to problems associated with external inputs in relation to accessibility, affordability and sustainability, efforts should be directed towards breeding for rust resistant varieties in Kenya.

Other cultural control methods, though not effective, were mentioned by a limited number of farmers. For instance, adjusting planting dates in a way that the susceptible reproductive stage occurs during the dry season was mentioned to reduce rust incidence. Early planting and use of early maturing varieties also helps the crop to escape the disease. In areas where rust is prevalent, wider spacing together with low plant densities can help the canopy to reduce leaf wetness periods as a result of dew, thus reducing ASR development. Intercropping was also mentioned as a means reducing disease incidences but research on the best companion crops needs to be conducted. Improving soil fertility, especially the major nutrients as a means of enhancing the crop vigour has also been reported to reduce disease levels though this has not been verified. However, cultural practices alone may not be sufficient in managing ASR, thus other feasible control methods including rust resistance and fungicide application need to be integrated.

#### **2.4.6 Other soybean production constraints**

Soybean production, marketing and consumption are limited by various factors ranging from biotic, abiotic and socio economic factors. The major biotic constraints as cited by the farmers were pests (aphids, thrips and whiteflies) and diseases (ASR, powdery mildew and Phytophthora root rot). Soybean is also sensitive to abiotic factors such as drought and water

logging. It is also affected by several socio economic problems that include lack of market, lack of appropriate processing and utilization methods, unavailability of improved seeds, lack of farm inputs, low yielding varieties, uncooked soybean seeds, poor seed viability and low demand for soybeans compared to other legumes, among others. The majority of respondents cited lack of markets as the major challenge facing soybean production. Given such circumstances, providing farmers with a ready market is likely to strengthen soybean productivity at farm level. The government supportive policies that will ensure importations from neighboring countries are reduced and promote local production are also necessary. Linking farmers with food and feed industries and other domestic buyers would also help in development of a market chain that is sustainable. At the same time, formation of farmers associations and gathering market information need encouragement to ensure a profitable soybean enterprise.

Although soybean usage is limited because of its cookability problems, it can be processed into various forms at household level (Fabiya, 2006). However, the majority of farmers indicated lack of knowledge in processing and utilization techniques. This problem was also observed by Chianu et al. (2009). It is, therefore, necessary to train farmers on various utilization methods that will increase awareness and consumption of nutritious soybeans through various products and recipes. Furthermore, development of simple processing technologies that will reduce soybean losses and develop new products would also improve marketing channels and increase the demand of soybean products.

Pest and disease incidences were common in some areas, thus efforts to develop integrated disease management requires farmers' participation to ensure their acceptance and adoption (Kiros-Meles and Abang, 2008). Despite, the introduction of improved varieties with resistance to pests and diseases in the country through TSBF-CIAT and other non-government organizations, seeds are not yet available in adequate quantities. Availability of improved seeds is important if soybean production is to be increased. This can be done successfully through formation of both formal and informal commercial seed production or multiplication systems and organized distribution programmes. Also dissemination of technical information to farmers on the production of the improved varieties needs to be done through the extension agents and other non-governmental service providers. In addition, farmers need to be linked with credit facilities that will enable them purchase farm inputs that they require for soybean production.

Farmers mentioned that current commercial varieties were low yielding. Breeding programmes have the potential of increasing soybean production through development of improved varieties that possess high yielding characteristics as well as other desirable attributes cited by the farmers. These traits include early maturity, drought tolerance, low shattering ability, resistance to lodging and nitrogen fixing ability, among others.

## **2.5 Conclusion**

From the survey, a total of 14 soybean varieties were documented to be grown by the farmers in Kenya. Farmers prefer local varieties because they possess desired traits such as moderate yield potential, early maturity, drought tolerance, and high grain quality. These traits are essential for genetic improvement of modern varieties, if they are to be adopted widely by farmers. Farmers also indicated several desired traits they would like in an ideal soybean variety including yield, maturity, adaptable and quality traits. These criteria were mainly based on cultural values, purpose of growing soybeans and the constraints that the farmers face during soybean production, marketing and consumption. Therefore incorporating the key farmers' desired traits in any soybean breeding programme will increase the adoption level of the improved varieties. In addition, development of soybean varieties that will satisfy the needs' of different groups of farmers in different regions is recommended.

Because of a low awareness of ASR, training is needed to improve farmers' knowledge particularly on disease identification, sources of planting seeds, and disease management. In addition, a participatory breeding programme that will focus on the resistant materials and development of integrated and sustainable disease management strategies that meets farmers' needs is required urgently. Farmers also cited several other challenges they face in soybean production, mainly lack of markets, lack of awareness on processing and utilization and seed unavailability among others. This situation needs urgent attention from the policy makers, researchers and extension agents in order to strengthen soybean production in Kenya.

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# **Appendix 1: Farmers' preferred soybean varieties, perceptions on Asian soybean rust (ASR) and other constraints facing soybean production in Kenya**

<b>Questionnaire No.</b>		<b>District</b>	
<b>Date of interview</b>		<b>Division</b>	
<b>Enumerator</b>		<b>Location</b>	
<b>AEZ</b>		<b>Sublocation</b>	
<b>Region</b>		<b>Village/unit</b>	

## **A: Biographic data**

1. Name of household head-----

2.

<b>a) Gender</b>	1. Male	2. Female	
<b>b) Marital Status</b>	1. Single	2. Married monogamous	3. Married polygamous
	4. Divorced	5. Widowed	
<b>c) Education level</b>	1. No formal education	2. Adult literacy classes	3. Primary Std 1-4
	4. Primary Std 5-8	5. Beyond Primary	
<b>d) Age</b>	1. Below 25	2. Between 25 - 35	3. Between 36-45
	4. Between 46-50	5. Above 50	

3. Name of respondent -----

4. Relation of respondent to the household head -----

5. What is the size of your land? ----- Acres

6. What is the size of land allocated to soybean production?

7. Which crops have you been growing for the last 5 years?

	<b>Crop</b>	<b>Rank</b>	<b>Acreage</b>	<b>Purpose*</b>
1				
2				
3				
4				
5				

\*Purposes: 1. Cash crop (income) 2. Food 3. Security/saving 4. Livestock feed 5. Others-----

## **B. Soybeans production information**

### **i) If soybeans is not among the crops grown in 6 above**

1. Have you ever grown soybeans? 1. Yes 2. No

2. If yes, why did you stop? -----

3. If no, why? -----

### **ii) If soybeans is among the crops grown in 6 above**

1. Which cropping system do you use for soybeans? 1. Mono crop (soybeans only) 2. Intercrop,
2. If intercropped, with which crops? 1. -----  
2. -----  
3. -----
3. What are reasons for intercropping?
4. What spacing did you use?
5. Which of the following inputs do you use in soybean production?

Input	Specific type used	Frequency of application	Amount used (per plant/area)	Reason for not using
Manure				
Fertilizer				
Pesticides				
Fungicides				
Other (specify)				For what purpose

### C. Common soybean varieties grown in Kenya

1. Which variety/varieties do you grow and what are their good and bad traits

	Variety	Good Traits	Bad Traits
1			
2			
3			

2. What is the source of your planting material 1. Neighbouring farmers 2. Agro vet 3. Market  
4. Research institutions 5. Seed companies 6. Others(specify)
3. Have you ever heard of improved soybeans varieties? 1. Yes 2. No
4. If yes; from whom did you hear? -----
5. If yes; have you ever planted the improved soybeans variety? 1. Yes 2. No
6. Where did you get your planting materials from? -----
7. If yes; how do you compare the improved varieties to traditional varieties?

Criteria	Comparison	
	Improved varieties	Traditional varieties
Yield		
Maturity		
Pest and disease resistance		
Drought tolerance		
Quality traits		
Nitrogen fixation		
Intercropping compatibility		
Seed viability		
Low shattering ability		
Standability		
Seed colour		
Others		

8. Reasons for not planting improved varieties?
- 9 Are you willingness to plant improved varieties or you are contented with the local variety? 1. willing 2. Contented



10. What are the most important traits you look for when selecting a soybean variety?

	Selection criteria	Rank
1	Yield	
2	Maturity	
3	Pest and disease resistance	
4	Drought tolerance	
5	Utilization	
6	Nitrogen fixation	
7	Intercropping compatibility	
8	Seed viability	
9	Low shattering ability	
10	Standability	
11	Seed availability	
12	Seed colour	
13	Quality traits	
14	Others	

#### D. Soybean Production, marketing and utilization constraints

1. What constraints do you face in soybeans production and marketing?

	Constraint	Rank	Suggested Solution
1			
2			
3			
4			
5			
6			
7			

#### E. Pests in soybean production

1. What are the common pests you've encountered in soybean production?

**a) Name** -----

Description -----

How the farmers has been controlling the pest-----

How effective is it 1. Effective 2. Not effective

Whether the farmer knows of a more effective way (not necessarily using it) 1. Yes 2. No

If yes, which one -----

Source of information -----

**b) Name** -----

Description -----

How the farmers has been controlling the pest-----

How effective is it 1. Effective 2. Not effective

Whether the farmer knows of a more effective way (not necessarily using it) 1. Yes 2. No

If yes, which one-----

Source of information -----

## F. Diseases in soybean production

2. What are the common diseases you've encountered in production

**a) Name** \_\_\_\_\_

Description \_\_\_\_\_

Causes of the disease \_\_\_\_\_

How the farmer has been controlling the disease-----

How effective is it     1. Effective                      2. Not effective

Whether the farmer knows of a more effective way (not necessarily using it) 1. Yes 2. No

If yes, which one                      -----

Source of information -----

**b) Name** \_\_\_\_\_

Description \_\_\_\_\_

How the farmer has been controlling the disease-----

How effective is it     1. Effective     2. Not effective

Whether the farmer knows of a more effective way ( not necessarily using it) 1. Yes 2. No

If yes, which one                      -----

Source of information -----

### 3 Evaluation of soybean genotypes for resistance to Asian soybean rust (ASR) and its correlation with selected agronomic traits in Kenya

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#### Abstract

ASR is becoming a serious threat to soybean production in Kenya. Several sources of ASR resistance have been identified in exotic germplasm but their reaction to local isolates is not known. This study evaluated plant introductions reported to carry single rust resistant genes (*Rpp1-4*), tolerant lines, genebank accessions, commercial varieties and advanced lines for ASR resistance, and its correlation with selected agronomic traits in Kenya. A total of 110 soybean genotypes were evaluated in three locations using an alpha lattice arrangement (10 x 11), replicated three times. ASR assessment was based on disease severity (1-9 scale), sporulation (1-5 scale), lesion type and area under disease progress curve (AUDPC) values. An additional evaluation was carried out in a screenhouse using artificial inoculation. Results indicated varying degrees of rust severity, AUDPC values, lesion type and sporulation among the genotypes. Out of 110 genotypes, five were classified as resistant, nine moderately resistant, while the rest were moderately or highly susceptible based on rust severity scores. Genotypes possessing *Rpp4* (G10428) and *Rpp2* (G8586) resistant genes and non-characterized genotypes MAK BLD 11.3, GC 00138-29 and Namsoy 4M were the most resistant, as indicated by low rust severity scores, low AUDPC values, red brown lesions and low sporulation scores. Other genotypes with known resistant genes which included G7955 (*Rpp3*), G58 (*Rpp1*), Tainung 4 (*Rpp1*), UG-5, UFV3, Maksoy 1N and Dowling were classified under moderately resistant category. Apart from advanced line BRS Sambimba, the other advanced lines, commercial varieties and genebank genotypes were classified as moderately to highly susceptible. Positive and highly significant correlation ( $P < 0.001$ ) observed between rust severity and rust sporulation indicates that reduction of this character has a significant contribution towards rust resistance. Resistant and moderately resistant genotypes identified in this study are recommended for further utilization in the breeding programmes to develop rust resistant varieties in Kenya.

### 3.1 Introduction

Soybean productivity is affected by several constraints including biotic, abiotic and socio economic stresses (Tefera et al., 2009). Among the biotic constraints, ASR caused by *Phakopsora pachyrhizi* Sydow is the most devastating foliar disease affecting soybean production worldwide (Calvo et al., 2008). In Kenya, ASR was first reported in 1996 and its presence is increasingly becoming a major problem in major soybean growing areas (Kawuki et al., 2003b). Severe yield reductions ranging from 10% to 80% due to ASR have been reported in Argentina, Asia, Brazil, Paraguay and other parts of Africa under disease-conducive conditions (Kawuki, et al., 2004; Yorinori et al., 2005). Bennett (2005) also reported that severely infected plants result in significantly fewer pods/plant, fewer seeds/pod, reduced number of filled pods/plant, reduced 1000 seed weight, reduced seed germinability and low oil content. Because of its fast spreading nature, ASR is likely to cause severe yield and quality losses depending on the growing season, location, variety, time of infection and favourable environment if adequate control measures are not taken (Kawuki et al., 2004).

Use of resistant cultivars is the most economical, long term and environmentally friendly method for managing ASR (Pham et al., 2010). As a result, soybean breeders have been screening thousands of soybean genotypes, cultivars, breeding lines and *Glycine max* Willd relatives in search of rust resistance sources for many years in Asia (Li, 2009a) and more recently in the United States (Miles et al., 2006) and Africa (Twizeyimana et al., 2008). Eight dominant race specific resistant genes to ASR at six loci *Rpp1-Rpp6*, *Rpp1b* and *Rpp?* *Hyuuga* have been identified in different plant introductions (Hartwig and Bromfield, 1983; Hartwig, 1986; Calvo et al., 2008; Garcia et al., 2008; Monteros et al., 2008; Chakraborty et al., 2009; Li et al., 2012). However, these sources of resistance are unstable and sometimes produce susceptible reactions when challenged with certain *P. pachyrhizi* isolates (Pham et al., 2009). Therefore, in a continuous search for more durable resistance, three recessive genes (*rpp2*, *rpp3*, *rpp5*) controlling ASR were identified (Calvo et al., 2008; Ray et al., 2011) but they have not been integrated into the breeding programmes. Partial or rate-reducing resistance to ASR has also been identified, but this too has not been exploited in breeding programmes because it is time consuming and its assessment is complicated (Kawuki et al., 2004; Hartman et al., 2005). Because of the shortcomings associated with specific genes and rate-reducing resistance, tolerance to rust (yield stability) was proposed as an alternative remedy to ASR (Hartman et al., 2005). Consequently, several tolerant lines with sufficient variation that could be exploited by breeders have been identified (Kawuki et al., 2004).

Although potential sources of resistant and tolerant lines to ASR have been identified, this resistance often breaks down from one geographical region to the other. For instance, only resistant genes *Rpp1* and *Rpp4* had resistant reactions to ASR in Nigeria (Twizeyimana et al., 2009), genes *Rpp2* and *Rpp4* were resistant in Brazil (Silva et al., 2008) and only *Rpp2* gene was resistant in Uganda (Oloka et al., 2008). This is as a result of the high variability and aggressiveness within *P. pachyrhizi* races (Hartman et al., 2005; Miles et al., 2006), susceptibility of different maturity groups, environmental effects (Kawuki, et al. 2004) and genotype x pathogen x environment interaction (Walker et al., 2008). For the purpose of utilizing identified resistant germplasm and/or identifying new sources of resistance in soybean breeding programmes, it is important to assess their reactions against local rust isolates. Therefore, the objectives of this study were to; (i) Evaluate previously identified rust resistant genotypes, tolerant lines, genebank genotypes, commercial varieties and advanced lines for rust resistance reactions, and (ii) Determine the correlation of ASR resistance reaction with selected agronomic traits.

### **3.2 Materials and methods**

#### **3.2.1 Soybean genotypes**

A total of 110 soybean genotypes divided into six sets were used in this study (Table 3.1). They included plant introductions with known rust resistant genes (*Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4*) and unknown resistant genes obtained from Asian Vegetable Research Development Centre (AVRDC) through the courtesy of Makerere University in Uganda (Set I); varieties/lines previously identified as tolerant or moderately resistant in Uganda and South Africa (Set II); advanced lines from Kenya Agricultural Research Institute (KARI-Njoro) and International Institute of Tropical Agriculture (IITA) (Set III); commercial varieties (Set IV); collections from farmers' fields (Set V); and genotypes from the National genebank of Kenya (Set VI). Variety 'Nyala' that is widely grown in Kenya was used as a susceptible check. All the genotypes were evaluated for rust resistance both in the screen house and field conditions.

Table 3.1: Soybean genotypes evaluated for rust resistance, previous reported rust reaction classification and their origin/source.

Set	Genotype/Genotype code	Resistance genes or susceptibility	Origin / Source	Reference
i	G10428 (PI 459025)	<i>Rpp4</i> resistance gene	Fujian China/ Uganda	(Pham et al., 2009).
	G58 (PI200492)	<i>Rpp1</i> resistance gene	AVRDC Taiwan, Japan/Uganda	(McLean and Byth, 1980; Pham et al., 2009; Yamanaka, 2010).
	G7955 (PI 462312) (Ankur)	<i>Rpp3</i> resistance gene	India / Uganda	(Pham et al., 2009).
	G8586(PI 230970)	<i>Rpp2</i> resistance gene	AVRDC Taiwan, Japan/Uganda	(Pham et al., 2009; Maphosa, 2010).
	G57 (Tainung 4)	<i>Rpp1</i>	AVRDC Taiwan	(McLean and Byth, 1980; Yamanaka, 2010).
	GC 00138-29	Resistant (Unknown gene)	AVRDC Taiwan, Japan/ Uganda	(AVRDC, 1989; Kiryowa et al., 2009; Yamanaka, 2010)
	UG 5	Resistant (Unknown gene)	Uganda	(Kiryowa et al., 2009)
	PI 200477A	Resistant		(Singh and Thapliyal, 1977)
ii	MAK BLD 11.3	Moderate resistant	Uganda	(Oloka et al., 2008; Kiryowa et al., 2009). Maphosa, <i>personal communication</i> <sup>2</sup> ).
	Maksoy 1N	Moderate resistant	Nigeria/Uganda	
	Maksoy 2N	Tolerant	Uganda	
	Namsoy 4M	Moderate resistant	Uganda	
iii	UFV3	Tolerant	Brazil/South Africa	(Jarvie, 2009). (Jarvie, <i>personal communication</i> <sup>3</sup> ).
	Dowling	Tolerant	South Africa	
iv	LS6161RR	Unknown	South Africa	(Mahasi et al., 2009).
	835/5/30, 911/6/3, 915/5/12, 916/5/19, 917/5/16, 931/5/34, 932/5/36, BRS 217 Flora, BRS MG46, BRS Sambaiba, SB-17, SB-20, SB-8, SB-37, SB-4, SB-19	Unknown	Advanced lines from KARI-Njoro and IITA	
v	Blackhawk, Bossier, Duicker, EAI3600, Gazelle, Hill, Nyala, SCS1, Sable	Susceptible	Commercial varieties from KARI-NJORO	
vi	Ex Japan, FH1, Ex-Kirinyaga and Kissi	Unknown	TSBF/Farmers collection	
vi	GBK 010246, 028397A, 028397B, 029511, 029570, 029571, 029573, 029574, 029575, 029577, 029600, 029610, 029611, 029612, 029614, 029616, 029617, 029620, 029621, 029622, 033203, 033204, 033205, 033206, 033207, 033208, 033209, 033210, 033211, 033213, 033214, 033215, 033217, 033218, 033220, 033221, 033222, 033223, 033224, 033225, 033226, 033229, 033230, 033231, 033232, 033233, 033234, 033236, 033237, 033241, 033242, 033243, 033245, 033246, 033247, 033248, 033249, 033250, 033251, 033252, 033253, 033254, 033255, 033257, 045342	Unknown	Genebank genotypes from the National genebank of Kenya	

AVRDC is the Asian Vegetable Research Development Centre; KARI is Kenya Agricultural Research Institute; IITA is the International Institute of Tropical Agriculture; TSBF is the Tropical Soil Biology Fertility

<sup>2</sup> Maphosa, M. Department of Agricultural Production, Makerere University, P.O. Box 7062, Kampala, Uganda.

<sup>3</sup> Jarvie, J.A. Pannar Seed Company, P.O. Box 19, Greytown 3250, South Africa.

### **3.2.2 Screen house experiment**

In this experiment, soybean genotypes were evaluated using inoculum prepared in the laboratory from naturally infected soybean leaves collected from variety Nyala grown in a field at KARI-Embu Research Station. Severely infected leaves were washed separately with sterile water, then blotted with tissue paper to remove excess water and to release old urediniospores (Yeh, 1983). The leaves were then sealed in transparent plastic bags to produce fresh urediniospores. Fresh urediniospores were mixed thoroughly with 0.1% Tween 20 (sodium monolaurate) containing sterile water and filtered through a 53- $\mu$ m pore size screen to remove any debris and spore clumps. Using a haemocytometer, the concentration of the urediniospores was adjusted to  $1 \times 10^6$  urediniospores per milliliter for inoculation (Twizeyimana et al., 2007).

Three to four soybean seeds of each test genotype were planted into 150x200 mm plastic pots filled with local topsoil mixed with manure and sand in a ratio of 3:2:1, respectively. This experiment was laid out in a completely randomized design replicated 3 times, with four pots treated as a replicate. Seedlings were later thinned to two plants per pot 2 weeks after emergence. At the V3 stage (three nodes on the main stem with fully developed leaves) and the R1 stage (beginning of bloom), the seedlings were inoculated with ASR urediniospores using a knapsack sprayer. After inoculation, soybean plants were covered with a plastic sheet 10-12 hours to increase the humidity level. Temperatures ranging between 22 and 28°C were maintained in the screenhouse, to provide an environment conducive for spore germination. ASR severity, presence of different types of lesions and sporulation were evaluated at the R2 (full bloom), R4 (full pod) and R6 (full seed) stages.

### **3.2.3 Field evaluation and experimental sites**

Field experiments were carried out in three locations, namely at KARI-Embu, KARI-Igoji and KARI-Mwea, under natural inoculum pressure. KARI-Embu is located in Kenya's Eastern Region at latitude 00° 30'S and longitude 37°42'E at an altitude of 1508 m above sea level, with an average rainfall of 1200-1495 mm per year. The mean temperature ranges between 14.1 and 25°C and the soil type is a Humic nitosol. KARI-Igoji is also located in the Eastern Region at a latitude of 00°34'S and a longitude of 37°19'E, at an altitude of 1189 m above sea level, with mean annual rainfall of 1095 mm and temperatures ranging between 20.9 to 22.9°C. The soil type is a Eutric nitosol. KARI-Mwea is situated in the Central Region at a latitude of 00° 37'S and a longitude of 37° 20'E, at an altitude of 1159 m above sea level. This site receives a mean

annual rainfall of 850 mm and the temperature ranges from 15.6°C to 28.6°C with a mean of 22.8°C. The soil types are nitosols. All the sites have a bimodal rainfall pattern, with long rains received between mid-March and June, and short rains in Mid-October to early January.

At KARI-Embu the evaluations were conducted for four consecutive seasons; during the long rains of 2010, the short rains of 2010, the long rains of 2011 and the cold season of June-August 2011. At KARI-Mwea and KARI-Igoji experiments were conducted for two seasons during the short rain of 2010 and the long rains of 2011. Data on cumulative rainfall (mm), maximum, minimum and mean temperatures (°C) were recorded during the experimental period in each cropping season, as presented in Table 3.2.

Table 3.2: Cumulative rainfall and temperatures during ASR evaluation trials for three cropping seasons in 3 locations.

Location	Cropping Season	Cumulative Rainfall (mm)	Temperatures during the growing season (°C)		
			Maximum	Minimum	Mean
KARI-Igoji	Long rains (2011)	137.0	23.73	17.9	20.8
KARI-Igoji	Short rains (2010)	173.0	23.12	17.2	20.2
KARI-Mwea	Short rains (2010)	191.7	29.00	17.7	23.4
KARI-Mwea	Long rains (2011)	496.9	28.60	18.8	23.7
KARI-Embu	Long rains (2010)	733.0	24.70	15.7	20.3
KARI-Embu	Short rains (2010)	251.8	25.90	14.4	20.2
KARI-Embu	Long rains (2011)	473.7	26.00	15.5	20.9
KARI-Embu	Cold season (2011)	141.7	23.65	14.2	18.9

### 3.2.4 Treatments, experimental design and planting

One hundred and ten soybean genotypes were sown in plots consisting of three rows of 2 m long, spaced at 0.3 m between rows and 0.15 m within the rows. Three seeds were planted in each hill and later thinned to one plant per hill 14 days after emergence to maintain the optimum plant population. The experiments were laid out in an alpha lattice arrangement (10x11) replicated three times. To ensure high and uniform disease pressure in the plots, spreader rows consisting of a highly susceptible variety (Nyala) were planted in the border rows surrounding all the test genotypes, as per the methodology described by Twizeyimana et al. (2007). Other agronomic practices of weeding, irrigating, and fertilizer applications were followed as recommended for each site.



### **3.2.5 Data collection**

#### **3.2.5.1 ASR severity**

Since soybean genotypes attain different crop stages at different times, rust severity data was assessed when each genotype attained a particular crop growth stage, to take care of the maturity-related variations in rust susceptibility (Oloka et al., 2008). ASR severity was scored in five plants selected randomly within a row during the R1, R2, R4 and R6 growth stages, as described by Fehr and Caviness (1977), depending on the season. Because ASR infections initially occurs at the lower part of the canopy and then progress upward (Kumudini et al., 2008), the assessment of the five plants was divided into three canopy sections (top, middle and bottom), with nearly equal numbers of nodes (Kawuki et al., 2004). In each canopy section, the area of leaf surface occupied with rust lesions was assessed using a scale of 1-9, as proposed by Subrahmanyam et al. (1995), as presented in Table 3.3. The mean leaf severity of the three canopy sections was computed to represent disease severity of the individual plants. For proper phenotypic classification of genotypes, rust severity scores were further grouped into 1= immune; 2.0-3.9 = resistant; 4.0-5.9 = moderately resistance; 6.0-7.9 = moderately susceptible and 8.0-9.0 = highly susceptible (Milena and Vello, 2010), when rated at the R6 growth stage. The R6 growth stage was used for phenotypic classification because it coincides with the period when soybean plants are severely infected (Kawuki et al., 2004). In addition, clear differences between susceptible and resistant genotypes are observed at this growth stage, thereby indicating the overall susceptibility or resistance level of a genotype.

Table 3.3: Description of the 9 point field scale for rust assessment

Score	Description	Disease severity (%) <sup>1</sup>
1	No disease symptoms	0
2	Lesions sparsely distributed, mainly on lower leaves	1-5
3	Many lesions on lower leaves, necrosis evident; very few lesions on the middle leaves	6-10
4	Many lesions on both lower and middle leaves; severe necrosis present on the lower leaves	11-20
5	Severe necrosis on the lower and middle leaves; few lesions present on top leaves, but not severe	21-30
6	Severe infection on the lower leaves; necrosis evident on middle leaves, with many lesions; lesions present on top leaves	31-40
7	Severe infection on lower and middle leaves; many lesions distributed on the top leaves	41-60
8	100% infection on the lower and middle leaves; lesions on the top leaves with severe necrosis	61-80
9	Almost all leaves withered with bare stems observed	81-100

1. Percentage of the leaf area infected by the disease

### 3.2.5.2 ASR reaction type

ASR resistance reaction type was recorded as immune (no visible infection), red brown lesions (no urediniospores or only few sporulating urediniospores), tan lesions (tan lesions with many urediniospores and prolific sporulation) or a mixture (a mixture of red brown and tan lesions on the same plant) (Bonde et al., 2006; Li, 2009b; Pham et al., 2009).

### 3.2.5.3 ASR sporulation

ASR sporulation levels were scored using a scale of 1-5 as described by Miles et al. (2008) where: 1 = no sporulation;

2 = Less than 25% of fully sporulating lesions;

3 = 26% to 50% of fully sporulating lesions;

4 = 51% to 75 % of fully sporulating lesions;

5 = Fully sporulating TAN lesions.

Days-to-maturity was recorded as the number of days from germination to 75% pods maturity. Plant height was measured as the distance in metres from the ground to the top of the main stem using a meter ruler. After harvesting, grain yields (kg ha<sup>-1</sup>) were estimated using the whole plot. 100 seeds were counted and their weight recorded in grams. Soybean seeds were later

analysed for protein and oil content using a Near-Infrared Spectroscopy system in ABSTCM limited laboratory<sup>4</sup>.

### 3.2.6 Data analysis

Data was subjected to analysis of variance using Genstat statistical package (12<sup>th</sup> edition) (Payne et al., 2009) for all the traits.

Area under disease progress curve (AUDPC) values were calculated using the formula below as presented by Kumudini et al. (2008);

$$\text{AUDPC} = \sum_{i=1}^n \left[ \frac{X_i + X_{i+1}}{2} \right] (t_{i+1} - t_i)$$

Where,  $X_i$  = the disease severity score at the  $i^{\text{th}}$  observation;

$t_i$  = the time (day) at the  $i^{\text{th}}$  observation;

$t_{i+1} - t_i$  = the interval (days) between two consecutive assessments

$n$  = the number of assessments.

To compare genotypes across the seasons, AUDPC were standardized by dividing AUDPC values with the total duration of the disease in each season (Mohapatra et al., 2008). The duration of the disease epidemic was 34, 28, 42 and 30 days for the KARI-Embu long rain seasons of 2010 and 2011, and the cold season of 2011, and for the KARI-Mwea long rains of 2011, respectively.

Pearson's phenotypic correlation estimates for ASR rust severity, sporulation scores and AUDPC with grain yield, days to 75% maturity, plant height, 100 seed weight, oil and protein content were computed using Genstat statistical package (12<sup>th</sup> edition).

## 3.3 Results

### 3.3.1 Screen house evaluations

ASR severity, sporulation and infection type of different soybean genotypes evaluated in the screen house for 2 seasons at KARI-Embu Research Station are presented in Table 3.4. Based on rust severity and sporulation scores, highly significant variations ( $P \leq 0.001$ ) were observed among soybean genotypes. Mean scores for rust severity ranged from 4.3 to 9.0 while sporulation scores ranged between 1.0 and 4.5 across the seasons. Both rust severity ratings

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<sup>4</sup> African Breeders Service Total Cattle Management Limited (ABSTCM, Ltd). P.O. Box 76478, 00508, Yaya, Nairobi, Kenya. Ndama place, Kabarnet Rd, Nairobi.

and sporulation scores were variable in the two seasons with the highest scores recorded in the first season. In this evaluation there were no categories of immune or resistant genotypes. However, about 9% of the genotypes were moderately resistant with severity scores of less than 6.0. About 6.4% had moderate susceptible infections with mean rust score of 6.0 to 7.0 while more than 90% were highly susceptible with rust severity scores ranging between 8.0 and 9.0.

Table 3.4: Soybean rust severity scores, sporulation, lesion type and rust reaction of genotypes evaluated in the screen house for 2 seasons at KARI-Embu.

Genotype code	Rust severity (1-9 score)			Rust sporulation (1-5 score)			Lesion type	Rust reaction classification
	Season 1	Season 2	Mean scores	Season 1	Season 2	Mean scores		
G10428	4.67	4.00	4.33	1.00	1.00	1.00	RB	MR
MAK BLD11.3	4.33	4.33	4.33	1.00	1.00	1.00	RB	MR
G8586	4.00	5.00	4.50	1.00	1.33	1.17	RB	MR
GC0013829	5.67	4.33	5.00	2.00	1.33	1.67	RB	MR
G58	5.67	5.33	5.50	2.00	1.00	1.50	RB	MR
G7955	5.67	5.33	5.50	1.00	1.33	1.17	RB	MR
Namsoy4M	6.00	5.00	5.50	2.33	1.00	1.67	RB	MR
Tainung	6.00	5.00	5.50	2.33	1.33	1.83	RB	MR
PI200477A	5.67	6.00	5.83	2.33	2.00	2.17	RB	MR
UFV3	6.33	5.33	5.83	2.00	1.67	1.83	RB	MR
UG-5	6.00	7.33	6.67	2.33	1.67	2.00	TAN	MS
SB-19	8.00	5.67	6.83	3.33	1.00	2.17	TAN	MS
Dowling	7.00	7.33	7.17	3.67	3.00	3.33	TAN	MS
Maksoy1N	7.67	6.67	7.17	3.00	2.33	2.67	TAN	MS
EXKirinyaga	7.00	8.00	7.50	3.67	3.67	3.67	TAN	MS
GBK 029511	7.33	8.33	7.83	2.33	3.67	3.00	TAN	MS
SB-8	8.33	7.33	7.83	3.67	3.00	3.33	TAN	MS
GBK 029574	7.33	8.67	8.00	3.67	4.00	3.83	TAN	HS
GBK 033215	7.33	8.67	8.00	3.67	4.67	4.17	TAN	HS
SB-17	8.67	7.33	8.00	4.00	3.33	3.67	TAN	HS
GBK 029600	8.67	7.67	8.17	3.00	3.33	3.17	TAN	HS
911/6/3	7.33	9.00	8.17	3.67	4.33	4.00	TAN	HS
Maksoy-2N	8.33	8.00	8.17	4.00	3.33	3.67	TAN	HS
GBK 029570	8.33	8.33	8.33	4.67	4.00	4.33	TAN	HS
932/5/36	8.33	8.33	8.33	3.33	3.67	3.50	TAN	HS
SB-37	8.33	8.33	8.33	4.33	4.67	4.50	TAN	HS
GBK 029575	8.67	8.33	8.50	4.33	3.67	4.00	TAN	HS
GBK 029611	8.67	8.33	8.50	3.00	4.00	3.50	TAN	HS
GBK 029612	8.67	8.33	8.50	4.33	3.67	4.00	TAN	HS

GBK 029621	8.67	8.33	8.50	3.33	3.33	3.33	TAN	HS
GBK 033209	8.67	8.33	8.50	4.33	4.00	4.17	TAN	HS
GBK 033226	8.67	8.33	8.50	3.67	3.00	3.33	TAN	HS
GBK 033242	8.67	8.33	8.50	4.00	3.67	3.83	TAN	HS
GBK 033246	8.67	8.33	8.50	4.33	3.67	4.00	TAN	HS
GBK 033249	8.67	8.33	8.50	4.00	3.33	3.67	TAN	HS
GBK 033250	8.67	8.33	8.50	4.33	4.00	4.17	TAN	HS
GBK 033252	8.33	8.67	8.50	4.33	4.00	4.17	TAN	HS
GBK 033253	8.67	8.33	8.50	4.67	4.00	4.33	TAN	HS
GBK 033254	8.67	8.33	8.50	4.33	3.67	4.00	TAN	HS
GBK 033255	8.33	8.67	8.50	4.00	3.67	3.83	TAN	HS
GBK 045342	8.67	8.33	8.50	4.67	3.67	4.17	TAN	HS
BlackHawk	8.33	8.67	8.50	4.00	4.67	4.33	TAN	HS
BRSSambaiba	8.67	8.33	8.50	4.33	3.67	4.00	TAN	HS
Kissi	8.67	8.33	8.50	4.33	4.00	4.17	TAN	HS
SB-20	8.67	8.33	8.50	4.33	3.67	4.00	TAN	HS
GBK 028397A	8.67	8.67	8.67	4.33	3.67	4.00	TAN	HS
GBK 029573	8.67	8.67	8.67	3.00	4.67	3.83	TAN	HS
GBK 029577	8.67	8.67	8.67	3.67	3.67	3.67	TAN	HS
GBK 029610	8.67	8.67	8.67	4.00	3.67	3.83	TAN	HS
GBK 029614	8.67	8.67	8.67	4.33	3.67	4.00	TAN	HS
GBK 033204	9.00	8.33	8.67	4.33	3.67	4.00	TAN	HS
GBK 033208	8.67	8.67	8.67	3.00	3.00	3.00	TAN	HS
GBK 033210	8.67	8.67	8.67	4.33	3.67	4.00	TAN	HS
GBK 033213	8.67	8.67	8.67	4.00	4.33	4.17	TAN	HS
GBK 033217	8.67	8.67	8.67	2.67	4.33	3.50	TAN	HS
GBK 033221	8.67	8.67	8.67	4.00	3.67	3.83	TAN	HS
GBK 033222	9.00	8.33	8.67	4.67	3.67	4.17	TAN	HS
GBK 033223	8.67	8.67	8.67	4.33	4.00	4.17	TAN	HS
GBK 033224	8.67	8.67	8.67	4.33	4.67	4.50	TAN	HS
GBK 033229	8.67	8.67	8.67	4.67	3.67	4.17	TAN	HS
GBK 033230	8.67	8.67	8.67	4.67	3.33	4.00	TAN	HS
GBK 033231	8.33	9.00	8.67	4.67	4.33	4.50	TAN	HS
GBK 033234	8.67	8.67	8.67	4.33	3.67	4.00	TAN	HS
GBK 033236	8.67	8.67	8.67	4.33	3.67	4.00	TAN	HS
GBK 033243	8.67	8.67	8.67	4.33	3.67	4.00	TAN	HS
GBK 033245	8.67	8.67	8.67	3.33	3.67	3.50	TAN	HS
GBK 033251	8.67	8.67	8.67	4.67	4.67	4.67	TAN	HS
GBK 033257	8.67	8.67	8.67	4.33	3.67	4.00	TAN	HS
835/5/30	8.67	8.67	8.67	4.67	5.00	4.83	TAN	HS
916/5/19	8.67	8.67	8.67	5.67	4.67	5.17	TAN	HS
BRS217Flora	8.67	8.67	8.67	4.67	4.33	4.50	TAN	HS
Duicker	8.67	8.67	8.67	4.33	4.00	4.17	TAN	HS

EAI 3600	8.67	8.67	8.67	3.00	4.67	3.83	TAN	HS
Sable	8.67	8.67	8.67	4.33	4.33	4.33	TAN	HS
SB-4	8.33	9.00	8.67	4.00	3.67	3.83	TAN	HS
GBK 010246	9.00	8.67	8.83	3.67	3.67	3.67	TAN	HS
GBK 029571	9.00	8.67	8.83	3.33	4.00	3.67	TAN	HS
GBK 029616	9.00	8.67	8.83	4.33	4.33	4.33	TAN	HS
GBK 029617	9.00	8.67	8.83	4.33	4.00	4.17	TAN	HS
GBK 029620	8.67	9.00	8.83	4.67	4.33	4.50	TAN	HS
GBK 029622	8.67	9.00	8.83	4.00	4.00	4.00	TAN	HS
GBK 033203	8.67	9.00	8.83	4.33	4.00	4.17	TAN	HS
GBK 033205	8.67	9.00	8.83	4.00	4.00	4.00	TAN	HS
GBK 033206	8.67	9.00	8.83	4.33	4.00	4.17	TAN	HS
GBK 033207	8.67	9.00	8.83	4.33	4.67	4.50	TAN	HS
GBK 033211	8.67	9.00	8.83	4.00	4.00	4.00	TAN	HS
GBK 033214	8.67	9.00	8.83	4.33	4.00	4.17	TAN	HS
GBK 033218	8.67	9.00	8.83	4.33	3.67	4.00	TAN	HS
GBK 033220	9.00	8.67	8.83	3.33	4.00	3.67	TAN	HS
GBK 033225	8.67	9.00	8.83	4.00	4.00	4.00	TAN	HS
GBK 033233	8.67	9.00	8.83	4.33	4.33	4.33	TAN	HS
GBK 033237	8.67	9.00	8.83	4.67	4.33	4.50	TAN	HS
GBK 033247	9.00	8.67	8.83	4.33	3.67	4.00	TAN	HS
GBK 033248	9.00	8.67	8.83	4.67	4.00	4.33	TAN	HS
915/5/12	8.67	9.00	8.83	4.00	5.00	4.50	TAN	HS
917/5/16	8.67	9.00	8.83	4.33	3.67	4.00	TAN	HS
931/5/34	8.67	9.00	8.83	4.00	3.67	3.83	TAN	HS
Bossier	8.67	9.00	8.83	4.33	4.67	4.50	TAN	HS
BRS MG46	8.67	9.00	8.83	4.00	4.67	4.33	TAN	HS
Ex-Japan	8.67	9.00	8.83	4.33	4.67	4.50	TAN	HS
FH-1	8.67	9.00	8.83	4.33	4.67	4.50	TAN	HS
Gazelle	9.00	8.67	8.83	4.33	4.33	4.33	TAN	HS
Hill	8.67	9.00	8.83	3.33	4.00	3.67	TAN	HS
GBK 033277	8.67	9.00	8.83	3.00	4.33	3.67	TAN	HS
GBK 028397B	9.00	9.00	9.00	4.00	4.67	4.33	TAN	HS
GBK 033232	9.00	9.00	9.00	4.33	3.67	4.00	TAN	HS
GBK 033241	9.00	9.00	9.00	5.00	4.00	4.50	TAN	HS
LS6161RR	9.00	9.00	9.00	4.00	4.00	4.00	TAN	HS
Nyala	9.00	9.00	9.00	4.33	3.67	4.00	TAN	HS
SCS-1	9.00	9.00	9.00	4.33	4.67	4.50	TAN	HS
mean	8.27	8.24	8.26	3.84	3.66	3.75		
Sed	0.80	0.49	0.07	0.92	0.66	0.08		
CV (%)	11.80	7.30	10.40	29.20	22.20	26.30		

MR is moderately resistant; MS is moderately susceptible; HS is highly susceptible; RB is red brown lesions; and TAN is tan reactions.

Soybean genotypes differed in their reactions to ASR. Plant introductions G10428 (*Rpp4*) and G8586 (*Rpp2*) exhibited moderate levels of resistance with rust severity scores of 4.3 and 4.5, respectively. MAK BLD 11.3 also had a rust score of 4.3. Plant introductions G7955 (*Rpp3*), G58 (*Rpp1*) and Tainung 4 (*Rpp1*) all had a moderate rust severity score of 5.5. Among the moderate resistant lines previously evaluated in Uganda and South Africa, GC 00138-29, PI 200477A, UFV3 and Namsoy 4M had rust severities ranging between 5.0 and 5.8. On the other hand, genotypes UG-5, Maksoy 1N, Maksoy 2N had high rust severity scores ranging between 6.7 and 8.2. The highest disease severity scores of 7.0 and above were observed mainly on genebank genotypes, advanced lines and commercial varieties, as well as landrace collections from farmers' fields. Commercial variety SCS-1 and LS6161RR from South Africa had the highest rust severity score (9.0), similar to the susceptible check variety Nyala.

It was also observed that genotypes with low rust severity scores had red brown reactions with low sporulation levels, while those with high rust severities had tan reactions with higher levels of spore production. For example, genotypes G10428 and G8586 with *Rpp4* and *Rpp2*, respectively had low rust severity scores that were associated with dark red brown lesions. These genotypes had low sporulation rates ranging from 1.0 to 1.2. Other genotypes with moderate rust severity scores ranging between 4.0 to 5.8 including G58 (*Rpp1*), G7955 (*Rpp3*), MAK BLD 11.3, GC 00138-29, UFV3, PI 200477A and Namsoy 4M expressed red brown lesions that differed in colouration, with sparsely sporulating uredinia with scores of less than 3. However, most of the commercial varieties, genebank genotypes and advanced lines developed higher levels of rust severity, which were related to high occurrence of tan reactions. In addition, these genotypes recorded the highest levels of sporulation with scores ranging from 3.0 to 4.5.

### 3.3.2 Field evaluations

Table 3.5 presents the analysis of variance for the rust severity and sporulation scores at different stages of soybean development (R2-R6 stage), and the AUDPC values. The Wald statistic for rust severity, sporulation scores and AUDPC values were highly significant ( $P \leq 0.001$ ) for genotypes and seasons at all three growth stages. The genotype x season interaction was also highly significant ( $P \leq 0.001$ ) for the rust severity scores, sporulation scores and AUDPC values when the data was combined.

Table 3.5: Combined analysis of variance for soybean rust severity and sporulation at different growth stages and AUDPC values

Source of variation	d.f.	Rust severity scores (1-9 score)			Rust sporulation scores (1-5 score)			AUDPC
		R2 stage	R4 stage	R6 stage	R2 stage	R4 stage	R6 stage	
Rep	2	11.623	8.339	1.523	6.2644	22.38	0.805	9.09
Variety	109	11.124***	16.549***	21.077***	2.8681***	7.179***	6.8**	14.90***
Season	3	506.544***	734.667***	565.9***	242.9846***	233.096***	159.742***	295.75***
Genotype x Season	327	1.824***	2.269***	1.922***	1.0055***	2.167***	2.442	1.68***
Residual	878	1.022	1.228	1.255	0.5294	1.151	2.238	1.00
Total	1319							

R2 =full bloom stage, R4 =full pod stage and R6 = full seed stage; AUDPC = Area under disease progress curve; \*\*\* and \* indicates significant at  $P \leq 0.001$  and  $P \leq 0.05$ , respectively.

### 3.3.2.1 Rust severity progress at different growth stages of soybeans

Rust severity scores were assessed at different growth stages of R1 (beginning bloom), R2 (full bloom), R4 (full pod) and R6 (full seed) among soybean genotypes at KARI-Embu Research station during the cold season of 2011. During the evaluations there were no rust symptoms at vegetative growth stages. However, clear rust symptoms were observed at the R1 stage but with relatively low severity scores ranging between 2.0 and 4.0 (Figure 3.1). At the R2 stage highly significant differences were noted among the genotypes with average rust scores ranging from 2.0-6.0. A sudden increase in rust severity scores was noted from the R2 to R4 stages, up to R6, especially for the susceptible genotypes. The highest rust severity scores (9.0) were recorded for the commercial varieties Nyala and Sable, while G8586 and GC 00138-29 developed the lowest rust severity scores at R6 growth stage.



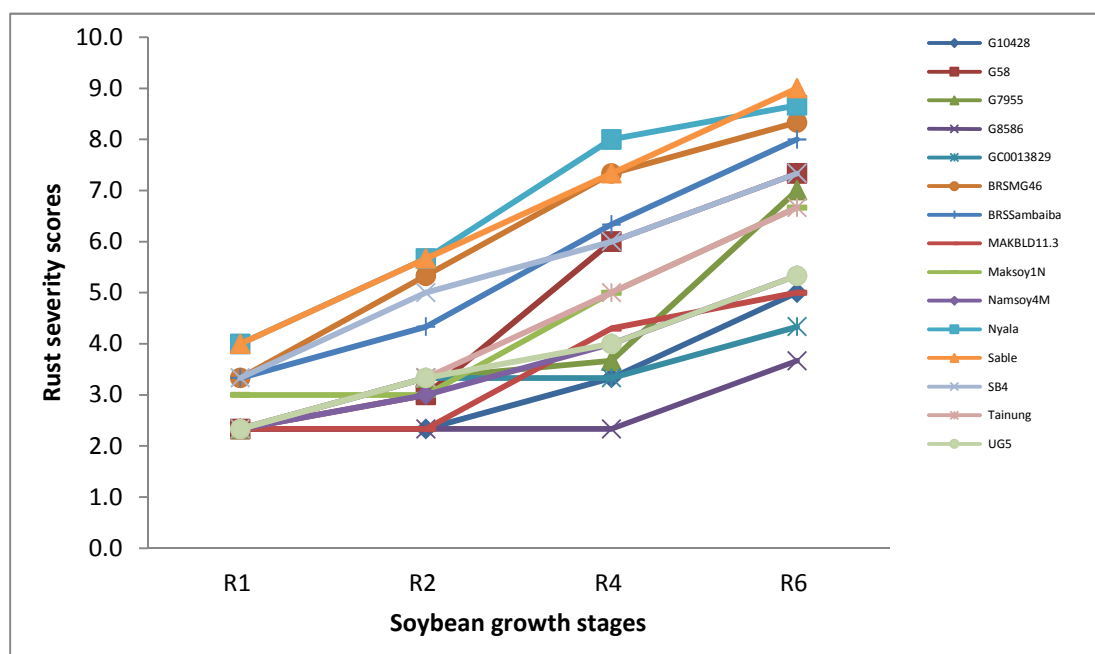


Figure 3.1: Rust severity recorded at different growth stages for selected soybean genotypes representing range of values observed at KARI-Embu Research Station during the cold season of 2011. R1=beginning bloom stage, R2 =full bloom stage, R4 =full pod stage and R6 = full seed stage.

### 3.3.2.2 Reaction of soybean genotypes to rust severity

From the results, only the mean rust severity scores at the R6 stage were presented in different locations and seasons because clear differences between resistant and susceptible genotypes were observed at this stage (Table 3.6). Highly significant variations ( $P \leq 0.001$ ) among different genotypes were observed for rust severity. Generally, rust severity scores in the field were slightly lower ranging from 2.7 to 8.3, compared to mean rust severity scores in the screenhouse, which were between 4.3 and 9.0, and provided a different classification of rust resistance for the same soybean varieties. Unlike the results from the screenhouse, field evaluations clearly defined genotypes into resistant, moderately resistant, moderately susceptible and highly susceptible. No immune rust reaction type was reported in this study (Figure 3.2). From the 110 genotypes; 5 were resistant, 9 were moderately resistant, and the rest were moderately to highly susceptible, when averaged across locations and seasons.

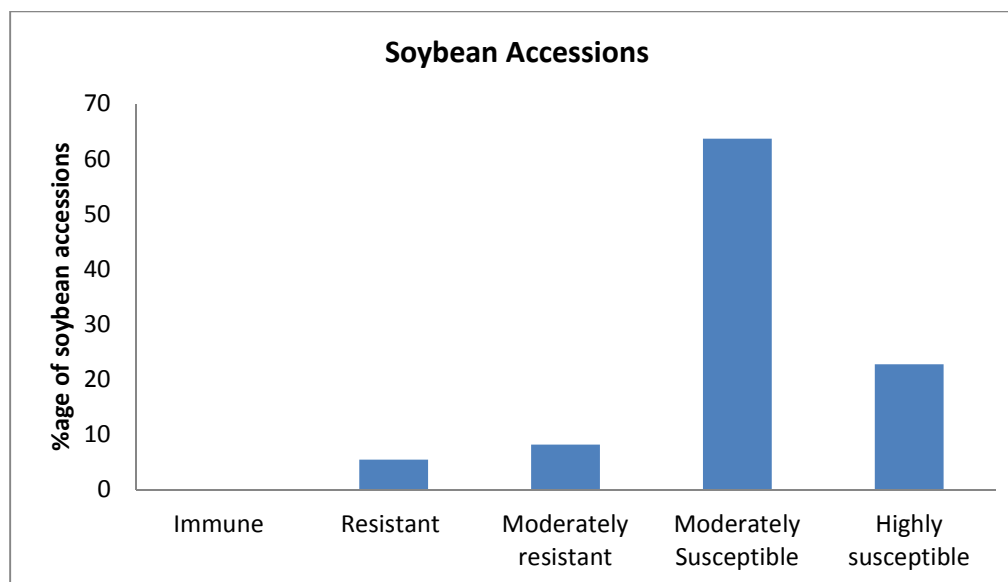


Fig 3.2: Rust severity frequency distribution of the 110 soybean accessions evaluated in the field.

Soybean genotypes known to have single resistance genes (*Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4*), unknown genes or genes for tolerance had differential responses to ASR. For instance, genotype G10428 (*Rpp4*) developed the lowest level of rust severity (2.7) and was classified as the most resistant. It was closely followed by genotype G8586 (*Rpp2*) with a rust severity score of 3.0, which was also classified as resistant. However, genotype G7955 (*Rpp3*), G58 (*Rpp1*) and Tainung 4 (*Rpp1*) had moderate rust severity scores of 4.8, 5.3 and 4.4, respectively. A few genotypes that were previously reported as tolerant in Uganda were classified as resistant in this study. These were MAK BLD 11.3 (2.7), Namsoy 4M (3.5) and GC 00138-29 (3.6). Other tolerant genotypes including UG-5, UFV3, Maksoy 1N, PI 200477A and Dowling exhibited moderate severity scores. Maksoy 2N was classified as moderately susceptible with a mean rust score of 6.0.

Among the advanced lines, BRS Sambaimba exhibited the lowest rust severity score of 5.3 and was consequently classified as moderately resistant. However, all the other advanced lines had higher rust scores, ranging from 6.5 to 8.1, and were grouped as moderately to highly susceptible. Similarly, all the commercial varieties, genebank genotypes and accessions from farmer's fields had higher rust severity scores ranging between 6.8 and 8.3. Genebank genotypes GBK 028397A, GBK 029620, GBK 033203 and GBK 033233 had the highest rust severity mean score of 8.3, higher than the susceptible check, variety Nyala (8.0).

Rust severity scores also varied significantly ( $P \leq 0.001$ ) across the locations and seasons. At Igoji, no ASR symptoms were observed in all the seasons, hence there were no reports on the disease. Similarly, at both KARI-Mwea and KARI-Embu Research Stations there were no incidences of rust infection during the short rains of 2010. However, clear rust symptoms were observed during the long rains of 2010 and 2011 and in the cold season of 2011 at KARI-Embu, and during the long rains of 2011 at KARI-Mwea. KARI-Embu recorded higher levels of rust severity (7.4) compared to KARI-Mwea (5.3) when data was collected for the same set of genotypes during the long rains of 2011. Within seasons, rust severity also varied significantly ( $P \leq 0.01$ ) at KARI-Embu. Considerably higher rust severity scores were recorded during the cold season of 2011, with a mean value of 8.4, compared to rust severity means of 7.5 and 7.4 recorded during long rains of 2010 and 2011, respectively.

Based on rust severities, the performance of soybean genotypes across the locations differed significantly ( $P \leq 0.001$ ). For instance, genotypes GC 00138-29, BRS Sambaiba, Dowling and Tainung 4 were scored as resistant at KARI-Mwea but moderately resistant at KARI-Embu when scoring was done at the same phenological stages during the long rain season of 2011. Other genotypes including Maksoy 2N, BRS 217 Flora, GBK 033243, a few advanced lines and commercial varieties were scored as moderately susceptible and highly susceptible at KARI-Embu, but were scored as resistant or moderately resistant at KARI-Mwea. A few genotypes including G10428, G8586, G58, Maksoy 1N, Namsoy 4M and MAK BLD 11.3 consistently maintained low levels of rust severity across the locations.

### **3.3.2.3 AUDPC values**

The mean AUDPC values significantly varied among soybean genotypes (Table 3.6). Genotypes with low rust severity scores consistently had the lowest AUDPC values, while those with high rust severity had high AUDPC values. Both resistant and moderately resistant genotypes recorded relatively low AUDPC values ranging from 2.12 to 3.69. High mean AUDPC values ranging from 4.12 to 6.67 were observed for moderately susceptible and highly susceptible genotypes.

Table 3.6: Soybean mean rust severity scores (1-9 score), rust reaction classification and AUDPC values for 3 seasons at KARI-Embu and 1 season at KARI-Mwea research station.

Genotype code	KARI-Embu 2010	Rust reaction	KARI-Embu 2011	Rust reaction	KARI-Embu cold season	Rust reaction	KARI-Mwea 2011	Rust reaction	mean score	Average Rust reaction	Combined AUDPC
MAK BLD 11.3	2.33	R	2.00	R	4.33	MR	2.00	R	2.67	R	2.12
Namsoy 4M	3.33	R	3.00	R	5.33	MR	2.33	R	3.50	R	2.75
G 10428	3.67	R	1.00	R	5.00	MR	1.00	R	2.67	R	2.16
G 8586	3.67	R	2.67	R	3.67	R	2.00	R	3.00	R	2.35
GC 00138-29	3.67	R	4.00	MR	4.33	MR	2.33	R	3.58	R	3.01
SB-19	3.67	R	3.33	R	5.67	MR	2.00	R	3.67	R	2.84
Tainung	3.67	R	5.00	MR	6.67	MS	2.33	R	4.42	MR	3.20
UFV3	4.00	MR	5.33	MR	6.00	MS	2.33	R	4.42	MR	3.24
G 7955	4.67	MR	4.00	MR	7.00	MS	3.67	MR	4.83	MR	3.69
PI 200477A	4.67	MR	3.00	R	8.67	HS	3.33	R	4.92	MR	3.69
Maksoy 1N	5.00	MR	3.67	R	6.67	MS	3.00	R	4.58	MR	3.18
SB-17	5.33	MR	5.33	MR	8.67	HS	5.33	MR	6.17	MS	4.57
EX-Kirinyaga	5.67	MR	6.33	HMS	8.33	HS	5.67	MR	6.50	MS	4.69
SB-8	5.67	MR	6.67	MS	8.00	HS	5.33	MR	6.42	MS	4.90
UG 5	5.67	MR	3.33	R	5.33	MR	2.00	R	4.08	MR	3.11
G 58	6.33	MS	3.33	R	7.33	MS	4.00	MR	5.25	MR	3.75
GBK 029600	6.67	MS	9.00	HS	9.00	HS	6.67	MS	7.83	MS	6.51
GBK 029612	7.00	MS	7.00	MS	8.33	HS	6.67	MS	7.25	MS	5.61
GBK 033204	7.00	MS	9.00	HS	9.00	HS	5.67	MR	7.67	MS	6.24
GBK 033209	7.00	MS	8.67	HS	8.67	HS	6.00	MS	7.58	MS	5.89
GBK 033222	7.00	MS	6.33	MS	9.00	HS	6.67	MS	7.25	MS	5.66
GBK 033242	7.00	MS	9.00	HS	9.00	HS	6.33	MS	7.83	MS	6.32
GBK 033246	7.00	MS	9.00	HS	9.00	HS	6.67	MS	7.92	MS	6.29
GBK 045342	7.00	MS	7.67	MS	8.67	HS	4.00	MR	6.83	MS	4.92
932/5/36 BRS	7.00	MS	6.67	MS	9.00	HS	4.33	MR	6.75	MS	4.49
Sambaiba	7.00	MS	4.33	MR	8.00	HS	2.00	R	5.33	MR	4.12
Dowling	7.00	MS	4.00	MR	8.67	HS	2.33	R	5.50	MR	3.96
SB-20	7.00	MS	8.00	HS	8.00	HS	3.00	R	6.50	MS	4.61
GBK 010246	7.33	MS	6.33	MS	9.00	HS	3.33	MR	6.50	MS	5.20
GBK 029511	7.33	MS	9.00	HS	9.00	HS	6.33	MS	7.92	MS	6.54
GBK 029575	7.33	MS	8.00	HS	8.33	HS	6.00	MS	7.42	MS	5.71
GBK 029611	7.33	MS	8.67	HS	9.00	HS	5.33	MR	7.58	MS	6.07
GBK 029621	7.33	MS	8.67	HS	9.00	HS	5.33	MR	7.58	MS	5.94
GBK 033208	7.33	MS	9.00	HS	8.67	HS	5.67	MR	7.67	MS	5.62
GBK 033221	7.33	MS	5.33	MS	9.00	HS	5.67	MR	6.83	MS	5.15
GBK 033226	7.33	MS	6.67	MS	8.67	HS	7.00	MS	7.42	MS	5.51

GBK 033249	7.33	MS	9.00	HS	9.00	HS	5.67	MR	7.75	MS	6.51
GBK 033253	7.33	MS	8.00	HS	9.00	HS	3.00	R	6.83	MS	5.20
EAI 3600	7.33	MS	8.33	HS	9.00	HS	5.33	MR	7.50	MS	5.63
GBK 029570	7.67	MS	8.00	HS	9.00	HS	4.67	MR	7.33	MS	5.81
GBK 029571	7.67	MS	9.00	HS	8.67	HS	6.33	MS	7.92	MS	6.55
GBK 029577	7.67	MS	8.00	HS	8.67	HS	5.67	MR	7.50	MS	5.37
GBK 033213	7.67	MS	9.00	HS	9.00	HS	6.33	MS	8.00	HS	6.37
GBK 033220	7.67	MS	9.00	HS	8.33	HS	6.67	MS	7.92	MS	6.49
GBK 033223	7.67	MS	9.00	HS	8.00	HS	6.00	MS	7.67	MS	6.23
GBK 033234	7.67	MS	7.33	MS	8.67	HS	6.33	MS	7.50	MS	6.07
GBK 033248	7.67	MS	8.33	HS	9.00	HS	5.00	MR	7.50	MS	5.52
GBK 033250	7.67	MS	9.00	HS	9.00	HS	6.00	MS	7.92	MS	6.32
GBK 033251	7.67	MS	8.33	HS	8.33	HS	6.33	MS	7.67	MS	5.75
GBK 033254	7.67	MS	8.67	HS	9.00	HS	7.00	MS	8.08	HS	6.60
GBK 033255	7.67	MS	7.00	MS	8.67	HS	6.00	MS	7.33	MS	5.57
BRS-217-Flora	7.67	MS	6.67	MS	9.00	HS	2.67	R	6.50	MS	5.10
Gazelle	7.67	MS	8.33	HS	8.67	HS	5.33	MR	7.50	MS	5.70
Kissi	7.67	MS	7.00	MS	8.67	HS	6.00	MS	7.33	MS	5.60
Maksoy 2N	7.67	MS	4.67	MS	9.00	HS	2.67	R	6.00	MS	3.90
SB-37	7.67	MS	8.00	HS	9.00	HS	5.33	MR	7.50	MS	5.65
GBK 029573	8.00	HS	8.33	HS	9.00	HS	6.33	MS	7.92	MS	6.27
GBK 029574	8.00	HS	7.67	MS	9.00	HS	6.67	MS	7.83	MS	6.28
GBK 029610	8.00	HS	7.33	MS	8.67	HS	7.00	MS	7.75	MS	6.34
GBK 029614	8.00	HS	8.00	HS	8.67	HS	3.67	R	7.08	MS	5.53
GBK 029616	8.00	HS	9.00	HS	9.00	HS	6.33	MS	8.08	HS	6.58
GBK 029617	8.00	HS	9.00	HS	9.00	HS	6.00	MS	8.00	HS	6.54
GBK 033210	8.00	HS	8.33	HS	9.00	HS	5.33	MR	7.67	MS	5.93
GBK 033215	8.00	HS	6.67	MS	9.00	HS	6.33	MS	7.50	MS	5.92
GBK 033224	8.00	HS	7.00	MS	9.00	HS	6.67	MS	7.67	MS	5.89
GBK 033229	8.00	HS	8.00	HS	9.00	HS	5.00	MR	7.50	MS	5.60
GBK 033232	8.00	HS	8.00	HS	8.33	HS	6.33	MS	7.67	MS	5.86
GBK 033243	8.00	HS	7.00	MS	9.00	HS	6.33	MS	7.58	MS	6.33
GBK 033245	8.00	HS	8.67	HS	8.67	HS	6.67	MS	8.00	HS	6.18
GBK 033247	8.00	HS	9.00	HS	9.00	HS	6.33	MS	8.08	HS	6.59
BlackHawk	8.00	HS	8.67	HS	9.00	HS	7.00	MS	8.17	HS	6.65
Duicker	8.00	HS	6.33	MS	8.33	HS	4.67	MR	6.83	MS	4.85
SB-4	8.00	HS	5.00	MR	7.33	MS	4.67	MR	6.25	MS	4.51
GBK 028397A	8.33	HS	8.67	HS	9.00	HS	7.00	MS	8.25	HS	6.53
GBK 029622	8.33	HS	9.00	HS	8.33	HS	6.00	MS	7.92	MS	6.39
GBK 033206	8.33	HS	8.33	HS	8.33	HS	6.33	MS	7.83	MS	6.04
GBK 033217	8.33	HS	9.00	HS	9.00	HS	4.67	MR	7.75	MS	6.16
GBK 033218	8.33	HS	8.67	HS	9.00	HS	6.67	MS	8.17	HS	6.76
GBK 033230	8.33	HS	8.67	HS	9.00	HS	6.33	MS	8.08	HS	6.03

GBK 033233	8.33	HS	9.00	HS	8.67	HS	7.00	MS	8.25	HS	6.68
GBK 033236	8.33	HS	9.00	HS	9.00	HS	6.33	MS	8.17	HS	6.53
GBK 033237	8.33	HS	7.33	MS	8.67	HS	7.00	MS	7.83	MS	6.42
GBK 033252	8.33	HS	8.67	HS	9.00	HS	5.33	MR	7.83	MS	6.09
GBK 033257	8.33	HS	9.00	HS	9.00	HS	6.33	MS	8.17	HS	6.65
835/5/30	8.33	HS	8.33	HS	9.00	HS	6.33	MS	8.00	HS	5.98
916/5/19	8.33	HS	8.00	HS	9.00	HS	5.67	MR	7.75	MS	5.76
BRS MG 46	8.33	HS	8.00	HS	8.33	HS	4.67	MR	7.33	MS	5.21
Ex-Japan	8.33	HS	7.33	HS	9.00	HS	6.00	MS	7.67	MS	5.72
Hill	8.33	HS	9.00	HS	9.00	HS	6.33	MS	8.17	HS	6.43
Sable	8.33	HS	8.33	HS	9.00	HS	5.00	MR	7.67	MS	5.47
GBK 028397B	8.67	HS	9.00	HS	9.00	HS	6.00	MS	8.17	HS	6.82
GBK 029620	8.67	HS	8.67	HS	9.00	HS	6.67	MS	8.25	HS	6.67
GBK 033203	8.67	HS	9.00	HS	9.00	HS	6.33	MS	8.25	HS	6.89
GBK 033207	8.67	HS	7.00	MS	9.00	HS	6.00	MS	7.67	MS	6.14
GBK 033211	8.67	HS	7.67	MS	8.67	HS	5.00	MR	7.50	MS	5.45
GBK 033214	8.67	HS	8.67	HS	8.67	HS	6.33	MS	8.08	HS	6.34
GBK 033231	8.67	HS	8.67	HS	9.00	HS	5.67	MR	8.00	HS	6.25
GBK 033241	8.67	HS	7.00	MS	8.33	HS	7.00	MS	7.75	MS	6.10
911/6/3	8.67	HS	7.67	MS	9.00	HS	5.67	MR	7.75	MS	5.94
LS6161RR	8.67	HS	8.00	HS	9.00	HS	5.67	MR	7.83	MS	5.71
SCS-1	8.67	HS	6.33	MS	8.67	HS	5.67	MR	7.33	MS	5.32
GBK 033205	9.00	HS	7.67	MS	9.00	HS	6.33	MS	8.00	HS	6.30
GBK 033225	9.00	HS	8.00	HS	8.67	HS	6.67	MS	8.08	HS	6.28
915/5/12	9.00	HS	8.00	HS	9.00	HS	4.67	MR	7.67	MS	5.74
917/5/16	9.00	HS	8.33	HS	9.00	HS	6.00	MS	8.08	HS	6.38
931/5/34	9.00	HS	7.67	MS	9.00	HS	5.33	MR	7.75	MS	5.78
Bossier	9.00	HS	7.00	MS	8.33	HS	5.00	MR	7.33	MS	5.56
FH-1	9.00	HS	8.00	HS	8.67	HS	6.00	MS	7.92	MS	6.00
Nyala	9.00	HS	8.00	HS	8.67	HS	6.33	MS	8.00	HS	6.21
GBK 033277	9.00	HS	9.00	HS	9.00	HS	6.00	MS	8.25	HS	6.32
Mean	7.452		7.352		8.433		5.315		7.14		5.53
Sed	0.94		1.10		0.50		0.81		0.09		0.43
CV (%)	15.40		18.40		7.30		18.60		15.70		18.00

R is resistant; MR is moderately resistant; MS is moderately susceptible; and HS is highly susceptible. AUDPC is area under disease progress curve.

### 3.3.2.4 Rust sporulation and rust reaction type

Rust sporulation differed significantly ( $P \leq 0.001$ ) among soybean genotypes, ranging from 1.0 to 4.4 across the seasons (Table 3.7). Significantly variable sporulation scores were also observed

in different seasons. The highest mean sporulation scores were recorded during the cold season of 2011 (3.67), followed by the long rain season of 2010 (3.48) while the long rain season of 2011 had the lowest values (2.32) at KARI-Embu. At KARI-Mwea mean sporulation scores were 2.45. Generally, genotypes with low rust severities had red brown lesions with low levels of sporulation. Namsoy 4M and MAK BLD 11.3 produced red brown lesions without sporulating uredinia. Similarly, genotypes UFV3, G10428, G8586, GC 00138-29, UG-5, Tainung and Maksoy 1N recorded low sporulation levels ranging from 1.3 to 1.8 with red brown lesions which differed in colour from light brown to dark brown lesions. On the other hand, plant introductions G7955, G58, variety Maksoy 2N and a few advanced lines, commercial varieties and genebank genotypes produced tan lesions with sparsely sporulating uredinia between 2.0 and 3.0. The other advanced lines, commercial varieties, genebank genotypes and accessions from farmers' fields had abundant sporulation, with scores ranging from 3.0 to 4.4, with a tan reaction type. The highest sporulation score (4.4) was recorded on the check variety Nyala. Two genotypes (PI 200477A and Dowling) exhibited mixed lesions with both red brown lesions and tan lesions observed on the same genotype.

Table 3.7: Soybean genotypes showing mean rust sporulation at KARI-Embu and KARI-Mwea, and lesion infection type.

Genotype code	Mean sporulation scores (1-5 scores)					Lesion type
	KARI-Embu 2010	KARI-Embu 2011	KARI-Embu cold season	KARI-Mwea 2011	Mean score	
MAK BLD 11.3	1.00	1.00	1.00	1.00	1.00	RB
Namsoy 4M	1.00	1.00	1.00	1.00	1.00	RB
SB-19	1.00	1.00	1.00	1.00	1.00	RB
Tainung	1.33	1.00	2.00	1.00	1.33	RB
UFV3	1.33	1.00	1.00	1.00	1.08	RB
GBK 029610	2.00	1.00	4.67	2.67	2.58	TAN
GBK 033210	2.00	1.00	4.67	2.67	2.58	TAN
GBK 033207	2.33	1.00	5.00	3.00	2.83	TAN
GBK 033217	2.33	1.00	2.00	2.33	1.92	RB
915/5/12	2.33	1.00	4.67	3.00	2.75	TAN
G 10428	2.33	1.00	1.00	1.00	1.33	RB
G 8586	2.33	1.00	1.00	1.00	1.33	RB
GC 00138-29	2.33	1.00	1.00	1.00	1.33	RB
Dowling	2.67	1.00	4.67	1.00	2.33	MIX
G 7955	2.67	1.00	3.67	2.00	2.33	TAN
Maksoy 1N	2.67	1.00	2.00	1.33	1.75	RB
GBK 033234	3.33	1.00	4.67	3.00	3.00	TAN

GBK 033249	3.33	1.00	2.33	2.67	2.33	TAN
UG 5	3.33	1.00	1.00	1.00	1.58	RB
GBK 033233	3.67	1.00	5.00	2.33	3.00	TAN
GBK 033247	3.67	1.00	3.00	2.33	2.50	TAN
GBK 033250	3.67	1.00	3.33	2.00	2.50	TAN
Ex-Japan	3.67	1.00	4.67	3.67	3.25	TAN
GBK 033277	3.67	1.00	2.00	3.00	2.42	TAN
G 58	4.00	1.00	3.00	1.67	2.42	TAN
GBK 010246	4.33	1.00	4.00	1.67	2.75	TAN
GBK 029600	4.33	1.00	2.33	3.33	2.75	TAN
GBK 033242	5.67	1.00	4.67	3.00	3.58	TAN
GBK 029614	3.33	1.33	4.67	1.67	2.75	TAN
GBK 029511	4.67	1.33	2.33	2.67	2.75	TAN
EAI 3600	4.67	1.33	2.00	2.67	2.67	TAN
GBK 033243	2.00	1.67	4.67	2.33	2.67	TAN
GBK 029621	2.67	1.67	2.33	2.33	2.25	TAN
GBK 029611	3.33	1.67	2.33	2.00	2.33	TAN
PI 200477A	3.33	1.67	5.00	1.67	2.92	MIX
SB-17	3.33	1.67	4.00	2.00	2.75	TAN
GBK 029616	3.67	1.67	2.33	2.67	2.58	TAN
GBK 033225	3.67	1.67	5.00	2.33	3.17	TAN
GBK 033229	3.67	1.67	2.67	2.67	2.67	TAN
SB-4	3.67	1.67	3.33	2.00	2.67	TAN
GBK 029577	4.33	1.67	4.00	3.00	3.25	TAN
GBK 029612	4.67	1.67	4.33	3.00	3.42	TAN
GBK 033221	4.67	1.67	4.00	2.00	3.08	TAN
GBK 033222	4.67	1.67	4.67	2.33	3.33	TAN
GBK 028397B	2.33	2.00	3.67	2.67	2.67	TAN
GBK 033223	3.00	2.00	4.00	1.67	2.67	TAN
GBK 033213	3.33	2.00	2.33	1.67	2.33	TAN
GBK 033254	3.67	2.00	5.00	4.00	3.67	TAN
SCS-1	3.67	2.00	2.00	3.33	2.75	TAN
Maksoy 2N	5.00	2.00	1.67	1.00	2.42	TAN
GBK 029617	2.00	2.33	5.00	2.33	2.92	TAN
GBK 029620	2.33	2.33	5.00	2.67	3.08	TAN
GBK 033230	2.33	2.33	3.67	2.67	2.75	TAN
GBK 033257	2.33	2.33	2.33	3.00	2.50	TAN
911/6/3	2.33	2.33	4.67	3.00	3.08	TAN
GBK 033204	3.00	2.33	5.00	2.33	3.17	TAN
GBK 033224	3.33	2.33	5.00	3.33	3.50	TAN
GBK 033248	3.33	2.33	3.00	2.67	2.83	TAN
GBK 033255	3.33	2.33	4.00	1.67	2.83	TAN
GBK 045342	3.33	2.33	4.33	1.67	2.92	TAN



Hill	3.33	2.33	2.33	3.00	2.75	TAN
GBK 029573	3.67	2.33	2.33	3.00	2.83	TAN
GBK 033220	3.67	2.33	4.67	4.33	3.75	TAN
GBK 033237	3.67	2.33	4.67	3.00	3.42	TAN
GBK 033241	3.67	2.33	4.67	3.33	3.50	TAN
916/5/19	3.67	2.33	4.00	3.33	3.33	TAN
BlackHawk	3.67	2.33	3.67	3.33	3.25	TAN
Sable	3.67	2.33	4.67	2.67	3.33	TAN
GBK 033208	4.67	2.33	2.00	3.00	3.00	TAN
Bossier	5.00	2.33	4.67	2.33	3.58	TAN
GBK 033226	5.67	2.33	4.33	3.00	3.83	TAN
BRS Sambaiba	4.33	2.67	4.33	2.00	3.33	TAN
SB-37	5.00	2.67	5.00	2.33	3.75	TAN
GBK 033203	2.33	3.00	5.00	3.33	3.42	TAN
GBK 033236	2.33	3.00	5.00	2.67	3.25	TAN
917/5/16	2.33	3.00	2.00	3.33	2.67	TAN
GBK 033245	3.33	3.00	4.33	3.33	3.50	TAN
GBK 0835530	3.67	3.00	2.00	3.00	2.92	TAN
GBK 033232	5.00	3.00	4.67	1.33	3.50	TAN
Duicker	5.00	3.00	4.00	2.33	3.58	TAN
GBK 028397A	2.33	3.33	4.33	3.00	3.25	TAN
GBK 033211	2.33	3.33	4.67	2.00	3.08	TAN
GBK 029571	3.33	3.33	2.33	3.00	3.00	TAN
GBK 029622	3.67	3.33	4.67	2.33	3.50	TAN
GBK 033205	3.67	3.33	5.00	3.67	3.92	TAN
Kissi	3.67	3.33	4.00	2.67	3.42	TAN
EX-Kirinyaga	4.00	3.33	4.67	2.33	3.58	TAN
GBK 033209	4.67	3.33	4.00	3.33	3.83	TAN
GBK 033214	5.00	3.33	5.00	3.00	4.08	TAN
GBK 033251	5.00	3.33	4.67	2.67	3.92	TAN
SB-8	5.67	3.33	4.33	2.67	4.00	TAN
931/5/34	1.00	3.67	4.00	2.67	2.83	TAN
GBK 033252	2.33	3.67	4.67	2.67	3.33	TAN
GBK 033246	2.67	3.67	3.67	2.33	3.08	TAN
LS6161RR	3.33	3.67	4.00	3.00	3.50	TAN
GBK 033215	3.67	3.67	5.00	3.00	3.83	TAN
GBK 033218	3.67	3.67	4.33	2.33	3.50	TAN
SB-20	5.33	3.67	4.33	1.33	3.67	TAN
Gazelle	6.00	3.67	4.67	3.00	4.33	TAN
GBK 033206	3.67	4.00	4.67	2.00	3.58	TAN
GBK 033231	3.67	4.00	4.67	2.67	3.75	TAN
GBK 029575	4.67	4.00	3.67	3.00	3.83	TAN
GBK 033253	4.67	4.00	4.33	1.00	3.50	TAN

932/5/36	5.00	4.00	1.33	3.00	3.33	TAN
BRS-217-Flora	3.33	4.33	2.00	1.67	2.83	TAN
Nyala	5.00	4.33	4.67	3.67	4.42	TAN
GBK 029574	3.67	4.67	5.00	3.00	4.08	TAN
FH-1	5.00	4.67	4.67	3.00	4.33	TAN
GBK 029570	3.33	5.00	5.00	2.00	3.83	TAN
BRS MG 46	4.67	5.00	4.67	2.67	4.25	TAN
Mean	3.48	2.32	3.67	2.45	2.98	
Sed	1.72	1.24	0.92	0.66	0.12	
CV (%)	60.60	65.50	30.80	32.80	50.20	

RB stands for resistance and red brown lesions; Tan is for a susceptible tan reaction; and Mix is a mixture of red brown and tan lesions on the same plant.

### 3.3.2.5 Evaluation of soybean genotypes for selected agronomic traits

Table 3.8 shows the performance of soybean genotypes for the following traits: maturity, plant height, yield, oil and protein content. Resistant genotypes G10428, G8586 and Nams soy 4M had slightly lower grain yields, 100 seed weight, protein and oil content compared to the susceptible check variety Nyala. Plants of genotypes G10428 and Nams soy 4M grew taller and matured later than Nyala across the locations and seasons. However, genotype G8586 recorded early maturity and slightly higher protein content scores than Nyala. Genotype G7955 had higher grain yields and protein content than Nyala, but only had an average oil content and moderate seed size, and medium maturity and tall plants. The advanced line BRS Sambaimba matured late, and recorded low yields and low protein content. On the other hand, the advanced line BRS MG46 recorded high yields, with good oil and protein content of 16 g kg<sup>-1</sup> and 33 g kg<sup>-1</sup>, respectively, although it was tall and late maturing. Commercial variety Nyala matured early, had a high seed weight, short plants and moderate performance with respect to oil content (15 g kg<sup>-1</sup>) and protein content (33 g kg<sup>-1</sup>).

Table 3.8: Selected soybean genotypes maturity, grain yield (kg ha<sup>-1</sup>) and quality traits.

Genotype	Days to 75% Maturity	Plant height (m)	100 seed weight (g)	Grain yield (kg/ha)	Protein content (g/kg)	Oil content (g/kg)
911/6/3	104.89	0.45	20.20	1975	31.49	16.65
835/5/30	102.44	0.31	20.51	1464	32.19	17.38
915/5/12	101.94	0.31	18.36	1912	32.35	16.93
916/5/19	100.89	0.39	19.81	2206	32.80	16.08
917/5/16	101.33	0.38	23.36	1828	32.87	15.65
931/5/34	101.89	0.29	18.73	1908	30.74	17.27
932/5/36	110.56	0.40	17.87	1813	30.00	16.70
BRS MG46	108.17	0.41	18.16	2321	33.20	15.96
BRSSambaiba	122.78	0.47	16.18	1216	28.85	16.82
G10428	111.61	0.48	15.59	1739	33.18	14.92
G58	97.44	0.40	24.25	1900	35.27	16.07
G7955	106.33	0.40	15.79	2199	32.91	16.47
G8586	97.83	0.45	14.01	1703	36.04	13.63
Gazelle	102.5	0.37	20.77	2011	29.72	16.80
Maksoy 1N	99.83	0.30	13.56	1611	34.96	11.94
Namsoy 4M	110.89	0.62	18.34	1699	34.71	15.36
Nyala	94.72	0.22	23.18	1747	33.37	16.11
SB-17	106.56	0.48	15.44	1413	35.23	14.31
SB-20	114.61	0.39	14.10	1267	34.54	14.30
SB-4	110.06	0.50	16.57	1221	34.48	15.49
Mean	96.88	0.34	18.29	1794	32.94	15.74
Sed	4.1	0.07	1.44	525.2	0.07	0.55
CV (%)	5.2	24.9	9.5	38.6	0.30	0.40

\*Maturity levels: early =≤100 days-to-maturity; medium=101to 110 days-to-maturity and late maturity = >110 days-to maturity.

### 3.3.2.6 Correlations between rust resistance reactions and selected agronomic traits

The correlation coefficients of the rust resistance reactions correlated with selected agronomic traits are presented in Table 3.9. Rust severity was positively correlated with 100 seed weight, AUDPC values, oil content and rust sporulation scores. However, the degree of correlation varied among these traits, with the highest degree of association recorded between AUDPC values ( $r=0.922$ ,  $P\leq 0.001$ ) with rust severity, and with rust sporulation scores ( $r=0.568$ ,  $P\leq 0.001$ ). 100 seed weight ( $r=0.321$ ) and oil content ( $r=0.266$ ) expressed significant but low positive correlation coefficient. Rust sporulation scores had positive and significant ( $P\leq 0.001$ ) associations with AUDPC values ( $r=0.451$ ), and with 100 seed weight ( $r=0.307$ ), and oil content ( $r=0.307$ ). Grain yield had a significant positive relationship with 100 seed weight ( $r=0.360$ ) and oil content ( $r=0.342$ ).

Table 3.9: Correlation coefficients of rust reaction with selected agronomic traits

	100 seed wt	75% maturity	AUDPC values	Grain yield	Plant height	Protein content	Rust severity	Rust sporulation	Oil content
100 seed wt	1.000								
75% maturity	-0.320	1.000							
AUDPC values	0.366***	0.018	1.000						
Grain yield	0.360***	-0.382	-0.038	1.000					
Plant height	-0.170	0.112	-0.319	0.136	1.000				
Protein content	-0.183	-0.160	-0.320	-0.083	0.201	1.000			
Rust severity	0.321***	0.089	0.922***	-0.043	-0.338	-0.282	1.000		
Rust sporulation	0.307***	-0.004	0.451***	0.066	-0.241	-0.311	0.568***	1.000	
oil content	0.537***	-0.164	0.331***	0.342***	-0.025	-0.609	0.266***	0.271***	1.000

AUDPC is the area under disease progress curve value; \*\*\* indicates significant at  $P \leq 0.001$ .

### 3.4 Discussion

This study revealed a wide range of rust resistance reactions with significant differences among the soybean genotypes based on rust severity, lesion type, sporulating rates and AUDPC over the seasons and locations. These differences could be attributed to differences in genetic backgrounds of soybean genotypes, probably a diverse array of physiological races of *P. pachyrhizi* and seasonal weather conditions during growth and development of soybeans. Nevertheless, the parameters used in this series of evaluations provided a good basis for classifying genotypes into resistant, moderate resistant, moderately susceptible and highly susceptible categories.

Among the plant introductions possessing known resistance genes, G10428 and G8586 were the most resistant genotypes because they recorded low rust severity scores, low AUDPC values, developed red brown lesions and scored low for production of urediniospores. This suggests that G10428 with *Rpp4* gene and G8586 with *Rpp2* gene are currently resistant to the *P. pachyrhizi* races present in Kenya. However, previous studies have reported varied results on the resistance levels of these genotypes in different parts of the world. For instance, genotype G8586 was reported to be resistant while G10428 succumbed to rust in Uganda (Oloka et al., 2008). Using a Japanese isolate of *P. pachyrhizi*, genotype G10428 was resistant (Yamanaka, 2010) and slightly resistant to a highly aggressive Brazilian isolate (BRP-2), while genotype G8586 was susceptible (Yamanaka, 2010; Yamanaka et al., 2011). This variation may be attributed to differences in rust isolates in the different locations indicating that specific resistance genes are susceptible to some physiological races and resistant to others. Despite

the resistance expressed by these two genotypes, their yields were lower and days-to-maturity longer, than the available commercial varieties. In addition, their greenish seed coat colour, poor grain quality traits, susceptibility to lodging and crop appearance were not appealing to farmers, as captured in the PRA survey conducted previously (Chapter 2). However, the genotypes could act as useful sources of resistance genes in soybean breeding programmes. Prior experience around the world suggests that to use these genotypes as sources of resistance genes would be attractive but they are unlikely to provide durable resistance.

Other genotypes including MAK BLD 11.3, GC 00138-29 and Namsoy 4M had higher resistance levels than some of the genotypes with known *Rpp* genes. Trials in Uganda showed that GC 00138-29 and Namsoy 4M had moderate resistance (Kawuki et al., 2003a; Oloka et al., 2008). In addition, GC 00138-29 had a resistant reaction in Japan (Yamanaka, 2010). However, the resistance genes in these genotypes have not been characterised. Characterization of these resistance genes would provide a better understanding of the type of resistance genes they confer, and the likelihood of these resistance genes being durable.

Plant introductions G7955 (*Rpp3*) and G58 (*Rpp1*) were not classified in the resistant category but as moderately resistant. In this evaluation they had moderate rust severity with tan lesions and sparsely sporulating urediniospores. This reaction was unexpected, but it suggests that the resistance genes in these genotypes were already susceptible to *P. pachyrhizi* physiological races present in Kenya. Slightly resistant, resistant and highly resistant reactions by G7955 have been reported when challenged with Brazil, Japanese and USA isolates, respectively (Paul and Hartman, 2009; Yamanaka, 2010; Yamanaka et al., 2011). On the other hand, G58 had immune reactions to Indian and 3 Mississippi isolates and high resistant reactions against Louisiana isolates and a Japanese isolate (Li, 2009b; Pham et al., 2009; Yamanaka, 2010). However, the resistance conferred by *Rpp1* in G58 has been matched by virulent races in several countries including Taiwan (McLean and Byth, 1980), Uganda (Oloka et al., 2008), Brazil, Paraguay, Thailand and Zimbabwe (Pham et al., 2009; Miles et al., 2011) and now in Kenya. These results suggest that the resistance genes *Rpp1* in G58 and *Rpp3* in G7955 may provide excellent but short-term resistance in some geographical regions until virulent races evolve in that region. Therefore, due to the rapid evolution of novel virulent races of *P. pachyrhizi* that overcome qualitative resistance genes, plant breeding programmes should focus on development of cultivars with durable resistance.

Genotypes UG-5, Tainung 4, UFV3, Maksoy 1N and Dowling provided moderate resistance to rust as indicated by rust severity scores of between 4.0 and 5.0, and low sporulation scores. They also developed red brown lesions, although lighter in colour than those observed in genotypes with *Rpp4* and *Rpp2* resistance genes. Previous studies have reported red brown reactions combined with low rates of sporulation as some of the attributes found with partial resistance genes (Hartman et al., 2005). Therefore, these genotypes classified under moderate resistance may possess partial resistance genes. To date partial resistance has not been well utilized by plant breeders because its assessment is time consuming and is complicated when evaluation is done in segregating populations and different maturity groups (Hartman et al., 2005). However, use of recurrent selection only 2-3 generations of screening for rust resistance could create highly resistant soybean with stable quantitative resistance. Genetic analysis studies are also needed to understand the mode of rust resistance inheritance for these genotypes and consequently develop durable rust resistant cultivars.

Apart from advanced line BRS Sambaimba, all the other advanced lines, genebank genotypes and commercial varieties, including Maksoy 2N from Uganda, were classified as moderately susceptible or highly susceptible. Farmers were aware that the local commercial varieties (Nyala and Gazelle) were highly susceptible to ASR, but they preferred them for their moderate yields, early to medium maturity, large seed size, moderate quality traits, seed availability and their good adaptation to the local environment (Chapter 2). Advanced line BRS Sambaiba showed moderate levels of resistance but it would not be accepted because of its late maturity and low yields. On the other hand, the advanced line BRS MG46 consistently produced a high yield, despite being moderately susceptible to ASR. Therefore, the advanced lines need to be bred for improvement in several key traits, if they are to meet the farmers' selection criteria.

Rust severity and sporulation scores in the field differed slightly from those recorded in the screenhouse evaluations, resulting in slightly different rust reaction classifications for each genotype. Lower rust severity ratings in the field were associated with the prevailing weather conditions, especially temperatures, rainfall and humidity, as well as the physiological stage of the soybean genotypes at the time of infection, and the lower level of pathogen inoculum present during the experimental period. In contrast, disease development in the screenhouse evaluation was dependent on the quality and quantity of the inoculum, age of soybean plants, inoculation procedures and the controlled environmental conditions before and after the inoculation that clearly facilitated rust development. Twizeyimana et al. (2007) reported similar

results when they compared screenhouse and field evaluations of soybean varieties for ASR resistance. In addition, inoculum used in this study was not purified and most likely it had a mixture of races that severely affected the resistance levels of soybeans in the screen house.

Rust severity, sporulation and AUDPC values differed across the locations and seasons. This could be attributed to variations in the inoculum pressure and the environmental conditions found in the different locations and seasons, and the variable expression of rust resistance by various genotypes. Another explanation could be that the virulence and aggressiveness of the races of *P. pachyrhizi* varied in space and time. Previous studies have reported the occurrence of various *P. pachyrhizi* races in different geographical areas including Nigeria and Uganda (Oloka et al., 2008; Twizeyimana et al., 2009) that differed in their virulence on soybean. Mixed lesion observed on leaves of the same genotype is also a sign of multiple races of *P. pachyrhizi* (Yamanaka, 2010); hence the assessment of *P. pachyrhizi* races is needed to identify pathogenic clusters present in Kenya. This information will help breeders to develop varieties with specific resistance to a pathotype group in a particular region.

The weather conditions experienced at different locations and in different seasons played an important role in the development of rust. Temperatures between 18 and 25°C (Del Ponte et al., 2006), coupled with a relative humidity of 75-80%, favour germination and infection of urediniospores (Park et al., 2008). Urediniospore germination is faster in regions with reliable rainfall distribution throughout the season than in regions with uneven rainfall distribution (Tschanz, 1984). Since the environmental conditions differed at KARI-Embu and KARI-Mwea, this could explain the differences in rust severity. At KARI-Embu, temperatures ranged between 15 and 25°C and rainfall was 473 mm during the long rain cropping season, which are ideal for development of ASR. On the other hand, KARI-Mwea in the same season had maximum temperatures beyond 25°C, which probably reduced urediniospore viability, germination and infection (Caldwell et al., 2005). In addition, dry conditions and high temperatures (29°C) in KARI-Mwea and its unreliable rainfall distribution (191 mm), especially during the short rains cropping season of 2010, were not conducive for germination and infection of urediniospores. The high rust severity observed at KARI-Embu suggests that this site provided a useful combination of high levels of rust inoculum and good conditions for rust infection and disease development. Therefore, KARI-Embu could be used as a hot spot for screening soybean genotypes for rust resistance, especially during the cold season of June to August.

Soybean growth stage plays an important role in the development of ASR (Tschanz et al., 1985). This study recorded the highest rust severity scores at the R6 stage (full seed formation stage). These results are in agreement with those reported by Maria et al. (2007) that ASR was more severe during the reproductive stages (flowering and pod-filling stages). Thus, it is important to rate genotypes for their rust resistance at the appropriate plant growth stage.

Understanding the relationship between phenotypic traits helps breeders to estimate the nature, size and the direction of genetic gains to be expected during plant selection (Selvaraj et al., 2011). In the present investigation, rust severity was positively correlated with AUDPC values, sporulation scores, 100 seed weight and oil content. The highest degree of association was observed between AUDPC values and rust severity, meaning that either of the two parameters can be used to evaluate genotypes for rust resistance. The positive correlation with sporulation scores suggested that high rust severity is associated with high rates of sporulation. Therefore, a reduction of sporulation scores should be given priority while selecting for ASR resistance.

The positive association between rust severity with oil content and 100 seed weight was unexpected because susceptible genotypes should have lower oil content and a reduced 100 seed weight (Bennett, 2005). A similar trend was observed between rust sporulation with 100 seed weight and oil content, which was equally unexpected.

### **3.5 Conclusion**

This study identified resistant and moderately resistant genotypes that were scored for low rust severity, low AUDPC values, red brown lesions and low sporulation levels. These included G10428, G8586, G7955, G58, Tainung 4, MAK BLD 11.3, Namsoy 4M, GC 00138-29, UG-5, UFV3, Maksoy 1N, PI 200477A, Dowling and BRS Sambaimba. Most of these genotypes, except BRS Sambaimba were exotic; hence they were not locally adapted to Kenyan environments and they performed poorly for many of the key traits required by farmers. However, they may be valuable to soybean breeders for the increased genetic diversity that they bring in, and they are likely to provide new, potentially useful, sources of resistance that may be introgressed into highly susceptible commercial varieties and advanced lines in Kenya.

Further studies are needed to characterise the resistance genes present in MAK BLD 11.3, Namsoy 4M and GC 00138-29 genotypes. Moderately resistant genotypes that exhibited red brown lesions and low sporulation scores probably carry partial resistance to ASR, and may be



durable resistance. Therefore, these genotypes could be utilized in a breeding programme to develop Kenyan soybean varieties with durable rust resistance, based on partial resistance.

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## 4 Combining ability for resistance to ASR and selected agronomic traits in soybeans

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### Abstract

Understanding the genetic mechanisms controlling inheritance of different traits is important for effective selection and breeding procedures. A study was conducted to determine the combining ability and type of gene action conditioning soybean rust resistance and other quantitative traits in soybeans using an 8x8 half diallel mating design. The F<sub>2</sub> populations, along with their parents, were evaluated for rust severity, sporulation, days-to-flowering, days-to-maturity, plant height and grain yield in two environments using an alpha lattice arrangement (6x6) replicated twice. Significant differences ( $P \leq 0.05$ ) were observed among soybean genotypes (parents and crosses) for all the traits under investigation, except days-to-maturity. General and specific combining ability (GCA and SCA) for all the traits were significant except GCA for grain yield and SCA for days-to-maturity. The GCA/SCA ratio were close to unity (0.72-0.98) for all traits except grain yield, indicating the preponderance of additive gene action in the inheritance of rust resistance and other agronomic traits over non-additive gene action. Hence there is a possibility of improving these traits through simple selection in the early generations. The GCA/SCA ratio for grain yield was close to zero (0.20) indicating the predominance of non-additive effects over additive effects in the inheritance of this trait. Parents G10428, G8586 and Namsoy 4M had the highest negative and significant GCA values, making them the best combiners for improving rust resistance across the environments. On the other hand, parents G7955, G8586 and G58 were the best combiners for early flowering. Parent Maksoy 1N effectively contributed towards early maturity, while parent G58 significantly contributed towards reduced plant height. This study confirmed the importance of additive gene action for improving rust resistance and selected agronomic traits, while non-additive gene action was predominant for grain yield in soybeans.

#### 4.1 Introduction

Soybean production is reduced by Asian soybean rust (ASR) caused by *Phakopsora pachyrhizi* (Calvo et al., 2008). It causes up to 80% yield losses depending on prevailing weather patterns, genotypes and growth of soybean during the time of infection (Yorinori et al., 2005). Such yield losses are mainly attributed to premature leaf defoliation, reduced green leaf area, reduced dry matter accumulation as well as reduced harvest index that inhibit full pod filling (Kumudini et al., 2008). Under favourable environmental conditions, ASR is likely to threaten soybean production in Kenya, if control measures are not put in place. Use of resistant cultivars and incorporating favourable resistance genes into the locally adapted susceptible commercial varieties and advanced lines is the most practical way of controlling ASR. Therefore it is necessary to understand the genetic mechanisms controlling ASR for the purpose of formulating selection and breeding strategies for the development of rust resistant cultivars (Ribeiro et al., 2009).

Choice of mating designs is important in the study of genetic mechanisms and various designs give different information. For example, diallel analysis plays an important role in crop improvement as it helps breeders to characterize the type and magnitude of gene action involved in the targeted characters (Iqbal et al., 2010). In addition, it provides useful information that can be used to select best parental combinations that will create sufficient genetic variability for the purpose of developing superior segregants or produce hybrid populations. Through the use of diallel analysis, breeders have gained knowledge of the general and specific combining ability (GCA and SCA), heterosis and maternal effects of the parental crosses (Arunga et al., 2010). For this reason, diallel analysis has been used by researchers to study the genetics of different traits in soybean (Mebrahtu and Devine, 2009; Maphosa et al., 2012b).

Genetic studies have been conducted in various parts of the world to understand the genetic mechanisms and other parameters associated with resistance to ASR, but the findings have been variable. For example, Bromfield and Hartwig (1980) studied the inheritance of ASR resistance in two  $F_2$  populations having PI 230970 and PI 230971 as the resistant parents. They reported that rust resistance was dominant and qualitatively inherited. Partial (incomplete) and complete dominance have also been reported to condition ASR resistance (Garcia et al., 2008; Ray et al., 2009). Recently, Ribeiro et al. (2007, 2009), and Maphosa et al. (2012b) reported that ASR resistance was predominantly controlled by additive gene action, while Kiryowa et al. (2009) reported the importance of both additive and non-additive genetic effects for rust resistance. Others have reported soybean cultivars with more than one major gene controlling

rust resistance, and the involvement of epistatic gene action (Garcia et al., 2008; Laperuta et al., 2008). Ribeiro et al. (2007) also detected non-allelic interaction but it did not play an important role in controlling rust resistance. This suggests that different selection procedures are needed to make genetic gains in rust resistance, depending on the genotypes used, the environment under which the experiments are conducted and the durability of resistance that is being selected for (Ribeiro et al., 2007, 2008, 2009; Kiryowa et al., 2009). These findings have raised more questions than answers and more genetic studies are therefore needed to understand the type of gene action controlling rust resistance in different soybean germplasm. In addition, no studies have been conducted on the genetic mechanisms controlling rust resistance and other important quantitative traits in soybean germplasm being used in Kenya. Such studies are important to provide information needed to breed soybeans for rust resistance and other economically important traits. Therefore, the objective of this study was to determine the type of gene action controlling rust resistance and selected agronomic traits.

## **4.2 Materials and methods**

### **4.2.1 Site characteristics**

This study was conducted in two locations, namely KARI-Embu and KARI-Mwea Research Stations in Eastern and Central Kenya, respectively. KARI-Embu is located at latitude 00° 30'S and longitude 37°42' E at an altitude of 1508 m above sea level, with a bimodal rainfall pattern of 1200-1495 mm annually. The long rains are received from mid-March to June while short rains are received from mid-October to early-January. The mean temperature ranges between 14.1 and 25°C and the soil type is a humic nitosol. KARI-Mwea is located at latitude 00° 37'S and longitude 37°20'E, with an altitude of 1159 m above sea level. This site has a mean annual rainfall of about 850 mm that is bimodal, with the long rains received between mid-March and June, and the short rains between mid-October and December. The temperatures range from 15.6 to 28.6°C with a mean of 22.8°C. The soils at KARI-Mwea are nitosols.

### **4.2.2 Soybean germplasm and diallel crosses**

The plant materials for this experiment included four soybean lines each reported to carry a single gene for resistance to ASR G58 (*Rpp1*), G8586 (*Rpp2*), G7955 (*Rpp3*), and G10428 (*Rpp4*); two moderately resistant lines, Namsoy 4M and Maksoy 1N, one advanced high yielding susceptible line (BRS MG46); and one adapted susceptible line (Nyala). The selection was based on rust resistance reactions in previous screening trials conducted in the 2010 to



2011 cropping seasons (Chapter 3). Other important characteristics and sources of the soybean germplasm are given in Table 4.1.

The eight parental lines were planted in crossing blocks in a screenhouse and in the field, at the KARI-Embu Research Station. In the screenhouse, each genotype was planted in 5 plastic pots on three planting dates at an interval of two weeks in order to synchronize flowering of varieties with different days-to-flowering. In the field, each genotype was planted in 5 rows of 2 m length, spaced at 0.5 m apart at three planting dates, at an interval of two weeks to ensure overlapping of flowering among genotypes. At flowering, all possible single crosses, excluding reciprocals, were made among the genotypes using an 8x8 half diallel mating design, following Griffing (1956) Model 1, Method 2. The resulting 28 F<sub>1</sub> populations were raised in the field and allowed to self-pollinate to produce the F<sub>2</sub> populations.

Table 4.1: Parental lines selected for genetic studies, their sources and agronomic characteristics

Parental lines	Origin/Source	*Maturity class	Seed coat colour	Yield range (ton/ha)	Average rust severity scores	Previous Soybean rust reaction
G58 ( <i>Rpp1</i> )	AVRDC/Uganda	Medium	Cream	1.9	5.3	MR
G8586 ( <i>Rpp2</i> )	AVRDC/Uganda	Medium	Green	1.7	3.0	R
G7955 ( <i>Rpp3</i> )	India/Uganda	Late	Cream	2.1	4.8	MR
G10428 ( <i>Rpp4</i> )	China/Uganda	Late	Green	1.7	2.8	R
Namsoy 4M	Uganda	Late	Cream	1.6	3.5	R
Maksoy 1N	Uganda	Medium	Cream	1.6	4.5	MR
BRS MG46	KARI-Njoro	Late	Cream	2.3	7.3	MS
Nyala	KARI-Njoro	Early	Cream	1.7	8.0	HS

AVRDC is the Asian Vegetable Research Development Centre; KARI is Kenya Agricultural Research Institute. \*Maturity class: early =<100 days-to-maturity; medium=101to110 days-to-maturity and late maturity = >110 days-to-maturity. Rust scores were based on a scale of 1-9 where 1=no disease while 9 is severely infected. R is resistant; MR is moderately resistant; MS is moderately susceptible; and HS is highly susceptible.

### 4.2.3 Field evaluation of the genotypes

The 8 parents together with 28 F<sub>2</sub> populations derived from diallel mating design were evaluated for resistance to ASR under field conditions at KARI-Embu and KARI-Mwea during the long

rains (April - August) cropping season of 2012. The trials were laid out in an alpha lattice arrangement (6x6) replicated twice. The planting dates (19<sup>th</sup> and 20<sup>th</sup> April, 2012) were selected to ensure that the most sensitive developmental stage of soybeans (R1) coincided with the environmental conditions conducive for the development of rust (cold season). Plants were grown in 2 m long plots, with four rows per plot spaced at 0.2 m within the row and 0.4 m between the rows. To ensure high and uniform disease pressure in the plots, spreader rows of a highly susceptible variety (Nyala) were planted after every three rows of the test materials and at the border rows surrounding the trial, according to the methodology described by Twizeyimana et al. (2007). During planting triple super phosphate (TSP: 46 % P<sub>2</sub>O<sub>5</sub>) at 13 kg Pha<sup>-1</sup> was applied while calcium ammonium nitrogen (CAN: 26 % N) at 12 kg N ha<sup>-1</sup> was used for top dressing. Weeding and other cultural practices were followed as recommended for each site.

#### **4.2.4 Data collection**

Data on rust severity was recorded at the R6 stage using a scale of 1-9 as proposed by Subrahmanyam et al. (1995) where; 1 = 0%, 2 = 1-5%, 3 = 6-10%, 4 = 11-20%, 5 = 21-30%, 6 = 31-40%, 7 = 41-50%, 8 = 61-80% and 9 = 81-100% of leaf surface occupied with rust lesions. For proper phenotypic classification of soybean genotypes, rust severity scores were further grouped into 1 = immune; 2.0-3.9 = resistant; 4.0-5.9 = moderately resistance; 6.0-7.9 = moderately susceptible and 8.0-9.0 = highly susceptible (Milena and Vello, 2010). Rust sporulation was rated using a scale of 1-5 as described by Miles et al. (2008) where; 1 = 0% (no sporulation); 2 = 1-25%; 3 = 26-50%, 4 = 51- 75%; and 5 = 75-100% of fully sporulating lesions. Data on plant height, days-to-75% maturity, days-to-50% flowering and grain yields were also recorded for each plot as explained in Chapter 3.

#### **4.2.5 Data analysis**

##### **4.2.5.1 Analysis of variance**

Data was subjected to analysis of variance (ANOVA) and residual maximum likelihood (REML) using Genstat statistical package (12<sup>th</sup> edition) (Payne et al., 2009) for all the traits measured, in order to test the significance of variation among the genotypes. Both REML and ANOVA outputs were similar; therefore an ANOVA model was used for this analysis as follows;

$$Y_{ijk} = \mu + G_i + \beta_j + \varepsilon_k + G_{\varepsilon ik} + \epsilon_{ijk}$$

Where;  $Y_{ijk}$  is the observed value of  $i$ th genotype ( $i=1$  to 36) in  $j$ th replicate ( $j=1$  to 2) for the  $k$ th environment ( $k=1$  to 2).  $\mu$  is the grand mean;  $G_i$  is the treatment effect for the  $i$ th genotype,  $\beta_j$  is the block effect for  $j$ th block;  $\epsilon_k$  is the environmental effect for the  $k$ th environment;  $G\epsilon_{ik}$  is the interaction term of  $i$ th genotype in  $k$ th environment; and  $C_{ijk}$  is the random error associated with the  $Y_{ijk}$  experimental unit.

#### 4.2.5.2 Estimation of general and specific combining ability

GCA and SCA values for each trait were calculated following Griffing's Model 1 (fixed genotype effects), Method 2 (parents and crosses) (Griffing, 1956) using Diallel SAS-05 program in SAS 9.2 version (SAS Institute, 2002; Zhang et al., 2005) as follows:

$$Y_{ijk} = \mu + g_i + g_j + S_{ij} + \epsilon_{ijk}$$

Where,  $Y_{ijk}$  = Observed value of the  $ij^{th}$  genotype in the  $k^{th}$  environment

$\mu$  = Overall mean;

$g_i$  = the GCA effects of the  $i^{th}$  parent;

$g_j$  = the GCA effects of the  $j^{th}$  parent;

$S_{ij}$  = the SCA effects for the cross between the  $i^{th}$  parent and the  $j^{th}$  parent

$\epsilon_{ijk}$  = experimental error associated with  $ij^{th}$  genotype in the  $k^{th}$  environment.

The relative importance of GCA and SCA were estimated using the general predicted ratio (GPR) for all the traits. This was computed as illustrated by Baker (1978);

$$\frac{GCA}{SCA} = \frac{2 \times MSQ_{GCA}}{2 \times MSQ_{GCA} + MSQ_{SCA}}$$

Where;  $MSQ_{GCA}$  and  $MSQ_{SCA}$  are the mean squares for GCA and SCA, respectively. A ratio for GCA/SCA that is close to 1 indicates the importance of additive effects in the inheritance of the trait. If the ratio is close to 0, then dominance effects are more important.

### 4.3 Results

#### 4.3.1 Analysis of variance

Combined analysis of variance revealed significant ( $P \leq 0.05$ ) genotypic differences between the 36 genotypes (parents and crosses) for all the traits studied except for days-to-maturity (Table 4.2). The environmental effect was also highly significant ( $P \leq 0.001$ ) for rust sporulation scores, days-to-flowering, days-to-maturity, plant height and grain yield, but was non-significant for rust severity scores. Genotype x environment interaction was significant for rust severity scores, rust

sporulation scores, days-to-flowering and grain yield. Due to significant genotype x environment, the data was analysed and presented for specific environments.

Table 4.2: Combined analysis of variance for rust severity, rust sporulation scores, days-to-maturity, flowering, plant height and grain yield from an 8 x 8 diallel cross in two environments.

Source of variation	d.f.	Mean squares					Grain Yield (kg ha <sup>-1</sup> )
		Rust severity	Rust Sporulation	Days to 50% flowering	Days to 75% Maturity	Plant Height (m)	
REP	1	0.250	0.250	0.000	11.960	134.820	247638 <sup>ns</sup>
Genotype	35	7.566***	2.755***	23.940**	54.380 <sup>ns</sup>	399.960***	2545476***
Environment	1	0.007	4.340***	1329.210***	14430.020***	4921.410***	19652967***
Genotype x Environment	35	1.646***	0.655**	18.790*	32.540 <sup>ns</sup>	45.880 <sup>ns</sup>	628039***
Residual	71	0.343	0.338	11.750	46.700	62.020	220910
Total	143						

\*\*\*Significant at P<0.001, \*\*Significant at P<0.01, \*Significant at P<0.05 and ns is non-significant

#### 4.3.2 Mean performance of the parents and the F<sub>2</sub> populations

The genotypic responses of soybeans for rust resistance using rust severity and rust sporulation scores are presented in Table 4.3. The rust severity scores for the parents ranged from 2.8 to 8.3, while sporulation scores ranged from 1.0 to 5.0. On average, parents G10428 and G8586 consistently had the lowest rust severity scores in the two environments (2.8) without sporulating pustules (1.0). Moderate rust resistant reactions (<4.8) with sparsely sporulating pustules (<1.8) were recorded for parents Namsoy 4M and G58 in the two environments. Parents G7955 had moderate rust reactions at KARI-Embu (5.0) but it recorded slightly higher rust severity scores at KARI-Mwea (5.5). The same parent had high sporulation levels (2.9) across the environments. Parent Maksoy IN on the other hand, recorded moderate susceptible rust severity (6.0) with a higher sporulation score of 2.8. The highest levels of rust severity were recorded for parents BRS MG46 (7.6) and Nyala (8.3) and the same parents produced high sporulation scores in the two environments (3.0-5.0).

Rust severity score for the F<sub>2</sub> population ranged from 2.2 to 8.3 while sporulation scores ranged from 1.0 to 5.0 across the environments. The F<sub>2</sub> population of cross G8586 x G10428 recorded the lowest rust severity (2.2) and sporulating scores (1.1). Other crosses with moderate rust severity scores (<4.0) and sparsely sporulating lesions (<2.0) were G10428 x Namsoy 4M and G8586 x G58. The F<sub>2</sub> population derived from BRS MG46 x Nyala had the highest rust severity (8.3) and rust sporulation scores (4.1). In general, about 64% of the F<sub>2</sub> populations had

moderate rust resistance reactions, while 4% expressed resistant reactions (Table 4.3). On the other hand, 11 and 6% of the F<sub>2</sub> populations showed moderate susceptible and highly susceptible rust reactions, respectively.

Table 4.3: Mean performance of 8 parents and 28 F<sub>2</sub> populations for rust severity, rust sporulation and average soybean rust reactions at KARI-Embu and KARI-Mwea Research Stations

Genotype	Rust Severity (1-9 scores)			Rust Sporulation (1-5 scores)			Soybean rust reaction
	KARI-Embu	KARI-Mwea	Across environments	KARI-Embu	KARI-Mwea	Across environments	
<b>Parents</b>							
BRS MG46	8.3	7.0	7.6	3.8	2.8	3.3	HS
<b>G10428</b>	<b>2.5</b>	<b>3.0</b>	<b>2.8</b>	<b>1.0</b>	<b>1.0</b>	<b>1.0</b>	<b>R</b>
G58	5.0	4.5	4.8	1.5	2.0	1.8	MR
G7955	5.0	5.5	5.3	2.5	3.3	2.9	MR
<b>G8586</b>	<b>2.5</b>	<b>3.0</b>	<b>2.8</b>	<b>1.0</b>	<b>1.0</b>	<b>1.0</b>	<b>R</b>
Maksoy 1N	6.0	6.0	6.0	2.5	3.0	2.8	MS
<b>Namsoy 4M</b>	<b>5.0</b>	<b>4.0</b>	<b>4.5</b>	<b>1.5</b>	<b>1.5</b>	<b>1.5</b>	<b>MR</b>
Nyala	9.0	7.5	8.3	5.0	5.0	5.0	HS
<b>Crosses</b>							
BRS MG 46 x Nyala	8.8	7.8	8.3	3.3	5.0	4.1	HS
G10428 x BRS MG46	5.0	6.0	5.5	2.0	3.0	2.5	MR
G10428 x Maksoy 1N	4.0	6.0	5.0	1.8	2.8	2.3	MR
<b>G10428 x Namsoy 4M</b>	<b>3.5</b>	<b>3.8</b>	<b>3.6</b>	<b>1.5</b>	<b>1.5</b>	<b>1.5</b>	<b>R</b>
G10428 x Nyala	6.0	4.0	5.0	2.8	2.3	2.5	MR
G58 x BRS MG46	6.3	6.0	6.1	2.5	2.3	2.4	MS
G58 x G10428	3.8	5.8	4.8	1.0	3.0	2.0	MR
G58 x Maksoy 1N	5.5	5.8	5.6	2.0	2.8	2.4	MR
G58 x Namsoy 4M	4.0	5.8	4.9	1.5	2.8	2.1	MR
G58 x Nyala	7.0	6.3	6.6	2.8	3.0	2.9	MS
G7955 x BRS MG46	7.0	6.3	6.6	3.3	4.3	3.8	MS
G7955 x G10428	4.0	4.8	4.4	1.5	2.3	1.9	MR
G7955 x G58	5.3	6.3	5.8	1.5	2.8	2.1	MR
G7955 x G8586	4.0	5.3	4.6	1.8	2.8	2.3	MR
G7955 x Maksoy 1N	5.0	7.3	6.1	2.0	3.3	2.6	MS
G7955 x Namsoy 4M	4.5	4.5	4.5	1.5	1.8	1.6	MR
G7955 x Nyala	6.8	6.3	6.5	3.5	3.0	3.3	MS
G8586 x BRS MG46	6.0	5.3	5.6	2.8	2.5	2.6	MR
<b>G8586 x G10428</b>	<b>2.3</b>	<b>2.0</b>	<b>2.2</b>	<b>1.0</b>	<b>1.3</b>	<b>1.1</b>	<b>R</b>
<b>G8586 x G58</b>	<b>3.3</b>	<b>4.5</b>	<b>3.9</b>	<b>1.5</b>	<b>2.5</b>	<b>2.0</b>	<b>R</b>
G8586 x Maksoy 1N	4.0	5.8	4.9	2.0	3.5	2.8	MR
G8586 x Namsoy 4M	3.8	4.5	4.1	1.5	2.0	1.8	MR
G8586 x Nyala	5.5	5.3	5.4	2.5	2.3	2.4	MR
Maksoy 1N x BRS MG46	7.5	4.3	5.9	3.3	1.8	2.5	MR
Maksoy 1N x Nyala	6.8	6.8	6.8	3.3	3.0	3.1	MS
Namsoy 4M x BRS MG46	6.0	3.5	4.8	2.5	1.3	1.9	MR
Namsoy 4M x Maksoy 1N	5.0	4.8	4.9	2.3	2.3	2.3	MR
Namsoy 4M x Nyala	6.5	5.0	5.8	2.8	2.5	2.6	MR
Mean	5.3	5.3	5.3	2.2	2.6	2.4	
Lsd(0.05)	1	1.3		0.7	1.5		
CV%	9.5	12.3		16.1	28.7		

R is resistant; MR is moderately resistant; MS is moderately susceptible; and HS is highly susceptible.

The mean performance of the parents and  $F_2$  population for other agronomic traits is presented in Table 4.4. Days-to-flowering varied among parents ranging from 34 to 38 DAP at KARI-Mwea and 35-48 DAP at KARI-Embu, with parent Nyala recording early flowering in the two environments. Similarly, days to 75% maturity varied, with earlier maturity recorded at KARI-Mwea than KARI-Embu. Parent G7955 recorded the least days-to-maturity while parent Namsoy 4M recorded the most days-to-maturity in the two environments. The  $F_2$  populations derived from cross G8586 x Maksoy 1N were early in maturity (94-106 DAP) while the progenies of cross G8586 x Namsoy 4M were late maturing (102-126 DAP) in both environments. Plant height ranged from 0.24 to 0.66 m with parents Nyala and Maksoy 1N recording the shortest plant heights in both environments. Parents Namsoy 4M on the other hand, recorded the highest plant height of 0.50 and 0.66 m at KARI-Embu and KARI-Mwea, respectively. The  $F_2$  populations of G7955 x Maksoy 1N and G58 x Nyala developed the shortest plants, while crosses Namsoy 4M x G7955 and Namsoy 4M x BRS MG46 developed the tallest plants.

With regard to grain yield, all the parental lines performed better than parent Nyala except Namsoy 4M at KARI-Embu, with parent BRS MG46 recording the highest yields in the two environments. Grain yields were higher at KARI-Mwea, ranging from 3083-4479 kg ha<sup>-1</sup> than at KARI-Embu, where yields ranged from 792-2604 kg ha<sup>-1</sup>. The highest yields were from the following crosses; G10428 x G8586 and G58 x Maksoy 1N at KARI-Mwea, and G8586 x Maksoy 1N and G10428 x BRS MG46 at KARI-Mwea. The grain yield for all the crosses was lower than for the parents in the two environments.

Table 4.4: Mean performance of 8 parents and 28 F<sub>2</sub> populations for days-to-flowering, days-to-maturity, plant height and grain yield at KARI-Embu and KARI-Mwea Research Stations.

Genotype	Days to 75% Maturity		Days to 50% Flowering		Plant height (m)		Yield (kg ha <sup>-1</sup> )	
	KARI-Embu	KARI-Mwea	KARI-Embu	KARI-Mwea	KARI-Embu	KARI-Mwea	KARI-Embu	KARI-Mwea
<b>Parents</b>								
BRS MG46	111.5	096.0	40.5	36.3	0.40	0.46	2292	4479
G10428	121.3	096.5	45.5	35.8	0.44	0.59	1833	3625
G58	111.5	096.8	44.5	34.0	0.35	0.45	1708	3417
G7955	107.5	095.3	38.5	38.3	0.34	0.41	2604	3229
G8586	111.3	098.0	41.0	35.0	0.48	0.67	2104	3229
Maksoy 1N	112.5	095.8	48.3	38.5	0.31	0.35	1750	3292
Namsoy 4M	121.3	106.5	45.5	36.0	0.51	0.66	0792	4250
Nyala	109.0	096.8	35.0	34.0	0.24	0.25	1500	3083
<b>F2 population</b>								
BRS MG46 x Nyala	109.3	096.5	45.5	36.3	0.38	0.65	1021	1177
G10428 x BRS MG46	126.5	097.0	46.0	37.3	0.44	0.57	1099	1521
G10428 x Maksoy 1N	124.0	095.3	44.5	35.8	0.39	0.47	0708	0688
G10428 x Namsoy 4M	120.5	099.3	44.5	35.0	0.50	0.63	0792	1229
G10428 x Nyala	117.5	097.3	41.0	37.3	0.45	0.52	0783	0990
G58 x BRS MG46	114.8	100.5	42.5	38.8	0.32	0.52	0500	1078
G58 x G10428	113.8	096.0	40.0	34.8	0.45	0.47	0708	0667
G58 x Maksoy 1N	118.0	096.0	42.5	34.3	0.35	0.46	0495	1861
G58 x Namsoy 4M	120.8	096.0	41.0	35.5	0.48	0.54	0715	0979
G58 x Nyala	114.5	096.8	37.0	34.3	0.33	0.45	0729	1184
G7955 x BRS MG46	116.0	095.8	38.5	34.0	0.34	0.57	0403	1427
G7955 x G10428	119.3	095.3	36.0	37.5	0.46	0.53	0750	0922
G7955 x G58	116.0	096.0	41.0	40.0	0.32	0.47	0535	0819
G7955 x G8586	122.8	096.0	44.5	34.5	0.37	0.41	0531	0750
G7955 x Maksoy 1N	119.3	096.3	40.0	32.8	0.24	0.31	0205	0708
G7955 x Namsoy 4M	113.5	0104	40.0	38.3	0.61	0.83	0906	0583
G7955 x Nyala	116.0	097.5	41.0	37.8	0.34	0.51	0979	1646
G8586 x BRS MG46	121.3	100.5	41.0	34.3	0.35	0.51	0854	1479
G8586 x G10428	119.0	099.0	55.5	36.5	0.46	0.69	0743	2979
G8586 x G58	116.5	096.5	36.0	34.5	0.37	0.55	0528	0917
G8586 x Maksoy 1N	106.5	094.5	38.0	37.5	0.39	0.46	1201	0993
G8586 x Namsoy 4M	126.5	102.5	47.0	35.8	0.47	0.62	0722	1326
G8586 x Nyala	126.5	101.5	46.5	37.5	0.44	0.51	0778	1120
Maksoy x BRS MG46	126.3	097.5	48.3	38.3	0.46	0.58	0578	1285
Maksoy 1N x Nyala	126.5	095.3	45.5	34.8	0.39	0.43	0625	0958
Namsoy x BRS MG46	121.3	101.3	41.5	41.8	0.57	0.76	0576	0910
Namsoy 4M x Maksoy 1N	127.8	099.8	44.0	39.8	0.55	0.60	0521	1215
Namsoy 4M x Nyala	120.5	104.8	40.0	36.8	0.56	0.65	0750	0903
Mean	118	97.9	42.4	36.4	0.53	9.53	0953	1692
Lsd(0.05)	018.7	05.6	09.4	02.7	0.19	5.86	585.6	1145.3
CV%	7.8	2.8	10.9	3.6	0.18	0.31	31.2	33.3

### 4.3.3 GCA and SCA estimates for ASR and selected agronomic traits

GCA mean squares were significant ( $P \leq 0.05$ ) for rust severity, rust sporulation, days-to-flowering, days-to-maturity and plant height but not for grain yields (Table 4.5). Specific combining ability mean squares were also significant ( $P \leq 0.05$ ) for all the traits except days-to-maturity. The general predicted ratios (GPR) for rust severity, rust sporulation, days-to-flowering, days-to-maturity and plant height ranged from 0.72-0.98, while for grain yield it was 0.20. The interaction of GCA effects with the environment was significant ( $P \leq 0.05$ ) only for rust severity, sporulation and days-to-flowering. Similarly, the interaction of SCA effect with the environment was significant ( $P \leq 0.05$ ) only for rust severity scores, rust sporulation and grain yields. Due to significant GCA x environment and SCA x environment interactions, the data was analysed and presented for specific environments.

Table 4.5: GCA and SCA mean squares for rust severity, sporulation scores, maturity, flowering, plant height and grain yields across the environments

Source of variation	d.f.	Rust severity (1-9 scores)	Rust sporulation (1-5 scores)	Days to 50% Flowering	Days to 75% Maturity	Plant height (m)	Grain Yield (Kg ha <sup>-1</sup> )
GCA	7	30.30***	9.09***	28.29*	95.86*	1202.42***	387472.50 <sup>ns</sup>
SCA	28	0.86***	0.61**	22.37*	40.57 <sup>ns</sup>	191.59***	3074682.26***
GCA x Environment	7	4.59***	1.03*	24.68*	32.81 <sup>ns</sup>	76.37 <sup>ns</sup>	124245.90 <sup>ns</sup>
SCA x Environment	28	1.03***	0.58*	17.34 <sup>ns</sup>	32.21 <sup>ns</sup>	40.86 <sup>ns</sup>	720482.84***
GPR <sup>a</sup>		0.98	0.97	0.72	0.83	0.93	0.20

\*\*\*Significant at  $P < 0.001$ , \*\*Significant at  $P < 0.01$ , \*Significant at  $P < 0.05$  and ns is non-significant

GPR<sup>a</sup> is the general predicted ratio (Baker, 1978).

### 4.3.4 GCA estimates of individual parents for ASR and other agronomic traits

The GCA values of individual parents for rust severity and rust sporulation are presented in Table 4.6. Based on the severity scale used in this study; the highest values correspond to greater disease severity and therefore, negative GCA values were desirable for rust resistance. In this study, GCA effects for rust severity ranged from -1.03 to 1.25, while for rust sporulation ranged from -0.55 to 0.84 across the environments. Among the parents, G10428, G8586 and Namsoy 4M exhibited highly significant ( $P < 0.001$ ) negative GCA effects (-1.03) for rust severity and rust sporulation across the environments. Parent G58 on the other hand, had inconsistent GCA effects in the two environments. This parent had significant and negative GCA effects for



rust severity and rust sporulation at KARI-Embu, but positive GCA estimates at KARI-Mwea. Relatively low positive GCA estimates for both rust severity and rust sporulation were observed for parents G7955 and Maksoy 1N across the environments. The highest and positive significant GCA effects ( $P < 0.001$ ) for both rust severity and rust sporulation were recorded for parents Nyala and BRS MG46 across the environments.

Table 4.6: Individual estimates of GCA effects for rust severity and rust sporulation scores from an 8 x 8 diallel cross at KARI-Embu and KARI-Mwea Research Stations.

Parents	Rust severity (1-9 scores)			Rust Sporulation (1-5 scores)		
	KARI-Embu	KARI-Mwea	Across environments	KARI-Embu	KARI-Mwea	Across environments
BRS MG46	1.56***	0.41***	0.99***	0.70***	0.26	0.48***
G10428	-1.32***	-0.74***	-1.03***	-0.64***	-0.46**	-0.55***
G58	-0.29**	0.26	-0.01	-0.43***	0.04	-0.19
G7955	-0.10	0.41**	0.16	-0.02	0.32*	0.15
G8586	-1.29***	-0.71***	-1.00***	-0.46***	-0.37*	-0.41**
Maksoy 1N	0.18	0.48**	0.33*	0.17*	0.20	0.18
Namsoy 4M	-0.50***	-0.87***	-0.69***	-0.33***	-0.65***	-0.49***
Nyala	1.75***	0.76***	1.25***	1.01***	0.66***	0.84***
LSD 0.05(gi)	0.22	0.28	0.77	0.15	0.31	0.36

\*\*\*Significant at  $P < 0.001$ , \*\*Significant at  $P < 0.01$  and \*Significant at  $P < 0.05$

GCA estimates of individual parents for days-to-flowering, days-to-maturity, plant height and grain yield are presented in Table 4.7. Parents with negative GCA effects for flowering, days-to-maturity and plant height were desirable as they represent early maturing, short varieties. Positive GCA effects were desirable for grain yield improvement. Across the environments, none of the parents had significant GCA effects for days-to-flowering, days-to-maturity and grain yield. However, significant GCA effects were observed in specific environments. For example, parent G7955 had significant and negative GCA effects for flowering at KARI-Embu. For flowering at KARI-Mwea, parents with significant negative GCA effects were G8586 and G58. The highest positive GCA effects for flowering were recorded for parents Namsoy 4M and BRS MG46 at KARI-Mwea.

For days-to-maturity, parent Maksoy 1N recorded the highest significant and negative GCA effects (-1.69) at KARI-Mwea while the highest positive and significant GCA effect for days-to-

maturity was observed for parent Namsoy 4M in the same environment. The same parent Namsoy 4M, had the highest positive and significant GCA values (11.99) for plant height in the two environments. On the other hand, parents Maksoy 1N, G58, G7955 and Nyala had significant negative GCA effects for plant height at KARI-Mwea. Parents Maksoy 1N and G58 also recorded significant negative GCA effects for plant height at KARI-Embu. For grain yield, none of the parents recorded significant positive GCA estimates in the two environments.

Table 4.7: Individual estimates of GCA estimates for flowering, days-to-maturity, plant height and grain yield from an 8 x 8 diallel cross at KARI-Embu and KARI-Mwea Research Stations

Parents	Days to 50%Flowering		Days to 75%Maturity		Plant height (m)		Grain Yield (kg ha <sup>-1</sup> )	
	KARI- Embu	KARI- Mwea	KARI- Embu	KARI- Mwea	KARI- Embu	KARI- Mwea	KARI- Embu	KARI- Mwea
BRS MG46	0.53	0.69*	-0.21	0.16	-0.9	4.21*	70.75	212.73
G10428	1.68	-0.18	1.67	-1.03	3.25*	2.04	82.46	120.71
G58	-1.88	-0.65*	-2.83	-1.16	-4.50***	-4.99*	-104.81	-91.53
G7955	-2.50*	0.22	-2.27	-0.97	-3.78***	-3.21	19.53	-196.13
G8586	1.25	-0.71*	0.23	0.59	-0.06	1.51	88.11	142.42
Maksoy 1N	1.43	0.04	1.54	-1.69*	-3.13*	-8.07**	-84.2	-81.76
Namsoy 4m	0.50	0.94*	2.95	3.78***	11.51***	12.46***	-122.83	-32.28
Nyala	-1.00	-0.34	-1.08	0.31	-2.38*	-3.97	50.99	-74.17
LSD 0.05(gi)	1.97	0.56	3.90	1.17	1.90	3.98	126.46	387.66

\*\*\*Significant at  $P < 0.001$ , \*\*Significant at  $P < 0.01$  and \*Significant at  $P < 0.05$

#### 4.3.5 Specific combining ability estimates for rust resistance and other agronomic traits

SCA effects for rust severity and rust sporulation scores are presented in Table 4.8. Generally, only a few  $F_2$  populations had significant SCA effects for rust severity scores, both site specific and across both environments. For example, across the environments, only the  $F_2$  population of crosses G10428 x G8586 and BRS MG46 x G58 had significant negative SCA effects. At KARI-Mwea, the  $F_2$  populations of crosses Namsoy 4M x BRS MG46 and BRS MG46 x Maksoy 1N had significant negative SCA effects. In the same environment, the  $F_2$  populations of crosses BRS MG46 x G10428 and G10428 x Maksoy 1N had significant positive SCA effects.

For rust sporulation, the SCA effects were significant and negative for the  $F_2$  populations of crosses BRS MG46 x Maksoy 1N and BRS MG46 x Namsoy 4M at KARI-Mwea and G7955 x

Namsoy 4M at KARI-Embu. F<sub>2</sub> populations with significant positive and SCA estimates across the environments were recorded for crosses BRS MG46 x G7955 and G8586 x Namsoy 4M.

Table 4.8: SCA estimates effects for rust severity and rust sporulation scores at KARI-Embu and KARI-Mwea Research Stations.

Crosses	Cross type	Rust severity			Rust sporulation		
		KARI-Embu	KARI-Mwea	Across environments	KARI-Embu	KARI-Mwea	Across environments
BRS MG46 x G10428	HS x R	-0.53	0.99**	0.23	-0.26	0.62	0.18
BRS MG46 x G58	HS x MR	-0.31	-0.01	-0.16*	0.02	-0.63	-0.31
BRS MG46 x G7955	HS x MR	0.25	0.09	0.17*	0.36	1.09*	0.72*
BRS MG46 x G8586	HS x R	0.44	0.21	0.33	0.30	0.02	0.16
BRS MG46 x Maksoy 1N	HS x MS	0.47	<b>-1.98***</b>	-0.75	0.18	<b>-1.29**</b>	-0.56
BRS MG46 x Namsoy4m	HS x MR	-0.34	<b>-1.38***</b>	-0.86	-0.07	<b>-0.95*</b>	-0.51
BRS MG 46 x Nyala	HS x H S	0.31	0.41	0.36	-0.81	1.84*	0.52
G10428 x G58	R x MR	0.07	0.90*	0.48	-0.14	0.84	0.35
G10428 x G7955	R x MR	0.13	-0.26	-0.06	-0.04	-0.20	-0.12
G10428 x G8586	R x R	-0.27	<b>-1.55**</b>	-0.91*	-0.11	-0.51	-0.31
G10428 x Maksoy 1N	R x MS	-0.15	0.93*	0.39	0.02	0.43	0.22
G10428 x Namsoy4m	R x MR	0.04	0.02	0.03	0.27	0.02	0.15
G10428 x Nyala	R x HS	0.44	-0.50	-0.03	0.09	0.13	0.11
G58 x G7955	MR x MR	0.35	0.24	0.29	-0.26	-0.20	-0.23
G58 x G8586	MR x R	-0.46	-0.38	-0.42	0.18	0.24	0.21
G58 x Maksoy 1N	R x MS	0.32	-0.32	0.00	0.05	-0.07	-0.01
G58 x Namsoy 4M	MR x MR	-0.50	1.02*	0.26	0.05	0.77	0.41
G58 x Nyala	MR x HS	-0.03	1.25	0.61	-0.19	0.38	0.09
G7955 x G8586	MR x R	0.10	0.21	0.15	0.02	0.21	0.12
G7955 x Maksoy 1N	MR x MS	-0.37	1.02*	0.33	-0.36	0.15	-0.10
G7955 x Namsoy 4M	MR x MR	-0.18	-0.38	-0.28	<b>-0.36*</b>	-0.51	-0.43
G7955 x Nyala	MR x HS	-0.09	0.41	0.16	-0.03	-0.59	-0.31
G8586 x Maksoy 1N	R x MS	-0.18	0.65	0.23	0.08	1.09*	0.58*
G8586 x Namsoy 4M	R x MR	0.25	0.74	0.50	0.08	0.43	0.26
G8586 x Nyala	R x HS	-0.03	0.78	0.38	0.03	0.22	0.13
Maksoy1N x Namsoy4m	MS x MR	0.04	-0.20	-0.08	0.21	0.12	0.16
Maksoy 1N x Nyala	MS x HS	-0.81	0.47	-0.17	-0.09	-0.47	-0.28
Namsoy 4M x Nyala	MR x HS	-0.75	-0.63	-0.69	-0.09	-0.31	-0.20
(Sij)Lsd 0.05		0.65	0.85	0.94	0.47	0.96	0.71

\*\*\*Significant at P<0.001, \*\*Significant at P<0.01 and \*Significant at P<0.05. R is resistant; MR is moderately resistant; MS is moderately susceptible; and HS is highly susceptible.

SCA effects for other agronomic traits are presented in Table 4.9. With regard to days-to-flowering, days-to-maturity and plant height only a few  $F_2$  populations had significant SCA effects across the environments. The  $F_2$  population of cross G58 x Nyala recorded the highest negative and significant SCA effects for days-to-flowering across the environments. Other  $F_2$  populations that had negative significant SCA effects for days-to-flowering were from crosses G8586 x Maksoy 1N and G58 x G8586 at KARI-Embu and G7955 x Maksoy 1N and Maksoy 1N x Nyala at KARI-Mwea. The highest significant and positive SCA effect for days-to-flowering was recorded for cross G8586 x G10428. For days-to-maturity, negative and significant SCA effects were recorded for G8586 x Maksoy 1N and G58 x Namsoy 4M at KARI-Embu and KARI-Mwea, respectively. On the other hand, cross G58 x BRS MG46 recorded the highest positive and significant SCA effect for days-to-maturity.

For plant height, G7955 x Maksoy 1N had the highest significant, negative SCA effects across the environments while Namsoy 4M x Nyala and BRS MG46 x Maksoy 1N recorded significant and positive SCA effects. Most of the  $F_2$  populations recorded significant and negative SCA effects for grain yield. The highest significant negative SCA effects were recorded for BRS MG46 x Nyala, Maksoy 1N x Nyala, Namsoy 4M x Nyala and G10428 x Nyala. None of the  $F_2$  populations had significant and positive SCA estimates for grain yield.

Table 4.9: SCA estimates for flowering, days-to-maturity, plant height and grain yield at KARI-Embu and KARI-Mwea Research stations.

Crosses	Days to 50% Flowering			Days to 75% Maturity			Plant height (cm)			Grain Yield (kg ha <sup>-1</sup> )		
	KARI-Embu	KARI-Mwea	Across Env	KARI-Embu	KARI-Mwea	Across Env	KARI-Embu	KARI-Mwea	Across Env	KARI-Embu	KARI-Mwea	Across Env
BRS MG46 x G10428	1.35	0.34	0.84	6.49	-0.09	3.20	-0.05	-2.84	-1.45	101.13	-269.37	-84.12
BRS MG46 x G58	1.41	2.31**	1.86	-0.76	3.53*	1.38	-4.64	-1.06	-2.85	-310.55	-499.84	-405.20
BRS MG46 x G7955	-1.96	<b>-3.32***</b>	-2.64	-0.07	-1.41	-0.74	-3.35	2.16	-0.60	-532.13*	-46.28	-289.20
BRS MG46 x G8586	-3.21	<b>-2.13*</b>	-2.67	2.68	1.78	2.23	-5.74*	-8.06	-6.90	-149.31	-332.74	-241.02
BRS MG46 x Maksoy1N	3.85	1.12	2.48	6.36	1.06	3.71	8.83*	8.02	8.43*	-253.04	-303.00	-278.02
BRS MG46 x Namsoy 4m	-1.96	3.71***	0.88	-0.04	-0.66	-0.35	5.02	6.11	5.57	-216.14	-727.48*	-471.81
BRS MG 46 x Nyala	6.53	1.03	3.78	-1.38	0.34	-0.52	-1.10	27.19*	13.04	-1251.08**	-3015.18***	<b>-2133.13***</b>
G10428 x G58	-2.25	-0.82	-1.53	-3.64	0.22	-1.71	4.29	-3.89	0.20	-113.93	-819.28	-466.61
G10428 x G7955	-5.62*	1.06	-2.28	1.30	-0.72	0.29	5.07	0.08	2.58	-196.61	-459.47	-328.04
G10428 x G8586	10.13**	1.00	5.56*	-1.45	1.47	0.01	0.94	11.61*	6.27	-272.15	1259.27**	493.56
G10428 x Maksoy 1N	-1.06	-0.5	-0.78	2.24	0.00	1.12	-2.49	-1.06	-1.78	-134.55	-808.21*	-471.38
G10428 x Namsoy 4m	-0.12	-2.16*	-1.14	-2.67	-1.47	-2.07	-6.22*	-5.59	-5.91	-12.59	-316.02	-164.30
G10428 x Nyala	-1.81	1.66	-0.08	-1.00	-0.59	-0.80	7.22	-0.98	3.12	-1018.89**	-2440.53***	<b>-1729.71**</b>
G58 x G7955	2.94	4.03***	3.48	2.55	0.16	1.35	-1.01	1.36	0.17	-224.60	-349.65	-287.13
G58 x G8586	-5.81*	-0.54	-3.17	0.55	-0.91	-0.18	0.19	4.64	2.42	-300.12	-590.99	-445.55
G58 x Maksoy 1N	0.50	-1.54	-0.52	0.74	0.88	0.81	0.59	5.22	2.91**	-160.81	577.63	208.41
G58 x Namsoy 4M	-0.06	-1.19	-0.63	2.08	<b>-4.59*</b>	-1.26	-0.47	-7.31	-3.89	98.30	-353.79	-127.74
G58 x Nyala	-8.38	-0.06	<b>-4.22*</b>	1.25	-1.47	-0.11	-4.53	-0.77	-2.65	-1134.97**	-2250.01**	<b>-1692.49**</b>
G7955 x G8586	3.32	-1.41	0.95	6.24	-1.59	2.32	-0.36	-10.64	-5.50	-421.01*	-653.05	-537.03
G7955 x Maksoy 1N	-1.37	-3.9***	-2.64	1.43	0.94	1.18	-10.88**	-11.31*	<b>-11.09*</b>	-575.09*	-470.54	-522.82
G7955 x Namsoy 4m	-0.43	0.68	0.13	-5.73	3.22	-1.26	12.06***	19.66**	15.86	164.93	-645.02	-240.04
G7955 x Nyala	1.00	0.06	0.53	7.31	0.97	4.14	-0.56	11.02	5.23	-1656.46***	-1705.29*	<b>-1680.88**</b>
G8586 x Maksoy1N	-7.12*	1.78*	-2.67	<b>-13.82*</b>	-2.38	-8.10	0.40	-1.28	-0.44	352.87	-524.35	-85.74
G8586 x Namsoy4m	2.82	-0.88	0.97	4.77	0.16	2.46	-6.49*	-5.56	-6.02	-87.67	-240.50	-164.08
G8586 x Nyala	7.75	2.13	4.94	16.56	3.78	10.17	-0.93	-10.52	-5.72	-1289.28***	-1892.79**	-1591.04*
Maksoy 1N x Namsoy 4m	-0.37	2.37**	1.00	4.71	-0.31	2.20	5.16	2.02	3.59	-116.75	-127.45	-122.10
Maksoy 1N x Nyala	-0.31	-3.38*	-1.84	16.63	-2.50	7.06	7.59	4.28	5.93	-1260.20***	-2340.93**	<b>-1800.56**</b>
Namsoy 4m x Nyala	-4.00	2.03	-0.98	3.28	1.72	2.50	19.07***	15.69	17.38*	-215.51	-3305.34***	<b>-1760.43**</b>
(Sij)Lsd 0.05	6.03	1.73	3.87	11.97	3.59	5.27	5.83	12.20	5.94	387.63	734.34	788.30

\*\*\*Significant at P<0.001, \*\*Significant at P<0.01 and \*Significant at P<0.05. Env. is the environment

#### 4.4 Discussion

In this study, significant differences observed among genotypes for rust reactions and other selected agronomic traits indicated the presence of genetic variability. This variability indicated the possibility of developing varieties that are resistant to ASR with improved attributes. The environmental effect on rust resistance reactions and other traits was also significant, indicating that the two environments were different from each other in terms of weather conditions, disease pressure and possibly different mixtures of physiological races of ASR. Furthermore, the performance of the genotypes for rust resistance and some selected agronomic traits were not consistent across the locations, indicating genotype x environment interactions. Similar observations were made for rust resistance reactions by Maphosa et al. (2012b) in Uganda.

GCA effects were significant for rust severity, rust sporulation, days-to-flowering, days-to-maturity and plant height, indicating the importance of additive gene action in conditioning inheritance of these traits in soybeans. The SCA mean squares were also significant for grain yields, rust severity, rust sporulation, days-to-flowering and plant height, which indicated the contribution of non-additive genetic effects controlling these traits. However, the GCA/SCA ratio was close to one for rust severity, rust sporulation, days-to-flowering, days-to-maturity and plant height. This indicated that additive gene action played a more significant role in the inheritance of these traits than non-additive gene action. The importance of additive gene action in controlling the inheritance of ASR resistance has previously been reported by Ribeiro et al. (2007; 2009), Pierozzi et al. (2008), Kiryowa et al. (2009) and Maphosa et al. (2012b). Similarly, the predominance of additive gene effects has also been reported by Shiv et al. (2011) for the traits days-to-flowering, days-to-maturity and plant height. Application of selection pressure to the segregating populations in this study from the best parental combinations should provide significant genetic gains and improved expression of desirable traits in the population under development.

The GCA/SCA ratio for grain yield was close to zero, indicating the predominance of non-additive gene action (dominance, or an epistatic effect) in the inheritance of grain yield. The involvement of non-additive gene action in the inheritance of soybean grain yields has previously been reported (Gadag et al., 1999). Kiryowa et al. (2009) also reported significant non-additive gene action (dominance effect) in controlling ASR inheritance. These variations are to be expected, depending on the genetic background of soybean genotypes used and the environments under which the studies were conducted. Therefore, in some cases, both gene

effects may be considered while breeding for rust resistance and other quantitative traits in soybeans. However, exploitation of non-additive gene effects is limited because breeding for soybean hybrids has not been fully realized. This is difficult to achieve because of the cleistogamous condition of soybean flowers, low rate of crossing from hand pollinations, poor seed set of crosses, and the lack of confirmed cytoplasmic male sterility (Singh and Hymowitz, 1999). However, the high GCA values for rust resistance genes suggest that a recurrent selection programme, aimed at accumulating additive genes for resistance is the most viable approach to develop rust resistant varieties. Recurrent selection is not common in soybeans because of the problems associated with the number of seed obtained after hand pollination. Use of male gametocides to induce male sterility provides an alternative route to enhanced cross pollination in soybean (Lai et al., 2004). Where SCA was identified as predominant, specifically with grain yields, then selection would be effective at later generations (Cho and Scott, 2000).

GCA and SCA mean squares significantly interacted with the environment, suggesting measureable levels of GCA x environment interactions, and SCA x environment interactions for some traits. This means that neither the additive nor the non-additive effects were stable across the environments, making it difficult to select superior parents/crosses that are widely adapted across the full range of environments. Similar observations were made by Kimani and Derera (2009) and Iqbal et al. (2010) for different traits in beans. Therefore, parents and their respective crosses need to be evaluated in several environments to obtain reliable genetic information for appropriate selection and breeding procedures while improving soybean traits.

To obtain successful crosses in hybridization and selection programme, breeders mainly rely on the mean performance of the parents and their respective GCA effects for different traits, which can easily be fixed especially for a self-pollinated crop like soybean. This study suggests that parents G10428 and G8586 which recorded the lowest rust severity and sporulation scores across the environments could effectively contribute towards rust resistance. Moderate rust reactions were also recorded for parents G58 and Namsoy 4M. In addition, both resistant and moderately resistant parents had significant and negative GCA effects, indicating highly favourable gene frequencies for rust resistance and their ability to transmit these rust resistance genes to their progenies (Christie and Shattuck, 1992). Significant and negative GCA effects also indicated that G10428, G8586 and Namsoy 4M were good sources of resistance genes, and that they were the best general combiners for rust resistance. Hence if utilized in a breeding

programme, selection of the enhanced rust resistance levels for the  $F_2$ s and the advanced generations would be possible.

This study also established that the susceptible parents Nyala and BRS MG46 had highly significant and positive GCA effects for rust traits, indicating unfavourable gene frequencies for rust resistance. These results were confirmed by high rust severities and sporulation scores for these parents across the environments. These parents had poor combining ability for rust resistance, and their  $F_2$  progenies had increased levels of susceptibility. As a result, using crosses with these parents would slow the selection progress of developing rust resistance cultivars. Similar findings were reported by Kiryowa et al. (2009).

In addition to excellent rust resistance, soybean farmers also prefer varieties with high yields and early maturity for increased number of harvests per year. Also, their desire for short plants is apparent to reduce yield and quality losses associated with lodging (Chapter 2). In this study, resistant, moderately resistant and susceptible parents also expressed relatively good combining ability for days-to-flowering, plant height and days-to-maturity. For instance, resistant parent G8586 and moderately resistant parent G58 contributed significantly towards early flowering at KARI-Mwea. The moderately susceptible parent, G7955, also demonstrated good combining ability for early flowering at KARI-Embu. The rust susceptible parent Maksoy 1N was the best combiner for early maturity. For plant height, parents Maksoy 1N, G58, G7955 and Nyala were good general combiners for short stature plant types. Therefore, inclusion of these parents in the breeding programme is likely to enhance early maturity and improve lodging resistance for rust resistant parents. Unfortunately, none of the parents contributed significantly towards grain yield improvements.

Generally,  $F_2$  populations of parents with desirable high negative GCA effects for rust resistance traits such as G10428, G8586, G58 and Namsoy 4M demonstrated good performance, though not in all the cases. For instance, the  $F_2$  populations of R x R (G8586 x G10428) developed high levels of rust resistance. This promising performance of the crosses of two parents with good combining ability for rust resistance is associated with additive gene action, or additive x additive gene action. Therefore, such crosses are likely to produce desirable segregants with improved rust resistance. In addition, pyramiding parents possessing resistance genes is likely to increase rust resistance (Maphosa et al., 2012a). In some cases, the  $F_2$  populations of MR x HS parents, such as Namsoy 4M x BRS MG46 and G58 x BRS MG46, also produced rust



resistant progenies, suggesting that resistant or moderately resistant parents were more variable for rust reactions than susceptible parents. Derera et al. (2008) reported similar results when breeding for grey leaf spot resistance in maize. Interestingly, a combination of highly susceptible and moderately susceptible parents; BRS MG46 x Maksoy 1N resulted in excellent resistance, with reduced rust severity and rust sporulation. The parent Maksoy 1N is known to carry some resistance to rust, as previously reported in Uganda (Oloka et al., 2008).

Regarding other selected agronomic traits, it was interesting to note that the  $F_2$  populations of some rust resistant or moderately resistant parents combined with susceptible parents were desirable. For example, the  $F_2$  population from cross G58 x Nyala contributed significantly towards early flowering progenies. Similarly, cross G8586 x Maksoy 1N contributed effectively towards early maturing progenies. In addition, cross G7955 x Maksoy 1N gave the shortest progenies. Such crosses would be desirable to the farmers as they prefer rust resistant, early maturing, short varieties.

#### **4.5 Conclusion**

Significant differences observed for both GCA and SCA suggests that additive and non-additive gene effects played an important role in controlling rust resistance and other selected agronomic traits in soybeans. A GCA/SCA ratio close to 1 for rust severity, rust sporulation, days-to-flowering, days-to-maturity indicated that additive gene action was predominant over dominance for rust resistance and other agronomic traits. This suggests that the best way to improve rust resistance would be to use recurrent selection to accumulate additive genes for these traits. GCA/SCA ratio for grain yield indicated the importance of non-additive gene action in the inheritance of this trait. Therefore, selection at later generations would be the best approach for improving grain yield in soybeans.

Parents G10428, G8586 and Namsoy 4M were the best combiners for improving resistance to ASR. Parents G7955, G8586 and G58 contributed towards early flowering, while parent Maksoy 1N was the best combiner for early maturity. Parents G58, Maksoy 1N, G7955 and Nyala significantly contributed towards short plant height. The  $F_2$  population of crosses G10428 x G8586, Namsoy 4M x BRS MG46 and BRS MG46 x Maksoy 1N recorded the highest significant negative SCA effects for rust severity, suggesting that they would produce the most promising rust resistant progenies. The  $F_2$  populations of crosses G58 x Nyala, G8586 x Maksoy 1N and

G7955 x Maksoy 1N were the best for early flowering, early maturity and reduced plant height, respectively.

Significant interaction of the genotype, GCA and SCA with the environment could be a major problem in the development of stable rust resistance varieties with other desirable attributes. It is therefore recommended that parents, together with their respective crosses, should be evaluated in a number of environments to obtain reliable genetic information necessary for effective selection and breeding procedures.

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## 5 Genotype x environment interaction and stability analyses for soybean grain yield in Kenya

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### Abstract

Genotype x environment interaction (GEI) is a major challenge in the breeding of novel crop varieties. Stability analysis is an important tool for plant breeders to identify and recommend widely or specifically adapted genotypes for a target set of environments. This study was conducted to determine the magnitude of GEI, identify high yielding and stable soybean genotypes and the most discriminating and representative environment(s) in Central and Eastern Kenya. A set of 30 soybean genotypes were evaluated in six environments using alpha lattice arrangement (6x5), replicated three times. Soybean yield stability analysis was carried out using additive main effects and multiplicative interaction (AMMI) model, and the genotype main effect and genotype x environment interaction (GGE) biplot statistic tools. The AMMI analysis revealed that the magnitude of the GEI sum of squares was approximately twice that of the genotypes, suggesting a significant variation in genotypic response across environments. Both AMMI and GGE biplot identified genotypes 916/5/19 and G7955 as high yielding and stable across the environments; hence they can be recommended for commercial production. Genotypes Sable and BRS MG46 were also high yielding but unstable, and hence they can be recommended specifically for Environments EM2 and MW2, respectively. Environment EM2 was identified as the most discriminating and representative environment, while Environments IG1 and MW1 were less favourable because of their low capacity to provide information on the genotype differences. Environment EM1 was better for discriminating genotypes but was a poor representative of the test environments, hence it should only be utilized for developing specifically adapted genotypes. Further analysis using GGE biplot approach suggested 3 putative mega-environments in Central and Eastern Kenya. However, more studies are needed to ascertain these mega environments, and to identify more sites in other regions for future soybean breeding in Kenya.

## 5.1 Introduction

Soybean occupies an important position among grain legumes for its economic benefits. In Kenya, interest in soybean is increasing, largely due to the recognition of its nutritive value for both humans and livestock. However, Kenya produces far less soybean than it consumes. Hence, there is a need to increase soybean production, primarily by breeding better soybean varieties. To satisfy this demand by farmers, processors and consumers, a breeding programme was initiated at KARI-Njoro and IITA to develop soybean lines with the traits of high yields, excellent seed quality and promiscuous nodulation for soil fertility improvement. Such advanced lines need to be evaluated for their yield performance in multi-locational trials before they can be recommended for the targeted environments (Dehghani et al., 2009). However, multi-locational trials expose soybeans to genotype by environment interaction (GEI).

GEI presents a problem to plant breeders when developing varieties for many agroecological zones. This is because GEI slows the genetic progress in breeding through reduced heritability estimates (Matheson and Raymond, 1986; Balestre et al., 2009). In such a situation, it becomes hard to make recommendations or select superior genotypes in a wide range of environments (Asrat et al., 2009). Therefore, plant breeders deal with the problem of GEI by selecting high yielding genotypes for specific environments, or by minimizing its effects by selecting genotypes that perform consistently in a range of environments. Several statistical techniques to measure phenotypic stability have been developed for studying GEI effects, and to facilitate variety recommendations in multiple environments. The most recent 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).

The AMMI and GGE biplot analyses represent excellent models for visual interpretation of the genotypes and environments, and their interaction (Miranda et al., 2009). The graphic analysis of AMMI model makes it easier to understand stability, genotypic performance, genetic divergence between genotypes, and the test environments favourable for variety performance (Miranda et al., 2009). The GGE biplot technique on the other hand, is effective for identifying superior genotypes for each environment, and mega-environments. It also 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). Evaluating genotypes in highly informative environments is an important aspect in plant breeding programmes for fast and effective selection while utilizing limited resources (Tukamuhabwa et al., 2012).

In recent years, application of both the AMMI model and GGE biplot has become common among plant breeders for interpreting GEI. The effectiveness of these statistical tools in analyzing multi-locational trials data has been well documented for many crops (Yan et al., 2007; Asrat et al., 2009; Jandong et al., 2011; Ahmadi et al., 2012). In Kenya however, the application of the AMMI model and the GGE biplot statistical tools in soybean breeding for analyzing multi-locational trials and identifying the best test environments has not been documented. This study therefore set out to; (i) Estimate the magnitude of GEI for soybean grain yield in Kenya, (ii) Identify high yielding soybean advanced lines with wide or specific adaptation using AMMI model and GGE biplot and (iii) Identify the most discriminating and representative environment for soybean testing in Kenya.

## **5.2 Materials and methods**

### **5.2.1 Soybean germplasm**

Thirty soybean genotypes were used in this study. These genotypes included; 14 advanced lines: 835/5/30, 911/6/3, 915/5/12, 916/5/19, 917/5/16, 931/5/34, 932/5/36, BRS 217 Flora, BRS MG46, BRS Sambaimba, SB-17, SB-20, SB-37 and SB-4 obtained from Kenya Agricultural Research Institute (KARI-Njoro) and International Institute of Tropical Agriculture (IITA); 4 rust tolerant varieties released in Uganda: Maksoy 1N, Maksoy 2N, Namsoy 4M and UG-5; 4 rust resistant plant introductions: G58, G8586, G7955 and G10428; 2 varieties collected from farmers' fields FH-1 and Ex-Japan (originally from Japan); and six commercial varieties Nyala, Gazelle, Bossier, EAI 3600, Sable and Duicker.

### **5.2.2 Site characteristics, experimental design and planting**

The 30 soybean genotypes were planted during two cropping seasons, i.e. short rains (October-December) and long rains (April to June) between 2010 and 2011 in three locations: KARI-Embu, KARI-Mwea and KARI-Igoji. These locations differed in terms of latitude, longitudes, altitude and rainfall distribution (Table 5.1). These locations represent the major soybean growing areas of Central and Eastern Kenya. All the genotypes were planted in plots consisting of three rows of 2 m long, 0.3 m inter-row spacing and 0.15 m intra-row spacing. The experiments were laid out in an alpha lattice arrangement (6x5), replicated three times. Three



seeds were planted in each hill and later thinned to one plant per hill at 14 days after emergence, to maintain a plant population density of 35,000 plants per hectare. During planting triple super phosphate (TSP: 46 % P<sub>2</sub>O<sub>5</sub>) was applied at the rate of 13 kg P ha<sup>-1</sup> while top dressing fertilizer calcium ammonium nitrogen (CAN: 26 % N) was applied at the rate of 12 kg N ha<sup>-1</sup>. Standard cultural practices including weeding were followed as recommended for each site.

### 5.2.3 Data collection

Data on yield per plot was converted into kg ha<sup>-1</sup> using the following formula:

$$Y = \frac{10,000 \times (X/1000)}{A}$$

Where Y = yield in kg ha<sup>-1</sup>

X = plot yield recorded in grams

A = Plot area = number of rows x row spacing x row length (3x0.3mx2m)

Table 5.1: Climatic characteristics of the six test environments

Location	Season	Environment code	Latitude	Longitude	Altitude (m)	Rainfall* (mm)
KARI-Igoji	Long rains (2011)	IG2	00° 34'S	37° 19' E	1189	137
KARI-Igoji	Short rains (2010)	IG1	00° 34'S	37° 19' E	1189	173
KARI-Mwea	Short rains (2010)	MW1	00° 37'S	37° 20' E	1159	191.7
KARI-Mwea	Long rains (2011)	MW2	00° 37'S	37° 20' E	1159	496.9
KARI-Embu	Short rains (2010)	EM1	31 °40 °S	105 °112 °E	1508	251.8
KARI-Embu	Long rains (2011)	EM2	31 °40 °S	105 °112 °E	1508	473.7

\* amount of rainfall received in each season

### 5.2.4 Data analysis

#### 5.2.4.1 Analysis of variance

A combined analysis of variance (ANOVA) across the environments (seasons and locations) was performed using Genstat (12<sup>th</sup> Edition) (Payne et al., 2009) to determine the effect of Environment, Genotype and GEI on soybean grain yields. Data for each environment was also analysed separately.

#### 5.2.4.2 AMMI model

Grain yield data was analysed using the AMMI model that combines into a single model analysis of variance (ANOVA) for Genotype and Environment main effects with principal component analysis (PCA) of the GEI, as shown below (Crossa, 1990; Sadeghi et al., 2011);

$$Y_{gjr} = \mu + a_g + \beta_e + \sum_n \lambda_n Y_{gn} \delta_{en} + P_{ge} + \sum_{ger}$$

Where,  $Y_{gjr}$  = is the mean yield ( $\text{kg ha}^{-1}$ ) of the  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  environment for  $r^{\text{th}}$  replicate;

$\mu$  = overall mean;

$a_g$  = Deviation of the  $i^{\text{th}}$  genotype from the overall mean;

$\beta_e$  = Deviation of the  $j^{\text{th}}$  environment from the overall mean;

$\lambda_n$  = Eigen value of the  $n^{\text{th}}$  interaction principal component analysis (PCA) axis;

$Y_{gn}\delta_{en}$  = the genotype and environment eigenvectors for the  $n^{\text{th}}$  PCA axis respectively;

$P_{ge}$  = Residual from the AMMI;

$\sum_{ger}$  = Error term.

In this model, AMMI analysis of variance and ranking of soybean genotypes per environment were presented to interpret the results. AMMI biplot showing the main effects (Genotype and Environment) and the first interaction principal components (IPCA 1) was also presented to assess the relationships among soybean genotypes, test environments and GEI for soybean grain yield.

#### 5.2.4.3 GGE biplot

Grain yield data was analysed according to the GGE biplot method using Genstat statistical package (12<sup>th</sup> Edition) (Payne et al., 2009). The GGE mathematical model based on PCA of environment-centered data (which contains G and GE as the main sources of variation) subjected to singular valued decomposition (SVD) was used for visualizing the relationship among soybean genotypes and the environments as described by Yan et al. (2000).

$$Y_{ij} - \mu - \beta_e = \lambda_1 Y_{i1} \hat{r}_{j1} + \lambda_2 Y_{i2} \hat{r}_{j2} + \varepsilon_{ij}$$

Where  $Y_{ij}$  = Yield grain mean ( $\text{kg /ha}^{-1}$ ) of the  $i^{\text{th}}$  line in the  $j^{\text{th}}$  environment;

$\mu$  = Overall mean

$\beta_e$  = Main effect of the Environment;

$\lambda_1$  and  $\lambda_2$  = Eigen value associated with IPCA1 and IPCA2 respectively;

$Y_{i1}$  and  $Y_{i2}$  = the scores of the IPCA1 and IPCA2 respectively for the  $i^{\text{th}}$  cultivar;  
 $\hat{\eta}_{j1}$  and  $\hat{\eta}_{j2}$  = the scores of the IPCA1 and IPCA2 respectively for the  $j^{\text{th}}$  environment;  
 $\varepsilon_{ij}$  = Error term associated with soybean line  $i$  in environment  $j$ .

GGE biplots based on average environment coordination (AEC) was used to determine yield performance and stability of 30 soybean genotypes. Environment-focused scaling was used to test the relationship of the test environments. A GGE polygon view was also used to identify high yielding genotypes in specific environments through analysis of the “which won where pattern” (Yan et al., 2000).

## 5.3 Results

### 5.3.1 Analysis of variance

The combined analysis of variance presented in Table 5.2 shows the effect of the genotype, environment and GEI on soybean yield variation. GEI was significant ( $P < 0.01$ ) while the environment and genotype main effects were highly significant at  $P < 0.001$ . The highest total variation was due to the environment, while genotype, and GEI accounted for only a small portion of yield variation.

Table 5.2: Combined analysis of variance for soybean grain yield (kg /ha<sup>-1</sup>) of soybean genotypes evaluated in six environments between 2010 and 2011 seasons

Source of variation	d.f.	Sum of squares	Mean squares
REP	2	20000000	9999000
Genotype	29	5210000	1766000***
Environment	5	700300000	140100000***
Genotype x Environment	145	100800000	695100**
Residual	358	179000000	500100
Total	539	1051000000	

\*\*\*Significant at  $P < 0.0001$ ; \*\*Significant at  $P < 0.01$

Table 5.3 shows the mean grain yield (kg /ha<sup>-1</sup>) of soybean genotypes evaluated in six environments. Commercial variety Sable produced the highest mean yield (2348 kg /ha<sup>-1</sup>) in all the six environments followed by BRS MG46 (2321 kg /ha<sup>-1</sup>) and Duicker (2217 kg /ha<sup>-1</sup>). Soybean genotypes performed differently in the various environments. The highest yielding genotypes were Sable, UG-5, SB-37 and 915/5/12 in environment EM1, IG1, IG2 and MW2,

respectively. Genotype BRS MG46 gave the highest yield in two environments, EM2 and MW1. MW2 was the highest yielding environment (3809 kg /ha<sup>-1</sup>), followed by EM2 (2632 kg /ha<sup>-1</sup>) while IG1 (705 kg /ha<sup>-1</sup>) and MW1 (500kg /ha<sup>-1</sup>) were the lowest yielding environments.

Table 5.3: Mean soybean grain yield (kg /ha<sup>-1</sup>) of 30 genotypes tested in six environments between 2010 and 2011 seasons.

Genotype	Genotype code	Environments						Mean
		EM1	EM2	IG1	IG2	MW1	MW2	
835/5/30	835	868	2241	315	1315	641	3407	1464
911/5/6	911	1071	3852	311	1815	320	4481	1975
915/5/12	915	1696	3352	259	870	482	<b>4815</b>	1912
916/5/19	916	2226	3593	593	1759	714	4352	2206
917/5/16	917	1900	2704	1000	1241	325	3800	1828
931/5/34	931	1305	2389	778	2056	588	4333	1908
932/5/36	932	1360	3000	556	1648	261	4056	1813
Bossier	BOS	1865	2852	1037	1148	613	4000	1919
BRS 217 Flora	BRF	1097	2500	630	2648	475	3833	1864
BRS MG46	BRM	1287	<b>4222</b>	1148	2111	<b>914</b>	4241	<b>2321</b>
BRS Sambaimba	BRS	1260	722	589	926	467	3333	1216
Duicker	DKR	2096	3000	1148	2074	386	4596	<b>2217</b>
EAI 3600	EAI	2472	3204	704	1019	627	3759	1964
Ex-Japan	EJP	1362	2000	463	1037	539	3093	1416
FH-1	FH1	651	2556	463	1556	314	3444	1497
G10428	G10	569	2222	1296	1796	697	3852	1739
G58	G58	1678	2852	1019	1111	405	4333	1900
G7955	G79	2120	4037	630	1852	614	3944	2199
G8586	G85	2076	1928	1111	1870	360	2870	1703
Gazelle	GZL	2211	3333	611	1407	394	4111	2011
Maksoy 1N	M1N	1129	2574	667	1481	370	3444	1611
Maksoy 2N	M2N	1012	1759	611	1444	691	3370	1481
Namsoy 4M	N4M	758	2444	629	1778	361	4226	1699
Nyala	NYL	2352	2778	667	1148	572	2963	1747
SB-17	SB-17	602	1759	704	1648	266	3500	1413
SB-20	SB-20	711	1519	389	1074	354	3556	1267
SB-37	SB-37	1277	2444	260	<b>2630</b>	391	3685	1781
SB-4	SB-4	952	1056	444	1241	324	3306	1221
Sable	SBL	<b>3242</b>	3907	722	1093	769	4352	<b>2348</b>
UG-5	UG-5	1256	2167	<b>1389</b>	1481	773	3204	1712
<b>Mean</b>		1482	2632	705	1543	500	3809	1778
<b>Lsd<sub>(0.05)</sub></b>		1064.9	1293	735.3	991.9	388	1611.3	463.6
<b>CV(%)</b>		44.0	30.1	33.8	39.3	39.3	25.9	39.8

### 5.3.2 AMMI analysis of variance

The AMMI analysis of variance results are presented in Table 5.4. The Genotypes, Environments and GEI were all highly significantly ( $P < 0.001$ ). Environment was the major cause of soybean grain yield variation, explaining 66.61% of the total sum of squares. Genotypic effects explained only a small portion (4.87%) of the total sum of squares, while 9.59% of the total sum of squares was attributable to GEI effects. The GEI sum of squares was approximately two times greater than that of Genotypes.

A full model with four IPCAs was fitted but only IPCA1 and IPCA2 were highly significant ( $P \leq 0.001$ ). The first principal component analysis (IPCA1) captured 47.75% of the GEI interaction sum of squares. This was closely followed by the second principal component analysis (IPCA2) which explained 29.22% of the GEI interaction sum of squares. When combined they explained about 77% of the total GEI interaction with 64 degrees of freedom. The mean squares for IPCA3 and IPCA4 were not significant and they cumulatively contributed 19.66% of the total GEI.

Table 5.4: ANOVA of AMMI model for grain yield ( $\text{kg /ha}^{-1}$ ) of 30 soybean genotypes grown at 6 environments between 2010 and 2011 seasons.

Source of variation	df	SS	MS	Explained % of Total SS	Explained % of GEISS
Treatments	179	852292794	4761412***	81.07	
Genotypes	29	51203491	1765638***	4.87	
Environments	5	700298416	140059683***	66.61	
Replication	12	45352151	3779346***		
GEI	145	100790886	695110***	9.59	
IPCA1	33	48132206	1458552***		47.75
IPCA2	31	29447866	949931***		29.22
IPCA3	29	12131316	418321 <sup>ns</sup>		12.04
IPCA4	27	7676145	284302 <sup>ns</sup>		7.62
Residuals	25	3403353	136134 <sup>ns</sup>		3.38
Error	348	153668430	441576		
Total	539	1051313375	1950489		

\*\*\*Significant at  $P < 0.001$ ; \*\*Significant at  $P < 0.01$ ; while ns is non-significant.

### 5.3.3 AMMI genotype ranking

AMMI model analysis revealed the best four selections from each test environment and these are presented in Table 5.5. Inconsistent ranking was observed for genotypes BRS 217 Flora, 915/5/12, Sable and UG-5 which were top ranked in IG2, MW2, EM1 and IG1 environments respectively. BRS MG46 was ranked the best in EM2 and MW1 environments. Advanced line 915/5/12 recorded the highest mean grain yields of 3809 kg /ha<sup>-1</sup> in environment MW2.

Table 5.5: First four AMMI selections per environment

Environment code*	Mean yield (t/ha)	IPCA Score	Rank			
			1	2	3	4
IG2	1543	28.9	BRS 217 Flora	SB-37	BRS MG46	931/5/34
IG1	705	23.18	UG-5	G10428	BRS MG46	Bossier
MW1	500	17.1	BRS MG46	UG-5	G10428	Duicker
MW2	3809	-4.77	915/5/12	Duicker	911/5/6	916/5/19
EM1	1482	-21.16	Sable	EAI 3600	Nyala	916/5/19
EM2	2632	-43.25	BRS MG46	G7955	Sable	911/5/6

\*Environment codes are as presented in table 1.

### 5.3.4 AMMI biplot analysis

The AMMI biplot of 30 soybean genotypes in 6 environments is presented in Figure 5.1. From the AMMI biplot analysis, the Environments MW1 and IG1 were grouped together, with mean yields less than the grand mean and negative IPCA1 scores. Environments EM2 and MW2 had high mean yields with high positive IPCA scores. Environments EM1 and IG2 were positioned close to the grand mean but they recorded the largest negative and positive IPCA1 values, respectively.

Soybean genotypes were less variable for both main effects (genotype and environment means) but highly variable with regard to IPCA1 interaction scores. All the genotypes had IPCA1 scores between 0 and  $\pm 20$ . There was a cluster with positive IPCA1 scores with yields close to the mean yield. This group included Namsoy 4M, SB-37, G10428 and 932/5/36. Similarly, genotypes UG-5, 917/5/16, G8586 and Nyala had yields close to the grand mean but with negative IPCA1 scores.

Another group consisted of soybean genotypes with IPCA1 scores close to zero. Genotypes 916/5/19, G7955, Duicker, G58 and Gazelle had mean yield above the grand mean and low

IPCA1 scores while genotypes SB-20, Maksoy 1N, 835/5/30 and Maksoy 2N had IPCA1 close to zero but yields below the grand mean.

Other genotypes had relatively low mean yields and IPCA scores between 10 and -20. These included BRS Sambaimba, SB-4 and Ex Japan and they were close to environments MW1 and IG1. Nyala and G8586 also had yields slightly below the grand mean and negative IPCA1 scores. Genotypes SB-17 and FH-1 recorded low yield and they performed better in environment IG2 which had the same positive interaction scores.

The other group consisted of genotypes with higher yields above the grand means. BRS MG46 exhibited the highest mean yields with high positive IPCA1 scores. Other soybean genotypes with higher positive IPCA1 values were 911/5/6, BRS 217 Flora, SB-37, 931/5/34, 915/5/12, 932/5/36 and G7955. In contrast, commercial varieties Bossier, EAI3600 and Sable had yield above the grand means but negative IPCA1 scores.

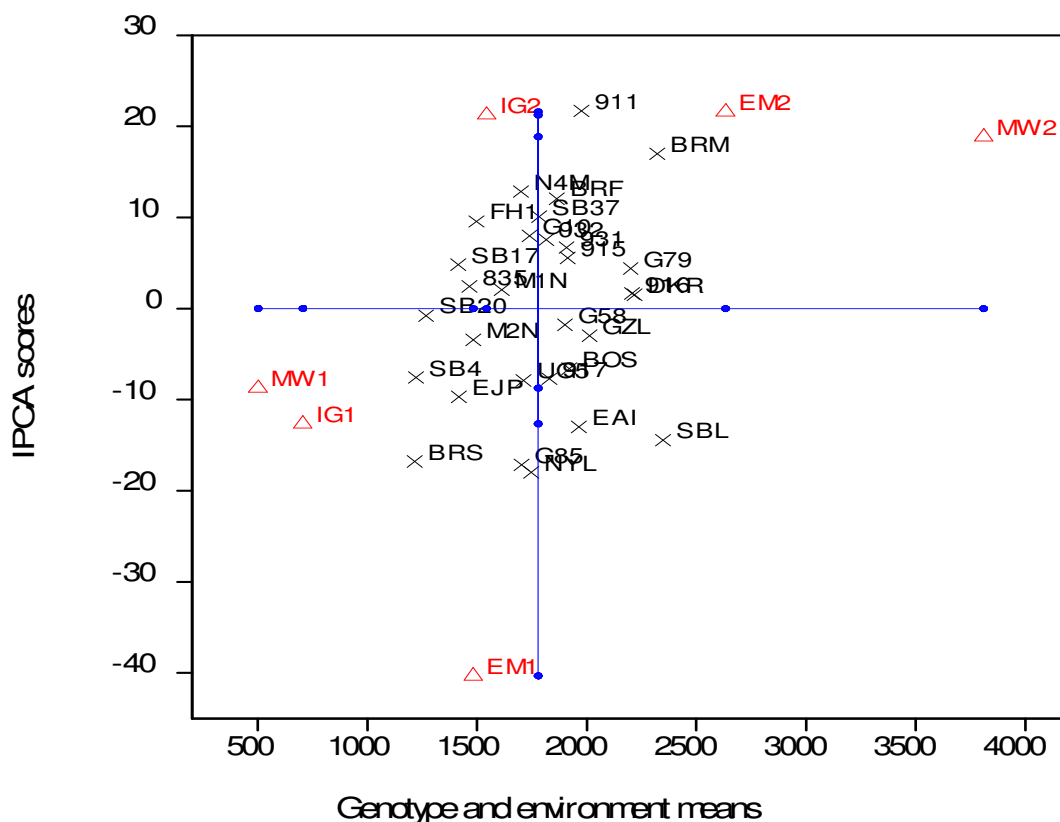


Figure 5.1: AMMI biplot showing the main effects and their interaction (IPCA1) effects of 30 genotypes and six environments. IPCA represents the Principal Component Analysis axis. Abbreviations for named environments and genotypes are as presented in Tables 5.1 and 5.3, respectively.

### **5.3.5 GGE bipot analysis**

From the GGE analysis, the first principal components explained 58.49%, while the second principal component captured 20.16%, cumulatively explaining 78.65% of the Genotype and GEI total sum of squares.

### **5.3.6 Best performing soybean genotypes**

The best performing genotypes in different environments were visualized using a polygon view in Figure 5.2. This polygon view was drawn by joining nine soybean genotypes at the furthest corners from the origin of the biplot. These were BRS 217 Flora, 911/5/6, BRS MG46, Sable, Nyala, BRS Sambaiba, SB-4, SB-17 and G10428. Nine perpendicular lines were later drawn to each of the polygon side passing through the origin of the biplot dividing the biplot into nine sectors.

The six environments appeared in 3 sectors of the polygon view. Environments IG1, MW1, EM1 and EM2 were in the same sector. Environments MW2 and IG2 were classified in the second and third sectors, respectively. Commercial variety Sable was the best performing genotype in Environments IG1, MW1, EM1 and EM2. Advanced line BRS MG46 followed by 911/5/6 performed best in Environment MW2, while BRS-217 Flora was the best performing genotype in Environment IG2. Other vertex genotypes like BRS Sambaiba, SB-4, SB-17 and G10428 did not fall under any of the test environments. The rest of the genotypes were located within the polygon, while Maksoy 1N was located close to the biplot origin.





Conversely, genotypes Sable, BRS MG46, 911/6/9 and EAI 3600 were among the high yielding genotypes but their yields were unstable because they were located far from the AEC line. Other genotypes had yields below the grand mean but their yields were stable. These included; SB-20, Maksoy 2N, Maksoy 1N and 835/5/30. On the other hand, genotypes BRS Sambaiba, SB-17, G8586, G10428 and BRS 217 Flora recorded the lowest yields and were position far away from AEC line.

Figure 5.3: GGE biplot for yield performance and genotype stability based on average environment coordination (AEC). PC1 and PC2 are the first and second principal components, respectively. Abbreviations for the names of environments and genotypes are as presented in Tables 5.1 and 5.3, respectively.

### 5.3.8 Relationship among the environments

environments, respectively. In this regard, Environment EM2 had the longest vector (largest PC1 scores) and PC2 scores close to zero compared to the other environments. This was followed by Environment MW2 with relatively low PC2 scores close to zero, and moderately low PC1 scores. Environment EM1 had large PC1 scores and high PC2 values. However, Environments IG1 and MW1 had the shortest vectors (small PC1 scores) and PC2 values close to zero while Environment IG2 had low PC1 scores but high PC2 scores.

The angle between the Environment vectors measures the correlation coefficient between the environments. An obtuse angle is a sign of negative correlations, whereas acute angles indicate positive correlations. A right angle depicts lack of correlation between environments. Figure 5.4 shows that environments EM1, MW1, IG1, EM2 and MW2 were positively correlated. However, Environments EM1 and IG2 were negatively correlated.

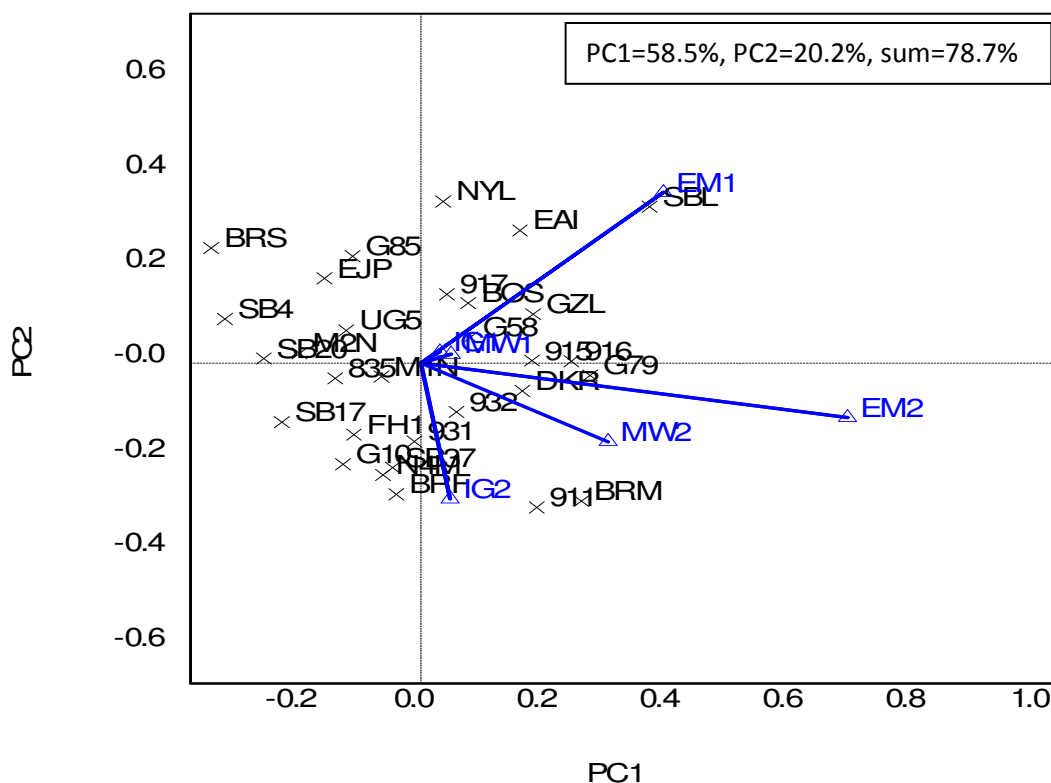


Figure 5.4: GGE biplot based on environment-focused scaling for six environments. PC1 and PC2 are the first and second principal components, respectively. Abbreviations for the names of the environments and genotypes are as presented in Tables 5.1 and 5.3, respectively.

## 5.4 Discussion

From the combined analysis of variance and AMMI analysis, soybean grain yield variation was highly influenced by the Genotype, Environment and GEI. Other studies have reported similar observations on soybean grain yield (Asrat et al., 2009; Jandong et al., 2011; Tukamuhabwa et al., 2012). Environment had the largest contribution to the total sum of squares indicating that the environments (location and season) selected for this study were highly diverse, and this was consequently the main effect contributing most variation for grain yield (Fekadu et al., 2009). The environment differences in terms of key climatic attributes (temperatures and rainfall distribution), altitude, soil fertility and diseases influenced the performance of soybean genotypes, justifying the need to identify high yielding genotypes that are stable in a wide range of environments, or to breed for specific adaptation to specific environments.

The magnitude of GEI sum of squares was approximately twice that of the Genotypes, indicating significant variation in genotypic response to diverse environments. For instance, a variable ranking of soybean genotype performances across the environments was observed for the advanced lines BRS 217 Flora, 915/5/12, Sable and UG-5, which had the highest grain yields in the Environments IG2, MW2, EM1 and IG1, respectively. This showed a cross over or qualitative type of GEI (Crossa, 1990), suggesting the presence of mega-environments where best performing genotypes could be selected more efficiently (Jandong et al., 2011).

In the AMMI analysis, the first two principal components (IPCA1 and IPCA2) were adequate in explaining the GEI for grain yield of the 30 genotypes in the six environments. These results are in agreements with those of Zobel et al. (1988), and of Yan and Rajcan (2002), who both found that the first two principal components were the best in predicting the AMMI model. Other researchers have suggested that the first four PCs were the best for predicting AMMI model (Sivapalan et al., 2000 and Sanni et al. 2009). However, in this study IPCA3 and IPCA4 were not significant.

From the AMMI biplot, Environments MW1 and IG1 were clustered together. These two environments are both characterized by short rains, but in different locations. On the other hand, genotypes had a relatively lower variability on the main effects suggesting that genotypes used in this study had similar response to the environment index, which could be attributed to the narrow genetic base among the advanced soybean lines that probably were derived from

related parental lines. Similar observations have been reported by Tukamuhabwa (2012) in Uganda.

Genotypes or environments located near the perpendicular lines in the biplot had comparable mean yields while high positive or negative IPCA score indicates high instability (Crossa et al., 1990). Therefore, genotypes Namsoy 4M, SB-37, G10428, 932/5/36, UG-5, 917/5/16, G8586 and Nyala had similar main effects. However, Namsoy 4M and Nyala were highly interactive with the environment because they had the highest positive and negative IPCA scores, respectively. In other words, they responded more to the environmental changes than the other genotypes. Similarly, Environments EM1 and IG2 had similar main effects but highly unstable.

In the AMMI biplot display, high yielding environments or genotypes are located on the right side of the perpendicular line while low yielding main effects are located on the left side (Sanni et al., 2009). In this regard, EM2 and MW2 were identified as the highest yielding environments. These two environments were characterized by reliable rainfall distribution and moderate temperatures throughout the experimental period, making them very suitable for soybean production. However, they were more responsive to the environmental effects; hence they were classified as unstable environments. In fact, it was in these environments that genotype BRS MG46 had its highest yield. On the other hand, Environments IG1 and MW1 had relatively smaller interaction effects, with low yielding potential. These two environments experienced short rains with very high temperatures during the experimental period, making them unsuitable for soybean production. The lowest yielding genotypes; BRS Sambaimba and SB-4, performed worst in these two environments. Environments EM1 and IG2 exhibited the largest interaction effects making them unpredictable sites for soybean production.

When a genotype with a large IPCA1 score interacts with an environment of a similar sign, it indicates a level of specific adaptation, while opposite sign depicts negative specific interaction (Zobel et al., 1988; Ebdon and Gauch, 2002; Muhe and Assefa, 2011). For instance, the advanced line BRS MG46 was identified as the best high yielding genotype, and it interacted positively with Environments EM2 and MW2, suggesting specific adaptation to these environments. However, it interacted negatively with Environments EM1, MW1 and IG1, which experience short rainy seasons, indicating that the quantity of rainfall received during the short seasons was not adequate for this genotype. Other medium yielding genotypes that largely interacted positively with Environments EM2 and MW2 were 911/5/6, BRS 217 Flora, 931/5/34

and 915/5/12. Genotypes SB-17, FH1 and Namsoy 4M also performed better in Environment IG2, which had the same positive interaction scores. In contrast, advanced lines BRS Sambaiba and SB-4 were low yielding and they were better adapted to low potential environments (MW1 and IG1) where they interacted with negative IPCA1 scores.

According to Ebdon and Gauch (2002), a genotype is considered stable if it is located close to zero level of the IPCA1 axis. Among the advanced lines, 916/5/19 was considered stable across a wide range of environments because it exhibited low IPCA1 scores. This genotype also delivered a higher mean yield, implying that such a genotype should be selected for future breeding, and could be recommended for release. This result illustrates the value of selecting genotypes with good yield performance and stability effectively (Dehghani et al., 2009). However, further improvement of this line is needed because it is highly susceptible to ASR. Other genotypes including SB-20, 835/5/30, Maksoy 1N and Maksoy 2N had IPCA1 score close to zero, confirming their stability across the environments, although their mean yields were below the grand means. Such genotypes would require breeding for increased yields before they could be released to farmers.

In GGE biplot analyses, the Genotype main effect and the genotype x environment effect were the major sources of variation important for genotype evaluation (Yan et al., 2000; Jandong et al., 2011). In the present study, the first two PCs of the biplot explained 78.65% of the total grain yield variation which was adequate for soybean evaluation. These findings are also supported by Yan et al. (2007), who reported that GGE biplot analysis was effective in regard to mega-environment, yield performance and stability analysis, as well as identification of the best test environments.

The GGE biplot aims to use the “which-won-where” pattern to facilitate identification of the most responsive genotypes (Yan et al., 2000). In this study, the most responsive genotypes were advanced line BRS 217 Flora, 911/5/6, BRS MG46, BRS Sambaiba, SB-4, SB-17 and G10428 and the commercial varieties, Nyala and Sable. Interestingly, these genotypes demonstrated either higher (sometimes the highest) or lower yields compared to the other genotypes in all the environments within the sector in which they fall (Yan, 2002). Other vertex genotypes including BRS Sambaiba, G10428, SB-4 and SB-17, which expressed highly responsive behavior but they did not fall under any of the test environments, indicating that they were not high yielding genotypes in any of the six environments.

The test environments appeared in three sectors of the polygon view, a sign of cross-over of GEI effects, suggesting the presence of three possible mega-environments in Central and Eastern Kenya. According to Yan and Rajcan (2002), a mega-environment refers to a cluster of environments having the same high performing genotype(s). For instance, the first sector had four environments (EM2, EM1, IG1 and MW1) with Sable as the winning genotype. Environment MW2 and IG2 appeared unique and they were classified in the second and third sector, with BRS MG46 and BRS 217 Flora performing the best, respectively. Mega-environments help plant breeders to select high yielding genotypes for a specific environment, making better use of GEI (Jandong et al., 2011). The other importance of mega-environments is that genotypes may be evaluated in a few representative environments, which will provide informative data representing GEI trials across a much larger number of environments. Therefore, Environments EM2, MW2 and IG2 may be used for evaluating soybean genotype in Central and Eastern Kenya. However, more studies are needed to confirm that these three environments are genuinely mega-environments that can represent the entire region.

A GGE biplot based on average environment coordination (AEC) was used to evaluate yield performance and stability of 30 soybean genotypes. According to Dehghani et al. (2009) both yield performance (large PC1 scores) and stability (PC2 close to zero) should be considered together for effective selection of desirable genotypes. Thus, genotypes G7955, 916/5/19, Duicker and 915/5/12 could be selected for future breeding or recommended for release because they were high yielding and stable. Other stable genotypes included Maksoy 1N, 835/5/30, Maksoy 2N and SB-20 but they were low yielding. Such genotypes would require further breeding for high yields before they are released to the farmers. Although genotypes Sable, BRS MG46, 911/5/9 and EAI 3600 recorded the highest grain yields, they were unstable across the test environments. These varieties would be recommended for specific environments or selected for their yield performance to improve low yielding genotypes in a soybean breeding programme.

According to Kaya et al. (2006) environments with longer vectors (large PC1 scores) have the ability to discriminate (informative) between genotypes for a given trait, while short vectors identifies environments with a poor ability to discriminate between genotypes. On the other hand, small PC2 values (PC2 scores close to zero) are good representative of the target environments and vice versa. Therefore, any test environment with large PC1 scores and PC2 scores close to zero are desirable. In this study, among the six environments, EM2 had the

longest vector, and PC2 scores close to zero. It was, therefore, identified as the most useful environment in terms of discriminating between genotypes and was the most representative of all the test environments. Similarly, Environment MW2 was fairly representative and moderately powerful in discriminating between genotypes, hence it was classified as a favourable environment. On the other hand, Environments IG1 and MW1 were classified as less favourable because of their low capacity to provide information on the differences between genotypes. These findings were supported by Yan and Tinker (2006). Environment EM1 was better for discriminating genotypes but a poor representative of the test environment. However, it was suitable for developing genotypes for specific environments (Mohammadi et al., 2009). Environments EM1, IG1, MW1, EM2 and MW2 were positively correlated, indicating that they discriminated between genotypes in a similar manner. On the other hand, Environments EM1 and IG2 were negatively correlated, indicating that they discriminated between genotypes in different ways.

This study identified EM2 as the most effective environment for discriminating between genotypes, and for being the most representative environment. Hence, soybean selections made in EM2 would be consistent across all the six test environments, reducing the costs and logistics of testing new soybean lines.

## **5.5 Conclusion**

In this study, GEI was tested for 30 soybean genotypes in six environments. The GEI was twice the main effect of Genotypes, indicating a significant variation of genotypes in multiple environments, thus justifying the need to run GxE trials in order to identify stable or specifically adapted soybean genotypes. Using both the AMMI model and GGE biplot, Genotypes 916/5/19 and G7955 emerged as the best lines, both in terms of mean yield performance and minimum GEI values. These genotypes are therefore recommended for commercial production in Central and Eastern Kenya. Genotypes Sable and BRS MG46 were the highest yielding genotypes, but were highly unstable; hence they can only be recommended for the specific environments in which they performed well, EM2 and MW2, respectively.

A GGE polygon view divided all the environments into 3 sectors. The first sector consisted of 4 environments (EM2, IG1, MW1 and EM1), with the variety Sable as the best performing genotype. Environments IG2 and MW2 were classified differently from all the other environments, with BRS MG46 and BRS 217 Flora as the best performing genotypes,



respectively. These results suggested that there are 3 mega-environments in Central and Eastern Kenya, but more studies are needed to confirm that these are mega-environments that can represent the region, and to identify other mega-environments in the other regions of the country. Environments EM2 was the most representative of all the test environments, as well as the most effective at discriminating between genotypes. This implies that selections made in this environment would produce the best genotypes that would perform consistently in all the six environments tested. This would be beneficial to the plant breeders for fast, accurate selection, combined with reduced field costs.

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## **6 General overview of the study**

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### **6.1 Introduction**

This study focused on pre-breeding experiments to identify ASR resistant soybean varieties with farmers' preferred traits that could increase soybean production in Kenya and reduce the current reliance on importations. The first step was to carry out a survey in the major soybean growing areas to identify farmers' preferred varieties, their perceptions of ASR and constraints facing soybean production in Kenya. The second step involved screening local and introduced soybean genotypes to identify resistance sources that could be used as the basis for a breeding program to develop ASR resistant varieties suitable for Kenya. In order to formulate effective breeding strategies, the third step was to determine combining ability for resistance to ASR and other selected agronomic traits in soybeans. Finally, stability analysis was carried out to identify stable or specifically adapted genotypes and suitable testing sites in Kenya. This chapter therefore highlights major findings from the study and the future breeding implications of the research.

### **6.2 Summary of the major findings**

The major findings of this study are highlighted as follows;

#### **6.2.1 Identification of farmers' preferred varieties, perceptions on soybean rust disease and key soybean production constraints in Kenya**

This study aimed at determining farmers' preferred traits, selection criteria and their knowledge on soybean rust and key constraints affecting soybean production and marketing. The results established that;

- Soybean is grown as a source of income, food and beverage, livestock feed, soil fertility improvement and green vegetables for fresh market.
- The majority of the farmers preferred local soybean varieties over the improved varieties because they possess more desired traits (such as yields, early maturity, drought tolerance and seed availability) than the improved varieties.
- There were differences in the desired traits by the farmers across the study regions.
- Farmers had several desired traits they would prefer in an ideal soybean variety including high yields, early maturity, adaptable and quality related traits.

- Inadequate information on the availability and production of the improved varieties and lack of resources to purchase improved varieties were the major limitations for growing improved soybean varieties.
- Aphids and thrips were the major pests in soybeans while ASR was identified as the most common disease in some counties.
- The majority of farmers were not aware of ASR.
- Farmers associated rust occurrence with environmental factors, weeds, physiological maturity and poor soil fertility conditions.
- ASR control measures were very minimal and sometimes non-existence
- Other major constraints cited by farmers included lack of markets, lack of awareness on processing and utilization, unavailability of seeds, lack of inputs, frequent dry spells and low yielding varieties.

#### **6.2.2 Evaluation of soybean genotypes for ASR resistance and its correlation with selected agronomic traits in Kenya**

This study aimed at identifying soybean germplasm with resistance to *P. pachyrhizi* isolates in Kenya. The main findings were;

- Fourteen soybean genotypes had relatively high levels of resistance to ASR that could be used in breeding programmes.
- Of the four known genotypes carrying single resistant genes, accessions G10428 (*Rpp4*) and G8586 (*Rpp2*) exhibited the highest level of resistance across the environments.
- Genotypes MAK BLD 11.3, GC 00138-29 and Namsoy 4M, with no known resistant genes also showed high levels of resistance.
- Genotype G7955 carries the *Rpp3* resistant gene, whereas G58 and Tainung 4 carry the resistant gene *Rpp1*. All three genotypes provided moderate resistance, which suggests that races with matching virulence genes are already in Kenya.
- Among the advanced lines, only BRS Sambaimba provided moderate rust resistance.
- None of the commercial varieties, genebank accessions and collections from farmers' fields were rust resistant.
- Rust severity, rust sporulation and AUDPC values varied considerably across the locations and seasons.
- ASR severity ratings were positively and highly correlated with AUDPC values and rust sporulation.

- Rust severity also expressed significant but low positive correlation coefficient with 100 seed weight and oil content

### **6.2.3 Combining ability for resistance to ASR and selected agronomic traits in soybeans.**

A genetic analysis was conducted using the parents and  $F_2$  progenies generated from an 8 x 8 half diallel mating design to determine the nature of gene action controlling resistance to ASR and selected agronomic traits in soybeans. The results showed that;

- Additive gene action played a significant role in controlling the inheritance of rust severity, rust sporulation, days to flowering, days to maturity and plant height.
- Soybean grain yield was predominantly governed by non-additive gene action.
- Parents G10428, G8586 and Namsoy 4M had good general combining ability for both rust severity and sporulation.
- Parents G7955, G8586 and G58 were desirable for early flowering, while parent Maksoy 1N contributed significantly towards early maturity.
- Parents G58, Maksoy 1N, G7955 and Nyala were good combiners for reduced plant height.
- None of the parents contributed significantly towards soybean grain yield improvements.
- $F_2$  populations derived from crosses G10428 x G8586, G58 x BRS MG46, Namsoy 4M x BRS MG46 and BRS MG46 x Maksoy 1N had the most promising progenies for rust resistance.
- $F_2$  populations of crosses G58 x Nyala and G7955 x Maksoy 1N overall the best for early flowering and reduced plant height across the environments, respectively.
- Crosses G8586 x Maksoy 1N and G58 x Namsoy 4M resulted in  $F_2$  progenies with early maturity.

### **6.2.4 Genotype x environment interaction (GEI) and stability for soybean grain yield in Kenya**

This study was conducted to determine the magnitude of GEI, to identify high yielding and stable or specifically adapted soybean genotypes, and to find suitable testing environments in Central and Eastern Kenya. Results established that;

- The effects on soybean yields due to Genotype, Environment and GEI were significant, with the highest variation (66.61%) being caused by the Environment. The magnitude of the GEI sum of squares was approximately twice that of the Genotype main effect.

- Soybean genotypes were ranked differently in different environments, suggesting a cross-over type of GEI.
- Genotypes Sable and BRS MG46 recorded the highest yields in some environments, but Using AMMI and GGE analysis showed that they were highly unstable in terms of a high GEI effect.
- The best genotypes were 916/5/19 and G7955 with high yields and yield stability across all the test environments.
- Environment EM2 (KARI-Embu, long rains) was the most representative of all the test environments, as well as the most effective in terms of discriminating between genotypes.
- Environment IG1 (KARI-Igoji, short rains) and MW1 (KARI-Mwea, short rains) were poor at discriminating between soybean genotypes.
- Environment EM1 (KARI-Embu, short rains) was good for discriminating genotypes but a poor representative of the test environments, therefore it is only suitable for developing specifically adapted genotypes.

### **6.3 Breeding implications and the way forward**

This study established that soybean was an important crop in Kenya with multiple uses. However, its production was allocated to smaller plots of land than most other food crops. The majority of the farmers continued to grow local varieties because they possessed several desired traits that were lacking in “improved” varieties released previously. Since the majority of farmers were willing to grow genuinely improved soybean varieties; future research should be conducted through participatory breeding approach in order to incorporate farmers’ needs. For instance, farmers’ preferred varieties may be used for selecting suitable parental materials for future breeding to develop varieties that will meet farmers’ need. The improved varieties on the other hand, possessed some desired traits that could be used as parents for introgressive breeding to improve trait deficits in the local varieties.

Farmers wanted several traits in soybean varieties, but these traits differed across the counties, indicating the importance of farmers’ involvement during variety development for enhanced acceptance and adoption. Overall, the traits included high yields, early maturity, ease of cooking, drought tolerance, shattering resistance, and pest and disease resistance. New soybean varieties should also have a high nitrogen fixing ability, to alleviate soil fertility problems for subsequent crops, intercropping compatibility to fit in with intercropping systems,

possess good quality traits (protein and oil content) that meet industrial requirements, and the stay-green trait for green pod sales. If Kenyan plant breeders can incorporate these traits into new soybean varieties, then some of challenges faced during soybean production, marketing and consumption would be solved. Efforts should also be made to address other socio-economic constraints by involving extension agents, microfinance institutions, and policy makers. There is also a need to link farmers to the markets offered by the processing industries, and to train farmers in the technologies of processing and utilization of soybean.

The majority of farmers were not aware of ASR, indicating a clear need for extension services to improve farmers' knowledge, particularly on disease identification and management strategies. The control measures employed by farmers were minimal and sometimes non-existent. This is partly because small-scale farmers cannot afford to buy fungicides and spraying equipment to control ASR, which is what the commercial farmers use to control it. In addition, cultural practices alone are not effective for controlling ASR. Furthermore, there are no available commercial rust resistant varieties. Therefore, breeding for rust resistant soybean cultivars would be the best option for managing ASR for small scale farmers in Kenya.

Soybean germplasm has been screened all over the world for ASR resistance. However, no good resistance has been found yet that is at a high level and is stable. Several factors are behind this problem, including the limited genetic diversity of soybean globally, the large number of hosts of *P. pachyrhizi*, the rapid evolution of *P. pachyrhizi* races, and the interaction between genotype, environment and pathogen. This study identified resistant and moderately resistant genotypes in the exotic germplasm and one advanced line. However, this resistance may be temporary because these varieties have not been grown widely, which would select for new races of *P. pachyrhizi* with virulence to match the resistance. This theory is confirmed by the fact that all the commercial varieties, advanced lines, genebank accessions and collections from the farmers' fields were susceptible to Kenyan rust races. Therefore, the resistant genotypes identified in this study could be used as sources of resistant genes or donor parents to improve the commercial varieties and advanced lines, using a backcrossing programme. But this approach is risky due to the rapid development of new virulent races of *P. pachyrhizi*.

The genetic studies indicated presence of sufficient genetic variability among the parental lines for improving rust resistance and other agronomic traits in soybeans. This study established that both additive and non-additive gene action were important for all the traits studied but that



additive gene action was predominant over non-additive gene action, as confirmed by GCA/SCA ratio. This indicated the possibility of high genetic gains due to additive gene effects of the genotypes used in this study because a strong GCA effect is a desirable predictor of segregants' performance. This shows that rust resistance, early flowering, early maturity and reduced plant height can be improved effectively through simple selection in early generations. A recurrent selection program using parents with excellent agronomic and quality traits would accumulate the additive genes governing these traits. In only 2-3 generations of screening for multiple traits concurrently plant breeders could create highly resistant soybean with stable quantitative resistance, combined with excellent agronomic and quality traits. In order to undertake this, the problem of increasing male sterility and the numbers of crosses that a breeder can successfully make needs to be solved, probably using male gametocides.

Non-additive gene action played a major role in controlling soybean grain yield. Therefore selection at advanced generations would be effective for substantial genetic gains in grain yield. In addition, breeding procedures such as bulk breeding and single seed descent methods would be suitable for improving soybean grain yields. Among the parental genotypes, G10428, G8586 and Namsoy 4M expressed good general combining ability for resistance to both rust severity and sporulation, indicating that they could contribute towards rust resistance. In addition, inclusion of G7955, Maksoy 1N, G58 and Nyala as parents in the breeding programme would incorporate the important traits of early maturity and lodging resistance.

The ultimate goal in any breeding programme is to develop high yielding, stable genotypes that are economically profitable. Using the AMMI and GGE biplot models, this study identified genotypes G7955 and 916/5/19 as high performing genotypes that were stable in all six test environments. These genotypes are therefore recommended for commercial production in Central and Eastern Kenya. Genotypes BRS MG46 and Sable were the highest yielding genotypes but also highly responsive to the environments. Therefore, these genotypes can only be recommended for specific environments or be utilized to improve yields in the breeding programmes. Genotype G7955 was moderately resistant to rust while 916/5/19, BRS MG46 and Sable were highly susceptible; hence they require further improvements on rust resistant. Environment EM2 was identified as the most suitable for testing soybean genotypes because it is a long rainy season, with temperatures ranging from 14-25°C and fertile soils (humic nitosols) suitable for soybean production.

In conclusion, development of rust resistant varieties with farmers' desired traits and yield stability needs to be given a priority to increase soybean productivity in Kenya. The efficiency of breeding soybeans for rust resistance and other agronomic traits will depend on a good understanding of the genetic variability present in soybean germplasm and the type of gene action controlling these traits. More emphasis should be directed towards developing varieties with partial or rate-reducing rust resistance. This form of resistance is more durable than single gene resistance, which is easily matched by rapid evolution of novel virulent races of *P. pachyrhizi*. Identification of varieties with long lasting resistance to ASR and desirable traits will ensure reduced reliance of fungicides and higher economic returns of a soybean farming that is sustainable.