

**SOYBEAN GENETIC DIVERSITY AND RESISTANCE TO SOYBEAN
RUST DISEASE**

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DECLARATION

This thesis is my original work, and has not been submitted to any other institution for a degree or any other academic award.

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DEDICATION

I dedicate this thesis to my parents Mr. and Mrs. Senoga, brothers, sisters, wife and to all my beloved friends.

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LIST OF ACRONYMS

ANOVA	Analysis of Variance
ASR	Asian Soybean Rust
AVRDC	Asian Vegetable Research and Development Centre
CTAB	Cetyl trimethylammonium bromide
CV	Coefficient of Variance
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
EM	Effective micro organisms
FAO	Food and Agriculture Organisation
GS	Genetic similarity
IBPGR	International Board for Plant Genetic Resources
IITA	International Institute of Tropical Agriculture
INTSOY	International Soybean program
IPGRI	International Plant Genetic Resources Institute
LSD	Least significant difference
MgCl ₂	Magnesium chloride
MUARIK	Makerere University Agricultural Research Institute Kabanyoro
NaCRRI	National Crops Resources Research institute.
NARO	National Agriculture Research Organisation
NTSYSpc	Numerical taxonomy and multivariate analysis system
PCR	Polymerase chain reaction
PIC	Polymorphism information content
QTL	Quantitative trait loci
RAPD	Random Amplified Polymorphic DNA
SAHN	Sequential Agglomerative Hierarchical and Nested clustering method
SSR	Simple sequence repeats
UPGMA	Unweighted Pair Group Method of the Arithmetic averages
USA	United States of America
USAID	United States Agency for International Development
USDA	United States Department of Agriculture

ABSTRACT

This study was conducted to enhance breeding for resistance to soybean rust, the greatest obstacle to soybean production in Uganda. To undertake this assignment, 91 exotic soybean accessions and 39 advanced breeding lines available from the National breeding programme were evaluated for soybean rust resistance. These evaluations were conducted at MUARIK and NaCRRI during 2011 and 2012. In addition, the 91 soybean accessions were assessed for phenotypic and genetic diversity. Similarity matrices were calculated from SSR data for 91 soybean accessions and clustered using NTSYSpc (Version 2.2, USA).

The genotyping of 91 soybean accessions with 10 standard SSR primers for soybean fingerprinting revealed an average of 5.3 alleles per locus. Thus significant genetic variation and high polymorphism were present among exotic soybean accessions indicating high divergence among evaluated genotypes hence could be used to broaden soybean genetic base. SSR markers revealed high polymorphism and confirmed known morphological trait differences among accessions. However, local soybean varieties had low genetic diversity as revealed by the dendrogram which clustered them into one group. Soybean accessions (PI 615437, PI 567053, PI 628861, PI 567056A, PI 635999, PI 578457A, PI 628959, Serenade, PI 628812, PI 567104B and PI 567025A) had yield of more than 3 tons ha⁻¹ which suggested that they had high yield potential.

Soybean accessions PI 462312 and PI 459025 with known specific resistance genes *Rpp3* and *Rpp4* respectively showed resistance to soybean rust at MUARIK and thus are recommended to be used in improving resistance in already released varieties in Uganda. There is need to evaluate them in other regions of Uganda to assess whether they have broad spectrum resistance. Rust severity was generally higher in late maturing soybean accessions compared to early maturing accessions. There was an increase in rust severity among soybean lines as they matured with the highest severity observed at R6. Soybean accessions PI 417126, PI 416826A and PI 615437 and the breeding lines BSPS 48A-27 and BSPS 9.2B showed an immune reaction to *P. pachyrhizi* at the two stations making them useful source of resistance to soybean rust. Therefore, there is need for hybridization between the resistant soybean accessions and the local adapted varieties to develop rust resistant varieties.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Origin, distribution and production of soybean

Soybean (*Glycine max* [L] Merrill) originated from China about 3000 years ago (Probst and Jude, 1973) and spread to other parts of the world (Purseglove, 1986). Soybean evolved from the wild forms *Glycine ussuriensis* and *Glycine soja* known to occur in China, Manchuria and Korea (Piper and Morse, 1992). It's from China that the crop spread to neighboring countries notably Mongolia, Japan and Indonesia (Singh, 1991), which constituted the secondary centers of domestication. Later, soybean spread to temperate and tropical regions of Asia, to include countries like India, Pakistan, Nepal and Burma (Gai and Zhao, 2001). Traders moved soybean from Asia to Europe from where it was introduced to USA (Kauffman, 1978). Soybean was introduced by missionaries to Africa (Mayo, 1945) and has spread to many African countries including: South Africa, Egypt, Nigeria, Zimbabwe, Kenya, Rwanda and Tanzania (FAO, 2002). In Uganda, soybean was first cultivated in 1913 (Rubaihayo, 1969). Expanded production was observed in late 1940s owing to Government's Department of Agriculture efforts to diversify agriculture and to promote soy-based health foods (Hittle, 1987).

Today soybean is a widely grown crop particularly in regions outside its centre of origin and domestication. By 2011, the world's leading soybean producing countries were USA (32%), Brazil (28%) and Argentina (21%). Other countries include China (7%); India (4%); Paraguay (3%); Canada (1%) with the remaining countries accounting for only 4% of soybean production. In Africa, the leading soybean producers include: Nigeria (617,000 tons); South Africa (430,000 tons); Uganda (180,000 tons); Zimbabwe (112,300 tons) and Egypt (52,500 tons) (FAO, 2011). In Uganda, the land areas under soybean production and yields have increased steadily. For example in 1995, 79,000 tons were produced from 72,000 ha; however, by 2000, 120,000 tons were produced from 106,000 ha and in 2010, 180,000 tons were produced from 150,000 ha (FAO, 2011). These figures illustrate the increasing interest to produce soybean and justify any research interventions to address its key production constraints.

1.2 Importance of soybean

Soybean is ranked as the most important grain legume worldwide (Lance, 2005; IITA 2009) due to its high quality protein and oil contents which constitute about 40% and 20% respectively (Anon, 2004). Soybean seeds also contain 25% carbohydrates, 6% ash and 9% water (USDA, 2009). Soybean is very important in diets of economically challenged communities as an alternative protein source, where animal proteins are not available and or limited (Anon, 1996). In practice, soybean can be consumed as a vegetable in form of fresh seeds, while the dried seeds are eaten whole, split or sprouted. Processed soybean gives several food products including soy mash relish, soy bread, biscuits, cookies, doughnuts, bagia, flour, soy nut butter, crisps, soy yogurt, soy milk and soy meal (Anon, 2004). Soymilk is very valuable in nutrition as a protein supplement for infant formula in form of curds and cheese (Pazdermuk *et al.*, 1997; Merritt and Jenks, 2004). The opening of the Africa Basic Foods in 1965 (the first soy-foods company in Uganda) with a focus to produce and market low-cost soy foods to combat widespread malnutrition then is testimony to the nutritional role of soybean.

Moreover, soybean consumption can be used as a therapy to several ailments including: reduction in risk of colon cancer (Symolon *et al.*, 2004) and heart diseases (Butcher *et al.*, 2002; Sacks *et al.*, 2006). Soybean oil is also used in the manufacture of paints, linoleum, oil clothing, printing ink, soap, cosmetics, insecticides and disinfectants (Myaka *et al.*, 2005) and as a source of bio-diesel (Roseli *et al.*, 2005). Soybean is an important cover crop and fixes soil nitrogen symbiotically ($60\text{--}168\text{ kgNha}^{-1}\text{yr}^{-1}$) which improves soil fertility (Rubaihayo *et al.*, 1980; Tanaka *et al.*, 1980). The food and non-food uses of soybean make it a highly competitive crop, making its production constraints a major concern especially soybean rust.

1.3 Soybean production constraints

Soybean growth, productivity and seed quality are adversely affected by a wide range of stresses that are either abiotic or biotic (Manavalan *et al.*, 2009; Tran and Nguyen, 2009; Gutierrez Gonzalez *et al.*, 2010). The crop is attacked by many insect pests notably; stink bug (*Nezera viridula*), armyworm (*Pseudaletia unipuncta*), soybean looper (*Pseudoplusia includens*), velvet bean caterpillar (*Anticarsia gemma*) and bean leaf beetle (*Cerotoma trifurcate*) (Musser and Catchot, 2008). Diseases of soybean include; bacterial pustule (*Xanthomonas campestris* pv *glycine*), bacterial blight (*Pseudomonas amygdali* pv. *Glycinea*), soybean mosaic virus, red leaf

blotch (*Phoma glycinicola*), frog-eye leaf spot (*Cercospora sojina*), purple stain (*Cercospora kikuchii*) and soybean rust (*Phakopsora pachyrhizi*) (Yang, 1991). By far, soybean rust is the most destructive foliar disease worldwide (Yamanaka, 2010) and can cause up to 80% yield loss in unprotected fields (Hyten *et al.*, 2007). In Uganda, this disease was first reported in 1996 (Anonymous, 2000) and since then it has been reported in several African countries including: Zimbabwe, Nigeria, Kenya, Rwanda, Zambia, South Africa, Cameroon, Mozambique and Benin Republic (Kawuki *et al.*, 2003).

1.4 Statement of the problem

Soybean rust disease caused by *Phakopsora pachyrhizi* has become an endemic major threat to soybean production in Uganda. The deployed resistance in varieties Maksoy 1N and Namsoy 4M has broken down due to emergence of more virulent and aggressive strains (Kiryowa *et al.*, 2008; Tukamuhabwa *et al.*, 2009). Moreover, *Phakopsora pachyrhizi* has been found to have multiple virulent and aggressive races in Africa and South America (Hartman *et al.*, 2004) which implies that chances of resistance breakdown are high (Miles *et al.*, 2006). In addition, repeated use of closely related parents in the breeding programme has narrowed the genetic base of the available germplasm consequently increasing its vulnerability to new races of soybean rust (Hyten *et al.*, 2006). The research conducted in this thesis, which involves exploitation of new exotic accessions with rust resistance is responding to this challenge.

1.5 Justification of the study

There is need to identify new sources of rust resistance from exotic accessions to cope with the highly variable rust pathogen (Tukamuhabwa and Maphosa, 2011). However, the genetic relatedness of soybean accessions with potential for rust resistance should be established before deploying them against soybean rust. This is important in order to increase the usefulness of the deployed resistant varieties. One option for establishing relatedness and/or similarity of parental materials is through their phenotypic/genotypic characterization. However, the commonly used phenotypic descriptors in soybean are less informative and limited, given the common ancestry of most commercial varieties (Priolli *et al.*, 2002). It's on this basis that DNA-based markers are used for genetic analysis in soybean (Kuroda *et al.*, 2009; Li *et al.*, 2009).

Most exotic soybean genotypes have narrow adaptation implying a need to screen and evaluate their performance under local conditions before their use in breeding programme. Therefore, characterisation of exotic soybean genotypes will enable the selection of diverse accessions for broadening the genetic base for soybean rust resistance breeding. The soybean breeding programme at Makerere University through the USDA, obtained 89 soybean accessions from the US soybean germplasm collection centre and Seed Co, Zimbabwe in an effort to broaden the genetic base for various traits with greater emphasis on soybean rust. In this study, the level of resistance to soybean rust, phenotypic and genetic diversity among the introduced soybean germplasm together with Ugandan germplasm were quantified.

1.6 General objective

To enhance soybean germplasm base and develop rust resistant varieties

1.7 Specific objectives

- (i) To determine the phenotypic and genetic diversity in exotic soybean germplasm
- (ii) To screen soybean germplasm for resistance to soybean rust in Uganda

1.8 Hypotheses

- (i) There is broad diversity among the exotic soybean germplasm
- (ii) Soybean germplasm is genetically variable for resistance to the rust disease in Uganda

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Genetic diversity and variability in soybean

The domestication of soybean (*Glycine max* [L] Merrill) from its wild progenitor (*Glycine soja*) occurred in China (Carter *et al.*, 2004; Chung and Singh, 2008). *Glycine soja* has smaller pods and seeds, viny and twining stems, and pronounced shattering at maturity. Hybridization between wild and cultivated species produces fertile progeny (Herman, 1962). Semi wild accessions can be distinguished from *G. max* and *G. soja* based on either phenotypic or genotypic data (Chen and Nelson, 2004). Major genetic bottlenecks occurred between wild and cultivated soybean species that were characterized with loss of genetic diversity (Li and Nelson, 2002; Xu and Gai, 2003). Using chloroplast microsatellites, eight haplotypes were found in cultivated soybean (Xu *et al.*, 2002). However, using DNA sequences from 102 genes, Hyten *et al.* (2006) reported that diversity in the wild species was halved in the domestication process and that 81% of the rare alleles were lost. Exportation of soybean from centers of origin to the New World has therefore resulted in significant loss of genetic diversity which justifies the need to broaden the current soybean germplasm base in order to sustain and/or increase production.

Quantification of germplasm genetic diversity is very vital for efficient selection of parental lines for crossing and/or for germplasm conservation (Tatineni *et al.*, 1996; Fu, 2003). Plant breeding has played a part in shaping genetic diversity of crops. For instance, soybean genetic diversity was enhanced through genetic recombination along the process of varietal improvement to meet agricultural, social and economic needs. Some varieties were artificially selected by farmers to suit their needs while others were naturally selected in response to geographical, climatic and edaphic factors (Hawkes, 1983). However, studies have indicated that only a few accessions have contributed majority of genes in current cultivars, leading to low genetic diversity in soybean varieties which is a major constraint to genetic improvement (Fu, 2006).

Uganda is no exception to this, thus the introduction of exotic and/or novel germplasm into local breeding programme would dramatically increase variability for systematic soybean genetic improvement including development of soybean rust resistant varieties. Moreover, exotic accessions are likely to have novel alleles for other traits of interest which can be incorporated into the breeding program. Previous studies have used molecular markers such as microsatellite

or simple sequence repeats (SSR) to quantify genetic diversity in accessions due to their robustness, highly polymorphic nature, reproducibility and ease of automation (Lee *et al.*, 2008; Kuroda *et al.*, 2009; Li *et al.*, 2009).

2.2 Phenotypic and molecular characterization of soybean germplasm

A number of studies have been carried out to determine the genetic diversity of cultivated soybean (Thompson *et al.*, 1998) using the coefficient of parentage (Cui *et al.*, 2000), morphological traits (Dong *et al.*, 2004), biochemical tests like isozymes (Hirata *et al.*, 1999) and molecular markers notably SSRs (Cho *et al.*, 2006; Wang *et al.*, 2006). Indeed, SSR markers were also used to compare genetic diversity among *G. soja* accessions collected from China and Korea (Lee *et al.*, 2008). Minimum overlap exists between molecular markers and agro-morphological traits which suggest that they are in linkage disequilibrium (Wang *et al.*, 2006). Hybridization among closely related soybean cultivars to produce commercial varieties reduces the genetic diversity leading to cultivars that are often indistinguishable based on morphological traits (Brown-Guedira *et al.*, 2000). Polymorphic DNA markers in combination with morphological traits provide the best estimate of the genetic diversity among the soybean cultivars. It's on this premise that this study employed both DNA-based markers and phenotypic descriptors to quantify the genetic diversity in the assembled soybean germplasm in Uganda.

2.3 History of soybean rust disease

Soybean rust (*Phakopsora pachyrhizi*) has for many years been limited to Asian countries mainly Taiwan, Thailand, India and Japan (Yorinori *et al.*, 2005). *Phakopsora pachyrhizi* was first reported in Japan in 1902 (Bromfield, 1984) and was initially limited to tropical and subtropical areas of Asia and Australia. The disease was later identified in the Philippines, China and several other countries in South East Asia in the last 60 years. This aggressive form of rust has spread to most of the soybean producing regions of the world. For instance, in Africa, soybean rust spread to Uganda (1996), Zimbabwe (1998), Zambia, Rwanda, Nigeria, West Africa, Mozambique, South Africa (2001) and Cameroon (Levy, 2005). Rust Spores are believed to have crossed from Asia to Africa via wind currents (Isard *et al.*, 2005; Schneider *et al.*, 2005). The pathogen was reported from Hawaii in 1994 and Paraguay in 2001 (Rossi, 2003). In South America, *Phakopsora pachyrhizi* was reported in Brazil and Argentina in 2002, Bolivia and Colombia in 2003 (Schneider *et al.*, 2005) as well as the Louisiana and Missouri of United States

in 2004 (Levy, 2005). This confirm that the rust pathogen, *Phakopsora pachyrhizi* has spread to all key soybean producing regions of the world, where it remains a formidable obstacle to soybean production.

2.4 Yield loss due to soybean rust disease

Soybean rust causes rapid reduction in green leaf area leading to premature defoliation (Ivancovich, 2005), reduction in number of filled pods, seed number and weight (Sinclair, 1982; Miles *et al.*, 2003). The extent of losses are related to time of disease onset, severity, yield components affected (Ogle *et al.*, 1979), and genetic makeup of plant. The earlier the leaves fall, the smaller will be the grain size hence resulting in high loss in yield and quality (Yang *et al.*, 1991).

Significant yield losses have also been reported in Asia (Sinclair and Hartman, 1999), South America (Yorinori *et al.*, 2005) and Africa (Caldwell and McLaren, 2004). Yield losses of 60-80% have been reported in eastern and southern parts of Africa (Levy, 2005). In Uganda, yield loss of 15-41% (Tukamuhabwa and Dashiell, 1999) and 60-90% (Anon, 2000) were recorded respectively. Yield losses (10 to 90%) have been observed in many countries, as nearly all cultivars grown in new locales of the disease are susceptible (Kawuki *et al.*, 2003). Soybean rust caused crop damage of 10% to 40% in Thailand, 10% to 90% in India, 10% to 50% in southern China, 23% to 90% in Taiwan, 60% to 90% in Australia, 40% in Japan and 75% in Brazil (Sinclair and Hartman, 1999; Yorinori *et al.*, 2005). Other studies have established that yield loss associated with soybean rust vary from negligible to 100% depending on prevailing conditions (Bonde *et al.*, 2006). The widespread distribution and potential for severe yield losses makes soybean rust one of the most serious foliar diseases of soybean worldwide (Miles *et al.*, 2005).

2.5 Symptoms and host range of soybean rust

The most common symptoms of soybean rust are foliar lesions which start as small, yellow specks on the lower leaf surface that enlarge and merge killing large leaf area and result into premature defoliation (Ivancovich, 2005). Mature lesions are either dark brown or tan in color, angular in shape and concentrated near leaf veins (Miles *et al.*, 2005). The rust fungus produces spores in cone-shaped pustules on the lower leaf surface where symptoms may be more apparent. Initially, pustules appear as small, raised blisters in mature tan lesions (Miles *et al.*, 2005) but as they mature, they produce large amounts of light-colored and powdery urediniospores

(Bromfield, 1984) that spread the disease under ambient conditions. Although rust pustules are most common on underside of leaves, they also develop on petioles, pods and stems. In general, rust infection begins from low to mid-canopy especially at flowering stage and moves up the plant as it matures, although plants are susceptible at any growth stage (Walker *et al.*, 2011).

Soybean rust infects over 150 host plant species (Lynch, 2006; Slaminko, 2008) including many wild, edible, forage and naturalized legumes (Rytter *et al.*, 1984; Ono *et al.*, 1992). The large number of host species serves as inoculum reservoirs and bridge hosts for *P. pachyrhizi* thus increasing the likelihood of pathogen build-up, survival and over wintering across seasons. Moreover, *P. pachyrhizi* is an obligate parasite and only survives in presence of its live plant hosts. Host reaction recorded 21 days of incubation post-inoculation, showed typical sporulating uredinia of soybean rust in pigeon pea (*Cajanus cajan*), crown vetch (*Coronilla varia*), lablab (*Lablab purpureus*), dum-cours (*Lespedeza cuneata*), lupin (*Lupinus* sp), lucerne (*Medicago sativa*), velvet bean (*Mucuna pruriens*), common bean (*Phaseolus vulgaris*), jack bean (*Canavalia ensiformis*), lima bean (*Phaseolus lunatus*), yellow sweet clover (*Melilotus officinalis*), medic (*Medicago lupulina*), desmodium (*Desmodium uncinatum*), kudzu vine (*Pueraria lobata*) and cowpea (*Vigna unguiculata*). Urediniospores from pustules on the above crops produced viable spores on susceptible soybean variety, Prima 2000 (Rytter *et al.*, 1984; Slaminko, 2008).

2.6 Life cycle and infection process of soybean rust

Phakopsora pachyrhizi, the causal pathogen of soybean rust produces urediniospores in multiple cycles throughout the crop growth season which are readily dispersed by wind currents (Bromfield, 1984) and thus spreading the disease within and between fields. The infection begins when urediniospores germinate to produce a single germ tube that grows across the leaf surface forming an appressorium which directly penetrates the epidermal cells through the cuticle (Koch and Hoppe, 1988). Uredinia develop 5 to 8 days after infection and the first urediniospores can be produced 9 days after infection (Koch *et al.*, 1983). Secondary uredinia may arise on the margins of the initial infections producing a vast number of spores. High spore production and long-distance spore spread allows soybean rust to buildup rapidly and persist making its control difficult (Hartman *et al.*, 1992). Successful infection depends on the availability of free moisture on plant surfaces for at least 6-12 hours, suitable temperatures of 15-28°C and high relative

humidity of 75 to 80% (Melching *et al.*, 1989). Most soybean growing regions in Uganda are characterized by these environmental conditions, which could in part explain the recurring soybean rust epidemics in areas where resistant varieties are absent.

2.7 Variation in *Phakopsora pachyrhizi* pathogen races

Genetic variation in *Phakopsora pachyrhizi* may be generated through parasexual recombination, migration, spontaneous mutation and somatic hybridization (McDonald *et al.*, 1989). These biological events are largely responsible for the evolution of races of most devastating plant pathogens. The pathogen also produces urediniospores asexually (Pivonia and Yang, 2004). The amount and distribution of genetic variation within and among pathogen populations is vital for designing control strategies. A large amount of pathogen genetic diversity over a small spatial area suggests rapid pathogen adaptation to changing environment while high genetic similarity among pathogen populations from widely separated geographical regions suggests pathogen long-distance dispersal and gene flow (Isard *et al.*, 2005). Both these phenomenon have strong implications of pathogen resistance breeding. For instance, they pose a threat to deployed disease resistant cultivars in a given area since immigrants may possess virulent genes that can overcome resistance of host cultivars (Hartman *et al.*, 2005; Bonde *et al.*, 2006). Moreover, virulence genes might be incorporated into local pathogen populations through parasexual recombination (Braisier, 1988). Highly polymorphic microsatellite markers specific to *P. pachyrhizi* have been developed to study the population biology of this fungus (Anderson *et al.*, 2008). Variable rust pathogen races (3-18) were identified in most parts of the world (Yamaoka *et al.*, 2002) but the genetic variation in *P. pachyrhizi* populations in soybean producing countries is not known.

2.8 Incidence of soybean rust in Uganda

Soybean rust was first reported in Uganda in 1996 and has become endemic in most soybean producing regions of the country due to its aggressiveness (Tukamuhabwa *et al.*, 2001; Levy, 2005). The pathogen was first observed at Namulonge and from there it spread to farmers' fields throughout the country infecting all commercial soybean cultivars (Tukamuhabwa *et al.*, 2001). It's likely that the rapid spread of soybean rust in Uganda was enhanced by the wide cultivation of susceptible varieties (Nam 1, Nam 2 and Kabanyolo 1) which were not previously bred for rust resistance and the availability of favorable environment for pathogen sporulation and spread.

National surveys conducted in major soybean growing regions of the country to determine the occurrence, incidence and severity of the disease among the agro-ecological zones established that incidence ranged from 24 to 100% in different soybean growing districts (Lamo, 2003). This was attributed to different rainfall patterns received in different regions, presence of alternative host species and the absence of highly resistant soybean varieties (Lamo, 2003). Commercial soybean varieties and promising lines were found to have 40% and 30% rust severity respectively (Kawuki, 2003).

2.9 Control strategies of soybean rust

A range of management options have been proposed for soybean rust (Anon, 2006) which include chemical fungicides and host plant resistance (Levy, 2003; Kawuki *et al.*, 2004). By far management of soybean rust has been focused on identification of sources of resistance (Twiyemizimana *et al.*, 2008). Varieties with specific resistance can be used against prevailing *P. pachyrhizi* pathotypes in a given area. Soybean genotypes with major resistance genes have been deployed to control soybean rust (Monteros *et al.*, 2007; Garcia *et al.*, 2008). However, there has been resistance breakdown associated with increased selection pressure in *P. pachyrhizi* populations resulting from the deployment of major resistance genes (Hartman *et al.*, 2005; Bonde *et al.*, 2006). Some breeding programs have been focused on selection for quantitative resistance to promote durable resistance to soybean rust (Ribeiro *et al.*, 2007). However, this remains a challenge due to the large number of genes involved and complicated assessment protocols for quantitative resistance.

2.9.1 Cultural control methods for soybean rust

Cultural practices that minimize conditions favorable for rust development (Hartman *et al.*, 1992) and optimize crop vigor have been advocated for (Desborough, 1984). These methods include: adequate row spacing, early planting, growing early maturing varieties (Akinsanmi *et al.*, 2001), crop rotation with non hosts, increasing field phosphorus levels (Anon, 2002), destruction of host weeds to reduce inoculum level and irrigating in mornings (Caldwell and Laing, 2002). It suffices to note that, cultural control methods have largely been ineffective since the conditions that promote crop development also influence soybean rust. In addition there is the issue of multiple host range (Slaminko, 2008) which limits its usefulness.

2.9.2 Chemical control of soybean rust

Foliar fungicides have been used to control soybean rust, but they are costly (Pedro *et al.*, 2008) and not affordable by subsistence soybean producers in most developing countries (Tukamuhabwa, 2001; Kawuki *et al.*, 2002; Miles *et al.*, 2003). Fungicides have also raised concerns about environmental pollution and toxicity to humans, livestock and non target organisms (Dorrance *et al.*, 2007). In addition, there is a possibility of development of fungicide-resistant pathogen strains (Marco *et al.*, 2008). The protectant fungicide Dithane M-45 was the earliest in control of soybean rust (Hartman *et al.*, 1992) and required frequent applications (7 to 10 days) (Patil and Anahosur, 1998). Other fungicides used include; Tridimefon, Difenconazole, Flusilazole and Carbendazim (Levy *et al.*, 2002). Early detection and proper application to cover the canopy is vital for efficient rust management. The required number of fungicide applications depends on time of rust occurrence and the weather conditions (Kawuki, 2002). Fungicides should be applied when the risk of infection is high and at the susceptible crop growth stages of R1 to R6 (Miles *et al.*, 2003; Levy, 2004).

2.9.3 Biological control of soybean rust

Serenade based on *Bacillus subtilis* (QST 713 strain) and Ballad based on *Bacillus pumilus* (QST 2808 strain) are biofungicides used to control soybean rust (Kloepper *et al.*, 2004; Leclere *et al.*, 2005). Foliar fertilizers with natural ingredients such as fish hydrosylate, neem oil, karanja oil, insecticidal soap, microbial-mineral blends, seaweed, calcium and humic acid are effective for disease suppression. In addition these mixtures supply nutrients and boost plant health (Destree, 2005). Composts, microbial inoculants and foliar bio stimulants are natural biological control strategies attracting attention in USA for their disease suppressive characteristics. They contain a diversity of microorganisms that regulate soil fertility, boost crop health and compete with plant pathogenic organisms for food resources in the root and leaf zones.

The physiological mechanisms for biological control include suppression, antagonism, competition and induced resistance which reduce pathogen ability to cause infection (Destree, 2005). Effective microorganism especially EM-Fermented Plant Extracts and EM-5 are used to control soybean rust through disease suppression. However, it is costly to manufacture, store, handle and apply the bio-fungicides in the field. This is because microbes are living and can easily be denatured by the adverse environmental conditions of rainfall and solar radiation.

2.9.4 Host plant resistance

The use of resistant varieties is a long term practical and economical means of managing soybean rust. However, most of the currently grown commercial soybean varieties in Uganda have little or no resistance to soybean rust. Soybean breeders are working to identify sources of resistance and incorporate into the current soybean varieties. Host plant resistance provides a cheaper, sustainable and environmentally friendly approach for managing soybean rust (Pedro *et al.*, 2008). Specific, partial and durable resistance mechanisms have been identified against *P. pachyrhizi* (Anonymous, 1992; Hartman *et al.*, 2005).

Through breeding, a number of varieties resistant to soybean rust with some having multiple resistance genes (Hartman *et al.*, 1992) have been identified and/or developed. However the durability of this resistance over localities is not stable and is difficult to predict due to lack of understanding of the fungus biology and genetic diversity (Tschanz and Shanmugasundaram, 1985). Soybean expresses three types of reactions against *Phakopsora pachyrhizi* pathogen (Bromfield, 1984). Immune varieties do not produce any visible symptoms while resistant varieties produce red-brown (RB) lesions with either no uredinia or only sparsely sporulating uredinia. Varieties expressing the susceptible TAN reaction have characteristic tan-colored lesions with abundant sporulation from many uredinia (Bonde *et al.*, 2006). Thus soybean varieties can be resistant, tolerant or susceptible to soybean rust.

2.9.4.1 Durable resistance to soybean rust

Durable resistance is associated with varieties having steady resistance to soybean rust across seasons, locations and to varying rust pathogen races (Miles *et al.*, 2006). This resistance reduces the amount and rate of rust development even when the infection type is similar to that of highly susceptible varieties (Bromfield, 1984). Durable resistance is likely to be effective against all rust races thus more useful than specific resistance. The quantification of durable resistance requires periodic rust severity assessments across cropping seasons for many years. In an effort to promote durability of resistance, breeding programs and selection criteria are now focusing on selection for quantitative resistance (Ribeiro *et al.*, 2007).

There is a need for the programme to develop commercial varieties with desirable agronomic traits and durable resistance to soybean rust (Kawuki *et al.*, 2003). This is due to the highly variable rust pathogen populations (Bonde *et al.*, 2006) present in many soybean growing

regions (Miles *et al.*, 2003; Hartman *et al.*, 2005). The use of durable/quantitative resistance ensures long lasting resistance of genotypes to soybean rust. The greater resistance of some varieties to soybean rust could be attributed to presence of quantitative resistance genes (Ribeiro *et al.*, 2007). Durable resistance is an evolutionary process thus would be more effective in disease management especially with available knowledge of soybean germplasm and *P. pachyrhizi* population genetic structure (Watson, 1981). Therefore early-generation soybean breeding lines should be screened against a range of diverse rust pathogen genotypes (Twiyemizimana *et al.*, 2007).

2.9.4.2 Partial resistance to soybean rust

Partial resistance to *P. pachyrhizi* is common in soybean (Wang and Hartman, 1992). Characteristically, partial resistance is associated with less lesion development during the season. Breeding lines and/or varieties displaying this characteristic are rated as moderately resistant (Hartman, 1995; Walker *et al.*, 2011). In inoculation studies, genotypes with RB reactions had longer latent periods, lower rates of increase in pustule number and smaller lesions compared with susceptible genotypes with TAN reaction (Bromfield, 1984). These phenotypic symptoms have enabled the identification of partially resistant soybean lines (Hartman *et al.*, 1991).

Variation in resistance to soybean rust is a more useful criterion for selection of genotypes for improvement (Tschanz and Wang, 1987). Genotypes should be screened for resistance against diverse rust pathogen populations (Hartman *et al.*, 2005). Several studies have been conducted to evaluate soybean for rust resistance under greenhouse conditions using either specific pathogen isolates (Miles *et al.*, 2006) or field pathogen populations (Bandyopadhyay *et al.*, 2006). However, the use of partial resistance in breeding programs has been limited due to time consuming evaluation processes (Anonymous, 1992; Hartman, 1995).

2.9.4.3 Specific resistance to soybean rust

Soybean rust resistance is sometimes controlled by single dominant genes (Tan *et al.*, 1991; Kiryowa *et al.*, 2005). Six single dominant genes *Rpp1*, *Rpp2*, *Rpp3*, *Rpp4*, *Rpp5*, and *Rpp?hyuuga* for specific resistance to *P. pachyrhizi* have been identified in different soybean genotypes (Hartman *et al.*, 2005; Hyten *et al.*, 2007; Garcia *et al.*, 2008). These genes are located at different loci and provide resistance to specific races of *P. pachyrhizi* (Hartman *et al.*, 2004; Bonde *et al.*, 2006). The single dominant genes may not be durable in commercial varieties and

they can be ineffective with diverse *P. pachyrhizi* isolates (Yorinori, 2004; Hartman *et al.*, 2005). Several major genes that confer resistance to soybean rust have also been identified in new plant introductions (Monteros *et al.*, 2007; Garcia *et al.*, 2008; Pierozzi *et al.*, 2008; Chakrobarty *et al.*, 2009).

Specific gene resistance to *P. pachyrhizi* is unlikely to provide lasting protection due to resistance break down (Miles *et al.*, 2006). This is associated with increased selection pressure in *P. pachyrhizi* populations (Bonde *et al.*, 2006) and high genetic variability of *P. pachyrhizi* races (Hartman *et al.*, 2005). Few moderately resistant soybean varieties (Maksoy 1N, Maksoy 2N, Maksoy 3N and Namsoy 4M) are available in Uganda (Tukamuhabwa, 2006), but these develop rust under severe rust pressure which results into yield losses. These varieties contain specific resistance genes to soybean rust (Tukamuhabwa and Maphosa, 2010) that are likely to break down as new *P. pachyrhizi* races proliferate in the region (Kiryowa *et al.*, 2005). This was initially observed with soybean varieties Komata and Ankur which were initially resistant to soybean rust but soon become susceptible due to resistance breakdown (Bromfield, 1984). Therefore, there is need to broaden the soybean germplasm base using genetic material from many possible sources for use in developing soybean rust resistant varieties.

CHAPTER THREE

PHENOTYPIC AND GENETIC DIVERSITY OF SOYBEAN GERMPLASM

3.1 Determination of phenotypic diversity of soybean germplasm

3.1.1 Introduction

Whether created by humans and/or by natural evolutionary forces, genetic variation provides for heterogeneous populations, which have great capacity to stabilise productivity over a range of changing environments (Chang *et al.*, 1979). Chang (1992) observed that with the unrelenting human population growth and environmental extremes, phenotypic variation in germplasm needs to be thoroughly exploited to meet the inevitable expansion in future food, feed and fibre needs. Thus, introducing novel germplasm sources into the Ugandan soybean breeding programme may provide the necessary genetic variability for systematic and/or sustainable development of soybean industry. This notion is based on the premise that soybean is a relatively new crop in Uganda (just over 100 years) and during then it hasn't benefited much from formal breeding.

Introduction of exotic soybean germplasm enables broadening of current genetic base (Chung and Singh, 2008) because it introduces novel alleles and/or allele combinations for desirable traits that can be subjected to selection (Guzman *et al.*, 2007). Thus, the knowledge of genetic diversity among these accessions is vital prior to their utilization in breeding. This information is important as it helps in setting priorities for germplasm conservation and also facilitates the selection of parental lines for introgression of useful genes (Fu, 2003). Therefore in this study, the phenotypic diversity of the 91 soybean accessions was determined.

3.1.2 The experimental site

This experiment was conducted at Makerere University Agricultural Research Institute (MUARIK). The institute is located in central Uganda about 20km north of Kampala at 0° 28' N and 32° 37' E at an elevation of 1200 meters above sea level. The area receives average total annual rainfall of 1160 mm with bimodal distribution. There is a general wet season, mild dry climate and slightly humid conditions with relative humidity of 45 to 75%. The average minimum and maximum temperatures at MUARIK are 16.5 °C and 27.5 °C respectively. These conditions favor optimal growth of soybean and severe rust epidemics (Kawuki, 2002) which enable varietal characterization and rust reaction assessment among exotic germplasm.

3.1.3 Materials and methods

A total of 91 exotic and local soybean accessions including 76 accessions from the US soybean germplasm collection centre, 12 from Zimbabwe Seed Company, one from Zambia and two local varieties from Uganda soybean breeding program were assembled and evaluated (Appendix 1). Each accession was planted in double row plots measuring 2 meters with about 30-35 plants per row and replicated twice in a randomized complete block design. A spacing of 60 cm between rows and 5 cm between plants in a row was adopted. The experiment was conducted twice during the first and second rains of 2011. Planting was done on 24th March 2011 and 7th September 2011 for the two seasons respectively.

3.1.3.1 Data collection

Data comprising of qualitative and quantitative traits were measured from ten randomly selected single soybean plants per plot following established procedures outlined in *Glycine max* descriptors of IBPGR, 1984. The qualitative traits included; hypocotyle color, cotyledon color, leaf color, stem color, growth habit, growth vigor, flower color, pubescence, pubescence density, type and color. Mature pod color, seed coat color, brilliance, hilum color, seed shape and size were also recorded. The entry across the two replicates for each of the qualitative traits was considered. The quantitative traits measured were; days to seedling emergence, hypocotyle length, leaf length and width (cm), number of nodes on the main stem, plant height (cm), number of branches per plant, days to 50% flowering, pod length and width (cm), pod clearance, number of pods per plant, number of seeds per pod, days to physiological maturity (R8), 100 seed weight (g) and yield (kg ha⁻¹). Pod shattering, nodulation and plant lodging were scored using a scale of 1-5 where 1 is low and 5 is high trait score (Helsel and Minor, 1993).

3.1.3.2 Data analyses

The generated data were analyzed using Genstat 13th Edition computer package software (Payne *et al.*, 2011). Quantitative data were subjected to ANOVA and significant mean values compared among varieties at 5% least significant difference. Shattering, lodging and nodulation scores were angular transformed to normalize them prior to analysis of variance.

3.1.4 Results

3.1.4.1 Vegetative qualitative traits

The descriptions of vegetative morphological traits of soybean accessions are presented in Appendix 1. The study showed a predominance of purple hypocotyle color in 53 soybean accessions, with 33 accessions having green hypocotyle while five were dark purple (Table 1). A total of 67 and 24 genotypes had green and purple cotyledons respectively. Green was dominant color of leaves among test materials with variation in color intensity where 42.9%, 16.5% and 40.7 % of genotypes had lighter green, green and dark green leaves respectively. Most of the accessions (72) had green stems while 19 had purple stems. Majority of genotypes (53.8%) had moderate growth vigor, 29.7% had vigorous growth while only 16.5% had poor seedling vigor.

A total of 47 accessions had medium leaves, 27 had large leaves while 17 accessions had small leaves. Most of the germplasm (56) were determinate, 34 were semi determinate while genotype (PI 567123A) was indeterminate. A total of 39 genotypes had broad ovate leaves, 48 had intermediate leaves while four genotypes (PI 628905, PI 567123A, PI 628929 and PI 417089) had narrow elliptic leaves. All germplasm had pubescence with variation in density where 18.7% and 22% had dense and sparse pubescence respectively while 59.3% had semi sparse pubescence. A total of 36 and 27 accessions had brown and light brown pubescence respectively while 28 accessions had grey pubescence. A total of 23.1% and 52.7% of genotypes had appressed and semi appressed pubescence respectively while 24.2% had erect pubescence.

3.1.4.2 Flower and seed traits

The flower and seed traits of the 91 soybean germplasm are presented in Appendix 2. Majority of the accessions (58) had purple flowers while 33 accessions had white flowers (Table 2). A total of 62.6% genotypes had brown mature pods, 26.4% had tan pods while 11% had black mature pods. Majority of the test materials (71) had cream seeds, eight genotypes (Gazelle, SCSI, Duiker, PI 462312, Safari, Santa, Solitaire and PI 605823) had yellow seeds, nine genotypes (PI 628887, PI 567056A, PI 567123A, PI 605838, PI 628933, PI 628959, PI 644103, PI 459025 and PI 628833) had green seeds, two genotypes (PI 416810 and PI 417089A) had black seeds while only one genotype (PI 416826A) had brown seeds. Based on seed coat pattern, 72.5% accessions had dark hilum, 26.4% had light hilum while only one accession (PI 606397B) had saddle seed coat pattern.

Table 1. Vegetative traits of 91 soybean accessions evaluated at MUARIK in 2011

Vegetative trait	Variation	No of Accessions	Percentage
Hypocotyle color	Green	33	36.3
	Purple	53	58.2
	Dark purple	5	5.5
Cotyledon color	Green	67	73.6
	Purple	24	26.4
Leaf color	Light green	39	42.9
	Green	15	16.5
	Dark green	37	40.7
Stem color	Green	72	79.1
	Purple	19	20.9
Seedling vigor	Poor	15	16.5
	Medium	49	53.8
	Vigor	27	29.7
Leaf size	Large	27	29.7
	Medium	47	51.6
	Small	17	18.7
Growth habit	Determinate	56	61.5
	Semi determinate	34	37.4
	Indeterminate	1	1.1
Leaf shape	Narrow	4	4.4
	Intermediate	48	52.7
	Broad	39	42.9
Pubescence	Present	91	100.0
	Absent	0	0.0
Pubescence density	Sparse	20	22.0
	Semi sparse	54	59.3
	Dense	17	18.7
Pubescence color	Grey	28	30.8
	Brown	36	39.6
	Light brown	27	29.7
Pubescence type	Erect	22	24.2
	Semi appressed	48	52.7
	Appressed	21	23.1

Majority of the soybean accessions (51) had brown hilum, 21 had black hilum, 12 had yellow hilum, four accessions (Solitaire, PI 628935, Roan and Maksoy 3N) had grey hilum, two accessions (PI 417503 and PI 628908) had buff hilum while only one accession (PI 459025) had green hilum. A total of 59 soybean germplasm had intermediate seed coat surface lusture, 18 shiny, 13 dull and one genotype PI 416826A was heavy brown. Most of the accessions (53.8%) had oval shaped seeds while 46.2% had round seeds. A total of 26 soybean genotypes had large seeds, 38 had medium seeds while 27 genotypes had small seeds. Most of the germplasm (57.1%) had medium quality seeds, 34.1% had good quality seeds while 8.8% had poor quality seeds.

3.1.4.3 Quantitative traits

The agro-morphological variations among 91 soybean germplasm are presented in Appendix 3. The differences in mean hypocotyl length, leaf length and width, days to flowering and physiological maturity, shattering, lodging, nodulation, number of nodes, branches, pods, seeds per pod, plant height, pod clearance, pod length, 100 seed weight and grain yield were highly significant ($p < 0.001$). However the differences in mean pod width among accessions were not significant ($p=0.015$). The average hypocotyle length for 91 soybean accessions was 3.8 cm, leaf length (11.4 cm), leaf width (7.0 cm), days to 50% flowering (44 days), physiological maturity (97 days), shattering (1.5), lodging (2.1), nodulation (3.6), number of nodes (11), branches (5), pods (41), seeds per pod (2.6), plant height (49.1 cm), pod clearance (8.7 cm), pod length (4.6 cm), pod width (0.9 cm), 100 seed weight (18.1 g) and grain yield (2134 kg ha^{-1}) (Table.3).

The longest hypocotyle (6.9 cm) was found in Santa while the shortest (2.7 cm) was found in PI 567104B (Appendix 3). Genotype PI 417089A had the highest leaf length (15.4 cm), while PI 628863 had the lowest (8.7 cm). Leaf width ranged from 5.4 cm to 8.8 cm for genotypes PI 416826A and PI 567025A respectively. Accession PI 416826A was the earliest to reach 50% flowering (32 days) while accessions PI 567102B, PI 644103 and PI 628929 took the longest duration (65 days). Genotype PI 417132 was the earliest to reach physiological maturity (87 days) while PI 628929 took 123 days to maturity (Appendix 3). The highest nodulation score (5) was found among accessions PI 471904, PI 628813, PI 628857, PI 567189A, PI 578457A, Maksoy 1N, PI 287197, PI 628812 and PI 459025 while the lowest nodulation score (2) was found among accessions SCSI, PI 628855 and PI 506491.

Table 2. Flower and seed traits of 91 soybean accessions evaluated at MUARIK in 2011

Flower and seed trait	Variation	No of accessions	Percentage
Flower color	Purple	58	63.7
	White	33	36.3
Mature pod color	Brown	57	62.6
	Tan	24	26.4
	Black	10	11.0
Seed coat color	Cream	71	78.0
	Yellow	8	8.8
	Green	9	9.9
	Black	2	2.2
	Brown	1	1.1
Seed coat pattern	Dark hilum	66	72.5
	Light hilum	24	26.4
	Saddle	1	1.1
Hilum color	Brown	51	56.0
	Black	21	23.1
	Yellow	12	13.2
	Grey	4	4.4
	Buff	2	2.2
	Green	1	1.1
Seed coat surface lusture	Intermediate	59	64.8
	Shiny	18	19.8
	Dull	13	14.3
	Heavy brown	1	1.1
Seed shape	Oval	49	53.8
	Round	42	46.2
Seed size	Large	26	28.6
	Medium	38	41.8
	Small	27	29.7
Seed quality	Good	31	34.1
	Medium	52	57.1
	Poor	8	8.8

Accession PI 567056A had the highest number of nodes per plant (18) while the lowest (7) was found in accessions PI 200526, PI 506764, PI 628935, PI 59172A, Edamame 1, PI 416826A, PI 506491 and PI 417126 (Appendix 3). The tallest soybean accession was PI 567102B (116.8 cm) while the shortest was PI 59172A (19.9 cm). Accessions PI 644103 and PI 628925 had the highest number of branches (8) while PI 605891A had one branch. The highest and the lowest pod clearance were found in genotypes PI 628925 (13.1 cm) and PI 628905 (5 cm) respectively. Genotype PI 567104B had the highest number of pods (94) while PI 506764 had the lowest number of pods (11). The highest pod length (5.3 cm) was found in accessions PI 628861 and Edamame 1 while the lowest (3.6 cm) was found in PI 567123A. Accession PI 417503 had the highest pod width (2.2 cm) while the lowest (0.6 cm) was found in accessions PI 567056A, PI 567123A, PI 567102B and PI 567104B. A total of 56 and 35 accessions had three and two seeds per pod respectively.

Table 3. Agro-morphological variation in soybean germplasm accessions

Descriptor	Min	Max	Mean	SE	LSD	%CV	F-prob
Hypocotyl length (HL) cm	2.7	6.9	3.82	0.04	0.11	2	<.001
Leaf length (LL) cm	8.7	15.4	11.43	0.55	1.53	9.6	<.001
Leaf width (LW) cm	5.4	8.8	6.99	0.56	1.10	11.3	<.001
Days to flowering (DF)	32	65	43.96	0.77	2.15	3.5	<.001
Days to physiological maturity (DPM)	87	123	96.87	1.85	5.15	3.8	<.001
Shattering S (1-5)	1	3	1.45	0.54	1.50	16	<.001 ^a
Lodging L (1-5)	1	4	2.08	0.64	1.78	15.9	<.001 ^a
Nodulation ND (1-5)	2	5	3.61	0.48	1.33	8.8	<.001 ^a
Number of nodes per plant (NN)	7	18	10.96	0.77	2.13	14	<.001
Plant height (PH)	19.9	116.8	49.08	4.27	11.90	17.4	<.001
Number of branches (NB)	1	8	4.52	0.54	1.51	24	<.001
Pod clearance (PC)	5	13.1	8.73	0.87	2.42	19.9	<.001
Pods per plant (PP)	11	94	41.3	7.39	20.58	35.8	<.001
Pod length (PL)	3.6	5.3	4.58	0.12	0.33	5.2	<.001
Pod width (PW)	0.6	2.2	0.93	0.18	0.51	39.3	0.015
Seeds per plant (SP)	2	3	2.61	0.16	0.45	12.5	<.001
100 seed weight (100SW)	7.9	31.7	18.09	1.13	3.15	12.5	<.001
Yield kg ^{ha} ⁻¹ (Yld)	621	3667	2134	402.20	1119.80	37.7	<.001

^a ANOVA conducted on angular transformed values; Min, Minimum; Max, Maximum; SE, Standard error; LSD, Least significant difference; CV, Coefficient of variance; F-Prob, Probability

Accession PI 628935 had the highest 100 seed weight (31.7 g) while PI 567123A had the lowest (7.9 g). Grain yield in kilograms per hectare (kg ha^{-1}) was high among genotypes Maksoy 3N (3667), PI 567025A (3609), PI 635999 (3582), PI 567056A (3569), PI 578457A (3369), PI 628959 (3306), PI 567104B (3234), PI 628861 (3178), PI 615437 (3176) and PI 567053 (3140) while genotype PI 506764 gave the lowest grain yield per hectare (621).

3.1.5 Discussion

Significant differences in means observed among the 91 soybean accessions for most of the evaluated quantitative traits showed wide range of variation among the phenotypic traits studied and high diversity among accessions. This variation was noticeable for: leaf color intensity, growth vigor, leaf size and shape, pubescence type, color and density, color of mature pod, seed coat and hilum, seed coat surface lusture, seed size and quality. The presence of such variability is the cornerstone for successful breeding (Chung and Singh, 2008). However, the specificity of some morphological traits for variety discrimination was limited. For example, the 91 soybean accessions could only be classified into two groups based on cotyledon color, stem color, flower color and seed shape.

The limited discrimination of phenotypic descriptor has been observed in a number of crops including cassava (*Manihot esculenta*), Asare *et al.*, 2011); Taro (*Colocasia esculenta*), Okpul *et al.*, 2005; papaya (*Carica papaya* L.), Somsri, 1999; cowpea (*Vigna unguiculata*), Naima *et al.*, 2009; European pea (*Pisum sativum* L.), Cupic *et al.*, 2009; french bean (*Phaseolus vulgaris*), Ashok *et al.*, 2008; sorghum (*Sorghum bicolor*) and millet (*Pennisetum glaucum*), Chakauya, 2002 which is somewhat consistent with this study. It's on this premise that DNA based markers are often advocated for in genetic diversity studies for both inbreeding and/or outcrossing crops.

Genotypes with traits better than local checks such as early maturity, high pod clearance, low pod shattering and lodging are suitable for breeding improved varieties with desirable traits. Some of the test accessions matured much earlier (2 to 7 days) than the local checks Maksoy 3N and Maksoy 1N implying that these accessions could be used in regions with short rain seasons and/or where soybean rust is prevalent. However, some accessions were highly susceptible to pod shattering and lodging which are undesirable traits with a potential of reducing the grain yield. Thus there is need to improve these accessions through breeding for resistance to pod shattering and lodging before the genotypes are recommended for adaptation trials. The local

check variety Maksoy 3N out yielded all accessions implying that all other accessions are inferior in terms of seed yield. They cannot be used to improve yield and need not to be tested further, but they could be used to improve traits such as early maturity, high pod clearance, low pod shattering and lodging where they are better than Maksoy 3N.

Eleven soybean accessions (PI 615437, PI 567053, PI 628861, PI 567056A, PI 635999, PI 578457A, PI 628959, Serenade, PI 628812, PI 567104B and PI 567025A) yielded more than 3 tons per hectare and thus could be used in breeding program to develop high yielding varieties with desirable traits. However, quantitative morphological traits have limitations including subjectivity in assigning values, limited to specific growth stage, and influenced by the environment and management practices. Therefore there is need to further evaluate these accessions in other soybean growing areas under the breeding programme. Soybean accessions with undesirable traits should not be tested further but should be kept in the gene bank.

3.2 Determination of genetic diversity in soybean germplasm using SSR makers

3.2.1. Introduction

Improvement of crops through the utilization of available genetic diversity in germplasm is very vital for a successful breeding program (Renganayaki *et al.*, 2001). Genetic diversity assessment is a crucial aspect of breeding that maximizes genetic improvement. Molecular markers are used to estimate genetic diversity among genotypes which provide a basis for selection of parents for crossing. Simple sequence repeats (SSR) or microsatellites have become genetic markers of choice in many plant species due to their advantages over other molecular markers. SSR markers are highly abundant in the genome, with large number of alleles per locus, have a high degree of polymorphism and codominant type of inheritance and are suitable for automation (Kuroda *et al.*, 2009). Since the use of SSR is based on the polymerase chain reaction (PCR) method, the technique is simple and only a small amount of DNA is required. In this study, we used SSRs markers to dissect the genetic diversity of soybean germplasm.

3.2.2 Materials and methods

The 91 soybean accessions including three local varieties planted in study one above were used in this study. Twenty days after germination, genomic DNA was extracted in bulk from young fresh leaves of seedlings using the CTAB method, as described by Doyle and Doyle (1990). DNA quality and concentration was determined using the NanoDrop ND-1000 spectrophotometer and concentrations adjusted to 10 ng μ L⁻¹. The DNA was assayed with ten SSR primer-pairs (Table 4). These markers are distributed on ten linkage groups and are highly informative according to previous studies (Song *et al.*, 2004).

PCR was performed in a Gene Amp 9700 (Bio-Rad, USA) thermocycler in a 20 μ l reaction volume containing 40 ng of template DNA, 0.5 μ M of each primer, 0.2 mM dNTPs, 1 U of *Taq* polymerase, 2.5 mM MgCl₂ and 1 X PCR buffer. Amplifications involved an initial denaturing cycle of 94°C for 2 minutes, followed by 35 cycles of 94°C for 35 s; annealing at 46°C for 55 s, extension at 72°C for 45 s and a final extension cycle at 72°C for 5 minutes (Bartlett and Stirling, 2003). The PCR amplicons were fractionated by electrophoresis on 3% (w/v) Metaphor (Lonza Bioscience, Singapore) agarose horizontal gel stained with Gel RedTM Nucleic Acid Stain (Biotium, USA), at 110 volts for 2 hours along with a 100-bp ladder as a size-standard. Gel images were taken using a Bio Doc-ItTM Imaging System (Bio-Rad, USA).

Table 4. SSR loci and primers sequence used for genotyping soybean germplasm

Primer	Linkage Group	Forward	Reverse	Motif	Position
Satt308	M	GCGTTAAGGTTGGCAGGGTGGAAAGTG (26)	GCGCAGCTTTATACAAAAATCAACAA (26)	(ATT)21	130.76
Satt009	N	CCAACITGAAATTACTAGAGAAA (23)	CTTACTAGCGTATTAACCCCTT (21)	(ATT)14	28.52
Satt173	O	TGCGCCATTATTCTTCA (18)	AAGCGAAATCACCTCCTCT (19)	(ATT)18	58.40
Sct_189	I	CTTTTCCTGGCAATGAT (17)	AAAATCGCAAAACCTTAGT (19)	(CT)17	113.77
Satt263	E	CACCCAATCATGATAGCATTTTAT (24)	CTCATGGAATTGTCTTTCAGTTTC (24)	(ATT)19	45.40
Satt406	J	GCGTGAGCATTTTTGTTT (18)	TGACGGGTTTAATAGCAT (18)	(ATT)31	38.19
Sat_074	F	GGGTGAGAAATACATGCAACTTACA (25)	GGGCATCAAAATTGATATTAAATGTCTAA (29)	(AT)30	142.35
Satt197	B1	CACTGCTTTTTCCCTCTCT (20)	AAGATACCCCAACATTATTTGTAA (25)	(ATT)20	46.39
Satt286	C2	GCGGCGTTAATTTATGCCGAAA (23)	GCGTTTGGTCTAGAATAGTTCTCA (24)	(ATT)17	101.75
Sat005	D1b+W	TATCCTAGAGAAGAACTAAAAAA (23)	GTCGATTAGGCTTGAAATA (19)	(ATT)19	75.29

Amplified fragments that displayed distinct sizes were considered to be different alleles and were scored for presence (1) or absence (0). The polymorphism information content (PIC) as a measure of allelic diversity at a given locus was calculated for the 10 loci, according to the formula referred by Wang *et al.* (2008); $PIC_i = 1 - \sum_{j=1}^n P_{ij}^2$, where P_{ij} is the frequency of the j^{th} allele for the i^{th} marker. The similarity matrices were calculated from the generated data and clustered using NTSYSpc software, version 2.2 (Rohlf, 2005) employing the Unweighted Pair Group Method of the Arithmetic average (UPGMA) to generate a dendrogram. The generated information was used to infer genetic diversity and phylogeny of the soybean accessions.

3.2.3 Results

The analysis of the 91 soybean accessions by 10 SSR primer pairs identified a total of 53 alleles. The number of alleles per locus ranged from 3 for Sct-189, Satt263 and Satt286 to 10 alleles for locus Satt173, with an average of 5.3 alleles per locus (Table 5). The average PIC of the ten markers was 0.769. Marker Satt263 revealed the highest PIC (0.834), while marker Satt197 had the lowest PIC (0.698). All the ten selected markers showed polymorphism among the soybean accessions. There was variation in total number of bands amplified (Figure 1). Marker Satt173 produced the highest number of fragments/alleles (457), while marker Satt263 produced the lowest number of alleles (116). Number of alleles produced per accession ranged from 1 to 46 with an average of 26 alleles per accession.

Table 5. SSR loci and summary statistics of soybean genotyped at ten loci

Primer	Allele per locus	Total fragments	PIC
Satt308	9	438	0.738
Satt009	4	161	0.821
Satt173	10	457	0.769
Sct-189	3	142	0.752
Satt263	3	116	0.834
Satt406	8	328	0.814
Sat-074	4	185	0.763
Satt197	4	209	0.698
Satt286	3	147	0.734
Satt005	5	230	0.766
Mean	5.3	241.3	0.769

PIC, polymorphic information content

A dendrogram constructed from the genetic distance matrix and sequential agglomerative hierarchical and nested (SAHN) clustering method using the unweighted paired group method with arithmetic average (UPGMA) and NTSYS-pc program (Rohlf, 2005) resolved the soybean accessions into six distinct groups at similarity coefficient level of 0.57 (Figure 2). A total of 31 accessions, including 25 from USA, five from Zimbabwe and one from Zambia were included in Group 1, which was further divided into two subgroups at the similarity coefficient level of 0.65. The first subgroup comprised 23 accessions including 19 from USA, three accessions (Duiker, Roan and Siesta) from Zimbabwe and one accession (Dinamri) from Zambia. The second subgroup comprised eight accessions, including six from USA and two accessions (Safari and Santa) from Zimbabwe. Group 2 comprised 12 accessions including 10 from USA and two accessions (SCSI and Soprano) from Zimbabwe. Three accessions PI 567053, PI 567102B and Serenade were clustered in group 3. Group 4 comprised eight accessions including seven from USA and one accession, Edamame from Zimbabwe.

Group 5 comprised 37 soybean accessions including 31 from USA, three from Uganda and three accessions from Zimbabwe. The group was sub divided into three sub groups at similarity coefficient of 0.67. Sub group 1 comprised 10 accessions including seven from USA and three local Ugandan varieties (Maksoy 1N, Maksoy 2N and Maksoy 3N). Sub group 2 comprised 24 accessions including 21 from USA and three accessions (Gazelle, Buffalo and Solitaire) from Zimbabwe.

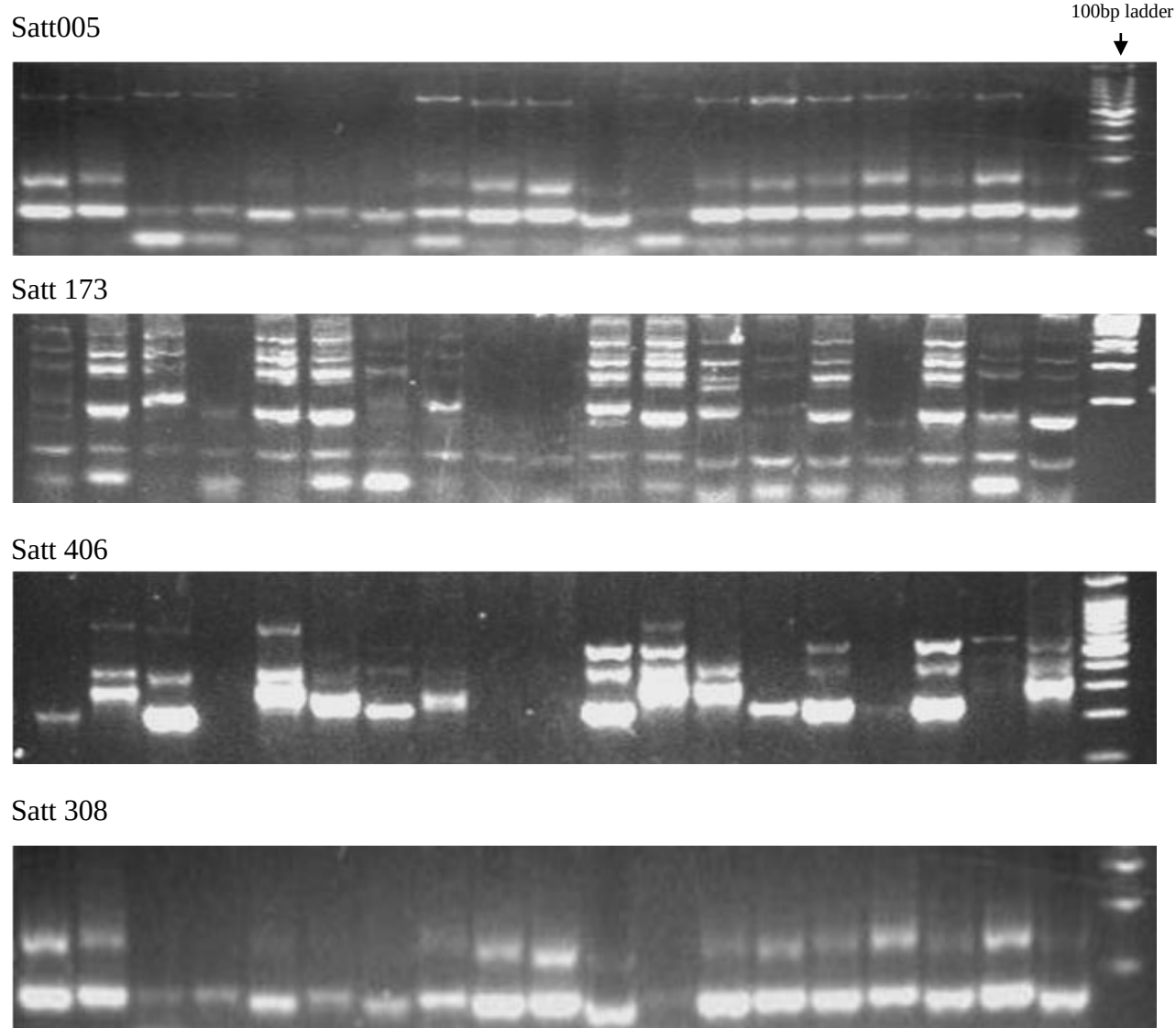


Figure 1. The electrophoretic patterns of DNA bands amplified by SSR markers from selected soybean accessions. From left to right are the soybean accessions Soprano, PI 644103, Safari, PI 416810, PI 605865B, PI 567102B, PI 417120, PI 567053, PI 605885B, PI 567059, PI 471904, PI 628850, Duiker, SCSi, PI 628903, Gazelle, PI 417132, PI 606405, PI 628863 and the 100bp ladder.

Sub group 3 comprised only three accessions (PI 506491, PI 417503 and PI 628932) from USA. Group 6 had only one accession (PI 628889) from USA. Soybean accessions (PI 685999 and PI 567025A, PI 59172A and PI 287197) had the highest genetic similarity (GS=98%) thus were genetically similar. However accessions PI 200526 and PI 628889 were the most genetically dissimilar (GS=44%).

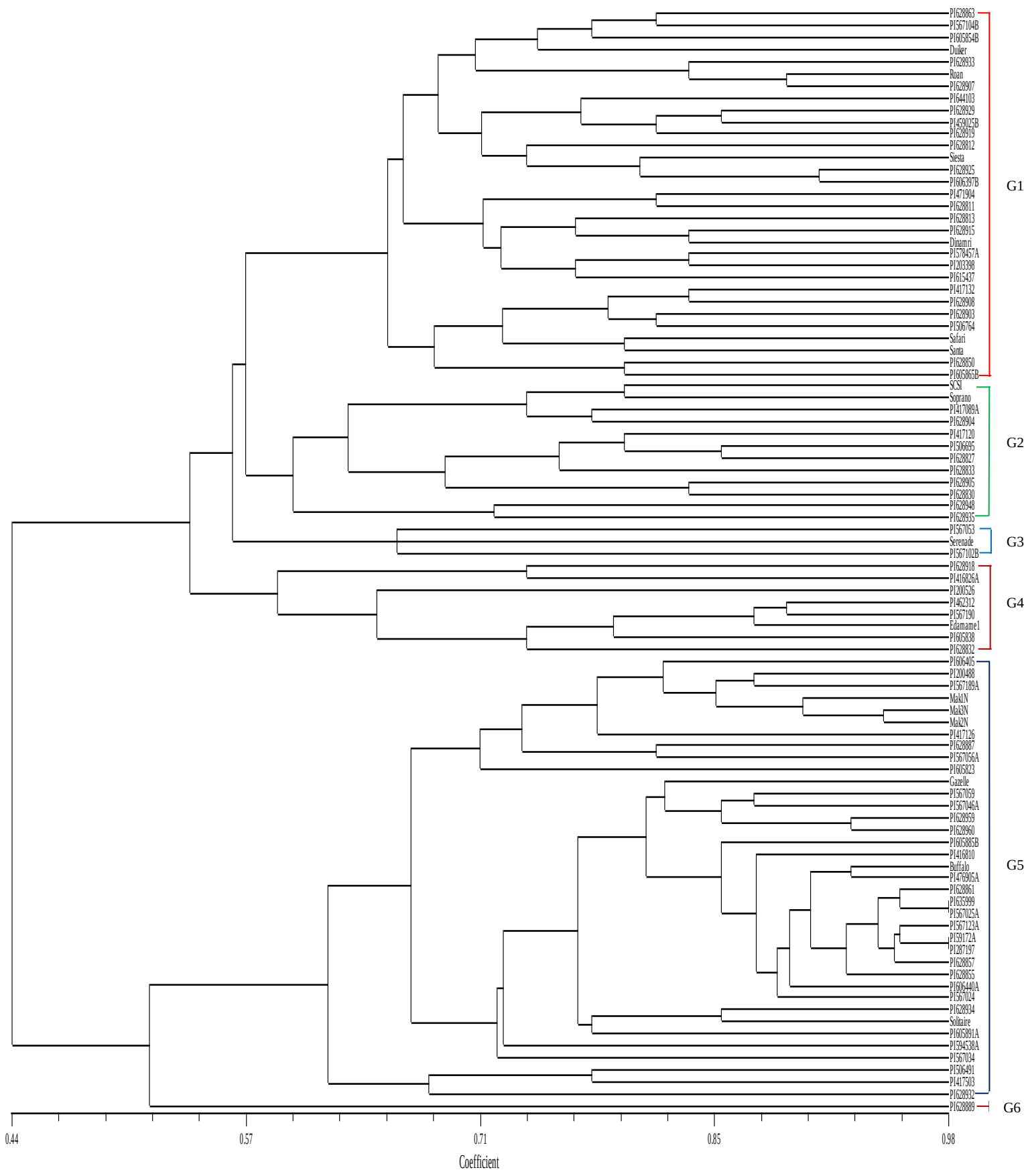


Figure 2. A dendrogram of genetic relationship among 91 soybean accessions based on 10 SSR loci. Six groupings (G1 to G6) are apparent at 0.57 coefficient.

3.2.4 Discussion

The major objective of SSR genotyping was to infer the genetic diversity available within the assembled exotic germplasm. This was achieved by assaying the 91 soybean accessions with 10 SSR markers that are scattered across the genome. This work was based on the premise that application of molecular markers in assessment of genetic diversity for germplasm improves the efficiency of parental selection for the breeding program (Fu, 2003; Li *et al.*, 2009). Moreover, molecular markers enable breeders to search for new sources of variation and to investigate genes controlling quantitative traits. SSR clustering confirmed known morphological trait differences among the soybean accessions.

Exotic soybean accessions from USA and Zimbabwe were scattered in all clusters of the dendrogram implying that they were genetically different, suggesting that they can broaden the genetic base of soybean germplasm in Uganda and facilitate development of new varieties with desirable traits. The local check varieties (Maksoy 1N, Maksoy 2N and Maksoy 3N) were grouped in the same cluster which implied that they were genetically related thus are likely to have shared similar pedigree. These local varieties are advanced soybean lines selected for high yielding trait.

The high level of polymorphism (77%) observed indicated high genetic diversity among the evaluated soybean genotypes and thus justifies their inclusion into the National soybean breeding programme for purposes of broadening the soybean germplasm base in Uganda. The study demonstrated that the selected SSR markers were highly informative and useful for characterization and estimation of genetic diversity in soybean despite its inbreeding nature. This is similar to the results obtained by Fu *et al.* (2007) while analyzing Canadian soybean cultivars and exotic germplasm with SSR markers. The generated information is important for breeders, as it helps in selection of parents that can be used in generating breeding populations with adequate variation for selection.

Several studies have been conducted to determine the genetic diversity among soybean cultivars. Li *et al.* (2010) genotyped 303 cultivated and wild soybean accessions with 99 SSR markers and reported an average of 21.5 alleles per locus. The genotyping of 1863 Chinese soybean landraces with 59 SSR loci revealed a mean of 19.7 alleles per locus (Li *et al.*, 2008). Guan *et al.* (2010) genotyped 205 Chinese soybean landraces using 46 SSR loci and reported mean of 16.2 alleles

per locus. The analysis of 129 Chinese soybean accessions using 60 SSR markers revealed an average of 12.2 alleles per locus and mean PIC value of 0.78 (Wang *et al.*, 2006). Abe *et al.* (2003) reported an average of 11.9 alleles per locus and genetic diversity of 0.78 among 131 Asian soybean accessions analyzed with 20 SSR loci. Chotiarnwong *et al.* (2007) reported an average of 11.8 alleles per locus and genetic diversity of 0.83 among 149 Thai indigenous and 11 recommended soybean varieties using 18 SSR markers. Narvel *et al.* (2000) reported an average of 10.2 alleles per locus among 39 elite soybean genotypes and 40 soybean accessions using 74 SSR markers. Diwan and Cregan (1997) reported an average of 10.1 alleles per locus and genetic diversity of 0.80 among 35 North American soybean genotypes using 20 SSR markers. The analysis of 40 soybean accessions including cultivars, landraces and wild soybean with 40 SSR markers revealed a mean of 6.6 alleles per locus (Wang *et al.*, 2010).

Its apparent from the above studies that: 1) soybean cultivars, landraces and wild relatives analyzed ranged from 11 to 1863; 2) SSR loci sampled ranged from 18 to 99; and 3) mean number of alleles per loci ranged from 6.6 to 21.5. In this study, we genotyped 91 soybean accessions with 10 SSR loci and obtained 5.3 alleles per locus, which is lower than that obtained in the previous studies but comparable to the study by Wang *et al.*, (2010), who genotyped 40 soybean accessions with 40 SSR markers and obtained 6.6 alleles per locus. These differences could be attributed to the number and location of loci in the soybean genome sampled. The genetic diversity (PIC) of 0.77 obtained was similar to that from the previous studies. These findings highlight the first systematic quantification of soybean genetic diversity in Uganda, and have laid a foundation for using this information for either selection of parental lines for crossing or for setting priorities for soybean germplasm conservation.

CHAPTER FOUR

ASSESSMENT OF EXOTIC SOYBEAN ACCESSIONS AND ADVANCED BREEDING LINES FOR RESISTANCE TO SOYBEAN RUST

4.1 Introduction

Soybean rust caused by *Phakopsora pachyrhizi* originated from Asia and spread throughout the world. The disease was first reported in Uganda in 1996 and has now been reported in most soybean growing countries on the African continent (Kawuki *et al.*, 2003; Levy, 2005). The disease has the potential of causing up to 100% yield loss depending on level of plant susceptibility and weather conditions (Mueller *et al.*, 2009). In terms of control, the use of fungicides has been successful (Dorrance *et al.*, 2007), though not sustainable with small-scale farmers in Africa who dominate soybean production. Host plant resistance is most suitable to manage soybean rust under such farming systems (Hartman *et al.*, 2005). Accordingly, this has necessitated the continued search for sources of resistance to the devastating soybean rust disease. Moreover, there is potential of resistance break down among the deployed rust resistant varieties as more virulent and aggressive pathogen strains emerge (Miles *et al.*, 2006).

Specific resistance genes to *P. pachyrhizi* *Rpp1*, *Rpp2*, *Rpp3*, *Rpp4*, *Rpp5* and *Rpp?hyuuga* have been identified in different soybean cultivars (Hyten *et al.*, 2007; Garcia *et al.*, 2008). These resistance genes have been used by breeding programmes located in regions where soybean rust has been endemic for long periods (Pham *et al.*, 2009). In Uganda, breeding for soybean rust resistance by Makerere University and the National Agricultural Research Organization has resulted in generation of new breeding lines whose reaction to soybean rust disease has not been determined.

With ever changing pathogen populations, it's vital that the soybean germplasm base be broadened to mitigate any new emerging races. It's against this backdrop that additional soybean accession were sought from USA, Zimbabwe and Zambia (as indicated in Chapter 3) and evaluated for possible sources of resistance to soybean rust to maximize its value for conservation and breeding. Thus in this research chapter, the reaction of advanced soybean breeding lines and exotic accessions to soybean rust is presented.

4.2 Materials and methods

This experiment comprised 91 exotic soybean accessions evaluated at MUARIK (Table 6) and 39 advanced lines breed for rust resistance evaluated at both MUARIK and NaCRRI (Table 7). NaCRRI is located at Namulonge in central Uganda about 25 km North of Kampala at 00°32'N, 32°37'E and 1150 meters above sea level. The area receives mean total annual rainfall of 1100 mm with bimodal distribution. There is a wet and mild dry season and slight humid conditions with mean relative humidity of 65%. The average minimum and maximum temperature are 16.0°C and 29.3°C respectively. Severe rust epidemics are observed at both sites during the seasons making the places suitable for rust assessment. At both locations susceptible varieties score up to 100% incidence and are associated with extensive lesion sporulation and defoliation.

The soybean accessions were each planted in double row plots of 5 m and replicated twice using a randomized complete block design with a spacing of 60 cm x 5 cm. The entire field was surrounded with susceptible variety Nam 1 to ensure early and optimum inoculum build up. The exotic soybean germplasm were planted on 24th March 2011 and 7th September 2011 for the two seasons respectively. On the other hand, advanced breeding lines were planted on 7th September 2011 and 3rd April 2012 for the two seasons respectively.

4.2.1 Data collection

Soybean rust severity was assessed at reproductive stages: R2 (full bloom), R4 (full pod formation) and R6 (full seed formation) using a scale of 1-9, where 1 = no disease and 9 = 90% and above disease severity (Kawuki *et al.*, 2003). Rust sporulation was scored among the soybean germplasm using a scale of 1-5 where 1= no infection and 5 = high sporulation. The soybean accessions and breeding lines were also assessed for rust reaction types based on lesion color on leaves according to Bromfield (1984). Soybean lines without any visible rust symptoms were considered to be immune (I), those with red-brown (RB) lesions were resistant while lines with tan-colored lesions (TAN) were susceptible (Miles *et al.*, 2005). Selected key quantitative traits were also recorded for the advanced breeding lines from 10 randomly selected plants including; plant height (cm), number of branches per plant, days to 50% flowering, days to physiological maturity (R8), pod clearance, number of pods per plant, number of seeds per pod, 100 seed weight (g) and yield (kg ha⁻¹). In addition, pod shattering, plant lodging and nodulation were scored using a scale of 1-5 where 1=no observed trait and 5= high observed trait.

4.2.2 Data analysis

The generated data were subjected to analysis using Genstat 13th edition (Payne *et al.*, 2011) and mean values compared among the soybean lines at 5% least significant difference. Rust severity, sporulation, pod shattering, plant lodging and nodulation scores were normalized by angular transformation prior to the analysis of variance. Rust severity and reaction types were compared among the soybean germplasm. Soybean varieties with low rust scores were considered to have a greater resistance potential against soybean rust.

4.3 Results

4.3.1 Reaction of exotic soybean accessions to soybean rust disease

The mean reaction of 91 exotic soybean accessions to soybean rust at MUARIK for two consecutive seasons of 2011 are presented in Table 6. There was progressive increase in rust severity among accessions as they matured. The differences in mean rust severity among accessions at full broom, full pod and full seed were highly significant ($p < 0.001$). The mean rust severity for 91 soybean accessions at full broom was 1.6, full pod (3.0) and full seed (4.4). At full broom stage (R2), soybean accessions (PI 416826A, PI 417126, PI 471904, PI 506491, PI 628812, PI 628863 and PI 628935) had the lowest rust severity score (1) while accession PI 628919 had the highest rust severity score (2.8). At full pod stage (R4), soybean accessions (PI 416810, PI 462312, PI 567025A, PI 615437 and PI 628935) had lowest rust severity score (1.8) while accessions PI 628855 and PI 628919 had the highest rust score (4.5). At full seed stage (R6), accessions (PI 416826A, PI 417126 and PI 615437) had lowest rust severity score (2.8) while accessions PI 605823 and PI 628813 had highest rust severity score (6.5). Full seed was therefore the most suitable stage for soybean rust expression and assessment.

Soybean accessions (PI 416826A, PI 417126 and PI 615437) had the lowest rust sporulation score (1) while accessions (Dinamri, PI 605823, PI 628855, PI 628861, PI 628919 and PI 628933) had the highest rust sporulation score (5). Mean rust sporulation for the 91 soybean accessions was 2.9. The differences in mean rust sporulation among the accessions were highly significant ($p < 0.001$). A total of 66 soybean accessions had TAN reaction thus were highly susceptible to soybean rust, while 22 accessions had RB reaction implying that they were moderately resistant to soybean rust. Only three accessions (PI 615437, PI 416826A and PI 417126) had immune reaction and were resistant to soybean rust.

Table 6. Reaction of exotic accessions to soybean rust at MUARIK in 2011

Plot No	Accession	RR2(1-9)	RR4(1-9)	RR6(1-9)	RS(1-5)	RRT
1	Buffalo	1.8	4.0	5.5	4.0	T
2	Dinamri	1.8	3.8	6.3	5.0	T
3	Duiker	1.8	3.0	4.0	2.8	T
4	Edamame 1	1.5	3.0	4.0	2.5	T
5	Gazelle	1.8	3.3	5.0	3.3	T
6	Maksoy 1N	1.5	2.8	4.8	3.3	T
7	Maksoy 3N	1.5	2.8	4.5	2.8	T
8	PI 200488	1.3	2.5	3.8	2.5	T
9	PI 200526	1.8	2.0	3.5	2.5	RB
10	PI 203398	1.5	3.5	4.5	3.0	T
11	PI 287197	1.8	4.0	6.0	3.8	T
12	PI 416810	1.3	1.8	3.0	2.0	RB
13	PI 416826A	1.0	2.0	2.8	1.0	I
14	PI 417089A	1.5	2.0	3.5	2.3	RB
15	PI 417120	1.3	3.0	4.5	3.0	T
16	PI 417126	1.0	2.0	2.8	1.0	I
17	PI 417132	1.5	2.5	3.5	2.3	RB
18	PI 417503	1.5	2.5	3.0	2.0	RB
19	PI 459025	1.5	2.5	3.5	2.3	RB
20	PI 462312	1.3	1.8	3.0	2.0	RB
21	PI 471904	1.0	2.0	3.3	2.3	RB
22	PI 476905A	1.5	3.0	4.5	2.8	T
23	PI 506491	1.0	2.3	3.8	2.5	RB
24	PI 506695	1.5	2.5	3.8	2.5	T
25	PI 506764	1.3	2.3	3.0	2.0	RB
26	PI 567024	1.3	2.5	3.5	2.5	T
27	PI 567025A	1.3	1.8	3.3	2.3	RB
28	PI 567034	1.5	2.5	3.0	2.0	RB
29	PI 567046A	1.8	2.5	4.0	2.8	T
30	PI 567053	1.5	3.0	4.8	3.3	T
31	PI 567056A	1.5	2.8	4.5	3.0	T
32	PI 567059	1.3	2.0	3.0	2.0	RB
33	PI 567102B	1.3	2.5	4.0	2.8	T
34	PI 567104B	1.8	3.0	5.0	3.5	T
35	PI 567123A	1.8	3.8	5.8	3.8	T
36	PI 567189A	1.3	2.3	3.3	2.3	RB
37	PI 567190	1.3	2.8	3.5	2.5	T
38	PI 578457A	1.5	2.8	4.5	3.0	T
39	PI 59172A	1.3	2.5	3.3	2.3	RB
40	PI 594538A	1.3	2.3	3.8	2.5	T
41	PI 605823	2.0	4.3	6.5	5.0	T
42	PI 605838	1.3	2.0	3.0	2.0	RB
43	PI 605854B	1.3	2.0	3.3	2.0	RB
44	PI 605865B	1.3	2.5	3.5	2.3	RB
45	PI 605885B	1.3	2.5	3.3	2.3	RB
46	PI 605891A	1.3	2.5	4.3	2.8	T
47	PI 606397B	1.5	2.5	3.5	2.5	T
48	PI 606405	1.3	2.5	3.0	2.0	RB
49	PI 606440A	2.0	4.3	5.3	3.5	T

50	PI 615437	1.3	1.8	2.8	1.0	I
51	PI 628811	2.3	3.5	5.0	3.5	T
52	PI 628812	1.0	2.0	3.0	2.0	RB
53	PI 628813	2.5	4.3	6.5	4.0	T
54	PI 628827	2.0	3.5	5.0	3.5	T
55	PI 628830	1.8	3.0	4.3	2.8	T
56	PI 628832	1.5	3.0	3.5	2.5	T
57	PI 628833	1.5	3.3	5.0	3.3	T
58	PI 628850	1.3	3.3	5.0	3.5	T
59	PI 628855	1.8	4.5	6.0	5.0	T
60	PI 628857	1.8	4.3	5.5	3.5	T
61	PI 628861	2.0	4.3	5.8	5.0	T
62	PI 628863	1.0	2.3	3.8	2.5	T
63	PI 628887	1.8	4.0	5.0	3.3	T
64	PI 628889	1.8	4.3	5.0	3.5	T
65	PI 628903	1.8	3.5	5.3	3.3	T
66	PI 628904	1.8	3.8	5.5	3.5	T
67	PI 628905	2.3	4.3	5.8	3.8	T
68	PI 628907	1.5	3.8	6.0	3.8	T
69	PI 628908	2.0	2.8	4.3	3.0	T
70	PI 628915	2.3	4.0	6.0	3.8	T
71	PI 628918	2.0	3.5	4.8	3.3	T
72	PI 628919	2.8	4.5	6.3	5.0	T
73	PI 628925	2.0	3.3	5.5	3.8	T
74	PI 628929	1.8	3.3	5.0	3.3	T
75	PI 628932	1.3	2.3	3.8	2.5	T
76	PI 628933	2.0	4.0	6.0	5.0	T
77	PI 628934	2.3	4.0	5.3	3.5	T
78	PI 628935	1.0	1.8	3.0	2.0	RB
79	PI 628948	1.3	3.3	4.5	3.3	T
80	PI 628959	1.8	4.3	5.5	3.5	T
81	PI 628960	2.3	3.5	5.5	3.8	T
82	PI 635999	1.3	2.3	3.8	2.5	T
83	PI 644103	1.5	3.5	4.3	2.8	T
84	Roan	2.0	3.3	5.5	3.5	T
85	Safari	1.5	3.5	5.0	3.3	T
86	Santa	1.8	3.5	5.0	3.3	T
87	SCSI	2.0	3.3	5.0	3.5	T
88	Serenade	2.0	3.8	5.8	3.5	T
89	Siesta	1.8	3.8	5.5	3.5	T
90	Solitaire	2.0	3.3	5.3	3.5	T
91	Soprano	1.8	3.8	5.3	3.5	T
Mean		1.6	3.0	4.4	2.9	
LSD		1.6	2.2	1.8	1.7	
%CV		16.6	15.9	10.9	12.4	
F probability ^a		<.001	<.001	<.001	<.001	

^a ANOVA conducted on angular transformed values; RR2, RR4 and RR6 are Rust severity at growth stages R2, R4 and R6 respectively; RS= Rust sporulation; RRT= Rust reaction type; I= Immune; RB= Red brown and T= Tan

4.3.2 Reaction of advanced breeding lines to soybean rust at MUARIK and NaCRRI during seasons 2011B and 2012A

The reaction of 39 advanced soybean breeding lines to soybean rust at MUARIK and NaCRRI during seasons 2011B and 2012A are presented in Table 7. The differences in mean rust severity were highly significant at full broom, full pod and full seed ($p < 0.001$). The mean rust severity among the 39 advanced breeding lines at full broom was 1.4, full pod (2.4) and full seed (3.9). At full broom stage, breeding lines BSPS 47B and BSPS 9.2B had the lowest rust severity (1) while lines BSPS 50B and D X T 8.11 had the highest rust severity (1.9). At full pod stage, breeding line BSPS 9.2B had the lowest rust severity (1.6) while line D X T 8.11 had the highest rust severity (3.3). At full seed stage, breeding line BSPS 9.2B had the lowest rust severity (2.6) while line BSPS 48A-1 had the highest rust severity (5.5). However at full seed stage, breeding line (NG 14.1-24) showed high resistance to soybean rust at NaCRRI with severity score 1.9 (Appendix 4) while breeding line (BSPS 9.2B) showed high resistance to soybean rust at MUARIK with severity score 2 (Appendix 5).

Advanced soybean breeding line (BSPS 9.2B) had the lowest rust sporulation (1.5) while breeding line BSPS 48A-1 had the highest rust sporulation (3.9). However breeding line BSPS 48A-27 had the lowest rust sporulation score (1.3) and immune reaction type at NaCRRI (Appendix 4) while line BSPS 9.2B had the lowest rust sporulation (1) and immune reaction type at MUARIK (Appendix 5). There were significant differences ($p < 0.001$) among the 39 soybean breeding lines with a mean rust sporulation score of 2.5. A total of 29 breeding lines had TAN reaction and were highly susceptible to soybean rust, while nine breeding lines had RB reaction implying that they were moderately resistant to soybean rust. Soybean breeding line (BSPS 9.2B) had immune reaction and was highly resistant to soybean rust. Higher disease severity was associated to genotypes with TAN reaction.

Table 7. Reaction of 39 advanced breeding lines to soybean rust at MUARIK and NaCRRRI in 2011B and 2012A

Plot No	Accession	RR2 (1-9)	RR4 (1-9)	RR6 (1-9)	RS (1-5)	RRT
1	BSPS 16A	1.5	2.3	4.4	2.8	T
2	BSPS 18.3	1.3	2.5	4.0	2.5	T
3	BSPS 182A	1.3	2.1	3.7	2.3	T
4	BSPS 24.2A-3	1.4	2.0	3.4	2.0	RB
5	BSPS 27.2B	1.1	2.5	3.8	2.4	T
6	BSPS 27A	1.8	2.6	4.7	3.3	T
7	BSPS 42	1.1	2.4	3.8	2.3	T
8	BSPS 43	1.5	2.6	3.8	2.4	T
9	BSPS 47B	1.0	1.8	3.0	1.8	RB
10	BSPS 48A-1	1.8	2.9	5.5	3.9	T
11	BSPS 48A-14	1.6	2.7	4.6	2.8	T
12	BSPS 48A-19	1.5	2.3	3.3	2.1	RB
13	BSPS 48A-21B	1.3	2.6	4.4	3.0	T
14	BSPS 48A-27	1.4	1.8	3.2	1.9	RB
15	BSPS 48A-5	1.4	2.1	3.8	2.5	T
16	BSPS 48B	1.1	1.9	3.1	1.9	RB
17	BSPS 48C	1.3	2.1	3.5	2.3	T
18	BSPS 50B	1.9	3.0	4.8	3.4	T
19	BSPS 62C-1	1.6	2.4	3.7	2.4	T
20	BSPS 7.6B	1.3	2.6	3.9	2.5	T
21	BSPS 70	1.6	2.4	4.1	2.9	T
22	BSPS 75B	1.3	1.9	3.1	1.8	RB
23	BSPS 9.2B	1.0	1.6	2.6	1.5	I
24	D X T 8.11	1.9	3.3	5.0	3.4	T
25	DXT SPS 16.6-2	1.4	2.2	4.0	2.6	T
26	GC X KAB	1.3	2.0	3.5	2.3	T
27	GC X WANDER	1.6	2.6	4.9	3.3	T
28	KAB X UG5	1.1	2.4	3.8	2.3	T
29	Maksoy 1N	1.6	2.8	4.1	2.8	T
30	Maksoy 3N	1.3	2.3	3.6	2.4	T
31	MNG 11.2	1.6	2.9	4.6	3.3	T
32	NG 14.1-24	1.5	2.2	2.9	2.1	RB
33	NG 14.1-6B	1.4	2.1	2.9	1.8	RB
34	NGDT 2.15-10	1.8	2.6	5.1	3.4	T
35	NGDT 2.15-4	1.5	2.5	4.9	3.3	T
36	NGDT 8.11	1.1	2.4	4.1	2.6	T
37	NGDT BLP12.4	1.4	2.2	4.1	2.9	T
38	NII X GC 20.2	1.6	2.9	4.3	2.9	T
39	NII x GC 4.1-2	1.3	2.4	3.3	1.9	RB
Mean		1.4	2.4	3.9	2.5	
LSD		1.0	1.2	1.2	1.4	
%CV		15.2	14.5	10.9	15.6	
F probability ^a		<.001	<.001	<.001	<.001	

^a ANOVA conducted on angular transformed values; RR2, RR4 and RR6 are Rust severity at growth stages R2, R4 and R6 respectively; RS= Rust sporulation; RRT= Rust reaction type; I= Immune; RB= Red brown and T= Tan

4.3.3 Agronomic traits of advanced soybean breeding lines evaluated at MUARIK and NaCRRRI during seasons 2011B and 2012A

Means of agronomic traits evaluated from advanced breeding lines at MUARIK and NaCRRRI in seasons 2011B and 2012A are presented in Table 8. The 39 soybean breeding lines had average plant height (75.3 cm), number of branches (3.5), days to 50% flowering (49 days), days to physiological maturity (102 days), nodulation (3.2), lodging (2.1), shattering (1.3), pod clearance (13.2 cm), number of pods (42.4 pods), seeds per pod (2.7), 100 seed weight (17.5 g) and grain yield (1502 kg ha⁻¹). The differences in mean plant height, number of branches, days to flowering and physiological maturity, nodulation, lodging, shattering, pod clearance, pods per plant, seeds per pod, 100 seed weight and grain yield were highly significant ($p < 0.001$).

Soybean breeding line BSPS 16A was the tallest (100.2 cm) while the shortest was the local check Maksoy 1N (36.2 cm). Maksoy 1N displayed the highest number of branches (6) while BSPS 48C and BSPS 7.6B lines had the lowest (1). Breeding line BSPS 42 was the earliest to reach 50% flowering (44 days) while BSPS 16A took 54 days to flower. Maksoy 1N was the earliest to reach physiological maturity (89 days) while breeding line BSPS 9.2B took the longest duration (112 days). However breeding line GC X KAB took the longest duration (108 days) to physiological maturity at NaCRRRI (Appendix 6) while BSPS 9.2B took 118 days to maturity at MUARIK (Appendix 7). Breeding line GC X KAB had the highest nodulation (4.1) while D X T 8.11 had the lowest (2.3). Lodging score was lowest (1) in Maksoy 1N while BSPS 16A had the highest (3.8). Seven breeding lines (BSPS 16A, BSPS 182A, BSPS 27A, BSPS 48A-27, BSPS 48B, BSPS 50B and NGDT BLP12.4) had the lowest shattering (1) while BSPS 70 had the highest shattering (2.1).

Breeding line BSPS 27A had the highest pod clearance (23.5 cm) while Maksoy 1N had the lowest (8.6 cm). Breeding line BSPS 50B had the highest number of pods (60) while line D X T 8.11 had the lowest (27 pods). Majority of the breeding lines (37) had three seeds per pod while two lines (BSPS 7.6B and D X T 8.11) had two seeds per pod. Breeding line BSPS 48A-14 had the highest 100 seed weight (20 g) while Maksoy 1N had the lowest (12.8 g). Breeding line BSPS 43 had the highest grain yield (1929 kg ha⁻¹) while line BSPS 9.2B gave the lowest grain yield (916 kg ha⁻¹). However soybean breeding line BSPS 48A-14 gave the highest grain yield (2244 Kg ha⁻¹) at MUARIK (Appendix 7).

Table 8. Means of agronomic traits of 39 advanced soybean breeding lines evaluated at MUARIK and NaCRRRI during seasons 2011B and 2012A

Plot No	Accession	PH	NB	DF	DPM	ND (1-5)	L(1-5)	S(1-5)	PC	PP	SP	100SW	Ykg/ha
1	BSPS 16A	100.2	3	54	109	3.5	3.8	1.0	15.7	47	3	16.3	1048
2	BSPS 18.3	77.4	3	54	106	3.5	2.0	1.4	14.4	39	3	17.5	1711
3	BSPS 182A	86.0	3	50	107	2.7	2.0	1.0	16.2	42	3	17.8	1801
4	BSPS 24.2A-3	78.8	3	49	104	3.2	1.9	1.1	12.3	45	3	18.5	1910
5	BSPS 27.2B	66.6	4	51	99	2.8	1.5	1.6	13.7	48	3	18.0	1514
6	BSPS 27A	86.6	2	53	109	2.9	2.4	1.0	23.5	33	3	18.0	1411
7	BSPS 42	65.2	3	44	98	2.5	1.6	1.3	13.9	29	3	17.2	1370
8	BSPS 43	72.3	4	52	105	3.5	2.0	1.3	13.6	47	3	17.0	1929
9	BSPS 47B	92.2	4	50	103	3.1	2.8	1.3	12.1	54	3	17.6	1326
10	BSPS 48A-1	87.8	4	53	107	3.5	2.3	1.1	13.3	47	3	18.1	1748
11	BSPS 48A-14	68.4	4	45	102	3.3	1.6	1.2	11.0	39	3	20.0	1856
12	BSPS 48A-19	80.9	4	46	102	2.9	2.8	1.6	10.0	48	3	18.3	1822
13	BSPS 48A-21B	79.3	4	53	106	3.3	2.0	1.1	12.9	38	3	16.8	1496
14	BSPS 48A-27	79.5	4	52	105	3.1	2.4	1.0	14.4	43	3	18.9	1767
15	BSPS 48A-5	74.4	5	51	101	2.9	2.1	1.2	11.3	42	3	17.6	1563
16	BSPS 48B	90.2	4	53	105	3.8	3.4	1.0	12.3	48	3	14.8	1234
17	BSPS 48C	82.2	1	48	107	3.3	2.3	1.3	18.1	33	3	18.7	1680
18	BSPS 50B	84.0	5	49	105	4.0	2.0	1.0	11.2	60	3	17.7	1562
19	BSPS 62C-1	57.1	3	48	102	2.6	2.0	1.3	14.1	32	3	17.2	1622
20	BSPS 7.6B	76.7	1	47	103	3.1	1.9	1.5	16.9	38	2	15.4	993
21	BSPS 70	71.1	3	49	103	3.0	1.8	2.1	14.4	39	3	15.3	1309
22	BSPS 75B	79.0	4	46	102	3.6	2.3	1.1	10.9	54	3	16.2	1436
23	BSPS 9.2B	91.3	3	50	112	3.6	3.1	1.3	20.4	43	3	16.8	916
24	D X T 8.11	64.9	2	51	93	2.3	1.4	1.6	15.7	27	2	16.5	1446
25	DXT SPS 16.6-2	70.1	4	48	95	3.0	2.1	1.1	11.8	34	3	18.7	1305
26	GC X KAB	70.7	4	48	107	4.1	2.1	1.6	11.4	54	3	19.4	1516
27	GC X WANDER	66.9	4	46	98	3.0	1.4	1.8	11.7	43	3	18.8	1168
28	KAB X UG5	68.3	5	49	105	3.5	2.0	1.5	11.2	56	3	17.8	1794
29	MAKSOY 1N	36.2	6	46	89	3.3	1.0	1.3	8.6	34	3	12.8	1473
30	MAKSOY 3N	74.9	4	46	102	2.4	1.9	1.7	9.5	48	3	19.3	1660
31	MNG 11.2	66.5	3	46	99	3.5	1.4	1.6	13.2	30	3	19.2	1293
32	NG 14.1-24	67.6	3	47	99	3.4	2.4	1.2	11.5	38	3	18.9	1389
33	NG 14.1-6B	70.4	4	47	99	2.8	1.9	1.5	9.9	48	3	18.6	1465
34	NGDT 2.15-10	82.9	4	47	100	3.5	2.2	1.1	12.4	42	3	16.3	1582
35	NGDT 2.15-4	76.9	3	47	103	3.1	2.2	1.1	13.8	42	3	15.5	1550
36	NGDT 8.11	68.6	4	48	96	2.7	1.9	1.3	11.6	37	3	18.7	1276
37	NGDT BLP12.4	67.2	5	49	99	3.3	2.1	1.0	9.9	50	3	16.1	1444
38	NII X GC 20.2	77.9	4	47	99	3.6	1.5	1.3	14.2	42	3	18.3	1580
39	NII x GC 4.1-2	80.2	4	49	103	2.7	2.6	1.6	12.0	42	3	17.8	1609
Mean		75.3	3.5	48.9	102.2	3.2	2.1	1.3	13.2	42.4	2.7	17.5	1501.9
LSD		9.9	0.7	2.1	2.6	1.5	1.4	1.0	2.1	10.8	0.2	1.6	436.0
%CV		13.3	19.8	4.4	2.6	15.1	17.6	16.1	16.4	25.9	7.6	9.2	29.5
F probability		<.001	<.001	<.001	<.001	0.002 ^a	<.001 ^a	<.001 ^a	<.001	<.001	<.001	<.001	<.001

^a ANOVA conducted on angular transformed values; PH, Plant height; NB, Number of branches; DF, Days to 50% flowering; DPM, Days to physiological maturity; ND, Nodulation; L, Lodging; S, Shattering; PC, Pod clearance; PP, Pods per plant; SP, Seeds per pod; 100SW, 100 seed weight; Yld, Yield in Kgha⁻¹

4.4 Discussion

Host resistance has been used to manage soybean rust in major soybean growing areas. This experiment evaluated 91 soybean accessions and 39 advanced breeding lines for resistance to soybean rust during seasons 2011B and 2012A. The results showed that a few soybean accessions and breeding lines were highly resistant to soybean rust while others were moderately resistant to soybean rust. However, majority of the soybean accessions were highly susceptible to soybean rust producing TAN lesions on the leaves.

The test materials included two accessions (PI 462312 and PI 459025) with known specific resistance genes *Rpp3* and *Rpp4* respectively. These two accessions also showed resistance to soybean rust under natural infestation at MUARIK. It therefore implied that these specific resistance genes could be used in improving resistance in released genotypes with desirable agronomic traits but susceptible to soybean rust disease such as Nam 1, Nam 2, Kabanyoro 1, Namsoy 3M and Namsoy 4M. The same genes could be used to improve Maksoy 1N, Maksoy 2N and Maksoy 3N whose resistance has broken down (Tukamuhabwa *et al.*, 2009). In previous studies, host-pathogen interaction that resulted in RB reactions tended to have longer latent periods, lower rates of increase in pustule number over time, and smaller lesions which are characteristic of partial resistance (Walker *et al.*, 2011). However, susceptible interactions resulted in TAN reaction (Bromfield, 1984; Miles *et al.*, 2006).

Three races of *P. pachyrhizi* have been reported in Uganda and there is possibility for existence of more races (Lamo, 2003). The presence of multiple virulent and aggressive rust pathogen races present a challenge of specific gene resistance break down among the soybean accessions (Hartman *et al.*, 2005; Tukamuhabwa *et al.*, 2009). This implies a need to screen the resistant accessions against all rust races in Uganda before being deployed against the pathogen.

Soybean accessions PI 417089A and PI 59172A previously found with RB resistance reaction to rust in the United States (Miles *et al.*, 2006) were also found to be resistant in this study. Miles (2003) reported a TAN reaction in soybean accessions PI 203398, PI 567024, PI 567046A, PI 567104B, PI 567123A and PI 594538A as it was observed in this study. Vuong *et al.* (2005) reported a TAN reaction in soybean accessions PI 417089A, PI 459025 and PI 462312, whereas a RB reaction was observed in this study. In addition, soybean accessions PI 594538A, PI 476905A, PI 567102B, PI 605823 and PI 605891A reported to have a RB reaction (Miles *et al.*,

2006) had a TAN reaction in this study suggesting resistance break down among genotypes. Soybean accessions PI 417126, PI 416826A and PI 615437 with RB, mixed and TAN reactions respectively (Miles *et al.*, 2006) were found with an immune reaction in this study. These findings reveal the existence of different rust pathogen races and resistance genes among host genotypes. Thus race by host plant interaction has to be addressed in all soybean rust breeding programmes and during variety registration before official variety release.

It suffices to note that the differences in reaction to soybean rust among soybean accessions are probably due to pathogenic variation in rust races that exist in different places (Hartman *et al.*, 2004) and the presence of different resistance genes among the soybean accessions which are known to react differently to *P. pachyrhizi* isolates (Garcia *et al.*, 2008). The results from the soybean rust screening trials conducted at MUARIK and NaCRRI are in agreement with the previous report by Hartman *et al.* (2005) which indicated that specific resistance genes to *P. pachyrhizi* can be challenged by some rust isolates. This affects breeding for resistance to soybean rust since resistance break down is common among soybean varieties with specific resistance genes as new virulent and aggressive pathogen races emerge (Miles *et al.*, 2006).

In general, mean rust severity and sporulation among soybean breeding lines were high at MUARIK and NaCRRI confirming them as hot spots for rust and appropriate sites for screening soybean germplasm for rust resistance. One limitation was that the rust races being evaluated in the field were not identified. Thus, there is need to routinely identify the rust pathotypes that exist in breeding trials at both MUARIK and NaCRRI. The mean rust severity on test materials was comparable across locations probably due to similar environmental conditions at both sites. This is similar to results by Kawuki *et al.* (2003) and Hartman *et al.* (2003) who observed that rust severity was influenced by location, moisture, relative humidity and temperature.

The soybean breeding lines BSPS 48A-27 and BSPS 9.2B that showed an immune reaction to *P. pachyrhizi* at NaCRRI and MUARIK respectively are suitable candidates for further evaluation for key agronomic and quality traits and if deemed suitable, can be considered for varietal testing on farmers fields for acceptability. Overall, soybean breeding lines (BSPS 9.2B, NG 14.1-24 and NG 14.1-6B) consistently showed low rust severity (< 3) at full seed stage (R6) compared to local checks Maksoy 1N (4.1) and Maksoy 3N (3.6) indicating resistance breakdown in Maksoy 1N which had been previously reported to be resistant to soybean rust (Kiryowa *et al.*, 2005).

The study showed that rust severity was generally higher in late maturing soybean accessions compared to early maturing accessions. This is in agreement with the previous results by Kawuki *et al.* (2003) who observed higher rust severity among late maturing soybean cultivars compared to early maturing cultivars. The early maturing soybean cultivars escape severe rust infection and therefore should be thoroughly screened for rust resistance under inoculation trials before being recommended. The hybridization between the resistant soybean accessions and the local varieties would be very important in the breeding efforts to develop rust resistant varieties that are adapted to Ugandan condition. The results indicated that some of the soybean accessions (PI 615437, PI 416826A and PI 417126) would be useful sources of soybean rust resistance genes for incorporation into high-yielding and adapted cultivars. The hypothesis that the soybean accessions are more resistant to soybean rust than the local varieties was accepted.

Agronomically, Maksoy 1N was the shortest variety while breeding line BSPS 16A was the tallest at both stations thus a useful source of genes for breeding tall varieties. The soybean breeding lines reached 50% flowering at the same period for both stations which showed that the photoperiods were similar at the two locations. The local check Maksoy 1N took the shortest duration to physiological maturity (89 days) compared to other breeding lines thus a useful source of genes for breeding early maturing varieties which are suitable to regions with short rain seasons. In general, the soybean breeding lines matured at the same duration at both locations. This is probably because NaCRRI and MUARIK are in the same geographical location with similar seasonal conditions. There is also a possibility that these lines were specifically selected for a particular maturity period. The soybean breeding lines had high nodulation capacity thus have the potential to fix large amounts of Nitrogen in the soil which improves the soil fertility. Most of the breeding lines were resistant to shattering while lines D X T 8.11, GC X WANDER, Maksoy 1N and MNG 11.2 were resistant to lodging making them important source of resistance genes to pod shattering and lodging.

The soybean breeding line BSPS 27A with the highest pod clearance (23.5 cm) is useful source of genes for breeding varieties with high pod clearance. Maksoy 1N with low pod clearance can be improved by crossing with this soybean line to improve on the pod clearance and ease of harvesting. Breeding line BSPS 50B with highest number of pods should be screened further in other locations in Uganda to determine its yield stability before release. Most of the breeding lines had three seeds per pod an indication of high grain yield potential. Breeding line BSPS

48A-14 had the highest 100 seed weight and large seeds which are desirable traits in soybean breeding for farmer acceptability. Breeding lines BSPS 182A, BSPS 24.2A-3, BSPS 43, BSPS 48A-14, and BSPS 48A-19 gave higher grain yield of over 1800 kg ha⁻¹ suggesting high potential for release as new commercial varieties. However these lines are still segregating with varying yields across seasons and locations thus a need to further purify and screen these lines in other locations in Uganda since uniformity is a major requirement for a variety before release. The local checks Maksoy 1N and Maksoy 3N had higher yield stability across seasons and locations compared to other breeding lines. Overall, the assembled germplasm harbours several key agronomic traits and thus qualify to be used as parental lines in the Ugandan soybean breeding programme.

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSION

5.1 Introduction

Soybean rust caused by *Phakopsora pachyrhizi* is a major threat to soybean production worldwide, and has potential of causing high yield losses among susceptible soybean varieties (Hartman *et al.*, 2005). Although many chemical fungicides are available and effective against soybean rust, their utility to resource poor farmers who dominate soybean production in Uganda is not feasible, thus host plant resistance is the best control measure against soybean rust. Previous breeding efforts (1996 to 2004) for host plant resistance to *P. pachyrhizi* in Uganda led to release of two moderately resistant varieties Maksoy 1N and Namsoy 4M with single resistance genes (Kiryowa *et al.*, 2005). However there is a threat of resistance breakdown among varieties as more virulent and aggressive pathogen races emerge (Hartman *et al.*, 2005; Miles *et al.*, 2006). This justifies a need to consistently screen soybean accessions for resistance to rust and undertaking scheduled monitoring of the pathogen population dynamics. This study was conducted to enhance the breeding efforts for host plant resistance to soybean rust through elucidation of diversity in soybean germplasm and assessing its reaction to soybean rust disease.

5.2 Discussion

There was significant variation among 91 soybean accessions for most of the evaluated traits, suggesting high phenotypic diversity among the soybean accessions which is vital for successful breeding (Chung and Singh, 2008). However, some morphological traits such as seed shape, color of the cotyledon, stem and flowers could only classify the 91 accessions into two groups. Some accessions matured much earlier than local checks Maksoy 3N and Maksoy 1N thus could be used in places with short rain seasons. However some accessions were susceptible to pod shattering and lodging implying a need to breed for resistance to these undesirable traits before genotypes are released. Maksoy 3N out yielded all accessions while 11 accessions (PI 615437, PI 567053, PI 628861, PI 567056A, PI 635999, PI 578457A, PI 628959, Serenade, PI 628812, PI 567104B and PI 567025A) had yield of more than 3 tons ha⁻¹ and thus could be used in breeding high yielding varieties. There is need to further evaluate these accessions in other soybean growing areas in Uganda to determine their adaptability and yield potential across diverse sites thus establish stable and/or location specific genotypes.

SSR analysis undertaken on 91 soybean accessions assayed at 10 loci confirmed known morphological trait differences among soybean accessions. High polymorphism (77%) with 5.3 alleles/loci was observed which indicated high genetic diversity among soybean accessions. The low genetic diversity among local soybean varieties justified a need for introduction of exotic accessions to expand the soybean germplasm base. Molecular markers enable breeders to search for new sources of variation and to investigate genes controlling quantitative traits thus improves the efficiency of parental selection for the breeding program (Li *et al.*, 2009). Therefore, inclusion of highly diverse germplasm in soybean breeding program may provide the genetic variability and new alleles required to permit continued progress and broad adaptation.

The study showed that 3.3% of soybean accessions were highly resistant to rust having immune reaction, 24.2% were moderately resistant having red brown reaction while 72.5% were highly susceptible to rust producing TAN lesions on leaves. Accessions PI 462312 and PI 459025 with known specific resistance genes *Rpp3* and *Rpp4* respectively also showed resistance to rust at MUARIK thus could be used to improve resistance in released, but susceptible varieties such as Nam 1, Nam 2, Kabanyoro 1, Namsoy 3, Maksoy 1N and Maksoy 2N.

Specific resistance genes to *P. pachyrhizi* can be challenged by some rust isolates (Hartman *et al.*, 2005) which affects the rate of genetic gain while selecting for soybean rust resistance. Thus development of durable “less-rusting” and “slow-rusting” cultivars is the best option for breeding rust resistant varieties in Uganda. Breeding lines BSPS 48C and GC X KAB had TAN reaction but with low rust severity score (3.5) suggesting suitability for developing varieties with durable resistance to soybean rust. Soybean rust resistance identified in Uganda will likely be of use in many African countries since *P. pachyrhizi* is a recent introduction in Africa.

Although a few soybean accessions and breeding lines showed resistance to soybean rust, many of them were susceptible to pod shattering and lodging which are undesirable traits in soybean production. Thus there is a need for hybridization with locally adapted varieties followed by backcrossing to remove undesirable while maintaining desirable traits. Soybean rust is known to infect the crop at any growth stage (Hartman *et al.*, 2006). However there was a general increase in rust severity among soybean genotypes as they matured with the highest severity observed at full seed (R6) hence the best stage for field screening of soybean rust in breeding trials. The rate of rust development under field conditions is influenced by the environmental conditions,

genotype and plant age with rapid increase in number of lesions during flowering and progressive increase up to maturity (Kawuki *et al.*, 2003; Hartman *et al.*, 2003). There is need for continued search for rust resistant genes to combat the risk of resistance breakdown among cultivars resulting from emergence of new races. Further studies should be conducted to understand the population dynamics of the pathogen and/or any other alternative host plants. Hybridization should also be carried out to incorporate resistance genes into locally adapted cultivars since the imported soybean accessions are not adapted to Ugandan conditions.

5.3 Conclusion

The field evaluations identified 24 soybean accessions and 10 advanced breeding lines with high to moderate levels of resistance to *P. pachyrhizi*. Soybean accessions PI 417126, PI 416826A and PI 615437 and the breeding lines BSPS 48A-27 and BSPS 9.2B showed steady resistance to *P. pachyrhizi* at the two stations making them useful source of resistance to soybean rust. Soybean accessions PI 462312 and PI 459025 with known specific resistance genes *Rpp3* and *Rpp4* respectively were also resistant in this study. Some of the accessions had RB lesions, which have been associated with single gene resistance (Hartman *et al.*, 2004), while others had TAN lesions with low visual rust severity. Soybean accessions with low lesion densities, low disease severity and low sporulation may be sources of partial resistance that may limit infection and lesion development, or may be a result of an incompatible reaction similar to that reported in *Rpp1* when challenged with a limited number of rust isolates. Single *P. pachyrhizi* isolate evaluations will need to be done on these accessions in order to evaluate their race specificity.

The soybean accessions with apparent resistance to soybean rust should be crossed with high yielding lines and adapted varieties. The derived populations will be used in breeding improved rust-resistant lines and for mapping their resistance genes. Some of the selected soybean accessions have the potential to provide soybean rust resistance genes that may be useful for incorporation into commercial soybean varieties to develop rust resistant genotypes. The commercial soybean varieties currently in use throughout Uganda soybean producing areas are moderate to very susceptible to soybean rust. Incorporation of rust resistance into these commercial varieties from identified sources in this research is imperative. The sources of resistance identified in this research may provide the resistance genes needed for future development of rust resistant soybean varieties.

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APPENDICES

Appendix 1. Vegetative traits of 91 soybean accessions evaluated at MUARIK during 2011 seasons

Plot No	Accession no	Origin	Hyp color	Coty color	Leaf color	Stem color	Seedling vigor	leaf size	Growth habit	Leaf shape	Pubescence	Pubescence density	Pubescence color	Pubescence type
1	Buffalo	Zimbabwe	G	G	LG	G	M	M	D	I	P	D	G	E
2	Dinamri	Zambia	P	G	LG	G	M	M	S	I	P	D	B	SA
3	Duiker	Zimbabwe	P	P	DG	G	M	M	D	B	P	S	G	A
4	Edamame 1	Zimbabwe	P	P	DG	P	V	M	D	I	P	SP	G	A
5	Gazelle	Zimbabwe	P	G	LG	P	M	M	S	I	P	SP	G	E
6	Maksoy 1N	Uganda	P	P	LG	P	M	M	D	I	P	SP	G	SA
7	Maksoy 3N	Uganda	P	G	LG	G	V	L	S	B	P	SP	B	E
8	PI 200488	USA	P	G	DG	P	M	M	D	B	P	S	LB	SA
9	PI 200526	USA	P	G	DG	P	M	M	D	I	P	D	B	E
10	PI 203398	USA	P	P	DG	P	M	L	D	B	P	SP	B	SA
11	PI 287197	USA	G	G	DG	G	M	L	D	B	P	SP	LB	E
12	PI 416810	USA	P	P	DG	G	M	S	S	I	P	SP	B	A
13	PI 416826A	USA	P	G	DG	G	M	S	D	I	P	SP	B	SA
14	PI 417089A	USA	P	G	DG	G	M	M	S	N	P	SP	LB	SA
15	PI 417120	USA	P	P	DG	G	M	M	S	I	P	SP	LB	SA
16	PI 417126	USA	G	G	DG	G	M	L	D	B	P	S	LB	SA
17	PI 417132	USA	DP	P	G	P	P	S	D	B	P	SP	LB	A
18	PI 417503	USA	DP	P	DG	P	M	S	D	B	P	S	G	SA
19	PI 459025	USA	P	P	DG	G	M	M	D	B	P	D	B	SA
20	PI 462312	USA	G	G	G	G	M	L	D	I	P	S	LB	SA
21	PI 471904	USA	P	G	LG	P	V	M	S	I	P	SP	B	SA
22	PI 476905A	USA	P	G	LG	G	M	M	S	I	P	SP	LB	SA
23	PI 506491	USA	P	P	DG	P	P	S	D	I	P	SP	B	SA
24	PI 506695	USA	DP	P	DG	P	M	M	D	B	P	SP	G	SA
25	PI 506695A	USA	DP	P	DG	P	P	M	D	B	P	S	G	A
26	PI 506764	USA	P	P	DG	P	P	M	D	B	P	S	G	A
27	PI 567024	USA	P	P	LG	P	V	M	S	I	P	S	B	SA
28	PI 567025A	USA	G	G	LG	G	V	L	S	B	P	SP	LB	E
29	PI 567034	USA	P	P	LG	G	M	M	D	B	P	SP	LB	SA
30	PI 567046A	USA	P	G	LG	G	V	L	S	B	P	S	LB	A
31	PI 567053	USA	P	G	LG	G	M	M	D	I	P	S	B	E
32	PI 567056A	USA	P	G	LG	G	V	M	S	I	P	SP	LB	SA

33	PI 567059	USA	P	G	LG	P	M	L	S	I	P	D	B	SA
34	PI 567102B	USA	P	G	LG	G	V	M	S	I	P	S	LB	E
35	PI 567104B	USA	P	G	LG	G	V	L	S	B	P	S	LB	E
36	PI 567123A	USA	P	G	LG	G	V	M	I	N	P	SP	B	SA
37	PI 567189A	USA	P	G	LG	G	V	L	S	B	P	D	B	SA
38	PI 567190	USA	G	G	DG	G	M	S	D	B	P	S	LB	A
39	PI 578457A	USA	P	G	LG	G	V	L	S	B	P	SP	LB	SA
40	PI 59172A	USA	DP	P	DG	G	M	S	D	I	P	S	LB	SA
41	PI 594538A	USA	G	G	DG	G	M	L	D	B	P	SP	LB	SA
42	PI 605823	USA	G	G	LG	G	V	L	S	B	P	SP	B	E
43	PI 605838	USA	P	G	G	G	M	S	S	I	P	S	B	SA
44	PI 605854B	USA	G	G	LG	G	M	M	S	I	P	S	B	SA
45	PI 605865B	USA	G	G	LG	G	M	M	D	I	P	SP	B	SA
46	PI 605885B	USA	G	G	LG	G	P	S	D	B	P	SP	LB	SA
47	PI 605891A	USA	G	G	DG	G	P	M	D	B	P	SP	G	A
48	PI 606397B	USA	G	G	LG	G	V	L	S	B	P	SP	B	SA
49	PI 606405	USA	P	G	G	G	M	M	S	I	P	D	LB	E
50	PI 606440A	USA	G	G	G	G	M	L	S	I	P	D	B	A
51	PI 615437	USA	G	G	LG	G	M	M	D	I	P	SP	B	SA
52	PI 628811	USA	G	P	G	G	M	L	D	B	P	SP	LB	E
53	PI 628812	USA	P	G	LG	G	P	S	S	I	P	D	G	A
54	PI 628813	USA	P	G	LG	G	V	L	D	I	P	D	G	A
55	PI 628827	USA	G	G	DG	G	V	S	D	I	P	SP	G	A
56	PI 628830	USA	G	G	LG	G	M	M	D	I	P	SP	B	E
57	PI 628832	USA	P	P	G	G	M	M	D	B	P	D	B	A
58	PI 628833	USA	G	G	DG	G	V	M	D	I	P	D	B	E
59	PI 628850	USA	G	G	LG	G	V	M	D	I	P	D	B	SA
60	PI 628855	USA	G	G	DG	G	V	M	D	I	P	SP	G	A
61	PI 628857	USA	G	G	DG	G	V	L	D	B	P	D	LB	E
62	PI 628861	USA	P	G	G	G	M	L	S	I	P	S	LB	SA
63	PI 628863	USA	P	P	DG	P	P	S	D	B	P	SP	LB	E
64	PI 628887	USA	P	G	DG	G	P	M	D	I	P	SP	G	SA
65	PI 628889	USA	G	G	DG	G	M	M	D	B	P	SP	B	E
66	PI 628903	USA	G	P	LG	G	M	M	D	I	P	SP	B	E
67	PI 628904	USA	G	G	DG	G	V	M	D	B	P	SP	G	A
68	PI 628905	USA	G	G	G	G	M	S	D	N	P	SP	B	SA
69	PI 628907	USA	P	P	LG	G	V	L	S	B	P	SP	G	SA

70	PI 628908	USA	G	G	G	G	V	M	D	B	P	S	G	E
71	PI 628915	USA	G	G	G	G	M	L	D	I	P	SP	G	SA
72	PI 628918	USA	G	G	G	G	P	S	D	I	P	SP	LB	SA
73	PI 628919	USA	P	G	LG	G	V	L	D	I	P	SP	B	E
74	PI 628925	USA	P	G	LG	G	M	M	D	I	P	SP	B	SA
75	PI 628929	USA	P	G	LG	G	M	L	D	N	P	SP	G	SA
76	PI 628932	USA	G	G	G	G	V	L	S	B	P	SP	G	SA
77	PI 628933	USA	P	G	LG	G	M	M	D	B	P	D	B	SA
78	PI 628934	USA	G	G	DG	G	M	M	D	I	P	D	B	E
79	PI 628948	USA	P	P	DG	G	M	M	D	I	P	SP	B	SA
80	PI 628959	USA	G	G	DG	G	M	M	D	B	P	D	B	SA
81	PI 628960	USA	P	G	LG	G	M	M	S	I	P	SP	B	E
82	PI 635999	USA	P	G	DG	G	V	L	S	B	P	SP	LB	SA
83	PI 644103	USA	P	P	LG	G	M	M	D	I	P	SP	G	SA
84	Roan	Zimbabwe	P	G	DG	G	V	L	S	B	P	SP	G	A
85	Safari	Zimbabwe	P	G	DG	P	P	M	S	I	P	SP	G	A
86	Santa	Zimbabwe	P	G	G	G	P	M	S	I	P	S	LB	A
87	SCSI	Zimbabwe	P	P	LG	P	P	S	D	I	P	SP	G	SA
88	Serenade	Zimbabwe	G	G	LG	G	P	M	D	I	P	S	LB	SA
89	Siesta	Zimbabwe	P	G	DG	G	M	L	D	B	P	SP	G	A
90	Solitaire	Zimbabwe	P	G	DG	G	M	S	S	B	P	SP	G	SA
91	Soprano	Zimbabwe	P	G	G	P	P	S	S	I	P	SP	G	A

G=Green
P=Purple
DP=Dark
purple

G= Green
P= Purple

LG= Light
green
G=Green
DG=Dark
green

G= Green
P= Purple

P= Poor
M= Medium
V= Vigorous

L= Large
M= Medium
S= Small

D= Determinate
S=semi
determinate
I=Indeterminate

N= Narrow
I= Intermediate
B= Broad

P= Present
O= Absent

S= Sparse
SP= Semi
sparse
D= Dense

G= Grey
B= Brown
LB= Light
brown

E= Erect
SA= Semi
Appressed
A= Appressed

Hyp - Hypocotyle; Coty - Cotyledon

Appendix 2. Flower and seed traits of 91 soybean accessions evaluated at MUARIK during 2011 seasons

Plot No	Accession no	Flower color	Mature pod color	Seed coat color	Seed coat pattern	Hilium color	Seed coat surface lusture	Seed shape	Seed size	Seed quality
1	Buffalo	W	BN	C	DH	BN	S	O	M	M
2	Dinamri	P	BN	C	DH	BK	I	O	M	M
3	Duiker	P	BN	Y	LH	Y	S	R	L	G
4	Edamame 1	P	BK	C	LH	Y	I	R	L	G
5	Gazelle	P	BN	Y	LH	Y	S	R	M	M
6	Maksoy 1N	P	BN	C	DH	BN	I	R	S	M
7	Maksoy 3N	P	BN	C	LH	GY	I	R	L	G
8	PI 200488	P	BN	C	DH	BN	I	O	L	M
9	PI 200526	P	BN	C	DH	BK	I	R	L	G
10	PI 203398	P	BN	C	DH	BN	I	O	L	G
11	PI 287197	W	T	C	DH	BN	I	R	S	M
12	PI 416810	P	BK	BK	DH	BK	D	O	L	G
13	PI 416826A	P	BK	BN	DH	BN	HB	O	M	M
14	PI 417089A	P	BN	BK	DH	BK	D	O	L	G
15	PI 417120	P	BN	C	DH	BN	I	O	M	M
16	PI 417126	W	BK	C	DH	BN	I	O	L	M
17	PI 417132	P	BN	C	DH	BN	I	O	M	M
18	PI 417503	P	T	C	LH	BF	D	O	L	M
19	PI 459025	P	T	G	DH	G	I	O	L	G
20	PI 462312	W	BN	Y	DH	BN	S	O	L	G
21	PI 471904	P	BN	C	DH	BN	I	O	S	M
22	PI 476905A	P	BN	C	DH	BK	D	O	S	M
23	PI 506491	P	BN	C	DH	BN	I	O	M	G
24	PI 506695	P	BK	C	LH	BN	I	O	L	M
25	PI 506695A	P	BK	C	LH	Y	I	R	L	M
26	PI 506764	P	BK	C	DH	BN	I	O	M	M
27	PI 567024	W	BN	C	DH	BN	I	O	M	M
28	PI 567025A	P	T	C	DH	BN	I	R	M	M
29	PI 567034	P	BN	C	DH	BN	I	O	S	M
30	PI 567046A	P	T	C	DH	BN	I	O	S	M
31	PI 567053	P	T	G	DH	BN	D	O	S	P
32	PI 567056A	P	BN	C	DH	BN	I	R	L	G
33	PI 567059	P	BN	C	LH	BN	I	O	S	P
34	PI 567102B	P	BN	C	DH	BN	I	O	S	M
35	PI 567104B	P	T	G	DH	BN	D	O	S	P
36	PI 567123A	P	T	C	DH	BK	D	O	S	M
37	PI 567189A	W	T	C	DH	BN	I	O	L	M
38	PI 567190	P	BN	C	DH	BK	I	O	M	M
39	PI 578457A	P	BN	C	DH	BN	I	R	M	M
40	PI 59172A	W	BN	C	DH	BN	I	O	L	M
41	PI 594538A	W	T	Y	DH	BK	S	R	S	M
42	PI 605823	P	BK	G	DH	BK	D	O	S	P
43	PI 605838	W	BN	C	DH	BK	I	O	L	G
44	PI 605854B	W	BN	C	DH	BN	I	O	L	G
45	PI 605865B	W	BN	C	DH	BK	D	O	S	M
46	PI 605885B	W	BN	C	LH	Y	I	R	L	G
47	PI 605891A	W	BK	C	S	BN	I	R	M	M

48	PI 606397B	P	T	C	DH	BN	I	O	S	M
49	PI 606405	W	BN	C	DH	BN	I	O	S	M
50	PI 606440A	W	BN	C	DH	BN	I	O	M	M
51	PI 615437	W	BN	C	DH	BN	D	O	M	P
52	PI 628811	P	BK	C	DH	BN	S	O	M	G
53	PI 628812	P	BN	C	LH	BN	I	R	S	I
54	PI 628813	W	BN	C	DH	BN	I	R	M	M
55	PI 628827	W	BN	C	DH	BN	I	O	L	M
56	PI 628830	P	BN	C	DH	BK	I	R	L	G
57	PI 628832	W	BN	G	DH	BK	I	O	M	G
58	PI 628833	W	T	C	LH	Y	I	R	S	M
59	PI 628850	W	BN	C	LH	Y	I	R	M	G
60	PI 628855	W	BN	C	DH	BN	I	R	M	G
61	PI 628857	P	T	C	DH	BK	I	R	L	G
62	PI 628861	P	BN	C	DH	BN	I	R	M	M
63	PI 628863	P	T	G	DH	BN	D	R	M	M
64	PI 628887	W	T	C	DH	BN	I	R	M	M
65	PI 628889	W	T	C	DH	BN	D	O	S	M
66	PI 628903	W	BN	C	DH	BN	I	R	S	M
67	PI 628904	W	T	C	DH	BN	I	R	M	M
68	PI 628905	P	BN	C	LH	BN	S	R	S	M
69	PI 628907	W	BN	C	LH	BF	S	R	M	M
70	PI 628908	W	BN	C	LH	BN	I	O	S	P
71	PI 628915	W	BN	C	DH	BN	I	O	M	M
72	PI 628918	P	BN	C	DH	BK	S	O	S	M
73	PI 628919	P	BN	C	DH	BK	I	O	S	M
74	PI 628925	P	BN	C	LH	Y	I	O	S	P
75	PI 628929	W	BN	C	LH	BN	I	R	M	G
76	PI 628932	P	BN	G	DH	BK	S	R	M	M
77	PI 628933	W	BN	C	DH	BK	I	R	L	G
78	PI 628934	P	BN	C	LH	GY	I	O	L	M
79	PI 628948	P	T	C	DH	BK	I	R	M	M
80	PI 628959	W	T	G	DH	BN	S	R	M	M
81	PI 628960	P	BN	C	DH	BN	S	R	M	G
82	PI 635999	P	BN	C	DH	BK	I	R	L	G
83	PI 644103	P	BN	G	DH	BN	D	O	S	P
84	Roan	P	T	C	LH	GY	I	O	M	G
85	Safari	P	BN	Y	LH	Y	S	R	M	G
86	Santa	P	T	Y	DH	BN	S	R	M	G
87	SCSI	P	BN	Y	LH	Y	S	R	M	G
88	Serenade	W	T	C	DH	BK	S	R	M	G
89	Siesta	P	BN	C	LH	Y	I	R	M	G
90	Solitaire	P	T	Y	LH	GY	S	R	M	G
91	Soprano	P	T	C	LH	Y	S	R	S	M
		W= White P= Purple	T= Tan BN= Brown BK= Black	C= Cream Y=Yellow G=Green BN=Brown BK=Black	LH= Light helium DH= Dark helium S= Saddle	Y= Yellow BF=Buff BN=Brown G=Green GY=Grey BK=Black	S= Shiny I=Intermediate D=Dull HB=Heavy brown	O= Oval R= Round	S= Small M=Medium L=Large	P= Poor M=Medium G=Good

Appendix 3. Means of agronomic traits for 91 soybean accessions evaluated at MUARIK during 2011 seasons

Plot No	Accession	HL	LL	LW	DF	DPM	S(1-5)	L(1-5)	ND(1-5)	NN	PH	NB	PC	PP	PL	PW	SP	100SW	Yld kg ha^{-1}
1	Buffalo	4.0	13.0	7.6	41	93	1.0	2.0	3.0	10	33.4	4	8.2	35	5.0	0.9	3	16.9	2419
2	Dinamri	4.0	12.5	7.3	55	112	1.0	2.0	4.3	11	55.7	6	12.4	46	5.2	1.0	3	14.6	2228
3	Duiker	5.2	10.8	6.7	42	98	1.0	1.3	3.0	10	41.2	5	9.9	41	4.7	2.1	3	18.4	2480
4	Edamame 1	4.5	9.3	6.3	33	88	1.3	2.0	3.3	7	27.6	3	9.2	18	5.3	1.2	3	31.1	1827
5	Gazelle	4.1	10.2	6.7	42	97	1.0	2.3	2.8	11	49.6	4	9.6	38	4.7	0.9	3	16.8	2472
6	Maksoy 1N	5.9	11.9	7.6	49	94	1.0	2.0	5.0	10	45.3	6	7.8	46	4.6	0.8	3	13.5	2568
7	Maksoy 3N	3.8	13.0	8.7	50	104	1.0	3.5	4.0	17	94.1	5	10.7	58	5.1	1.0	3	22.5	3667
8	PI 200488	2.8	11.6	7.3	34	89	2.5	1.5	3.0	9	30.6	3	7.5	26	4.3	0.9	2	23.2	1463
9	PI 200526	3.6	11.3	6.7	33	93	1.5	1.0	3.8	7	24.6	3	7.7	14	4.8	1.0	2	25.6	1063
10	PI 203398	4.0	12.5	7.6	38	91	1.3	1.0	4.3	10	39.7	3	7.6	35	4.7	1.0	2	20.9	2498
11	PI 287197	3.5	12.4	7.2	41	91	1.8	1.0	4.8	9	40.0	5	7.7	35	4.3	0.9	2	15.7	1401
12	PI 416810	3.5	12.5	5.9	36	90	2.8	3.0	3.3	12	54.8	4	6.5	40	4.8	1.0	2	25.6	1926
13	PI 416826A	4.2	8.8	5.4	32	89	2.3	1.5	3.3	7	24.6	2	7.2	13	4.1	0.9	2	17.7	1016
14	PI 417089A	3.4	15.4	8.2	39	94	2.3	3.0	4.0	13	56.9	3	6.8	41	4.8	1.1	2	27.4	2011
15	PI 417120	3.7	9.8	5.5	37	88	1.8	1.8	2.8	11	46.2	3	8.6	29	4.3	0.9	3	15.3	1622
16	PI 417126	4.6	11.4	8.2	36	94	2.8	1.0	2.8	7	26.7	2	6.3	20	4.1	1.1	2	26.2	1094
17	PI 417132	3.8	10.2	6.0	34	87	3.0	1.3	3.5	8	26.6	3	8.5	22	4.8	1.0	2	26.5	873
18	PI 417503	4.1	10.7	6.2	33	88	1.8	1.8	3.8	11	29.6	2	5.8	29	4.2	2.2	2	20.5	1299
19	PI 459025	3.9	12.0	8.4	40	94	1.0	1.5	4.8	8	31.4	3	9.8	20	5.0	1.0	2	26.4	1858
20	PI 462312	4.0	11.6	6.5	39	95	1.0	2.0	4.0	9	37.5	4	5.5	34	4.5	1.0	2	22.5	2757
21	PI 471904	4.5	11.3	6.5	44	94	2.5	3.3	4.8	13	70.2	5	9.1	59	4.7	0.8	3	15.6	2680
22	PI 476905A	3.7	12.6	7.6	41	88	2.5	3.0	4.0	10	48.8	3	7.9	33	4.5	0.9	2	12.6	1524
23	PI 506491	4.3	11.3	6.4	33	92	2.3	1.5	2.3	7	26.7	3	9.2	24	4.6	1.0	2	25.1	1294
24	PI 506695	3.8	10.5	6.5	35	89	2.0	1.0	4.3	10	27.6	3	7.1	26	4.5	1.0	2	25.5	1065
25	PI 506764	3.9	10.9	6.6	34	93	1.8	1.0	3.8	7	21.4	3	6.5	11	4.7	1.1	2	28.0	621
26	PI 567024	4.0	10.9	6.4	47	98	2.0	2.5	4.3	12	61.7	5	11.1	45	4.5	0.9	3	16.1	2599
27	PI 567025A	3.5	12.9	8.8	48	96	1.5	3.5	4.0	14	85.8	6	8.3	69	4.7	0.8	3	16.1	3609
28	PI 567034	4.0	11.4	6.7	36	90	2.3	2.3	3.3	10	43.8	3	8.8	28	4.3	0.8	2	15.7	1062
29	PI 567046A	3.2	12.3	7.7	56	100	2.0	3.8	3.8	15	86.0	5	12.9	46	4.5	0.8	3	13.7	2540
30	PI 567053	3.4	11.2	6.5	49	101	2.3	3.3	4.3	14	70.4	5	10.6	62	4.3	0.7	3	12.9	3140
31	PI 567056A	2.8	10.9	6.3	59	105	1.3	3.5	4.3	18	93.3	6	12.7	77	3.9	0.6	3	10.2	3569
32	PI 567059	3.8	12.6	7.6	43	95	2.3	2.8	4.3	13	59.5	4	9.1	49	4.5	1.0	2	20.0	1835

33	PI 567102B	3.7	12.7	7.2	65	117	1.8	4.3	4.3	17	116.8	7	9.3	76	3.7	0.6	2	9.1	1555
34	PI 567104B	2.7	12.2	7.7	61	111	1.3	3.8	4.0	17	82.0	6	9.4	94	3.9	0.6	3	9.8	3234
35	PI 567123A	3.3	10.7	6.0	61	104	1.5	4.0	3.0	14	95.0	6	12.6	65	3.6	0.6	3	7.9	2645
36	PI 567189A	4.3	12.2	8.1	49	92	1.5	2.8	4.8	11	61.3	5	11.8	42	4.2	0.8	2	16.8	1592
37	PI 567190	2.8	10.8	7.1	42	89	1.5	1.8	4.3	9	37.1	4	7.4	30	5.2	1.1	3	19.9	2031
38	PI 578457A	4.7	12.0	7.7	48	103	1.8	3.0	4.8	17	95.2	6	11.4	61	4.6	0.9	3	16.5	3369
39	PI 59172A	4.4	10.2	6.3	36	92	2.0	1.3	3.0	7	19.9	3	6.6	17	4.6	1.1	2	29.1	714
40	PI 594538A	3.3	12.6	7.3	42	96	1.0	2.0	3.3	10	39.3	6	6.1	52	4.8	1.0	2	21.9	2794
41	PI 605823	3.7	12.0	7.4	57	106	1.0	2.3	3.8	13	63.9	6	9.7	74	4.6	0.9	3	12.5	2935
42	PI 605838	4.9	10.8	6.4	47	91	1.8	2.5	3.8	12	58.3	5	9.0	49	4.4	0.8	2	13.7	2420
43	PI 605854B	4.0	12.3	7.8	47	95	1.5	3.8	3.8	11	50.3	4	11.8	44	4.4	0.9	3	19.2	2768
44	PI 605865B	4.0	12.4	7.8	45	96	1.0	2.8	4.3	11	48.7	4	11.0	41	4.5	0.9	3	18.3	1710
45	PI 605885B	3.6	10.0	5.7	42	90	1.8	2.8	4.3	11	51.0	4	9.7	38	4.4	0.7	2	15.0	1759
46	PI 605891A	2.8	9.2	6.8	33	91	1.3	1.3	2.8	9	28.6	1	5.9	17	4.6	1.1	3	23.3	1298
47	PI 606397B	4.0	11.1	7.3	42	92	1.8	2.0	2.8	11	51.6	4	10.4	27	4.5	0.9	3	18.9	2315
48	PI 606405	3.2	10.2	6.4	47	89	1.8	3.0	2.8	10	43.8	5	7.6	41	4.1	0.8	2	14.9	2219
49	PI 606440A	3.1	13.9	7.4	35	89	1.3	2.5	2.8	12	52.4	3	6.2	38	4.8	0.9	3	17.2	1895
50	PI 615437	3.6	13.2	8.5	43	94	1.5	3.8	4.0	12	59.7	4	12.1	37	4.5	0.9	3	19.0	3176
51	PI 628811	2.8	11.6	7.4	59	108	1.0	2.3	3.3	14	66.8	7	12.4	66	4.5	0.7	2	14.8	2162
52	PI 628812	3.4	11.1	6.7	42	95	1.5	1.5	4.8	11	42.2	3	8.1	49	4.2	0.8	3	15.9	3053
53	PI 628813	4.2	11.7	6.9	53	109	1.0	2.0	4.8	11	55.7	5	12.4	38	4.5	0.7	3	11.7	1774
54	PI 628827	3.1	10.2	6.2	38	90	1.0	1.8	2.8	8	30.3	4	8.1	26	4.4	0.9	3	18.3	1694
55	PI 628830	3.7	12.5	7.5	40	97	1.0	2.0	3.0	10	39.4	5	7.0	42	4.8	0.9	3	17.2	2586
56	PI 628832	4.0	10.5	6.2	39	90	1.3	1.8	3.0	8	30.4	3	7.3	22	4.4	0.9	3	17.9	1324
57	PI 628833	3.6	11.8	6.9	39	92	1.0	1.3	3.8	9	31.3	5	7.6	34	4.8	0.9	3	18.8	2954
58	PI 628850	3.4	11.2	6.6	47	97	1.3	2.5	3.5	10	48.5	5	8.1	37	4.3	0.8	3	13.6	2260
59	PI 628855	2.9	9.2	6.1	38	90	1.0	1.8	2.3	9	39.2	4	7.6	35	4.8	1.0	2	20.4	2283
60	PI 628857	4.0	12.4	7.6	41	94	1.0	2.0	4.8	12	42.3	6	7.4	49	4.9	1.0	3	18.3	2931
61	PI 628861	4.0	12.7	7.5	42	96	1.0	2.5	4.0	13	66.8	4	10.6	44	5.3	0.8	3	17.8	3178
62	PI 628863	4.7	8.7	5.9	40	93	1.5	1.0	3.5	8	25.3	4	6.4	25	4.6	1.0	3	18.2	1445
63	PI 628887	3.9	10.1	6.5	44	98	1.3	2.5	3.3	11	34.0	6	7.1	43	4.8	0.9	2	17.2	2067
64	PI 628889	3.2	11.7	6.6	40	91	1.0	2.3	2.8	10	34.2	5	5.5	41	4.1	0.8	2	16.9	1925
65	PI 628903	3.7	11.5	7.2	49	103	1.5	1.5	4.0	12	50.9	6	8.0	43	4.5	0.9	3	14.5	2382
66	PI 628904	3.5	10.0	6.8	42	93	1.3	1.3	2.8	9	37.0	3	9.8	26	4.2	0.9	3	14.6	1393
67	PI 628905	3.1	11.4	6.7	41	94	1.3	1.3	3.8	11	33.6	5	5.0	39	4.8	0.9	3	17.6	2516

68	PI 628907	4.1	11.5	6.9	46	101	1.0	1.8	4.3	11	49.5	5	10.0	46	4.4	0.7	3	14.1	2648
69	PI 628908	5.2	10.0	6.9	41	96	1.0	2.0	3.3	10	36.1	4	8.0	39	4.2	0.8	3	16.3	2626
70	PI 628915	3.0	12.1	7.4	54	105	1.0	2.3	3.8	12	65.5	6	10.6	38	5.0	0.8	3	12.5	1433
71	PI 628918	4.5	9.7	5.6	39	91	1.5	1.8	3.3	9	37.9	4	7.2	31	4.6	1.0	2	18.7	1410
72	PI 628919	4.0	12.4	7.0	54	107	1.0	2.3	3.8	10	58.8	6	10.5	46	5.1	1.0	3	16.5	2585
73	PI 628925	3.3	12.6	7.4	58	114	1.3	1.3	3.8	12	58.0	8	13.1	59	4.8	0.8	3	12.4	1699
74	PI 628929	3.4	12.6	7.0	65	123	1.0	2.0	3.8	15	59.8	7	10.7	72	4.6	0.9	3	12.1	1420
75	PI 628932	3.0	11.8	7.3	40	94	1.0	1.8	3.0	8	35.6	5	7.7	29	4.4	1.0	2	23.1	1931
76	PI 628933	3.7	11.6	7.1	46	107	1.3	1.3	3.3	10	40.5	7	6.9	37	5.0	0.9	3	15.4	2514
77	PI 628934	3.8	11.8	6.6	40	99	1.0	1.5	3.3	9	38.7	5	9.1	33	4.7	0.9	3	18.3	2268
78	PI 628935	2.9	11.2	7.1	37	94	2.8	1.0	3.8	7	23.2	3	7.1	20	4.6	1.1	2	31.7	914
79	PI 628948	3.3	11.4	6.7	43	91	1.0	2.5	3.3	9	45.7	4	8.5	35	4.7	1.0	3	19.3	1911
80	PI 628959	2.9	11.6	7.5	47	101	1.0	1.8	3.3	11	42.2	7	6.9	59	4.9	0.9	3	15.7	3306
81	PI 628960	4.5	11.2	6.7	48	103	1.0	2.5	3.8	15	77.3	5	12.1	60	4.6	0.9	3	16.7	2254
82	PI 635999	4.6	12.1	8.0	46	97	1.8	2.3	3.8	14	66.2	4	9.8	48	4.8	1.0	3	22.9	3582
83	PI 644103	3.8	14.0	8.0	65	108	1.0	1.8	4.0	15	61.5	8	10.0	91	4.7	1.0	3	12.2	1332
84	Roan	5.2	11.1	6.6	45	99	1.0	1.3	3.8	9	39.9	4	9.3	28	5.1	1.1	3	19.9	1817
85	Safari	4.6	11.5	6.8	47	104	1.3	2.3	4.3	13	53.2	5	7.5	65	4.6	0.9	3	16.1	2494
86	Santa	6.9	11.2	7.1	49	104	1.0	1.5	3.3	9	43.4	5	8.0	37	4.7	1.0	2	18.7	1209
87	SCSI	3.8	9.9	6.4	46	102	1.3	1.5	2.3	13	56.3	5	7.4	53	4.5	0.9	3	16.8	2617
88	Serenade	3.5	11.4	7.0	45	105	1.3	1.3	3.3	12	56.2	4	8.2	37	5.1	1.1	3	19.6	3005
89	Siesta	4.2	10.5	7.3	44	101	1.0	1.5	2.5	9	36.7	4	7.9	33	4.9	1.0	2	18.2	1602
90	Solitaire	3.7	9.8	6.9	41	101	1.0	1.3	2.8	12	50.9	4	7.7	41	4.6	1.0	2	18.6	2785
91	Soprano	4.0	11.2	7.2	48	103	1.0	1.8	4.0	14	59.6	4	8.8	52	4.6	0.9	3	15.4	2369
	Mean	3.8	11.4	7.0	44.0	96.9	1.5	2.1	3.6	11.0	49.1	4.5	8.7	41.3	4.6	0.9	2.6	18.1	2134.0
	LSD	0.1	1.5	1.1	2.2	5.2	1.5	1.8	1.3	2.1	11.9	1.5	2.4	20.6	0.3	0.5	0.5	3.1	1119.8
	%CV	2.0	9.6	11.3	3.5	3.8	16.0	15.9	8.8	14.0	17.4	24.0	19.9	35.8	5.2	39.3	12.5	12.5	37.7
	F probability	<.001	<.001	<.001	<.001	<.001	<.001 ^a	<.001 ^a	<.001 ^a	<.001	<.001	<.001	<.001	<.001	<.001	0.015	<.001	<.001	<.001

^a ANOVA conducted on angular transformed values; HL, Hypocotyle length; LL, Leaf length; LW, Leaf width; DF, Days to 50% flowering; DPM, Days to physiological maturity; S, Shattering; L, Lodging; ND, Nodulation; NN, Number of nodes; PH, Plant height; NB, Number of branches; PC, Pod clearance; PP, Pods per plant; PL, Pod length; PW, Pod width; SP, Seeds per pod; 100SW, 100 seed weight; Yld, Yield in Kgha⁻¹

Appendix 4. Reaction of 39 advanced breeding lines to soybean rust at NaCRRRI in 2011B and 2012A

Plot No	Accession	RR2 (1-9)	RR4 (1-9)	RR6 (1-9)	RS(1-5)	RRT
1	BSPS 16A	1.5	2.0	4.3	2.8	T
2	BSPS 18.3	1.5	2.8	4.5	3.3	T
3	BSPS 182A	1.5	2.0	3.0	1.8	RB
4	BSPS 24.2A-3	1.0	1.8	2.6	1.5	RB
5	BSPS 27.2B	1.0	2.5	3.5	2.5	T
6	BSPS 27A	1.5	1.9	4.1	3.0	T
7	BSPS 42	1.1	2.0	2.9	1.8	RB
8	BSPS 43	1.8	2.5	4.0	2.8	T
9	BSPS 47B	1.0	1.4	2.6	1.8	RB
10	BSPS 48A-1	1.8	2.4	5.0	3.8	T
11	BSPS 48A-14	1.5	2.0	3.6	2.0	T
12	BSPS 48A-19	1.5	1.9	2.6	1.8	RB
13	BSPS 48A-21B	1.0	1.9	3.5	2.3	T
14	BSPS 48A-27	1.5	1.5	2.3	1.3	I
15	BSPS 48A-5	1.5	1.8	3.1	2.3	RB
16	BSPS 48B	1.0	1.5	3.3	1.8	RB
17	BSPS 48C	1.3	1.9	2.9	2.0	RB
18	BSPS 50B	1.8	3.0	5.0	3.8	T
19	BSPS 62C-1	1.8	2.5	3.4	2.3	RB
20	BSPS 7.6B	1.0	2.3	3.5	2.5	T
21	BSPS 70	1.8	2.3	4.3	3.3	T
22	BSPS 75B	1.5	2.0	2.8	1.5	RB
23	BSPS 9.2B	1.0	1.8	3.3	2.0	RB
24	D X T 8.11	1.8	3.3	5.0	3.5	T
25	DXT SPS 16.6-2	1.1	1.5	3.3	2.3	RB
26	GC X KAB	1.0	1.5	3.3	2.3	RB
27	GC X WANDER	1.5	2.3	4.8	3.3	T
28	KAB X UG5	1.0	2.3	3.5	2.3	T
29	Maksoy 1N	1.6	2.4	3.6	3.0	T
30	Maksoy 3N	1.0	1.8	3.1	2.3	RB
31	MNG 11.2	1.5	2.5	5.0	3.8	T
32	NG 14.1-24	1.6	1.8	1.9	1.8	RB
33	NG 14.1-6B	1.3	1.8	2.4	1.5	RB
34	NGDT 2.15-10	1.8	2.3	4.6	3.0	T
35	NGDT 2.15-4	1.3	2.0	4.3	3.0	T
36	NGDT 8.11	1.0	2.1	3.6	2.3	T
37	NGDT BLP12.4	1.5	1.9	3.4	2.8	T
38	NII X GC 20.2	1.5	2.5	3.8	2.8	T
39	NII x GC 4.1-2	1.3	2.5	2.4	1.5	RB
Mean		1.4	2.1	3.5	2.4	
LSD		1.4	1.8	1.6	1.9	
%CV		15.5	16.2	10.4	15.7	
F probability ^a		0.013	0.017	<.001	<.001	

^a ANOVA conducted on angular transformed values; RR2, RR4 and RR6 are Rust severity at growth stages R2, R4 and R6 respectively; RS, Rust sporulation; RRT, Rust reaction type; I, Immune; RB, Red brown and T, Tan

Appendix 5. Reaction of 39 advanced breeding lines to soybean rust at MUARIK in 2011B and 2012A

Plot No	Accession	RR2 (1-9)	RR4 (1-9)	RR6 (1-9)	RS(1-5)	RRT
1	BSPS 16A	1.5	2.5	4.5	2.8	T
2	BSPS 18.3	1.0	2.3	3.5	1.8	RB
3	BSPS 182A	1.0	2.3	4.4	2.8	T
4	BSPS 24.2A-3	1.8	2.3	4.3	2.5	T
5	BSPS 27.2B	1.3	2.5	4.0	2.3	T
6	BSPS 27A	2.0	3.3	5.3	3.5	T
7	BSPS 42	1.1	2.9	4.6	2.8	T
8	BSPS 43	1.3	2.8	3.5	2.0	RB
9	BSPS 47B	1.0	2.3	3.4	1.8	RB
10	BSPS 48A-1	1.9	3.5	6.0	4.0	T
11	BSPS 48A-14	1.6	3.4	5.6	3.5	T
12	BSPS 48A-19	1.5	2.8	4.0	2.5	RB
13	BSPS 48A-21B	1.5	3.3	5.4	3.8	T
14	BSPS 48A-27	1.3	2.1	4.1	2.5	T
15	BSPS 48A-5	1.3	2.5	4.4	2.8	T
16	BSPS 48B	1.3	2.3	3.0	2.0	RB
17	BSPS 48C	1.3	2.4	4.1	2.5	T
18	BSPS 50B	2.0	3.0	4.5	3.0	T
19	BSPS 62C-1	1.4	2.3	4.0	2.5	T
20	BSPS 7.6B	1.5	3.0	4.3	2.5	T
21	BSPS 70	1.5	2.5	4.0	2.5	T
22	BSPS 75B	1.0	1.9	3.4	2.0	RB
23	BSPS 9.2B	1.0	1.5	2.0	1.0	I
24	D X T 8.11	2.0	3.3	5.0	3.3	T
25	DXT SPS 16.6-2	1.8	2.9	4.8	3.0	T
26	GC X KAB	1.5	2.5	3.8	2.3	RB
27	GC X WANDER	1.8	3.0	5.0	3.3	T
28	KAB X UG5	1.3	2.5	4.0	2.3	T
29	Maksoy 1N	1.5	3.3	4.6	2.5	T
30	Maksoy 3N	1.6	2.8	4.0	2.5	T
31	MNG 11.2	1.8	3.3	4.3	2.8	T
32	NG 14.1-24	1.4	2.6	3.9	2.5	RB
33	NG 14.1-6B	1.5	2.5	3.4	2.0	RB
34	NGDT 2.15-10	1.8	3.0	5.5	3.8	T
35	NGDT 2.15-4	1.8	3.0	5.5	3.5	T
36	NGDT 8.11	1.1	2.6	4.6	3.0	T
37	NGDT BLP12.4	1.3	2.5	4.8	3.0	T
38	NII X GC 20.2	1.8	3.3	4.8	3.0	T
39	NII x GC 4.1-2	1.3	2.3	4.1	2.3	T
Mean		1.5	2.7	4.3	2.7	
LSD		1.4	1.7	1.5	1.8	
%CV		14.4	12.7	9	13.6	
F probability ^a		0.001	0.004	<.001	<.001	

^a ANOVA conducted on angular transformed values; RR2, RR4 and RR6 are Rust severity at growth stages R2, R4 and R6 respectively; RS, Rust sporulation; RRT, Rust reaction type; I, Immune; RB, Red brown and T, Tan

Appendix 6. Means of agronomic traits of 39 advanced soybean breeding lines evaluated at NaCRRI during seasons 2011B and 2012A

Plot No	Accession	PH	NB	DF	DPM	ND (1-5)	L(1-5)	S(1-5)	PC	PP	SP	100SW	Y kg/ha
1	BSPS 16A	90.6	3	52	105	3.0	3.5	1.0	14.1	55	3	15.8	1039
2	BSPS 18.3	63.6	2	54	103	4.0	2.0	1.3	14.8	41	3	15.7	1775
3	BSPS 182A	76.2	2	49	105	1.9	1.8	1.0	13.9	46	3	16.5	1502
4	BSPS 24.2A-3	75.9	3	48	102	3.0	1.8	1.1	11.8	43	3	17.6	1833
5	BSPS 27.2B	55.6	4	52	97	2.3	1.3	2.3	12.6	48	3	17.3	1484
6	BSPS 27A	79.3	1	52	107	2.6	2.8	1.0	21.5	35	3	17.2	1302
7	BSPS 42	57.8	2	46	96	2.4	1.4	1.6	12.5	30	3	16.6	1427
8	BSPS 43	68.7	3	50	104	3.5	2.0	1.5	11.6	51	3	16.4	2234
9	BSPS 47B	80.6	3	48	102	2.6	3.1	1.3	11.4	50	3	16.9	1323
10	BSPS 48A-1	86.6	4	51	107	2.9	2.3	1.0	13.0	53	3	17.5	1656
11	BSPS 48A-14	62.3	3	44	101	3.0	1.5	1.3	11.6	35	2	18.4	1469
12	BSPS 48A-19	80.9	3	45	101	2.3	3.0	1.8	9.7	53	3	16.1	2132
13	BSPS 48A-21B	73.5	3	51	106	2.9	2.3	1.0	13.6	40	3	16.3	1615
14	BSPS 48A-27	79.4	4	50	106	2.5	2.4	1.0	15.7	46	3	17.4	1824
15	BSPS 48A-5	74.6	5	50	102	2.6	2.5	1.3	10.8	50	3	16.7	1651
16	BSPS 48B	75.7	4	53	103	3.3	2.8	1.0	11.8	55	3	14.0	1336
17	BSPS 48C	76.4	1	48	105	2.4	1.9	1.3	18.6	36	3	18.2	1458
18	BSPS 50B	79.1	5	48	102	3.5	2.0	1.0	10.1	67	2	17.0	1374
19	BSPS 62C-1	51.3	3	47	99	1.9	1.6	1.6	14.4	26	3	16.6	1865
20	BSPS 7.6B	76.6	2	49	103	3.5	1.8	1.5	13.6	45	3	15.1	1040
21	BSPS 70	61.5	3	47	101	3.0	1.3	2.5	14.0	44	2	15.1	1488
22	BSPS 75B	75.8	3	45	100	3.0	1.9	1.0	9.8	59	3	15.6	1411
23	BSPS 9.2B	87.8	3	52	106	3.8	2.8	1.3	15.4	55	2	17.4	857
24	D X T 8.11	67.1	2	51	92	3.0	1.3	1.8	13.5	36	3	16.7	1695
25	DXT SPS 16.6-2	67.1	4	47	94	2.8	2.1	1.1	10.9	38	3	18.7	1417
26	GC X KAB	67.1	4	48	108	4.0	3.0	2.3	10.9	58	3	17.8	1476
27	GC X WANDER	68.7	3	47	98	3.0	1.8	1.5	12.7	46	3	18.4	1066
28	KAB X UG5	69.8	5	47	107	3.5	2.8	1.8	10.9	62	3	16.2	1924
29	Maksoy 1N	32.6	6	46	90	3.3	1.0	1.4	8.0	32	3	13.0	1464
30	Maksoy 3N	75.1	4	46	102	2.0	1.9	1.8	9.0	53	3	18.4	1630
31	MNG 11.2	65.6	3	46	100	4.0	1.0	1.8	12.9	37	3	18.9	1190
32	NG 14.1-24	60.6	2	46	97	3.5	2.1	1.3	11.4	41	3	17.6	1469
33	NG 14.1-6B	67.7	3	46	97	3.0	1.9	1.3	8.7	49	3	17.3	1724
34	NGDT 2.15-10	80.5	4	47	100	3.4	2.0	1.1	11.1	56	3	16.1	1615
35	NGDT 2.15-4	65.6	2	48	102	2.9	2.0	1.0	14.5	43	3	15.8	1406
36	NGDT 8.11	71.5	4	47	96	2.5	1.6	1.4	10.8	37	3	18.4	1172
37	NGDT BLP12.4	67.2	5	49	99	3.1	1.9	1.0	9.0	63	3	15.5	1620
38	NII X GC 20.2	74.0	4	47	101	3.5	1.8	1.5	13.2	47	3	18.4	1443
39	NII x GC 4.1-2	76.3	4	48	102	2.0	2.4	1.3	11.6	48	3	17.6	1672
Mean		70.9	3.3	48.2	101.2	2.9	2.0	1.4	12.4	46.3	2.7	16.8	1514.8
LSD		12.5	0.9	2.1	3.5	2.3	2.0	1.5	2.6	15.5	0.2	2.3	596.3
%CV		12.6	18.7	3.2	2.5	17.1	18.1	15.8	15.1	23.9	6.4	9.9	28.1
F probability		<.001	<.001	<.001	<.001	0.059 ^a	<.001 ^a	<.001 ^a	<.001	<.001	<.001	<.001	0.008

^a ANOVA conducted on angular transformed values; PH, Plant height; NB, Number of branches; DF, Days to 50% flowering; DPM, Days to physiological maturity; ND, Nodulation; L, Lodging; S, Shattering; PC, Pod clearance; PP, Pods per plant; SP, Seeds per pod; 100SW, 100 seed weight; Yld, Yield in Kgha⁻¹

Appendix 7. Means of agronomic traits of 39 advanced soybean breeding lines evaluated at MUARIK during seasons 2011B and 2012A

Plot No	Accession	PH	NB	DF	DPM	ND (1-5)	L(1-5)	S(1-5)	PC	PP	SP	100SW	Y kg/ha
1	BSPS 16A	109.8	2	56	113	4.0	4.0	1.0	17.2	40	3	16.8	1057
2	BSPS 18.3	91.3	3	54	108	3.0	2.0	1.5	14.1	36	3	19.2	1647
3	BSPS 182A	95.9	3	51	108	3.5	2.3	1.0	18.5	38	3	19.1	2101
4	BSPS 24.2A-3	81.6	4	50	106	3.4	2.1	1.0	12.8	46	3	19.4	1987
5	BSPS 27.2B	77.7	5	49	102	3.3	1.8	1.0	14.8	48	3	18.6	1544
6	BSPS 27A	93.8	2	55	112	3.1	2.0	1.0	25.4	30	3	18.8	1520
7	BSPS 42	72.6	3	43	100	2.6	1.8	1.0	15.4	28	3	17.9	1313
8	BSPS 43	75.9	4	54	107	3.5	2.0	1.0	15.6	43	3	17.7	1625
9	BSPS 47B	103.9	4	52	105	3.5	2.5	1.3	12.7	58	3	18.4	1330
10	BSPS 48A-1	89.0	5	55	107	4.1	2.3	1.1	13.6	40	3	18.7	1840
11	BSPS 48A-14	74.5	4	46	103	3.5	1.6	1.1	10.5	43	3	21.5	2244
12	BSPS 48A-19	80.9	5	47	103	3.5	2.6	1.5	10.4	43	3	20.6	1512
13	BSPS 48A-21B	85.1	4	55	106	3.6	1.8	1.3	12.2	36	3	17.3	1378
14	BSPS 48A-27	79.7	4	53	105	3.6	2.5	1.0	13.0	39	3	20.3	1710
15	BSPS 48A-5	74.1	4	52	101	3.3	1.8	1.1	11.8	35	3	18.6	1475
16	BSPS 48B	104.8	4	53	106	4.3	4.0	1.0	12.8	41	3	15.6	1132
17	BSPS 48C	88.0	2	48	108	4.3	2.8	1.3	17.7	31	3	19.2	1901
18	BSPS 50B	88.9	4	50	107	4.5	2.0	1.0	12.3	52	3	18.4	1749
19	BSPS 62C-1	62.9	4	49	105	3.3	2.4	1.0	13.8	37	3	17.9	1379
20	BSPS 7.6B	76.8	1	45	103	2.8	2.0	1.5	20.1	30	2	15.8	945
21	BSPS 70	80.8	4	51	105	3.0	2.3	1.8	14.8	34	3	15.5	1130
22	BSPS 75B	82.2	4	46	103	4.1	2.6	1.1	12.0	48	3	16.8	1461
23	BSPS 9.2B	94.8	2	49	118	3.5	3.5	1.3	25.4	31	3	16.2	976
24	D X T 8.11	62.7	3	51	95	1.5	1.5	1.5	17.9	19	2	16.4	1196
25	DXT SPS 16.6-2	73.2	4	50	95	3.3	2.0	1.0	12.8	30	3	18.8	1194
26	GC X KAB	74.2	4	48	107	4.3	1.3	1.0	11.9	51	3	21.0	1557
27	GC X WANDER	65.2	4	45	98	3.0	1.0	2.0	10.7	40	3	19.2	1270
28	KAB X UG5	66.9	5	51	104	3.5	1.3	1.3	11.6	50	3	19.3	1663
29	Maksoy 1N	39.9	6	45	89	3.4	1.0	1.3	9.3	36	3	12.6	1483
30	Maksoy 3N	74.8	5	47	103	2.8	2.0	1.6	10.0	43	3	20.1	1689
31	MNG 11.2	67.5	4	47	98	3.0	1.8	1.5	13.5	22	3	19.5	1396
32	NG 14.1-24	74.6	4	48	101	3.4	2.8	1.1	11.6	34	3	20.3	1309
33	NG 14.1-6B	73.2	4	48	102	2.5	2.0	1.8	11.2	47	3	19.9	1205
34	NGDT 2.15-10	85.3	4	47	100	3.6	2.4	1.1	13.7	28	3	16.5	1549
35	NGDT 2.15-4	88.1	4	47	104	3.3	2.4	1.3	13.2	41	3	15.1	1693
36	NGDT 8.11	65.7	4	49	96	2.9	2.1	1.1	12.4	38	3	19.0	1380
37	NGDT BLP12.4	67.2	5	50	99	3.5	2.3	1.0	10.9	37	3	16.7	1268
38	NII X GC 20.2	81.8	3	48	97	3.8	1.3	1.0	15.3	38	3	18.3	1717
39	NII x GC 4.1-2	84.1	4	49	103	3.4	2.8	2.0	12.5	37	3	18.1	1546
Mean		79.7	3.8	49.5	103.3	3.4	2.2	1.2	14.0	38.4	2.8	18.2	1488.9
LSD		14.1	0.9	3.4	2.5	1.7	1.7	1.3	2.7	14.5	0.3	2.1	641.7
%CV		12.6	17.7	4.8	1.7	11.7	15	14.5	13.7	27	7.3	8.4	30.8
F probability		<.001	<.001	<.001	<.001	<.001 ^a	<.001 ^a	0.001 ^a	<.001	<.001	<.001	<.001	0.021

^a ANOVA conducted on angular transformed values; PH, Plant height; NB, Number of branches; DF, Days to 50% flowering; DPM, Days to physiological maturity; ND, Nodulation; L, Lodging; S, Shattering; PC, Pod clearance; PP, Pods per plant; SP, Seeds per pod; 100SW, 100 seed weight; Yld, Yield in Kgha⁻¹