SOYBEAN RUST DIVERSITY AND ADAPTATION OF ELITE SOYBEAN LINES TO THE UGANDAN ENVIRONMENT

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DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other University

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DEDICATION

I dedicate this work to my parents; Mr. Opio John Richard and Mrs. Evasta Opio, my brother, sisters, fiancée and my dear friends.

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Table of Contents

List of Tables

List of Figures

List of Plates

Abstract

Soybean rust (*Phakopsora pachyrhizi* Sydow) is a major threat to soybean production worldwide. The objectives of this study were to; a) assess *P. pachyrhizi* diversity in the major soybean growing areas of Uganda usin SSR markers; b) assess elite soybean lines for resistance against geographically diverse rust bulk isolates under screen house conditions; c) determine the adaptation and stability of selected elite soybean lines in five diverse locations of Uganda. Rust isolates were collected from five locations that are the major soybean growing areas. Total DNA of the rust isolates was extracted. Twenty four elite soybean lines were inoculated with rust isolates from four locations (Iki-Iki, Mubuku, NaCRRI and Nakabango) using detached leaf technique. To determine the adaptation and stability of the 24 elite soybean lines, a study was conducted for four consecutive seasons.

A dendrogram constructed from the similarity data using NTSYSpc Version 2.2 showed that the isolates from the five locations were grouped into two clusters. Three distinct reaction types (tan, reddish brown and mixed) were detected. Genotype Maksoy 3N showed reddish brown reaction type for isolates from three sites (Iki-Iki, Mubuku and Nakabango). Maksoy 3N had the lowest mean rust score of 3.1; followed by DXT 5.16 that had a mean score of 3.5. Mubuku and Iki-Iki had the highest mean rust scores of 4.7 and 3.7 respectively while NaCRRI had the lowest mean score of 2.8. Season 2011A had the lowest mean score of 2.8; followed by season 2010A (3.3), 2010B (4.1) and season 2011B had the highest (5.0). Combined yield of the 24 soybean genotypes for three seasons across the five locations indicated that Maksoy 3N had the highest mean yield of 1703 kg ha^{-1}; followed by DXT 3.11 (1576 kg ha^{-1}), DXT SPS 7.11-1 (1549 kg ha^{-1}) and DXT SPS 2.15-12 (1432 kg ha^{-1}).

The scatter plot from GGE analysis indicated that all the five locations were in one mega environment with the best genotype being Maksoy 3N. Comparison biplots showed that DXT 5.16 is the most stable genotype while Maksoy 3N is the most ideal genotype and Nakabango, the most ideal environment.

This study showed that the SSR markers developed by Anderson *et al.* (2008) can be used to study rust diversity in Uganda. The 24 elite soybean lines showed different reaction types when inoculated with bulk rust isolates from different locations in Uganda. Field observations showed that soybean rust was the major soybean disease in all soybean growing areas in Uganda. Maksoy 3N that was included as the highest yielding recommended variety in Uganda had the highest yields and was the most ideal genotype. All the test locations were grouped into one mega environment by GGE biplot. In future rust diversity studies, the number of fields sampled should be increased and the rust isolates should be collected from specific plants per location within the fields. Maksoy 3N should be used to improve other soybean varieties for soybean rust resistance and yield through hybridization.

CHAPTER ONE

INTRODUCTION

1.1 Origin and distribution of soybean

Soybean (*Glycine max* (L.) Merrill) belongs to the family *Fabacae* and subfamily *Papilionoidae* and is an important crop believed to have originated from China. The first domestication of soybean occurred in the eleventh century in China (Lance and Garren, 2005), from where it spread to other parts of the world (Probst and Jude, 1973). From Asia, the crop was introduced into Europe, America and later to Africa. Soybean is believed to have been first cultivated in Uganda in about 1913 (William and Akiko, 2009). Cultivation of the crop in Uganda started in the central parts of the country and spread to other regions later.

1.2 Importance of soybean

Soybean grains contain about 40% protein, 20% oil, an optimal supply of essential amino acids and nutrients, and a high calorie value (Singh *et al*., 2008). It is therefore an important food and feed resource (Tukamuhabwa, 2001). A large variety of fresh, fermented and dried food products can be prepared from soybean (Lance and Garren, 2005). The crop has other agronomic values in form of improving soil fertility through nitrogen fixation and enhanced moisture retention (Graham and Vance, 2003). It disrupts the life cycle of several pests and diseases of cereals and cassava when grown as intercrops (Pandey, 1987). Soybean based activities support livelihoods of many rural communities through provision of employment and hence incomes.

1.3 Soybean production

The total world soybean production is estimated at 260.9 million metric tons (MT). The world's leading producer is USA which produces about 32% of the world soybean (83.2 million MT). Brazil is second with 29% of soybean produced (74.8 million MT) while Argentina is third with 19% (48.9 million MT). China produces 6% (14.5 million MT), India, 5% (12.2 million MT), Paraguay 3% (8.3 million MT). The remaining countries account for 6% (15.7 million MT) of the global soybean output (FAO, 2011).

In Africa, total soybean production rose from 0.574 million MT in 1994 to1.59 million MT in 2009; representing 0.7% of the world production. The three leading African countries in soybean production are Nigeria (610, 000 MT), South Africa (516,000 MT) and Uganda (180,000 MT) (FAO, 2011). Total soybean output in Uganda in 2001 was 144,000 MT which rose to 180,000 MT in 2011 (FAO, 2011). A report by Uganda Cooperative Alliance in 2009, recorded that northern region produced 15,729 MT, eastern region (5,809 MT), western region (1,886 MT) and Central region (192 MT). This report also showed the leading districts in soybean production in Uganda were Oyam (8,030 MT), Apac (3,225 MT), Tororo (2,180 MT) and Lira (2,045 MT) (Anon, 2009).

1.4 Soybean production constraints in Uganda

Although soybean production has increased in Uganda, the yield has remained low; about1200 kg ha^{-1}. This is very low compared to yields in other major producers in Africa estimated at about 2000 kg ha^{-1} (FAO, 2011). The low yields are attributed to several factors including poor soil fertility, inappropriate management practices, low use of improved varieties and attack by pests and diseases (AVRDC, 1987). In the tropics, there are also production constraints such as

poor adaptation and short seed longevity period (Hartman *et al.*, 1991). Currently, soybean rust disease caused by the fungus *Phakopsora pachyrhizi* is the single most soybean yield reducing constraint in Uganda (Kawuki, 2002).

1.5 Origin and distribution of soybean rust

Soybean rust (*Phakopsora pachyrhizi* Syd. & P. Syd) was first reported in Japan in 1902 (Hennings, 1903) and much later in Hawaii in 1994 (Killgore *et al*., 1994). It was reported for the first time in Uganda in 1996 at Namulonge Agricultural and Animal Production Research Institute (NAARI), in central Uganda that later spread to all the major soybean growing areas (Tukamuhabwa and Dashiell, 1999; Kawuki, 2002). In the same year, it was reported in Kenya and Rwanda (Tukamuhabwa and Maphosa, 2011). From 1997, the fungus spread very fast and was reported for the first time in several African countries (Levy, 2005). It was reported in Zambia and Zimbabwe in 1998 (Hartman, 2012), Nigeria in 1999; Mozambique in 2000; South Africa in 2001 (Tukamuhabwa and Maphosa, 2011). Around this time, it was reported in Southern America. It was reported in Paraguay in 2001 (Morel and Yorinori, 2002) and Argentina and Brazil in 2002 (Rossi, 2003; Yorinori *et al*., 2005). By this time, it had become endemic in almost all regions of Uganda (Kawuki *et al*., 2003). It was reported for the first time in United States of America and Colombia in 2004 (Schneider *et al*., 2005; Hartman, 2012), Ghana and Democratic Republic of Congo in 2007 (Bandyopadhyay *et al*., 2007; Ojiambo *et al*., 2007).

1.6 Statement of the problem

A number of management options have been recommended to mitigate the effects of soybean rust disease. Chemical control, cultural practices and deployment of resistant varieties have been

widely used in areas where the disease is prevalent. Fungicide applications in early vegetative stages, although effective in reducing disease severity, have not been effective in improving yield. The reason being that timing of fungicide application is critical; delaying application after the disease establishment results in inconsistent yields (Miles *et al*., 2003). The high costs and possible environmental hazard make fungicides unsuitable for managing the disease in developing countries such as Uganda. Cultural practices like destruction of alternate hosts, timely irrigation, early planting and growing early maturing cultivars can also reduce the incidence of the disease (Akinsanmi *et al.,* 2001; Caldwell *et al*., 2002). However, the rapid spread by wind-borne urediniospores and the large number of host species increases chances of soybean rust survival making cultural practices relatively ineffective (Hartman *et al*., 2005).

Growing of resistant cultivars still remains the most viable strategy to manage soybean rust in Africa and other countries in the developing world (Hartman *et al.,* 2005; Twizeyimana *et al*., 2008). However, breeding for resistance to soybean rust disease is still a great challenge due to lack of resistant parental lines in most breeding programs. Specific rust resistance genes (*Rpp1, Rpp2, Rpp3, Rpp4, Rpp5* and *Rpp?*) have been identified in soybeans (Hartwig, 1986; Garcia *et al*., 2008; Hartman, 2012). Use of these genes for development of rust-resistant soybean varieties has been complicated by the high variability within *P. pachyrhizi*. After deployment, single resistance genes succumb to certain isolates of the pathogen (Hartman *et al.*, 2005). Moreover, the existence of multiple virulence factors in the rust pathogen implies that resistance based on single genes will not be durable given the polycyclic nature of the pathogen (Hartman *et al*., 2005).

Soybean varieties Maksoy 1N and Namsoy 4M were released in 2004 and are widely grown in Uganda. These were initially resistant to soybean rust but are now showing signs of succumbing to the disease. Inheritance studies for soybean rust resistance in these two varieties showed that they possess specific resistance genes (Kiryowa *et al.*, 2005). Deployment of durable resistance genes that will remain effective for a long time requires that the diversity of the pathogen is known. Using RAPD markers, three pathogen clusters of soybean rust were reported by Lamo (2004) on Ugandan isolates of *P. pachyrhizi.* Occurance of these clusters may signify functional diversity within the pathogen. Therefore, before any resistance genes are deployed against rust in Uganda, they should be thoroughly screened to take care of all possible diversity within the pathogen population. Unfortunately, no comprehensive study has been done to conclusively determine the current pathogen diversity in Uganda. In this study, nine SSR markers mapped by Anderson *et al*. (2008) were used to assess the pathogen diversity in the major soybean growing areas of Uganda. The study also sought to identify elite soybean lines with stable broad resistance to soybean rust, soybean pests and diseases and high stable yields.

1.7 Justification of the study

SSR molecular markers are now available to standardise rust diversity studies (Anderson *et al*., 2008). However, the SSR markers have not been used for studying diversity in *P. pachyrhizi* in Uganda. Therefore there is need to clearly and accurately understand the pathogen diversity in Uganda using these markers. Knowledge of pathogen diversity allows development of varieties with a combination of resistance genes that match the prevailing *P. pachyrhizi* pathotypes in the different geographical zones (Twizeyimana *et al.*, 2009). One major desirable attribute being sought by the Ugandan soybean breeding programme is wide adaptation of the genotypes. There

is need to identify genotypes that have a wide adaptation to the diverse environmental conditions in the major soybean growing areas in Uganda in addition to resistance to soybean rust. However, to ensure durable resistance of the genotypes to soybean rust, it is important to know the response of genotypes to the geographically diverse rust races in the major soybean growing areas. It is therefore important to evaluate high potential genotypes in the major soybean growing areas of Uganda.

1.8 Overall objective

To develop high yielding rust-resistant soybean genotypes that are stable in the major soybean producing areas of Uganda

1.9 Specific objectives

- To determine *P. pachyrhizi* diversity in the major soybean growing areas of Uganda using SSR markers.
- To identify elite soybean lines with resistance against geographically diverse bulk rust isolates under screen house conditions.
- To determine the adaptation and stability of selected elite soybean lines in five diverse environments of Uganda.

1.10 Hypotheses

- Simple sequence repeat markers are polymorphic and therefore usable in determining *P pachyrhizi* diversity in Uganda
- Resistance of elite lines to bulk rust isolates is dependent on the level of rust diversity and origin

- Performance of elite soybean lines is stable across soybean growing areas in Uganda.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diseases of soybean

Diseases rank first among the production constraints of soybean worldwide (Yorinori, 1988). The majority of diseases are caused by fungal pathogens namely; soybean rust (*Phakopsora pachyrhizi*: Sydow and Sydow), brown leaf spot (*Septoria glycines*), bacterial pustule (*Xanthomonas campestris* pv. *glycines*), red leaf blotch (*Phoma glycinicola*), anthracnose (*Colletotrichum dematium* f. *truncatum*), damping-off (*Rhizoctonia solani*) and charcoal rot (*Macrophomina phaseolina*). Soybean rust is one of the most economically important soybean diseases and causes the most damage of all the pathogens known to attack soybean (Bonde *et al*., 2006).

2.2 Symptoms, crop damage and host range of *Phakopsora pachyrhizi*

Soybean rust symptoms initially appear as small water soaked lesions on the abaxial surface of the leaves with a size ranging from 2 to 5 mm^2. The lesions assume a polygonal shape and are usually confined to the veins or close to the veins. Within each lesion, there are one or several uredinia (Miles *et al.*, 2005). During the early stages of manifestation, the lesions may be confused with those of bacterial pustule (*Xanthomonas campestris* pv. *glycines*) until uredinia complete development and discharge of spores. The lesions then gradually increase in size and turn from gray to tan or reddish-brown (Miles *et al.*, 2005). Depending on the *Phakopsora pachyrhizi* race and soybean genotype, lesions develop into one of the three infection types; Hypersensitive Reaction (HR), Reddish-Brown (RB) and Tan reaction (TAN). The HR infection type is characterized by no visible symptoms while the RB type is characterized by reddish-

brown lesions and possesses about 0-2 uredinia per lesion. The TAN infection type is characterized by tan-coloured lesions and each lesion has about 2-5 uredinia (Bromfield, 1984). These different infection types can appear concurrently on a leaf on the same plant. The disease attacks any above ground part of the plant but most of the symptoms are observed on the leaves.

Yield losses due to soybean rust ranging from 10% to 100% have been reported for individual fields and experimental plots in different countries (Levy *et al*., 2002; Morel and Yorinori, 2002). In Uganda, Tukamuhabwa and Dashiell (1999) observed a yield loss of 15 – 41% while a yield loss of 60 – 90% was reported by the Uganda Seed Project (Anon, 2000). Yield losses are a result of fewer and smaller seeds, early maturity and increased pod abortion (Miles *et al.*, 2003). The rapid spread of *Phakopsora pachyrhizi* and the potential for severe yield loss makes this the most destructive foliar disease of soybean (Miles *et al.*, 2005). The damage caused to the soybean plants include rapid deterioration of the leaf tissue, resulting in drying and premature fall of leaves; hence poor grain formation. This in turn leads to small grains and consequently loss in yield and quality (Sinclair, 1982; Yang *et al.*, 1991).

The host range of *Phakopsora pachyrhizi* is quite broad. It has been reported on more than 95 species of plants from more than 42 genera including soybean and related *Glycine* species (Ono *et al*., 1992; Slaminko *et al*., 2008). This also includes wild and cultivated legumes (Hartman *et al.*, 2005). Such a broad host range though unusual is attributed to *P. pachyrhizi* ability to directly penetrate leaf tissue. Furthermore, this makes management of soybean rust a challenge as there are many bridge species where the pathogen can survive between growing seasons.

2.3 Infection and spread of soybean rust

Soybean rust disease becomes severe depending on a number of factors. Two important factors are temperature and leaf wetness duration that together determine the suitability of infection periods. Another moisture related factor affecting epidemic development is the timing of the first rains and amount of rainfall. The most intensive rust epidemic has been observed where the mean daily temperature is moderate; usually less than 28^{o}C; with precipitation and long periods of leaf wetness (Tschanz and Wang, 1980). Soybean rust development is inhibited by dry conditions and mean daily temperatures greater than 30^{o}C and less than 15^{o}C. Casey (1979) observed that development of a severe rust epidemic requires about 10 hours/day of leaf wetness and mean daily temperature of 18^{o}C to 26^{o}C. Moisture on the plant surface is a prerequisite for urediniospores germination, infection and development. The optimum urediniospores germination temperature has been variously reported as 21-27^{o}C (Kitani and Inoue, 1960), 12-21 ^{o}C (Casey, 1979) and 19-23^{o}C (Bromfield *et al.*, 1980).

The infection process starts with urediniospores germination to produce a single germ tube that grows across the leaf surface, until an appressorium is formed. Penetration of epidermal cells is direct through the cuticle by an appressorial peg. Most rust pathogens enter the leaf through stomatal openings and penetrate cells once inside the leaf. However, soybean rust is unique in its ability to directly penetrate the epidermis (Miles *et al.*, 2005). Direct penetration of epidermal cells and the non-specific induction of appressoria in the infection process of *P. pachyrhizi* may explain the broad host range of the pathogen which has consequences for development of resistant varieties (Koch and Hoppe, 1988). Uredinia develop 5 to 8 days after infection by urediniospores. The first urediniospores can be produced as early as 9 days after infection and

continues for up to 3 weeks (Marchetti *et al.*, 1975; Koch *et al.,* 1983). Secondary uredinia form on the margins of the initial infections for an additional 8 weeks (Miles *et al.*, 2005). Thus, from an initial infection, sporulation can be maintained up to 15 weeks. Even under dry conditions this extended sporulation capacity allows the pathogen to persist and remain a threat.

2.4 Soybean rust diversity

The soybean rust consists of two species; the eastern hemisphere group (*Phakopsora pachyrhizi*) and the western hemisphere one (*Phakopsora meibomiae*). The former species is very aggressive, variable and virulent than the western hemisphere one. To determine pathogen diversity, a set of different host plants is challenged with single spore urediniospores. These host plants are referred to as race differentials. The aggressiveness is then quantified based on their characteristic infection patterns (Lin, 1966; Bromfield *et al.*, 1980); that is to say HR, RB and TAN.

In Japan, 18 pathogenic races were identified using a set of differential varieties composed of nine cultivars of soybean and two accession lines of *G. soja* (Yamaoka *et al*., 2002). Soybean rust diversity can also be studied using molecular markers. Limited research however, has been undertaken to understand the diversity of soybean rust at molecular level yet it is a fundamental aspect in understanding the fungus biology. In Uganda, the only diversity study done by Lamo (2004); showed limited diversity of three clusters using RAPD markers and 19 race differentials. Seven pathotype clusters were identified in Nigeria when 116 rust isolates collected from different fields in three agroecological zones were inoculated on eight soybean genotypes using the detached leaf technique (Twizeyimana *et al*., 2009). Freire *et al*. (2008) undertook a diversity study using internal transcribed spacer regions (ITS 1 and ITS2) of the nuclear ribosomal DNA

of rust isolates to understand the phylogeography of Brazilian and South African isolates. The study revealed that independent sequence alignments of the ITS1 and ITS2 identified 27 and 19 ribotypes, respectively. Anderson *et al*. (2008) developed highly polymorphic SSR markers that are specific to *P. pachyrhizi* that has made it possible to easily determine the pathogen diversity. Twizeyimana and Hartman, (2012) carried out a pathogenic variation of *Phakopsora pachyrhizi* isolates in soybean in the United States and identified three pathotypes among 72 U.S. isolates based on the virulence of the isolates on race differentials.

In Uganda, soybean rust diversity has dramatically changed since 2004 as evidenced by tendency of resistance breakdown of commercial varieties (Maksoy 1N and Namsoy 4M). The apparent lack of universal race differentials has meant genetic diversity estimates cannot be standardized. Yet this is important for global efforts in the management of soybean rust. Therefore use of SSR markers that are highly polymorphic will allow for greater understanding of the pathogen diversity in Uganda and allow for informed resistance breeding.

2.5 Soybean rust control

2.5.1 Chemical control

Several studies on the efficacy of fungicides for control of soybean rust have been conducted. Early research from Asia indicated that mancozeb was effective (Hartman *et al*., 1992). Additionally, fungicide trials in India (Patil and Anahosur, 1998) and Southern Africa (Levy *et al*., 2002) identified several triazole compounds and triazole mixes that could manage the disease. Other compounds that reduce disease severity have been identified although their efficacy has been inconsistent. For example studies in Zimbabwe and South Africa showed that fungicide application before flowering did not increase the yield of soybean (Miles *et al*., 2003).

In Uganda, Kawuki (2002) evaluated three fungicides (Dithane M-45, Saprol and Folicur) under three different spray regimes for management of soybean rust. The results demonstrated that Dithane M-45 offered the best protection with 5 sprays at a weekly interval from disease on-set while Saprol and Folicur showed best protection with 2 and 3 sprays from disease on-set to full seed formation. Although fungicide application is the strategy commonly used in soybean rust management, it is costly (Miles *et al.*, 2003; Pedro *et al.*, 2008) and associated with environmental hazards (Bromfield, 1984). Additionally, fungicide use is not a viable option in subsistence soybean production systems in most developing countries (Miles *et al.*, 2003).

2.5.2 Cultural control

Some cultural practices can be used in the control of soybean rust. For example, destruction of host weeds and increased phosphorus levels in the fields have been reported to reduce the incidence of soybean rust. Where the crop is grown under irrigation, water should be supplied in the middle of the day so that the leaves dry before dew sets in (Caldwell *et al*., 2002). Early planting and growing early maturing soybean cultivars may also limit disease progress and development. For example in Nigeria, disease severity was higher on the medium-maturing varieties and those planted late in the season (Akinsanmi *et al*., 2001).

2.5.3 Host resistance

Host plant resistance would provide a cheaper, environmentally friendly and sustainable approach for managing soybean rust under resource poor agricultural systems that characterize the agricultural landscape of Uganda. Specific resistance to *P. pachyrhizi* is known and a number of single dominant genes have been identified as *Rpp1* (McLean and Byth, 1980), *Rpp2*

(Bromfield *et al.*, 1980), *Rpp3* (Bromfield and Hartwig, 1980; Bromfield *et al.*, 1980), *Rpp4* (Hartwig, 1986), *Rpp5* (Garcia *et al*., 2008) and *Rpp?* (Hyuuga) (Monteros *et al*., 2007; Monteros *et al*., 2010). Numerous soybean genotypes have also been reported to show resistance reaction. For example 3 TGx breeding lines in Nigeria (Twizeyimana *et al*., 2008; Hartman, 2012); UG5 and Maksoy 3N in Uganda (Maphosa *et al*., 2012). However the resistance genes in these genotypes have not been identified. Non-soybean sources of resistance have also been reported; for example in *Glycine* species, *Pueraria* species and other legume species (Hartman, 2012). These genes however condition resistance to a limited set of rust isolates. The availability of resistant varieties is the most desirable solution, because its adoption by farmers is simple, cheap and better for the environment (Pedro *et al.*, 2008).

2.6 Significance of the environment on soybean performance

The ability of developed varieties to adapt to a wide range of target environments is the ultimate goal of plant breeders. The yielding ability of a variety is the result of its interaction with the prevailing environment. Environmental factors such as soil characteristics and types, moisture, fertility, temperature and day length vary over the years and locations. Varieties react differently in different environments due to genotype × environment interaction (GEI). It is important to study the response of genotypes to different environments and eventually select varieties suitable for specific environments. Ideally, varieties that are adapted to diverse environments would be most suitable. Understanding and predicting crop response to environment is very important in a plant breeding programme.

The additive main effects and multiplicative interaction (AMMI) model offers an appropriate statistical analysis of yield trials that may have a GEI (Zobel *et al.*, 1988). Gauch (1992) compared different methods of analyzing multi-locational yield trial data. These included analysis of variance (ANOVA), linear regression, shifted multiplicative model (SHMM), AMMI and principal component analysis (PCA); he then concluded that AMMI model is the best to distinguish clearly between the main and interaction effects. However, genotype main effect plus genotype by environment interaction (GGE) biplot developed later is more superior to AMMI in mega-environment analysis and environment evaluation (Yan and Ma, 2006). The GGE biplot has many visual interpretations than AMMI; particularly allows visualization of any crossover GEI. This part of GEI is usually essential to a breeder. GGE biplot is more logical and biological than AMMI in terms of explanation of PC1 score, which represents genotypic effect rather than additive main effect (Yan *et al*., 2000).

CHAPTER THREE

ASSESSMENT OF *PHAKOPSORA PACHYRHIZI* DIVERSITY IN THE MAJOR SOYBEAN GROWING AREAS OF UGANDA USING SSR MARKERS

3.1 Introduction

Specific resistance to *P. pachyrhizi* is known and some single dominant genes have been identified as *Rpp1*, *Rpp2*, *Rpp3*, *Rpp4, Rpp5* and *Rpp?* (Hartman *et al*., 2005). These genes condition resistance to a limited set of rust isolates (Twizeyimana *et al*., 2008). Therefore it is important to determine the pathogen genetic diversity in the major soybean growing areas in Uganda to deploy effective resistance. This can best be achieved using molecular markers because they are more informative and reproducible. However, utilization of SSR markers to study genetic diversity of rust fungi is still in its infancy. Standard molecular markers such as RAPD, AFLP, RFLP and SSR have been successfully used to estimate genetic diversity of important rust pathogens such as *Puccinia recondita* (Kolmer *et al.,* 1995), *P. striiformis* (Steele *et al.,* 2001; Justesen *et al.,* 2002), *Melampsora epitea* (Pei *et al*., 1997), *Cronartium ribicola* (Kinloch *et al.,* 1998), *C. flaccidum* (Moricca and Ragazzi, 1998) and *Peridermium pini* (Hantula *et al.,* 1998; Moricca and Ragazzi, 1998).

The purpose of the current study was to use the SSR markers in assessing *P. pachyrhizi* diversity in the major soybean growing areas of Uganda. Knowledge about genetic diversity and structure of pathogen population is crucial to better understand variations observed among isolates of *P. pachyrhizi* and can be used to investigate pathogen evolution, design disease management strategies and development of new varieties. Despite the importance of soybean rust disease, no

comprehensive molecular study has been done to determine rust diversity in the major soybean growing areas of Uganda using SSR markers.

3.2 Materials and Methods

3.2.1 Collection of soybean rust isolates

Soybean rust isolates were collected from five locations in Uganda (NaCRRI, MUARIK, Nakabango, Iki-Iki and Mubuku); that are the major soybean growing areas of Uganda. Details of the locations are presented in Table 1.

Table 1: Description of experimental sites

Location	Position	Location in Uganda	Altitude (m.a.s.l)	Mean annual temperature (^{o}C)	Mean Annual rainfall (mm)
NaCRRI	0^{o}32'N/32^{o}37'E	Central	1,160	22.6	1,400
Nakabango	0^{o}29'N/33°14'E	Eastern	1,210	22.8	1,400
Iki-Iki	1^{o}06'N/34^{o}00'E	Eastern	1,156	24.7	1,200
Ngetta	2°17'N/32°56'E	Northern	1,103	24.7	1,200
Mubuku	0^{o}13'N/30^{o}08'E	Western	1,007	27.8	750

Source: Meteorological stations at the study sites; masl = metres above sea level

Urediniospores were collected using handheld Liliput® vaccum pump from one to several leaves bearing uredinia. The rust isolates from each location were bulked in vials as bulk isolate as shown on Plate 1.

a b

Plate 1: a) Soybean rust isolate collection at MUARIK using a Liliput® vacuum pump; (b) vial next to a collected bulk rust isolate

3.2.2 DNA Extraction from the bulk isolate

Total genomic DNA of *P. pachyrhizi* was extracted from the collected bulk isolates from each of the five locations according to Frederick *et al.* (2002). *Phakopsora pachyrhizi* urediniospores were ground in 100 µL of extraction buffer (89 mM Tris- HCl (pH 8.0), 45 mM boric acid, 0.05 mM EDTA, and 1.0% (vol/vol) β-mercaptoethanol) in microcentrifuge tubes using a plastic pestle. The samples were incubated at 75 °C for 15 minutes and later centrifuged for 15 minutes at 13,000 g to pellet debris. The supernatant was transferred to new tubes and stored at -20 °C as DNA extracts (Frederick *et al.*, 2002).

A set of 9 SSR primers developed by Anderson *et al*. (2008) (PP003, PP004, PP010, PP014, PP15, PP016, PP017, PP019 and PP021) were used for characterisation of the bulk soybean rust isolate. The 9 SSR primer pairs were obtained from Molecular and Cell Biology Laboratory, University of Cape Town, South Africa. Table 2 shows the 9 primers, their sequence and expected size range of associated amplification products.

Table 2: Primers, Primer sequences and expected allele size range of the SSR used in the rust diversity study

	Forward	**Reverse**	**Expected allele size range in bp**
PP003	GGCTCAGTCAAAGCATCCTC (20)	ATCAATTCTGGCCTGGTGAG (20)	(186–196)
PP004	ACTGTTCGGTTCGGTTTCAG (20)	CTTGGTTAAATGCCAAGCTTG (21)	(235–253)
PP010	CTGAGTGAAATCACGCTGAGA (21)	GGCAGGTGATTCGTAGAGTCTAC (23)	(205–281)
PP014	CAGCGATCAGGTTCAGAAATC (21)	CCATCAGAGTTGTTGGCTCTC (21)	(279–293)
PP015	CAACCACGTGCACAACTATTC (21)	CCACCTCCTTTGAATCCTCA (20)	(462–471)
PP016	CAGGAAGACTCCAGAACTGTGC (22)	CCAAGGACACTTCTAGTCCTTC (22)	(320–348)
PP017	CGAGCCATTGCCCCAAGTTTG (21)	CAGTTAGATGAGCCTGAGGAC (21)	(208–216)
PP019	CCAAGTGCTGCAAATCAAGC (20)	GCTCTAACTAGAGCCCTTGTG (21)	(194–198)
PP021	CAACGGCAAAAGACCTAGGTAC (22)	GCGCAGCCCTAACTACAATAC (21)	(335–338)

Source: Anderson *et al.* (2008).

The reactions were carried out in PCR thermocyclers; Cycler (Bio-Rad, California, USA) and GeneAmp 9700 (Applied Biosystems, California, USA). The amplification was done with an initial denaturing phase at 94^{o}C for 1 minute, followed by 35 cycles at 94^{o}C for 25 seconds, annealing at 48^{o}C for 45 seconds and extension at 72°C for 50 seconds. A final extension step at 72°C for 5 minutes was followed by termination of the cycle at 4°C. Reactions were performed in 20 µl reaction mix consisting of 1 × reaction buffer (Promega, Madison, USA), 2.0 mM $MgCl_2$, 0.4 mM of dNTPs, 0.5 µM of each primer, 5 units of Taq DNA polymerase (Promega, Madison, USA), PCR water and 3 ng of DNA. The PCR amplicons were fractionated on 4% Metaphor (Lonza Bioscience, Singapore) agarose horizontal gel stained with GelRed^{TM} Nucleic Acid Stain (Biotium, USA) in 1X TAE buffer at 140 V for 90 minutes. Gel images (Figure 1) were taken using a BioDoc-It^{TM} Imaging System (Bio-Rad).

3.3 Data analysis

The banding pattern of each isolate was visually assessed and scored. Only clearly identified and consistent bands were scored. Each band generated by a primer was considered as a locus with two alleles: presence (1) or absence (0) of a band to create a binary matrix. The binary matrix was analyzed with the computer program NTSYSpc version 2.2 (Applied Biostatistics Inc., USA). A UPGMA cluster analysis was then performed.

3.4 Results

Isolate from Nakabango showed distinct banding pattern with primers PP003 and PP010 (Figure 1). Primers PP014 and PP019 showed similar banding pattern for all the five rust isolates at different locations. This banding pattern was also showed by primers PP004 and PP010.

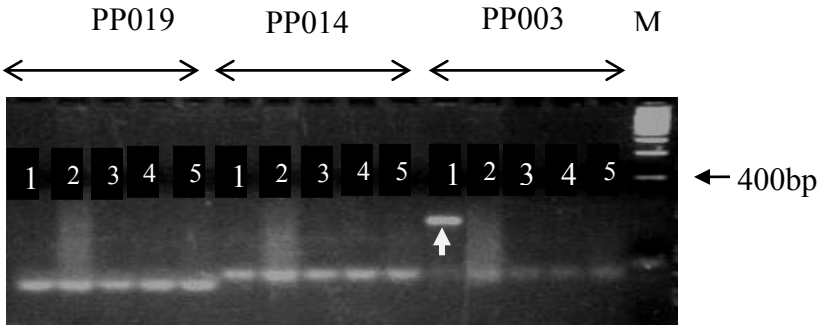

Fig. 1a

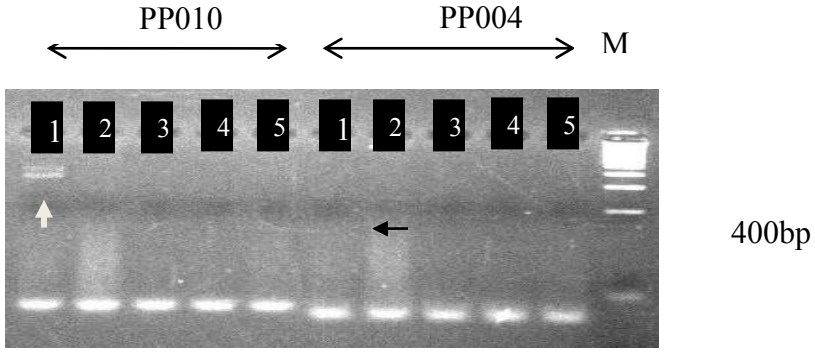

Fig. 1b

Figure 1: SSR polymorphism in isolates of *P. pachyrhizi* generated with primers PP003, PP014 and PP019 (a); and PP004 and PP010 (b). 1-Nakabango; 2-Mubuku; 3-Iki-Iki; 4- NaCRRI and 5-MUARIK. M- 1kb molecular weight ladder

A dendrogram constructed from the similarity data of the isolates from the five locations grouped the isolates into two clusters (Figure 2). The first cluster consisted of isolates from MUARIK, Iki Iki and Mubuku and the other cluster consisted of isolates from NaCRRI and Nakabango. From the dendrogram, rust isolate from Nakabango was genetically unique.

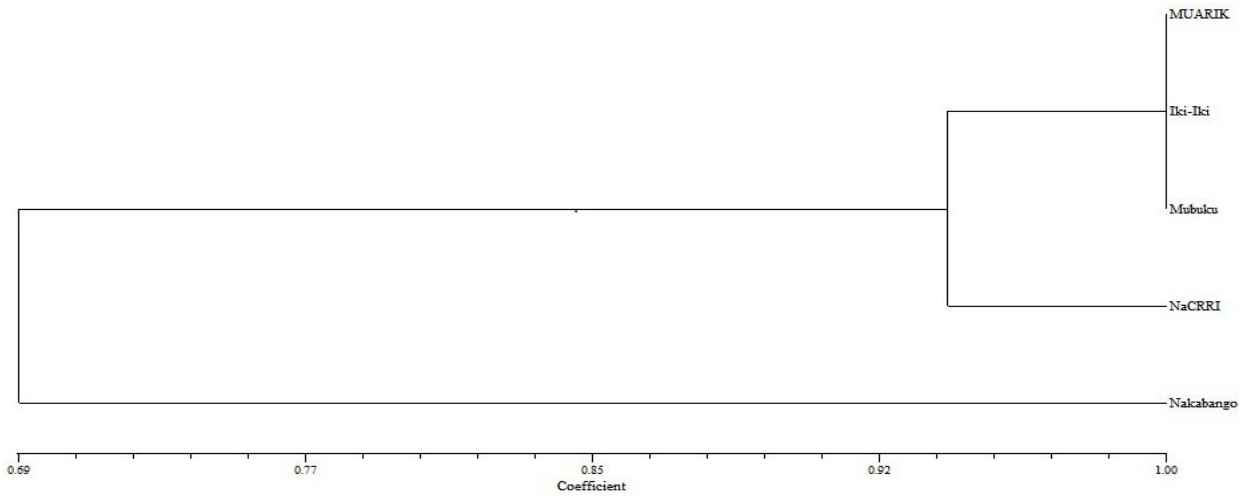

Figure 2: Dendogram of isolates constructed using SSR markers from five locations in Uganda

3.5 Discussion

Genetic diversity of *P. pachyrhizi* pathogen has been reported in several countries including Taiwan, India, Australia, Nigeria and also Uganda (Lamo, 2004; Twizeyimana *et al.,* 2009). This study was undertaken to characterize the degree of genetic differentiation in *P. pachyrhizi* collected from major soybean growing areas in Uganda. It was observed that *P. pachyrhizi* isolates collected from five locations were variable with only two major clusters. This is contrary to Lamo (2004) who identified three clusters of the rust isolates in Uganda using RAPD and race differentials. The reduction in the number of clusters could be due to failure of other rust clusters to survive in the environmental conditions of Uganda. The low rust diversity could partly be due to its mode of reproduction that is asexual through the production of dikaryotic urediniospores. The method of DNA analysis could have also contributed to this low diversity. The dendrogram also showed that Nakabango rust isolates are genetically unique from the other four sites. However the genetic diversity of the rust isolates was not linked to geographical locations of the study locations.

A previous study by Twizeyimana *et al.* (2009) showed more genetic variation of the pathogen within each soybean field and very low genetic diversity among fields in Nigeria. The limited genetic variation among field populations could be attributed to the nature of dispersion of *P. pachyrhizi* that migrate over hundreds or thousands of kilometers. The genetic diversity between and within soybean fields could not be rigorously tested by the present data set, but with addition of more isolates from each field and increasing the number of isolates, it may be possible to test this hypothesis.

A study on genetic structure of *P. pachyrhizi* showed that genetically diverse populations of the pathogen were responsible for the soybean rust epidemics in Nigeria (Twizeyimana *et al.,* 2009). These findings have implications on the management of soybean rust. Deployment of resistant varieties is the most sustainable and effective approaches to control soybean rust. Therefore, a broad selection of isolates can be used to effectively screen soybean germplasm for resistance to soybean rust.

No comprehensive study had been done in Uganda to assess *P. pachyrhizi* diversity using standard SSR markers that were developed by Anderson *et al.* (2008). This study therefore has showed that SSR markers can be used to test for genetic diversity of *P. pachyrhizi* in Uganda.
The results of this study can provide valuable information for future work on genetic diversity and population structure of the soybean rust fungus and can serve as a baseline for monitoring population evolution of *P. pachyrhizi* in Uganda. The Nakabango rust isolates were unique from the rest. The epidemiological implication of this uniqueness is not known. Broadly however, this simply emphasizes the need for testing promising lines in several environments.

CHAPTER FOUR

ASSESSMENT OF SOYBEAN LINES FOR RESISTANCE TO BULK ISOLATES OF *PHAKOPSORA PACHYRHIZI*

4.1 Introduction

Soybean rust disease is an aggressive foliar disease caused by an obligate biotrophic fungus, *Phakopsora pachyrhizi*. It is considered to be the most destructive foliar disease in soybeans (*Glycine max* (L.) Merr.). Breeding for resistance to soybean rust is currently the most economic and strategically means of managing the disease. Resistance to rust is conferred mainly by major genes *Rpp1, Rpp2, Rpp3, Rpp4* and *Rpp5* and have been identified to show resistance to specific races of soybean rust (Bromfield and Hartwig, 1980; Hartwig, 1986; McLean and Byth, 1980; Monteros *et al.,* 2007; Garcia *et al.,* 2008). However, many other sources of resistance like *Rpp6* to *Rpp*? are not yet genetically characterized. In addition, two Ugandan soybean genotypes (Maksoy 3N and UG 5) have showed a resistance reaction to soybean rust. UG 5 is suspected to possess R*pp1* and R*pp3* genes (Hartman, 2012). However use of these major resistance genes has been complicated by the high variability within *P. pachyrhizi* that causes rapid breakdown of resistance. In fact there is no major gene that is resistant to all *P. pachyrhizi* isolates (Hartman, 2012).

4.2 Materials and Methods

Twenty four elite soybean genotypes were used in this study (Table 3). A set of three soybean plants for each of the 24 genotypes were planted in 10 litre buckets in the screen house in MUARIK and arranged in a completely randomized design.

Table 3: Elite soybean genotypes used for resistance study and advanced yield trials

No.	Genotypes	Origin	Remarks
1	DXT 1.23	Uganda	F8 line
2	DXT SPS 1.33-1	Uganda	F8 line
3	DXT 1.62	Uganda	F8 line
4	DXT SPS 2.15-12	Uganda	F8 line
5	DXT 3.9-3	Uganda	F8 line
6	DXT 3.11	Uganda	F8 line
7	DXT 3.12	Uganda	F8 line
8	DXT SPS 3.17-2	Uganda	F8 line
9	DXT SPS 3.17-5	Uganda	F8 line
10	DXT 3.21	Uganda	F8 line
11	DXT 4.8	Uganda	F8 line
12	DXT 5.15	Uganda	F8 line
13	DXT 5.16	Uganda	F8 line
14	DXT SPS 7.11-1	Uganda	F8 line
15	DXT SPS 8.10-6	Uganda	F8 line
16	DXT 9.12	Uganda	F8 line
17	DXT 9.18	Uganda	F8 line
18	DXT 9.24	Uganda	F8 line
19	DXT 10.9	Uganda	F8 line
20	DXT SPS 16.6-2	Uganda	F8 line
21	DXT SPS 16.9-2	Uganda	F8 line
22	Duiker	Zimbabwe	Soybean rust susceptible check I
23	Nam I	Colombia	Soybean rust susceptible check II
24	Maksoy 3N	Uganda	Soybean rust resistant check

Two weeks later, soybean rust isolates were collected from the field for inoculation. Soybean rust isolates were harvested using a handheld Liliput® vacuum from random soybean leaves at the R6 stage from four locations that represent the major soybean growing areas in Uganda (NaCRRI, Nakabango, Iki-Iki and Mubuku). These locations are described in Table 1. Rust isolates were not collected from the fifth site (Ngetta) because rust was not severe. Isolates were selected from about five leaves and bulked. The four bulk rust isolates were then inoculated on the 24 elite soybean lines using the detached leaf technique at the V2 stage within 48 hours of collection from the field (Twizeyimana *et al.*, 2008).

For each isolate, freshly harvested field spores were mixed with distilled deionised water containing the surfactant Tween-20 at 0.5ml/l. Urediniospore suspensions were diluted to a concentration of 50 000 spores per millimeter using a haemocytometer. Leaves at two trifoliate stage were detached from the seedlings and artificially inoculated with 1.5 ml of spore suspension on the abaxial leaf surface using a Canyon® (Model 5A, England) hand sprayer. Each of the inoculated detached leaves was carefully placed in 9-cm-diameter petri dish with the adaxial side placed on the moist filter paper as illustrated in Plate 2. After inoculation, the leaves were covered with black polythene bags for 24 hours at 22^{o}C-24^{o}C to maintain high relative humidity necessary for infection. After 24 hours, the polythene bags were removed for the rest of the experimental period.

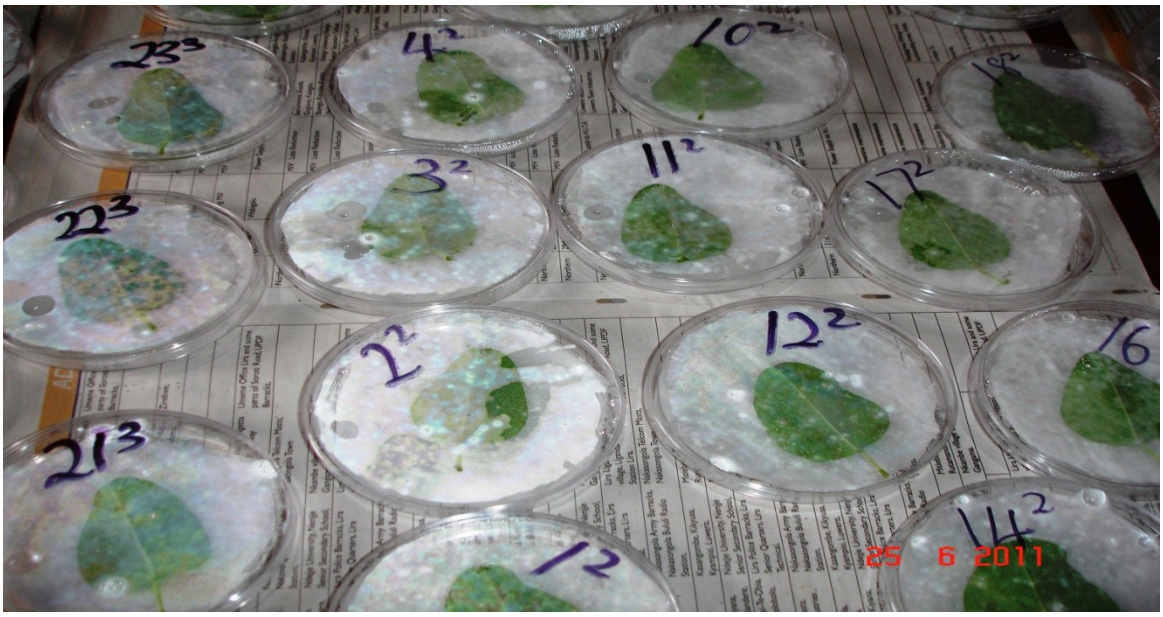

Plate 2: Detached leaves inoculated with rust isolates in petri dishes

4.3 Data collection and analysis

The data recorded from the study included reaction type; Immune (I) or Redish-Brown (RB) or Tan cloured (TAN) or Mixed reaction with both RB and TAN (MX), lesion number and frequency of lesions with uredinia. This was done using ×10 magnification lenses. Data were collected after five days of inoculation on a three day interval up to the 16th day after inoculation and subjected to analysis of variance in GENSTAT 13th Edition (Payne *et al*., 2010).

4.4 Results

4.4.1 Reaction type

Results of reaction type are summarized in Table 4. Based on reaction type, the 24 elite soybean lines responded differently to the four isolates; 16 genotypes showed tan lesion colour for isolates from all the four sites. This is a typical reaction for susceptible genotypes. Maksoy 3N showed Reddish brown reaction type with isolates from two sites (Iki-Iki and Mubuku) while isolates from NaCRRI and Nakabango showed a tan reaction type. For Mubuku isolates, genotypes DXT 1.2, DXT 9.12 and DXT 9.24 showed mixed reaction while DXT SPS 3.17-5 and Maksoy 3N showed reddish-brown reaction type (Plate 3). On the other hand, three genotypes (DXT SPS 1.33-1, DXT 1.62 and DXT 3.12) showed immune reaction type for isolates from NaCRRI.

Table 4: Reaction types of 24 elite soybean lines after inoculation with rust isolates from four locations in Uganda

Genotypes	**Bulk isolates**				
	Iki-Iki	Mubuku	NaCRRI	Nakabango	Comments
DXT 1.23	T	MX	T	T	
DXT SPS 1.33-1	T	T	I	T	
DXT 1.62	T	T	I	T	
DXT SPS 2.15-12	T	T	T	T	All T

DXT 3.9-3	T	T	T	T	All T
DXT 3.11	T	T	T	T	All T
DXT 3.12	T	T	I	T	
DXT SPS 3.17-2	T	T	T	T	All T
DXT SPS 3.17-5	T	RB	T	T	
DXT 3.21	T	T	T	T	All T
DXT 4.8	T	T	T	T	All T
DXT 5.15	T	T	T	T	All T
DXT 5.16	T	T	T	T	All T
DXT SPS 7.11-1	T	T	T	T	All T
DXT SPS 8.10-6	T	T	T	T	All T
DXT 9.12	T	MX	T	T	
DXT 9.18	T	T	T	T	All T
DXT 9.24	T	MX	T	T	
DXT 10.9	T	T	T	T	All T
DXT SPS 16.6-2	T	T	T	T	All T
DXT SPS 16.9-2	T	T	T	T	All T
Duiker	T	T	T	T	All T
NAM 1	T	T	T	T	All T
Maksoy 3N	RB	RB	T	T	

T-Tan, RB- Reddish Brown, MX- Mixed (Tan and Reddish brown), I-Immune

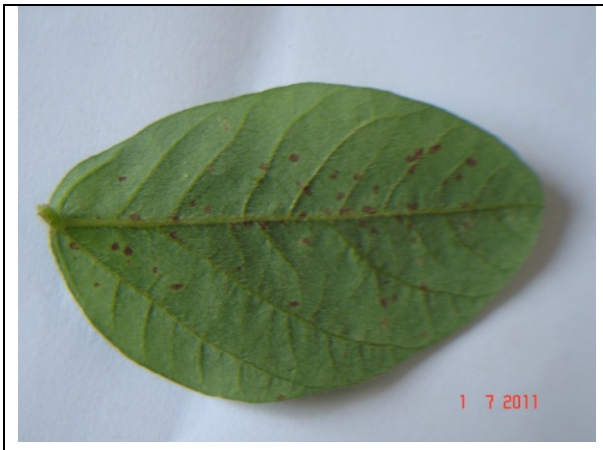	
Reddish-Brown reaction of Maksoy 3N leaf	Tan reaction of Duiker leaf

Plate 3: Reaction types of two soybean leaves

4.4.2 Number of lesions per leaf

Analysis of variance for number of lesions per leaf showed that location effect was significant (p=0.01) while genotypes were not significant for lesion number per leaf; implying that isolates from the different locations infected all genotypes differently. Genotypes DXT 3.9-3 and DXT SPS 3.17-5 had a mean of 41 lesions; followed by Duiker (35), DXT 9.12 (34) and DXT 3.21 (33). On the other hand, Maksoy 3N showed the lowest mean number of 5 lesions; followed by DXT SPS 1.33-1 (10), DXT 5.16 (13) and Nam I (13) as summarized in Table 5.

Table 5: Lesion number per leaf of 24 elite soybean lines after inoculation with rust bulk isolates from four locations in Uganda

Genotypes	**Bulk isolates**				
	Iki-Iki	Mubuku	NaCRRI	Nakabango	Mean
DXT 1.23	17	20	35	29	25
DXT SPS 1.33-1	9	4	0	27	10
DXT 1.62	10	61	0	23	24
DXT SPS 2.15-12	5	46	1	9	15
DXT 3.9-3	5	79	45	35	41
DXT 3.11	5	33	29	9	19
DXT 3.12	6	43	0	11	15
DXT SPS 3.17-2	6	28	1	33	17
DXT SPS 3.17-5	9	54	53	49	41
DXT 3.21	17	35	45	35	33
DXT 4.8	8	27	44	24	26
DXT 5.15	9	14	54	40	29
DXT 5.16	6	20	1	25	13
DXT SPS 7.11-1	7	18	15	43	21
DXT SPS 8.10-6	8	59	0	13	20
DXT 9.12	6	44	59	26	34
DXT 9.18	8	26	57	27	29
DXT 9.24	11	64	0	19	24
DXT 10.9	3	19	69	21	28
DXT SPS 16.6-2	1	42	24	39	27
DXT SPS 16.9-2	6	73	10	21	28
Duiker	4	56	56	23	35
NAM 1	1	23	0	27	13
Maksoy 3N	4	4	11	2	5

Mean	7	37	25	26	24
CV%	103.3	73.8	151.7	83.2	126.4
l.s.d	12.2	45.1	63.1	34.8	24.2

4.4.3 Number of lesions with uredinia

Both genotypes and location effect were not significant for number of lesions with uredinia. Table 6 shows a summary of mean number of lesions with uredinia among the 24 genotypes with isolates from different locations. The genotypes DXT 3.9-3, DXT SPS 3.17-5, DXT 3.21, DXT 9.12, DXT 9.24, DXT SPS 16.6-2 and Duiker had a mean of 1 uredinia/leaf with isolates from at least one location. The rest of the genotypes did not have any lesions with uredinia.

Table 6: Number of lesions with uredinia of 24 elite soybean lines after inoculation with rust isolates from four locations in Uganda

Genotypes	**Locations**				
	Iki-Iki	Mubuku	NaCRRI	Nakabango	Mean
DXT 1.23	0	1	0	0	0
DXT SPS 1.33-1	1	0	0	0	0
DXT 1.62	1	0	0	0	0
DXT SPS 2.15-12	0	0	0	0	0
DXT 3.9-3	0	0	0	2	1
DXT 3.11	0	0	1	0	0
DXT 3.12	0	0	0	0	0
DXT SPS 3.17-2	0	0	0	0	0
DXT SPS 3.17-5	1	1	0	0	1
DXT 3.21	0	2	0	0	1
DXT 4.8	1	0	0	0	0
DXT 5.15	0	0	0	0	0
DXT 5.16	0	0	0	0	0
DXT SPS 7.11-1	0	0	0	0	0
DXT SPS 8.10-6	1	0	0	0	0
DXT 9.12	0	1	2	0	1
DXT 9.18	0	0	0	0	0
DXT 9.24	0	2	0	0	1
DXT 10.9	0	0	0	0	0
DXT SPS 16.6-2	0	0	1	2	1
DXT SPS 16.9-2	0	0	0	0	0
Duiker	0	1	1	0	1

NAM 1	0	0	0	0	0
Maksoy 3N	0	0	0	0	0
Mean	0	0	0	0	0
CV%	284.4	300.5	437.6	561.8	392.8
l.s.d	0.8	1.9	1.4	1.5	1.4

4.5 Discussion

Successful development rust resistance in soybean requires exposure of breeding lines to as much diversity of the pathogen as possible. Use of host plant resistance offers a long term solution to management of soybean rust disease. This study employed the detached leaf technique to rapidly screen soybean lines for resistance to soybean rust. The technique is simple and rapid; its results easily match those obtained under field conditions (Twizeyimana *et al*., 2008).

The reaction types obtained from the four isolates from different locations indicate that each isolate is distinct which is suggestive of high pathogen diversity in Uganda (Yamanaka *et al.,* 2010). Most of the genotypes resulted into a TAN reaction type for isolates from all the locations; which implies high genetic relatedness and relatively similar level of aggressiveness of the pathogen on the study soybean genotypes. However, of the three locations, isolates from NaCRRI caused Immune reaction type among some genotypes; implying that these isolates are less virulent compared to the others collected from the other three locations.

On the other hand, isolates from Mubuku showed three distinct reaction types (TAN, RB and MX); which is indicative of a mixture of races with heterogeneous virulence compared to isolates from the other three locations. However isolates from Nakabango resulted in a TAN reaction type on all the soybean genotypes implying high level of aggressiveness and existence

of possibly a single race. The MX reaction type for isolates from Mubuku is indicative of a mixture of pathogen races at this location (Miles *et al*., 2008).

Maksoy 3N, a released commercial variety resistant to soybean rust exhibited a RB reaction type for isolates from two locations (Iki-Iki and Mubuku) while isolates from NaCRRI and Nakabango produced a TAN reaction type. The RB reaction type of Maksoy 3N is indicative that this genotype inhibits the spread of the soybean rust from the point of infection on soybean leaves resulting in resistance reaction. This is in agreement with the works of Maphosa *et al.* (2012) where Maksoy 3N showed a reddish brown reaction type when inoculated with isolates from these locations. This implies that Maksoy 3N could possess a specific resistance gene that suppresses development of rust on its leaves. However, the TAN reaction type on this variety from the Iki-Iki and Mubuku isolates points to existence of isolates capable of breaking resistance in Maksoy 3N. This means that Maksoy 3N should be used cautiously as it may soon succumb to the disease as pathogen isolates spread more in the country. Therefore, efforts to search for more sources of resistance to soybean rust should be a continuous process.

Different soybean genotypes showed varying number of lesions per leaf for all the isolates from the different locations. Isolates from Mubuku had the highest number of lesions per leaf; suggesting high aggressiveness of these isolates compared to other location. This corroborates field results where Mubuku had the highest rust mean score compared to the other locations (Table 7). Maksoy 3N had the lowest number of lesions per leaf; implying that it possesses resistance to soybean rust. Maksoy 3N showed a RB reaction type that in turn reduces the number of lesions on the leaf (Bromfield and Hartwig, 1980; Marchetti *et al.,* 1975). Such

genotypes that possess partial resistance should be identified and utilized in breeding programms because it is more durable compared to single gene resistance that condition resistance to a limited set of rust isolates (Hartman *et al.,* 2005).

CHAPTER FIVE

EVALUATION OF ELITE SOYBEAN LINES FOR ADAPTATION IN UGANDA

5.1 Introduction

The major focus of soybean breeding programme in Uganda is development of superior soybean varieties with either wide adaptation or specific adaptation to different regions. Some of the traits desirable in a good soybean variety include early maturity, resistance to pod shattering, resistance to lodging, high seed yield, improved nutritional composition, resistance to pests and diseases. The hardest task is to identify an individual with all the above traits. This is further complicated by the interaction between genotype and environment (Kaya *et al*., 2002). In Multi-environment trials (METs), genotypes are evaluated for stability performance of varying environmental conditions (Yan *et al.,* 2000). Each genotype that is grown in different environments will frequently show significant fluctuations in performance. GEI reduces the genetic progress in plant breeding programs and therefore must be either exploited by selecting superior genotype for each specific target environment or avoided by selecting widely adapted and stable genotype across wide range of environments. The objective of this study was therefore to determine adaptation of 24 elite soybean lines to local environmental conditions to make recommendations on the most suitable soybean line(s) for production and identify the most important factor(s) affecting soybean yield in Uganda.

5.2 Materials and methods

The study was conducted at five locations that represent the major soybean growing regions of Uganda; NaCRRI (central), Nakabango and Iki- iki (eastern), Ngetta (northern) and Mubuku (western) (Table 1). These locations have different climatic conditions and therefore may influence soybean growth and development of soybean rust differently. Mubuku irrigation scheme would additionally facilitate assessment of the adaptability of the elite lines under irrigation conditions. The 24 selected elite soybean lines (Table 3) were planted in a Randomized Complete Block Design (RCBD) with three replications. Each entry was represented by three rows measuring 5 m long with spacing of 60 cm between rows and 5 cm between plants within a row. The study was conducted for four consecutive seasons; first rains of 2010 (2010 A), second rains of 2010 (2010 B), first rains of 2011 (2011 A) and second rains of 2011 (2011B). The yield data were recorded for only three seasons (2010B, 2011A and 2011B). The trial was kept weed free by constant weeding. No agrochemicals were used on the trials to control pests. Means for each parameter per location and season of the 24 genotypes across the three seasons were compiled.

Parameters measured

The trials were assessed for resistance to the following diseases: soybean rust, soybean bacterial pustule, red leaf blotch and soybean mosaic virus. A scale of 1-5 adopted from Iqbal *et al.* (2004) was used for all the diseases where 1= highly resistant, 2= resistant, 3=moderately resistant, 4= susceptible and 5= highly susceptible. Lodging was rated on a scale of 1 to 5, with 1 being the most resistant or up right and 5 indicating that the soybeans are completely prostrate on the ground (Helsel and Minor, 1993). Another parameter that was recorded was nodulation; that is to

say ability of the different soybean genotypes to freely form nodules in their roots without inoculation. Nodulation was assessed using a scale of 1-5, where 1 is a genotype without root nodules and 5 is a genotype with more than 50 root nodules. The number of days after planting for the soybean genotypes to flower and attain physiological maturity was also recorded. At maturity, each genotype was harvested separately, threshed and yield per plot determined by weighing. Plot yields were consequently adjusted to 12% moisture content before computing yield per hectare.

5.3 Data analysis

Analysis of variance was performed initially for each of the parameters measured above in the different locations. Yield data were analysed using both AMMI and GGE models to determine GEI, genotype stability and winning cultivars in the five locations using GENSTAT 13th Edition.

5.4 Results

5.4.1 Genotype reaction to soybean rust disease

Mean rust severity scores on soybean genotypes for the five locations during the four seasons are presented in Table 7. Genotypes with the lowest rust severity scores included Maksoy 3N (3.1) and DXT 5.16 (3.5). On the other hand, Duiker had the highest mean score of 4.0; followed by DXT 10.9 and Nam I. They all had a mean score of 3.8. Rust severities were significantly different across the different locations ($p<0.001$) and seasons ($p<0.001$). Mubuku and Iki-Iki had the highest mean scores of 4.7 and 3.7 respectively while NaCRRI had the lowest mean score of 2.8. Season 2010A had the lowest mean score of 2.8; followed by season 2011A (3.3), 2010B (4.1) and season 2011B had the highest (5.0).

Table 7: Soybean Rust disease scores of 24 elite soybean lines tested at five locations in Uganda during four seasons (2010 A, 2010 B, 2011 A and 2011B).

	Location					
Genotype	Iki-Iki	Mubuku	NaCRRI	Nakabango	Ngetta	Mean score
DXT 1.23	3.7	4.9	2.7	3.8	3.4	3.7
DXT SPS 1.33-1	3.7	4.9	3.0	3.6	3.4	3.7
DXT 1.62	3.8	4.5	2.8	3.5	3.5	3.6
DXT SPS 2.15-12	4.0	4.4	2.8	3.3	3.4	3.6
DXT 3.9-3	3.5	4.9	2.9	3.8	3.4	3.7
DXT 3.11	3.7	4.7	2.7	3.4	3.4	3.6
DXT 3.12	3.6	4.8	2.9	3.4	3.4	3.6
DXT SPS 3.17-2	3.5	5.0	3.0	3.6	3.4	3.7
DXT SPS 3.17-5	3.5	4.6	2.8	3.8	3.4	3.6
DXT 3.21	3.5	4.6	2.8	3.5	3.4	3.6
DXT 4.8	3.8	4.8	2.8	3.4	3.4	3.6
DXT 5.15	3.5	4.8	2.8	3.6	3.4	3.6
DXT 5.16	3.8	4.5	2.7	3.4	3.4	3.5
DXT SPS 7.11-1	3.7	4.5	2.9	3.5	3.4	3.6
DXT SPS 8.10-6	3.6	5.0	2.9	3.5	3.4	3.7
DXT 9.12	3.7	4.6	2.9	3.4	3.4	3.6
DXT 9.18	3.5	4.9	2.8	3.4	3.4	3.6
DXT 9.24	3.7	4.6	2.7	3.5	3.4	3.6
DXT 10.9	3.8	4.9	3.0	3.9	3.4	3.8
DXT SPS 16.6-2	3.8	4.4	3.0	3.7	3.4	3.7
DXT SPS 16.9-2	3.5	4.6	2.9	3.4	3.4	3.6
Duiker	3.9	5.0	3.3	4.4	3.4	4.0
NAM 1	4.2	4.4	3.1	4.0	3.3	3.8
Maksoy 3N	3.5	3.5	2.1	2.9	3.3	3.1
Mean	3.7	4.7	2.8	3.6	3.4	3.6
CV %	8.0	11.3	16.8	12.9	2.2	34.0
l.s.d	0.09	0.17	0.38	0.15	0.02	0.50

Score scale of 1-5; where 1 is the least (close to no attack) and 5 being the highest possible attack

5.4.2 Other diseases

The mean scores of red leaf blotch and soybean mosaic virus disease are summarized in Table 8. Red leaf blotch disease was absent in all locations except NaCRRI while it had a mean score of 2.3. Genotype DXT SPS 16.6-2 and DXT SPS 16.9-2 had the lowest mean score of 1.3.

Genotype DXT 1.23 had the highest mean scores of 1.6 (Table 8). On the other hand, soybean mosaic virus disease was present in all the five locations; although its severity was low. Most of the genotypes had low mean scores of soybean mosaic virus disease except Nam I which had a mean score of 1.3.

Table 8: Red leaf blotch and soybean mosaic virus disease resistance of 24 elite soybean lines tested at different locations in Uganda

Genotype	**Diseases mean scores**	
	Red Leaf Blotch	Soybean Mosaic Virus
DXT 1.23	1.6	1.1
DXT SPS 1.33-1	1.4	1.1
DXT 1.62	1.5	1.1
DXT SPS 2.15-12	1.5	1.1
DXT 3.9-3	1.4	1.0
DXT 3.11	1.4	1.1
DXT 3.12	1.5	1.0
DXT SPS 3.17-2	1.5	1.0
DXT SPS 3.17-5	1.4	1.0
DXT 3.21	1.5	1.1
DXT 4.8	1.4	1.2
DXT 5.15	1.4	1.0
DXT 5.16	1.4	1.2
DXT SPS 7.11-1	1.4	1.2
DXT SPS 8.10-6	1.5	1.0
DXT 9.12	1.4	1.1
DXT 9.18	1.4	1.0
DXT 9.24	1.4	1.1
DXT 10.9	1.4	1.2
DXT SPS 16.6-2	1.3	1.1
DXT SPS 16.9-2	1.3	1.1
DUIKER	1.5	1.1
NAM 1	1.4	1.3
Maksoy 3N	1.4	1.1
Mean	1.4	1.1
CV %	27.7	24.3
l.s.d	0.16	0.11

Score scale of 1-5; where 1 is the least (close to no attack) and 5 being the highest possible attack.

5.4.3 Agronomic traits

Table 9 is a summary of means of the agronomic traits which included lodging, nodulation, flowering dates and maturity periods. There was a significant difference among the genotypes ($p<0.001$) and locations ($p<0.001$) for lodging resistance. The results showed that genotypes DXT 10.9, Duiker and Nam I were the most resistant to lodging, each with a mean score of 1.0. Genotypes with the highest scores were DXT SPS 1.33-1, DXT 3.11, DXT 5.15 and DXT 9.18 which had a mean of 2.0. Nodulation results revealed that there were significant genotype ($p< 0.009$) and location ($p<0.001$) effects on nodulation of the soybean genotypes. Genotypes DXT 3.12 and Duiker had the lowest nodulation scores of 2.3 while genotypes DXT 9.18 and Maksoy 3N had the highest mean scores of 3.1 and 3.0 respectively. There was a significant difference among the genotypes ($p<0.001$) and seasons ($p<0.001$) for days to flowering with Duiker flowering earliest (37 days). Most genotypes flowered between 40-46 days after planting. On the other hand, the results showed that genotype DXT SPS 16.9-2 reached physiological maturity after 88 days; followed by genotypes DXT 1.23 and DXT 3.12 that matured after 89 days. DXT SPS 16.6-2 took the longest time to reach physiological maturity (102 days); followed by DXT SPS 2.15-12, DXT 4.8 and DXT 5.16 all matured in 99 days.

Table 9: Lodging, nodulation scores, flowering dates and maturity period of the 24 elite soybean lines tested at different locations in Uganda during four seasons (2010A, 2010B, 2011A and 2011B).

	Agronomic traits			
Genotype	Lodging	Nodulation	Days to flowering	Days to maturity
DXT 1.23	1.4	2.8	38	89
DXT SPS 1.33-1	2.0	2.7	41	94
DXT 1.62	1.2	2.9	39	94
DXT SPS 2.15-12	1.1	2.7	46	99
DXT 3.9-3	1.6	2.9	41	92
DXT 3.11	2.0	2.6	41	97
DXT 3.12	1.9	2.7	41	89

DXT SPS 3.17-2	1.8	2.7	42	93
DXT SPS 3.17-5	1.6	2.5	43	93
DXT 3.21	1.7	2.3	44	95
DXT 4.8	1.5	2.7	42	99
DXT 5.15	2.0	2.9	44	91
DXT 5.16	1.6	2.8	41	99
DXT SPS 7.11-1	1.5	2.8	43	97
DXT SPS 8.10-6	1.8	2.8	43	91
DXT 9.12	1.8	2.7	43	93
DXT 9.18	2.0	3.1	43	93
DXT 9.24	1.6	2.9	45	97
DXT 10.9	1.0	2.4	38	93
DXT SPS 16.6-2	1.2	2.4	46	102
DXT SPS 16.9-2	1.5	2.4	42	88
Duiker	1.0	2.3	37	94
NAM 1	1.0	2.9	41	97
Maksoy 3N	1.6	3.0	41	96
Mean	1.6	2.7	42	94
CV %	45.4	35.5	7.49	3.76
l.s.d	0.29	1.75	1.27	2.00

Score scale of 1-5. Where 1 is the least (close to no lodging) and 5 being the highest possible lodging state

5.4.4 Genotype seed yield

Combined seed yield results of the 24 soybean genotypes for the three seasons across the five locations are summarized in Table 10. Maksoy 3N had the highest mean yield of 1703 kg ha^{-1}; followed by DXT 3.11 (1576), DXT SPS 7.11-1 (1549) and DXT SPS 2.15-12 (1432). Mubuku had the highest mean yield (2313); followed by NaCRRI (1493), Nakabango (1270), Iki-Iki (795) while Ngetta was the worst performing site (688). Season 2011A had the highest mean seed yield of 1809 kg ha^{-1}; followed by season 2010B (1411) and 2011B had the lowest yield of 715 kg ha^{-1}.

Table 10: Seed yield performance in kg ha^{-1} of 24 elite soybean lines tested in five locations in Uganda across three seasons (2010B, 2011A and 2011B).

Genotype	**Location**					
	Iki-Iki	Mubuku	NaCRRI	Nakabango	Ngetta	Mean yield
DXT 1.23	706	1903	1259	1203	501	1114
DXT SPS 1.33-1	776	2366	1333	1307	773	1311
DXT 1.62	825	2315	1296	1469	721	1325
DXT SPS 2.15-12	843	2565	1750	1288	715	1432
DXT 3.9-3	640	2176	1491	1412	614	1267
DXT 3.11	943	2787	1778	1479	894	1576
DXT 3.12	628	1954	1417	1287	568	1171
DXT SPS 3.17-2	812	2343	1519	1222	574	1294
DXT SPS 3.17-5	847	2406	1333	1177	587	1270
DXT 3.21	894	2722	1315	1251	677	1372
DXT 4.8	845	2481	1472	975	775	1310
DXT 5.15	747	2241	1537	1412	893	1366
DXT 5.16	687	2250	1306	1244	821	1261
DXT SPS 7.11-1	975	2648	1778	1558	785	1549
DXT SPS 8.10-6	587	2147	1583	1365	676	1272
DXT 9.12	791	2000	1639	1295	805	1306
DXT 9.18	873	2287	1704	1275	674	1363
DXT 9.24	833	2157	1546	1233	697	1293
DXT 10.9	944	1926	1454	1008	537	1174
DXT SPS 16.6-2	888	2741	1296	1014	538	1295
DXT SPS 16.9-2	691	1917	1231	1200	589	1126
DUIKER	672	1991	1435	894	544	1107
NAM 1	671	2343	1463	1019	628	1225
Maksoy 3N	961	2843	1898	1892	922	1703
Mean	795	2313	1493	1270	688	1312
CV %	42.2	20.2	28.7	29.7	28.4	47.0
l.s.d	110.4	435.5	398.0	350.7	87.0	570.9

The ANOVA results generated by AMMI for genotype yields are summarized in Table 11. The model partitioned main effects into genotypes, environments and GEI with all the components showing significant effects at $p<0.05$ with the exception of GEI that was not significant. The environment had the greatest effect that accounted for 90.1% of the treatment SS; genotypes accounted for 5.3% and GEI had the least effect and accounted for only 4.7% of the treatment

SS. AMMI analysis also showed that first IPCA captured 53.1% of the interaction sum of squares and the second IPCA accounted for 23.9% of the interaction sum of squares. The combined mean square for the three IPCA axes was 9.6 times that of residual mean square, while the first IPCA was 5.3 times that of the residual mean square. The second IPCA and third IPCA mean squares were 2.6 and 1.8 times the residual mean square respectively. The AMMI model also contained 40.9% of the treatment sum of squares while the block contained 1.6% and the error contained 57.5%. The treatment and block sum of squares combined made 42.5% of the total sum of squares. IPCA 1 and, IPCA 2 and IPCA 3 were not significant.

Table 11: The analysis of variance for AMMI of the 24 elite soybean lines tested over five locations in Uganda during 2010B, 2011A and 2011B seasons

Source	Df	SS	MS	F	F_prob
Total	1079	440696781	408431		
Treatments	119	180419190	1516128	5.68	0.00000
Genotypes	23	9478416	412105	1.54	0.04891
Environments	4	162548024	40637006	59.19	0.00000
Block	10	6865599	686560	2.57	0.00447
Interactions	92	8392751	91226	0.34	1.00000
IPCA 1	26	4459413	171516	0.64	0.91556
IPCA 2	24	2003655	83486	0.31	0.99943
IPCA 3	22	1279740	58170	0.22	0.99995
Residuals	20	649943	32497	.12	1.00000
Error	950	253411991	266749		

The AMMI biplot provided a visual expression of the relationships between the first interaction principal component axis (IPCA1) and means of genotypes and environments (Figure 3). The AMMI biplot showed four groupings of genotypes; DXT 10.9(G19) and Duiker (G22), generally low yielding and unstable; DXT 1.23(G1) and DXT SPS 16.9-2(G21), low yielding and stable. The other two groups included DXT 3.11(G6) and DXT SPS 7.11-1(G14) that had moderate yield and stable and Maksoy 3N (G24) that was high yielding but unstable. Mubuku showed high yields and moderate stability while Iki-Iki and Ngetta were low yielding environments. However

Ngetta was more stable than Iki-Iki. NaCRRI and Nakabango had moderate yields but Nakabango was very unstable.

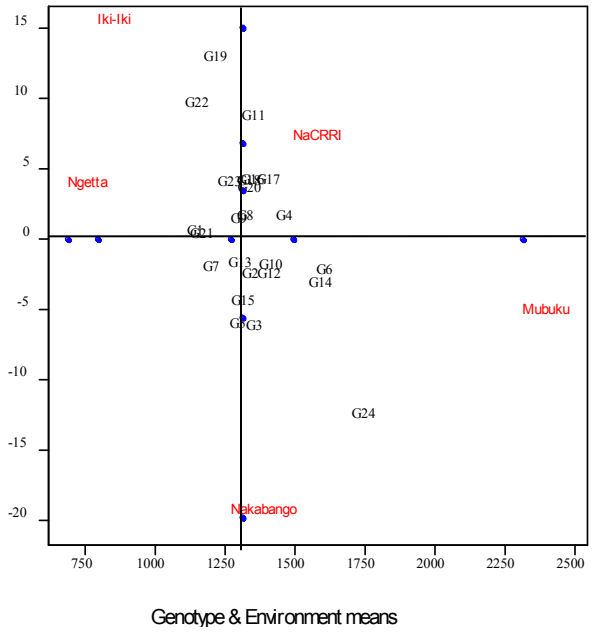

Figure 3: AMMI biplot of IPCA1 scores versus yield means for 24 soybean genotypes and five environments for 2010B, 2011A and 2011B seasons.

DXT 1.23	G9-DXT SPS 3.17-5	G17-DXT 9.18
G2-DXT SPS 33-1	G10-DXT 3.21	G18-DXT 9.24
G3-DXT 1.62	G11-DXT 4.8	G19-DXT 10.9
G4-DXT SPS 2.15-12	G12-DXT 5.15	G20-DXT SPS 16.6-2
G5-DXT 3.9-3	G13-DXT 5.16	G21-DXT SPS 16.9-2
G6-DXT 3.11	G14-DXT SPS 7.11-1	G22-DUIKER
G7-DXT 3.12	G15-DXT SPS 8.10-6	G23-NAM 1
G8-DXT SPS 3.17-2	G16-DXT 9.12	G24-Maksoy 3N

Based on the first IPCA, the genotypes with highest GEI were DXT SPS 16.6-2, DXT 3.21 and DXT 9.12 with interaction scores of -16.96, -12.71 and 10.60 respectively while the least interactive genotype was DXT 5.16 with IPCA 1 score of 0.61. The environments were also highly interactive with Mubuku having the highest IPCA 1 score of -27.87 while the least interactive environment was Iki-Iki with IPCA score of -0.36 (Table 12).

Table 12: AMMI mean yield and IPCA 1 scores for the 24 elite soybean lines grown in five locations in Uganda during 2010B, 2011A and 2011B seasons

Genotype codes	Genotypes	Mean yield (kg ha^{-1})	IPCA1
G1	DXT 1.23	1114	6.81
G2	DXT SPS 1.33-1	1311	-1.82
G3	DXT 1.62	1325	0.67
G4	DXT SPS 2.15-12	1432	-4.02
G5	DXT 3.9-3	1267	4.78
G6	DXT 3.11	1576	-6.32
G7	DXT 3.12	1171	8.27
G8	DXT SPS 3.17-2	1294	-1.85
G9	DXT SPS 3.17-5	1270	-5.62
G10	DXT 3.21	1372	-12.71
G11	DXT 4.8	1310	-7.44
G12	DXT 5.15	1366	5.26
G13	DXT 5.16	1261	0.61
G14	DXT SPS 7.11-1	1549	-2.48
G15	DXT SPS 8.10-6	1272	6.24
G16	DXT 9.12	1306	10.60
G17	DXT 9.18	1363	2.49
G18	DXT 9.24	1293	4.20
G19	DXT 10.9	1174	5.85
G20	DXT SPS 16.6-2	1295	-16.96
G21	DXT SPS 16.9-2	1126	6.75
G22	DUIKER	1107	2.81
G23	NAM 1	1225	-4.26
G24	Maksoy 3N	1703	-1.86
Environment			
	Iki-Iki	795	-0.36
	Mubuku	2313	-27.87
	NaCRRI	1493	9.32
	Nakabango	1270	12.01
	Ngetta	688	6.89

When genotype ranking was performed, Maksoy 3N (G24) was ranked best in four environments and second best in one environment. DXT 3.11 (G6) was best in one environment; second in two and third best in two environments. Genotype DXT SPS 7.11-1 (G14) was second best in two environments and third best in two other environments (Table 13).

Table 13: Genotype ranking in the five environments

Environment	Environmental Mean	Genotype ranking			
		1st	2nd	3rd	4th
Nakabango	1270	G24	G14	G6	G12
NaCRRI	1493	G24	G14	G6	G16
Ngetta	688	G24	G6	G14	G4
Iki-Iki	795	G6	G24	G14	G4
Mubuku	2313	G24	G6	G20	G10

A scatter plot (Figure 4) indicated that all the five locations were in one mega environment with the best genotype (vertex cultivar) being Maksoy 3N (G24).

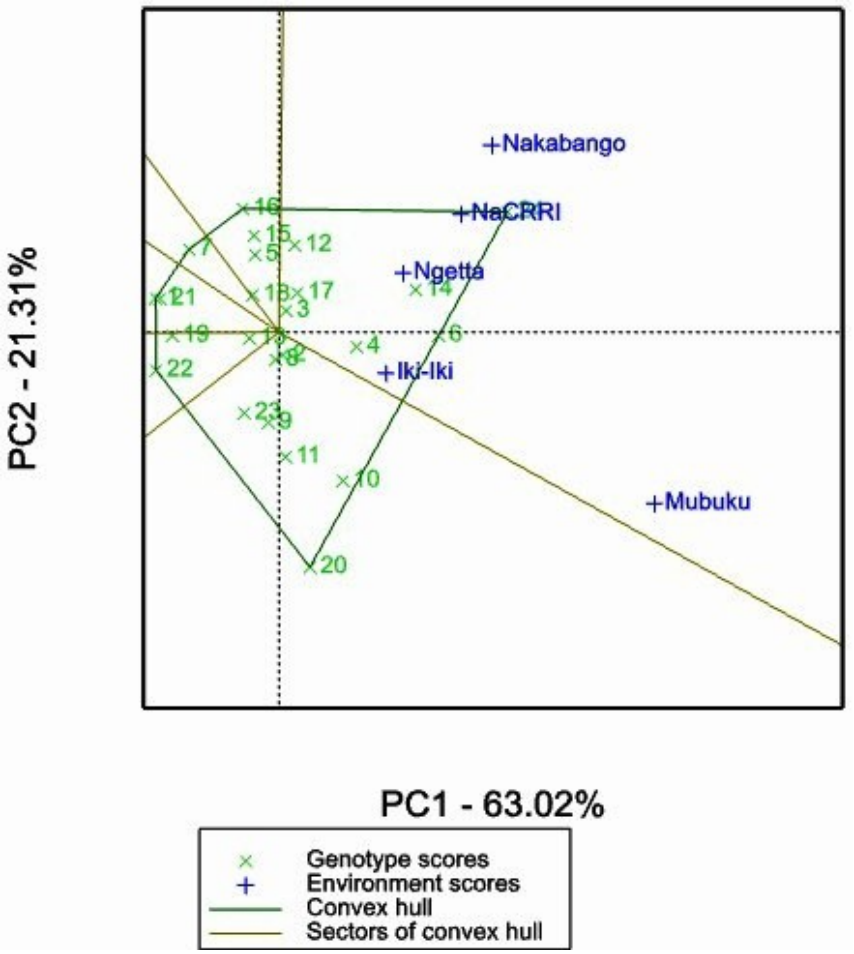

Figure 4: A scatter plot showing PC2 verses PC1 for 24 soybean genotypes and five environments for 2010B, 2011A and 2011B seasons.

On the other hand, a comparison biplot that is genotype focused (Figure 5) showed that genotype DXT 5.16(G13) is the most stable genotype while Maksoy 3N(G24) is the most ideal genotype, followed by DXT 3.11(G6), DXT SPS 7.11-1(G14), and DXT SPS 2.15-12(G4).

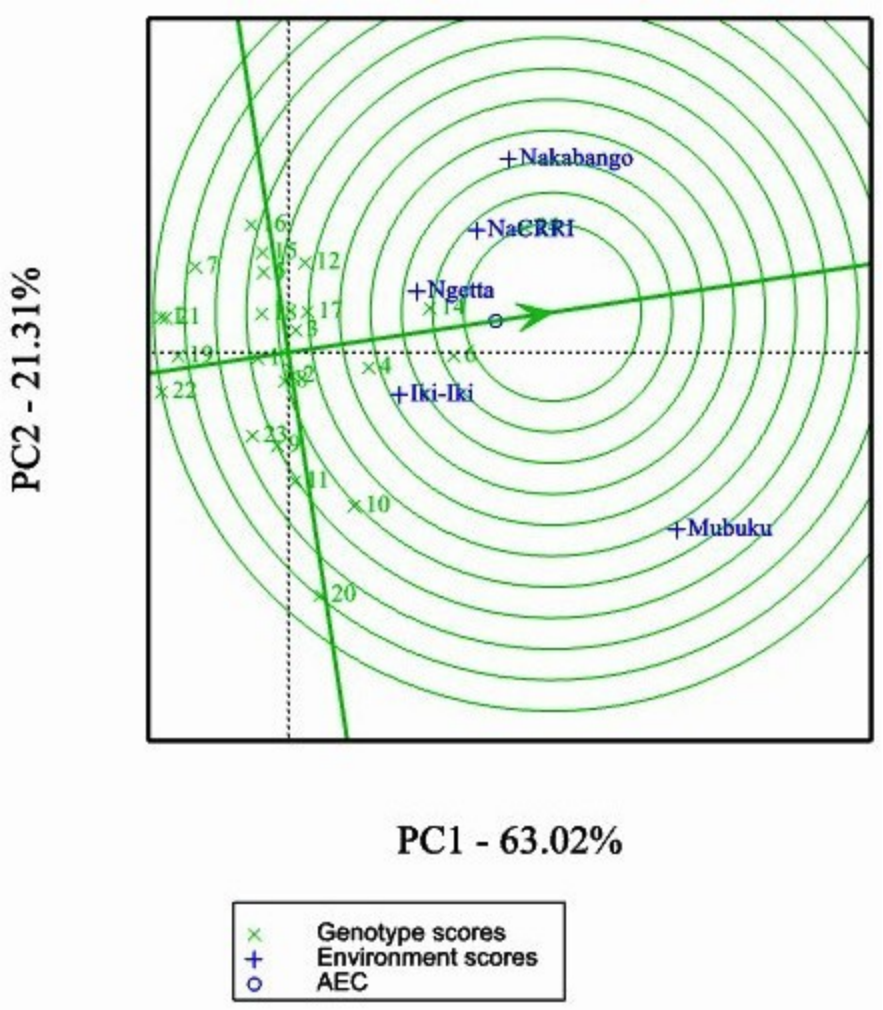

Figure 5: Genotype focused comparison biplot showing PC2 verses PC1 for 24 soybean genotypes and five environments for 2010B, 2011A and 2011B seasons.

Figure 6 shows an environment focused comparison biplot where Nakabango was the ideal environment, followed by NaCRRI, Mubuku, Ngetta and Iki-Iki.

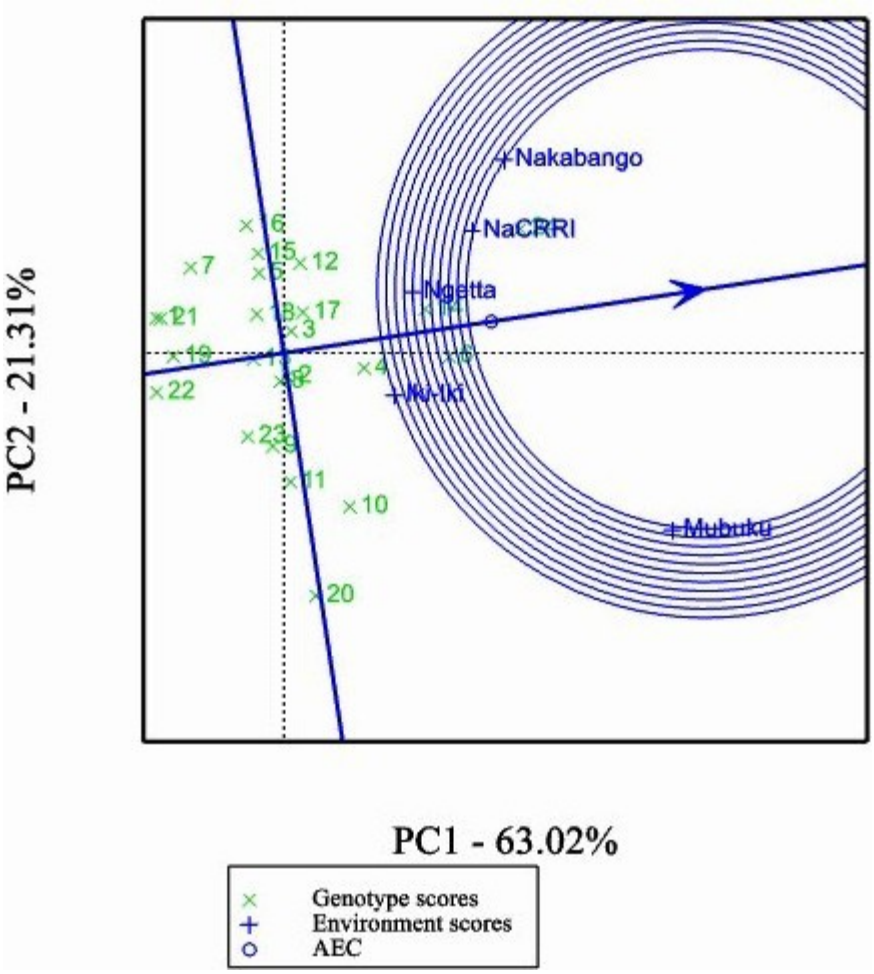

Figure 6: Environment focused comparison biplot showing PC2 verses PC1 for 24 soybean genotypes and five environments for 2010B, 2011A and 2011B seasons.

5.5 Discussion

Use of resistant varieties is the most cost effective and environmentally friendly means of managing soybean diseases especially for developing countries like Uganda. However, these varieties should be adapted to a wide range of target environments if they are to offer maximum benefits to the country. Field observations showed that soybean rust was the major soybean disease in all soybean growing areas. Earlier findings also reported that soybean rust was the most important and destructive foliar disease of soybeans (Kawuki, 2002; Twizeyimana *et al.,*

2008). The results of the study showed that mean rust severity scores for the four seasons were significantly different among genotypes ($p<0.008$) and highly significant among locations ($p<0.001$) and across seasons ($p<0.001$). Genotypes Maksoy 3N, DXT 3.11 and DXT 5.16 were among the genotypes with low rust severity; suggesting that they possess resistance genes to soybean rust. Maksoy 3N is one of the recommended commercial varieties grown in Uganda and was included in the study as a resistant local check. It is therefore recommended for use in crosses for development of other new varieties. The other two genotypes with low rust severity (DXT 3.11 and DXT 5.16) should be tested on farmers' fields for acceptability if they are to be released for commercial production. All the elite lines showed lower rust severity compared to the highly susceptible local checks Duiker and Nam I except DXT 10.9; suggesting effective response in selection for resistance to soybean rust disease.

Mubuku and Iki-Iki had the highest mean rust scores of 4.5 and 3.6 respectively while NaCRRI and Ngetta had the lowest mean score of 3.1 and 3.2 respectively. Earlier studies also reported low rust severity in Ngetta (Kawuki, 2002; Asiimwe, 2012). Similarly a survey carried by Lamo (2004) indicated that Lira district where Ngetta is located had the lowest rust incidence and severity while Kasese district where Mubuku is located had the highest compared to other districts in Uganda. However, Tukamuhabwa *et al*. (2011) observed that NaCRRI had the lowest soybean rust severity, though earlier studies by Kawuki (2002) and Asiimwe (2012) indicated that NaCRRI had the highest rust severity which is contrary to the current study.

Soybean rust development is known to be influenced by environmental factors such as rainfall, temperature, leaf wetness, relative humidity and dew point. Therefore the high soybean rust severity at Mubuku could have been due to the high relative humidity, resulting from flood

irrigation which maintains a high level of relative humidity; combined with temperature that greatly favour soybean rust development. The results showed that season 2011A had the lowest mean score of 2.2; followed by season 2010A (3.0), 2010B (4.6) and season 2011B which had the highest (5.0). This corroborates with earlier findings by Asiimwe (2012) where soybean rust severity is higher in second rains (B) than in the first rains (A) during the year; because of accumulated rust inocula in the area from the first rains.

The red leaf blotch was absent in all the locations except at NaCRRI suggesting that for any new variety to be released in future should be screened against this disease at NaCRRI. This is probably because this site has a high level of innoclum since it has been used for testing different soybean genotypes.

Lodging results showed that Duiker and Nam I had the lowest mean scores across the four seasons. This could be attributed to their short stature which does not favour lodging. However, genotype DXT 10.9 which is not short had a low score as well, suggesting that it has a strong and firm stem. Field observations showed that all the genotypes were taller in Mubuku compared to other sites. Additionally, the results showed that most of the genotypes in Mubuku were not upright because of adequate supply of irrigation water throughout the season that led to excessive growth of the genotypes. Such results seem to suggest that adequate moisture in the soil during the growing season lead to excessive growth of the soybean genotypes.

The GGE genotype focused comparison biplot also showed that early maturing genotypes were also low yielding and unstable. Therefore early maturity seems to be associated with low and unstable yields. Among the locations, Mubuku had the highest seed yield. The high seed yield in Mubuku could have been due to optimal supply of water to the crop through irrigation. In

addition to the longest period to physiological maturity, Mubuku had the highest mean seed yield (2313 kg ha^{-1}). This was followed by NaCRRI (1493 kg ha^{-1}). Ngetta recorded the lowest yield of 688 kg ha^{-1}. Earlier studies by Tukamuhabwa *et al*. (2012) also showed that Ngetta was the lowest yielding environment. These results seem to suggest that presence of moisture in the soil during the season delays maturity but increases seed yield of soybeans. Nakabango was the most ideal environment as earlier observed by Tukamuhabwa *et al*. (2012) and is therefore recommended as a primary testing centre for new soybean genotypes.

The environmental mean yield and biplots also showed that yields were generally lower in the second rains than in the first rains of the year. This suggests that the conditions in the first season were more favorable for soybean production; most probably due to higher rainfall in this season. Another factor that greatly affected the yields of the genotypes was the high soybean rust severities during the second rains. It was clearly observed that seasons with high rust severities, the yield was remarkably low. For example season 2011A had a low rust severity of 3.3 and the yield was 1809 kg ha^{-1} while 2010 B had a mean rust severity score of 4.1 and a yield of 1411 kg ha^{-1}. The yield in 2011B was 715 kg ha^{-1} with a mean rust severity score of 5.0. This implies that moisture stress and high soybean rust severities greatly affect soybean yields in Uganda as was observed by Kawuki (2002).

The analysis of variance for AMMI of the 24 elite soybean lines showed that the interactions (IPCA 1, IPCA 2 and IPCA 3) were not significant. This implies that the soybean lines were more homogenous and there was no interaction of these lines with the test environments.

Both AMMI and GGE biplots showed that genotypes DXT 3.11 and DXT SPS 7.11-1 had stable moderate yield. These are desirable traits in plant breeding suggesting that the two genotypes can

be retained to hybridise low yielding soybean genotypes. Genotype DXT 3.11 had higher yield than Maksoy 3N during seasons with low rust severities. This observation implies that Maksoy 3N is more resistant to rust compared to DXT 3.11. Maksoy 3N that was included as the highest yielding recommended variety in Uganda yielded higher than all the test genotypes as was earlier observed by Tukamuhabwa *et al*. (2012). Since Maksoy 3N was more superior to all the test genotypes, there is need for more breeding work to develop more superior genotypes for release to farmers for production.

CHAPTER SIX

6.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

Soybean rust (*Phakopsora pachyrhizi* Sydow) is a major threat to soybean production worldwide. The objectives of this study were to: (i) assess *Phakopsora pachyrhizi* diversity using SSR markers, (ii). assess elite soybean lines for resistance to bulk rust isolates and (iii). determine the adaptation and stability of selected elite soybean lines in Uganda. The study has shown that the population of *P. pachyrhizi* in Uganda is variable with two major clusters. This diversity has disease management implications.

The knowledge of reaction of elite soybean lines with field isolates of *P. pachyrhizi* from different locations is important for successful breeding and management of soybean rust. Additionally, the reaction of the elite soybean lines can help in identification of resistant genotypes and matching the diverse *P. pachyrhizi* in a given location (Miles *et al.,* 2011). The reaction types obtained from the four bulk isolates is suggestive of diversity within the pathogen (Yamanaka *et al.,* 2010). However reaction types may also suggest that the test genotypes have varying levels of resistance. The reddish brown reaction type of Maksoy 3N is indicative that this genotype prevents the spread of the soybean rust from the point of infection on the soybean leaves hence its resistance to rust. This may also imply that Maksoy 3N could possess specific resistance genes that suppress development of rust on its leaves.

The results also showed that each location either has a different race composition or dissimilar virulence to which genotypes responded differently. Maksoy 3N (local check) performed better

than all the other genotypes and can therefore be used as a source of rust resistance in breeding programmes. As a commercial cultivar released in Uganda in 2010, Maksoy 3N already has several agronomic traits desirable by end users. However the resistance gene present in Maksoy 3N has not been ascertained. Specific resistance presents a challenge to soybean breeding because it is only efficient in suppressing rust in specific locations; hence can easily be broken down. Prospecting and exploration for other sources of resistance and strategies such as partial resistance and tolerance is highly recommended because of durability.

Soybean rust development is known to be influenced by environmental factors such as rainfall, temperature, leaf wetness, relative humidity and dew point. The high soybean rust severity at Mubuku could have been due to the high relative humidity and temperatures. The flood irrigation used in Mubuku maintains a high level of relative humidity. In addition, this location has high temperatures throughout the season. It was clearly evidenced that soybean grain yield was very low in seasons with high rust severity. This clearly shows the significance of rust disease to soybean production.

6.2 Conclusions

This study showed that the SSR markers developed by Anderson *et al.* (2008) can be used to study rust diversity in Uganda.

The 24 elite soybean lines showed different reaction types when inoculated with bulk rust isolates from different locations in Uganda.

Field observations showed that soybean rust was the major soybean disease in all soybean growing areas in Uganda. This is in agreement with earlier findings that confirmed that soybean

rust is the most important and destructive foliar disease of soybeans. Results suggest that Maksoy 3N possesses very effective resistance genes to soybean rust disease. All the elite lines in the study had lower rust severity compared to the highly susceptible local checks Duiker and Nam I except DXT 10.9; suggesting effective response in selection for resistance to soybean rust disease. Maksoy 3N that was included as the highest yielding recommended variety in Uganda had the highest yields and was the most ideal genotype. This calls for further breeding work to develop high yielding varieties that have resistance to soybean rust and possible replacement of Maksoy 3N in case the resistance breaks down in future. All the test locations were grouped into one mega environment by GGE biplot.

6.3 Recommendations

In future rust diversity studies, the number of fields sampled should be increased and the rust isolates should be collected from specific plants per location within the fields. Investigations using molecular markers should also be supplemented with phenotypic markers. Race differentials need to be developed to facilitate soybean rust diversity studies. There is need for the soybean breeding program to collect more sources of resistance genes and use them to improve the available soybean varieties in Uganda whose resistance seems to be breaking down due to high disease pressure.

Genetic analysis of rust resistance in Maksoy 3N should be done in order to identify the resistance gene and other important parameters like gene effects. Since Maksoy 3N is resistant to soybean rust and high yielding, it is recommended that more progenies be generated by crossing it with other high yielding but susceptible genotypes. Genotype DXT 3.11 is high yielding in rust

free seasons but not resistant to rust. Therefore it can be used to improve yields of low yielding genotypes that are tolerant / resistant to soybean rust.

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Genetic analysis of rust resistance in Maksoy 3N should be done in order to identify the resistance gene and other important parameters like gene effects.

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