MAKERERE  **UNIVERSITY**

ENHANCING GENETIC RESISTANCE TO SOYBEAN RUST DISEASE

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DECLARATION

The work presented here is my own and has not been submitted to any other university for the award of a PhD degree

Signed…….. Date…….

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This thesis is submitted with our approval as supervisors

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DEDICATION

To my parents Gibson and Matilda Maphosa, for their uncompromising principles that have guided my life and for leading all their children to academic pursuits

To my sisters and brothers, for their abundant support and setting a good precedence

To my academic supervisors, for making my study worthwhile and serving as inspirational role models

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ABSTRACT

Soybean (*Glycine max* L.) is increasingly playing an important nutritive role in the food and feed industry in most countries. However, the crop is currently threatened by soybean rust disease (*Phakopsora pachyrhizi*). The rapid spread of soybean rust, coupled with its potential for causing severe yield losses, makes it an important disease in soybean growing countries. A major impediment to breeding for resistance to soybean rust is the lack of stable sources of resistance to the highly variable pathogen in different soybean producing areas.

Therefore the study sought to assess comparative virulence of five diverse field isolates from major soybean producing areas in Uganda, and identify soybean lines with resistance to isolates of soybean rust in seedling and adult plants under screen house and field conditions, respectively. When inoculated with the five field isolates, all twelve lines evaluated showed diverse and mixed reactions, suggesting each location differed in soybean rust races and/or virulence. Experimental sites where many diverse soybean lines are grown every year had the greatest diversity of soybean rust. The effectiveness of specific resistance genes was restricted to certain locations and gene R*pp2* previously resistant was ineffective producing a susceptible tan reaction at the seedling stage. A positive correlation between mean lesion density at the seedling stage and adult plant severity indicated that using field isolates to screen for seedling resistance can be a useful breeding approach to extrapolate resistance in adult plants. Overall, these results emphasise the relevance of using field isolates from the target areas to evaluate lines for soybean rust resistance. Soybean genotypes Maksoy 3N, UG5, G7955 and GC00138-29 were identified as ideal sources resistance to soybean rust that can be used across the selected sites.

The impact of soybean rust (*Phakopsora pachyrhizi*) on soybean yields has been extensively studied. However, few studies have evaluated early generation segregating material under field conditions for soybean rust resistance to facilitate selection. The objective of the second study was to estimate combining abilities for soybean rust resistance in the F_2 and F_3 populations at MUARIK and F_3 populations simultaneously across five locations. Combining ability for soybean rust resistance was estimated from a half diallel cross of eight soybean lines using disease severity and sporulation rates as indices for resistance. A consistent contribution of additive gene action was observed at MUARIK across F_2 and F_3 despite high environment contribution to both severity and sporulation rate. The simultaneous evaluation of F_3 populations in five diverse locations produced similar results with significant GCA effects for both traits. There were, however, greater genotypic effects compared to environmental effects for soybean rust severity and sporulation across the five test environments, although genetic systems of severity and sporulation rate acted independently. Additive and complementary additive gene effects were the most common form of GCA controlling resistance. Specific combining ability did not always contribute to soybean rust resistance. The positive correlation between parental severity, sporulation rate performance and GCA estimates suggested that selection of parents for soybean rust resistance breeding can be based on parental performance. Parental line UG 5 was the most outstanding producing the greatest number of resistant populations. This study underscores the importance of additive gene effects in the control of soybean rust severity and sporulation rate.

The problem of soybean rust is compounded by its high pathogenic variability which overcomes single gene resistance present in most cultivars. Few studies have, however, been undertaken to use mapped simple sequence repeat markers for gene pyramiding to enhance rust resistance. The study validated use of identified simple sequence repeat

markers for gene pyramiding and determined the most effective pair wise gene combination for three independent resistance genes; R*pp2*, R*pp3* and R*pp4*. Markers Satt460 and AF162283 were polymorphic for three resistance genes between the parents and therefore used in F_2 and $F_{2:3}$ family selection. In the F_2 generation, soybean plants (homozygous or heterozygous at both loci) with two gene combinations had relatively lower disease severity and sporulation than the parents suggesting complementary epistatic gene action for resistance. Homozygous $F_{2:3}$ families similarly showed lower severity, lesion density and sporulation. Gene R*pp3* interacted and contributed positively to resistance with various genetic backgrounds for most parameters measured compared to R*pp2* and R*pp4* resistance genes. Overall, the results suggest that marker gene pyramiding is feasible and can substantially increase resistance to soybean rust through reduced severity and reduced frequency of uredinia with sporulating lesions.

High potential genotypes UG5, Maksoy 3N, G7955 and GC00138-29 are ideal sources of soybean rust resistance in Uganda. These findings also imply that breeding for soybean rust has to focus on other strategies such as partial resistance and tolerance that are polygenic and have broad ASR effectiveness.

PUBLICATIONS DECLARATION

This thesis is based on the following scientific publications:

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CHAPTER ONE

INTRODUCTION

1.1 Economic and Nutritional importance of Soybean

Soybean (*Glycine max* (L) Merrill) is one of the most important and versatile crops worldwide. Its high quality protein content (40%), oil content (20%), and numerous bioactive factors make soybean a highly desirable crop with a potential of improving diets of millions of people (Singh *et al*., 2008; Hartman *et al*., 2011). Moreover, in farming systems, the crop is known to improve soil properties such as fertility through nitrogen fixation and enhancing moisture retention. The combination of improved soil properties and the ability to break life cycles of pests and diseases makes soybean an ideal crop in cereal rotation programmes (Waymark, 1997).

Globally, soybean production constitutes 6% of all total arable land in the world and has the highest percentage increase in area under production than any other crop (Hartman *et al*., 2011). The leading soybean producers in the world are USA (33%), Brazil (29%) and Argentina (19%) (SoyStats, 2012). Africa accounts for 0.4-1% of the total world soybean production and the potential is yet to be realised although its utilisation is rapidly increasing. The main soybean producers are Nigeria, South Africa, Uganda and Zimbabwe, however, most of the continent is suitable for soybean production (Kalapo, 2011). The discrepancies in production between the leading soybean producers and Africa are mainly due to biotechnological innovations embraced by those countries.

Grain soybean is the primary source of vegetable protein for feed supplement and accounts for much of the world's oil supply (Sudarić *et al*., 2011). The demand for the crop is expected to increase due to its potential to improve the dietary quality of the vast majority

of people and livestock. This has significant implications given that most of the staple diets in Sub-Saharan Africa comprise of starchy foods and animal protein is generally expensive. The unparalleled amino acid content and profile, and protein productivity per unit area imply that soybean could be more important in developing countries faced with malnutrition. Therefore, any production constraints that threaten soybean need to be adequately addressed to promote adoption and/or sustain production.

1.2 Asian Soybean Rust as a constraint to soybean production

Despite the importance of soybean a number of biotic factors especially foliar diseases reduce yields and thus farmer income (Hartman *et al*., 2011). Bacterial pustule (*Xanthomanas axonopodis* pv *phaseoli*), frog eye leaf spot (*Cercospora sojina*), red leaf blotch (*Ductuliochaeta glycines*) and soybean rust (*Phakospora* spp) are important disease in most soybean producing areas. Of these diseases, soybean rust is the most important foliar disease in soybean production that causes yield losses of up to 80% in unprotected fields (Kato *et al*., 2006; Hyten *et al*., 2007; Kato and Yorinori, 2008). Two obligate *Phakopsora species*; *P. meibomiae* (Authur) and *P. pachyrhizi* (Sydow and Sydow) cause soybean rust, but the latter is more aggressive and economically important species in most soybean producing areas (Bonde *et al*., 2006). Soybean rust caused by *P. pachyrhizi* also called Asian soybean rust (ASR) is a relatively new disease in Africa having been reported in the late 1990's (Twizeyimana *et al*., 2008). However, its rapid spread coupled with the potential of causing severe yield losses makes it a very important disease of soybean. Soybean rust moved from Asia through air-borne urediniospores from India to Central Africa, then from Africa to South America leaving a trail of devastation for soybean growers (Isard *et al*., 2006).

Symptoms of soybean rust are either tan or red-brown depending on the host-pathogen interaction. Typically rust lesions consist of polygonal pustules (2-5 mm^2) under the leaf (abaxial) surface with circular ostioles producing urediniospores. Yield losses result from decreased photosynthetic capacity hence low number of filled pods per plant, pods per plant, seeds per plant, weight of seed per plant and 100 seed weight (Hartman *et al*., 1991; Kumudini *et al*., 2010).

Ideal environmental conditions have caused soybean rust disease to become endemic in most soybean growing areas in tropical Africa (Kawuki *et al*., 2003; Twizeyimana *et al*., 2007). Disease incidence is favoured by a hot, humid environment which leads to reduced photosynthetic area on the leaves and premature defoliation. Unlike other rust pathogens with a narrow host range, soybean rust has more than 100 hosts including cultivated legumes such as common beans (*Phaseolus vulgaris*), scarlet runner bean (*P. coccineus*), limabean (*P. lunatus*), cowpea (*Vigna unguiculata*), pigeon pea (C*ajanas cajan*), field pea (*Pisum sativum*), and lentil (*Lens culinaris*) which act as inoculum reservoirs and bridge hosts, further exacerbating the problem of soybean rust (Hartman *et al*., 2004; Anon, 2007). In Uganda, most functional hosts that allow soybean rust reproduction such as common bean, cowpea are available throughout the year due to high rainfall which makes management of rust a challenge of great importance. Since ASR is an obligate parasite, urediniospores are harboured by these live hosts before moving to the soybean crop during the growing season (Pante and Esker, 2008; Goellner et al., 2010).

1.3 Geographical Distribution of Asian Soybean Rust

Asian Soybean Rust occurs in Africa, Asia, South America and North America (Ivancovich, 2008). Soybean rust was first observed in 1903 in Japan and spread to most Australasian countries such as China, Taiwan, Thailand and Australia (Bromfield, 1984).

The disease was for a long time restricted to South East Asia, Australia and India. The arrival period of ASR in Africa is unknown (Levy, 2005) but suggestions are that aerial urediniospores spread from India to Central Africa causing the first outbreaks in Africa (Isard *et al*., 2006). In Africa, the devastating effects of ASR were reported in Uganda, Kenya and Rwanda in 1996; Zambia and Zimbabwe in 1998; Mozambique in 2000 (Levy, 2003; Levy, 2004, Nigeria in 2001 (Akinsanmi *et al*., 2001) South Africa in 2000 (Pretorius *et al*., 2007), Democratic Republic of Congo and Ghana in 2007 (Bandyopadhyay *et al*., 2007; Ojiambo *et al*., 2007) . All these areas have a warm tropical environment which promotes the proliferation of the pathogen. Similarly, air currents are thought to have moved urediniospores from Africa to South America, where ASR symptoms were observed in Paraguay in 2001; Brazil in 2002; Argentina in 2002; Bolivia in 2003 (Ivancovich, 2008; Junior, 2008); Colombia and Uruguay, 2004 (Stewart *et al*., 2005) . In North America, ASR was identified in Hawaii in 1994 and spread to continental US in 2004 (Schneider *et al*., 2005; Junior, 2008). The wide spread nature of soybean rust and its rapid spread suggests that it is global problem that requires urgent attention.

1.4 Statement of the Problem

Soybean rust is the greatest threat to soybean production worldwide. The pathogen is highly variable and the rapid spread of ASR epidemics during favourable conditions coupled with the potential of causing severe yield losses makes it an important disease (Kato *et al*., 2006). Decreased photosynthetic capacity and premature defoliation caused by ASR precludes grain filling resulting in reduced 100 grain weight, number of filled pods, seeds per plant and subsequently yield (Bennett, 2005).. However, the extent of yield loss depends on prevailing weather conditions during the cropping season, varieties grown i.e. whether there are susceptible or resistant, aggressiveness of the pathogen and physiological growth stage of crop when it is attacked (Tukamuhabwa and Maphosa,

2011). However, there are no universally accepted sources of resistance due to its high diversity that easily overcomes classical sources of specific gene resistance.

In Uganda, yield loss associated with ASR were assessed using soybean fungicide treatments in three susceptible commercial varieties and ranged between 27-36% and <10% in elite resistant lines (Kawuki *et al*., 2003); 15-41% in susceptible lines (Tukamuhabwa and Dashiell, 1999). In Zimbabwe, yield losses ranged from 60-80%, South Africa 10-80% and 100% in monocultural systems (Caldwell and Laing, 2001). According to Akinsamni *et al*. (2001) yield in TGx soybean lines ranged from 28-49% in Nigeria. In Paraguay and Brazil, yield losses of 60% and 30-75% respectively were experienced in 2001 (Yorinori *et al*., 2005). In Argentina, direct field evaluation in some provinces resulted in yield losses of between 17-28% (Formento, 2008). Fungicide treatment experiments done at Asian Vegetable Research Development Centre (AVRDC) , Taiwan indicated yield losses ranging from 23 to 50% (Yang, 1991). However, losses of up to 100% have been encountered in areas without chemical protection. Similarly, in China losses of 30 to 50% were reported under heavy infestation (Yu *et al*., 1994). In India losses of up to 80% were experienced between 1994-5 in the state of Karnataka (Patil and Basavaraja, 1997).

In the United States, limited information is available of the actual losses due to ASR, however, yield losses greater than 10% were detected during fungicide trials in South Carolina (Mueller, 2008). From these yield loss studies done worldwide it is evident that ASR has capacity to cause substantial yield losses if mitigation strategies are not in place. Moreover, this threat of ASR is even greater in tropical countries such as Uganda where environmental conditions for the proliferation of rust exist throughout the year. Therefore

it is imperative to deal with the problem of rust decisively using all available means at our disposal.

As the area under soybean production continues to increase particularly in warmer regions of Africa and South America, ASR is expected flourish and become more severe as it has long been a member of tropical and sub tropical fungal flora (Yang, 1991). More so, several studies have shown that in most soybean producing countries rust has become endemic and is likely to persist all year round on alternative hosts making it agronomically difficult to eliminate. Fungicides are expensive and are not very effective at preventing epidemics as Bonde *et al*. (2006) noted yield losses of up to 50% under severe rust epidemics with chemical control. Other legumes that also form an integral part of the cropping system such as cowpea, pigeon pea and common beans are functional hosts of ASR which makes control a great challenge (Anon, 2007; Slaminko *et al*., 2008). Cultural practices such as changing planting dates, row width and rotation sequences have little or no effect on soybean rust development (Shaner *et al*., 2005).

1.5 Justification of the Study

Few studies have evaluated segregation progeny aimed at identifying high potential soybean rust resistant genotypes. Soybean improvement is largely dependent on the generation of bi-parental crosses and advancement of segregating progenies to homozygosis. In this context, combining ability studies provide a guide for selecting elite parents and desirable combinations to be used in the formulation of a systematic breeding project. Diallel analysis when used in combining ability studies allows for the evaluation and identification of more promising crosses with superior segregating lines (Hakizimana *et al*., 2004; Awan *et al*., 2005; Mebrahtu and Devine, 2009). These cultivars identified can be used as parents in breeding programmes to seek the development of soybean

cultivars with resistance to soybean rust. In a diallel, general combining ability (GCA) characterises the average performance of a genotype in a series of hybrid combinations and is mainly associated with additive gene action. Specific combining ability (SCA) is used to characterise the performance of a specific hybrid combination in relation to the average of its parents and is predominantly associated with genetic effects involving dominance (Lopes *et al*., 2001). These parameters facilitate selection of desirable parents and cross combinations for ASR resistance breeding.

The observation that expression of some resistance genes is influenced by the genetic background in which there are introgressed makes it worthwhile to test the effectiveness of available resistance genes in crosses with local market class varieties (DeLucia *et al*., 2008; Yamanaka *et al*., 2010). Though some resistance genes are overcome by specific pathogen races, their interaction with certain backgrounds has shown to confer greater resistance (DeLucia *et al*., 2008). Furthermore, the aggressiveness of African and other "New World" races towards accessions with single gene resistance makes it imperative to determine effective gene and background combinations which could enhance the resistance and safeguard against changes in soybean rust race populations (Hartman *et al*., 2004). The prospects of carrying out marker assisted selection to enhance soybean rust resistance genes are now possible due to the availability of a dense soybean molecular map (Song *et al*., 2004). DNA markers can be used as a precise and efficient tool for multiple gene identification and genotype selection (Yamanaka *et al*., 2008). All the six resistance genes, R*pp1*, R*pp2*, R*pp3*, R*pp4*, R*pp5* and R*pp?*$_{Hyuuga}$ have been mapped and simple sequence repeat markers designed to facilitate for their selection (Hyten *et al*., 2007; Monteros *et al*., 2007; Garcia *et al*., 2008; Hyten *et al*., 2009). During field trials of 2008/9 in NaCRRI, Uganda, these genes were quite effective at managing soybean rust though R*pp3* and R*pp4* are relatively less effective as compared to R*pp1* and R*pp2* (Oloka

et al., 2008). However, none of the local varieties contains any of the identified classical resistance genes, suggesting need to increase diversity of major genes available for breeding purposes. Given that specific resistance genes have been overcome by changes in pathogen populations in some soybean producing areas, marker assisted gene pyramiding can be used to further enhance resistance against soybean rust and guard against changes in rust populations (Yamanaka *et al*., 2010; Lemos *et al*., 2011).

Soybean expresses TAN and red brown (RB) reaction phenotypes in the presence of ASR (Bromfield and Hartwig, 1980). Genotypes with Red Brown (RB) lesions have less sporulation and sparse uredinia whereas tan lesions are characterised by profuse sporulation. A locally available genotype UG5 with RB lesions has heritable resistance showing great potential towards contributing to improvement in disease management worldwide (Kawuki *et al*., 2003; Twizeyimana *et al*., 2007). There is, however, no information regarding whether the resistance genes in UG5 are different from *Rpp1-5*, *Rpp Hyuuga* already described. Allelic tests on UG5 can contribute important information on the identity of this resistance gene which is effective against most geographically diverse ASR races (Kawuki *et al*., 2003; Twizeyimana *et al*., 2007; Paul and Hartman, 2009). Information on allelic characterisation of such new sources of resistance is important as soybean breeders worldwide are looking for new sources of resistance to curtail soybean rust disease (Ribeiro *et al*., 2008). This can further rationalise the breeding process by preventing the over deployment of a single gene locus to manage ASR.

Therefore, the objectives of this study were to determine the response of selected resistance gene sources to soybean rust in five geographically diverse regions of the country and identify parents capable of originating superior individuals with comprehensive resistance through combining ability analyses. In addition, it validated

identified simple sequence repeats linked to three resistance genes for pyramiding the soybean rust resistance genes. The study also sought to determine the most effective gene combination(s) conferring the greatest resistance and conduct allelic tests on UG5 using known selected sources of resistance.

1.6 Overall Objective

The goal of the study was to develop genotypes with broad and more effective resistance to field isolates of soybean rust in Uganda

1.6.1 Specific Objectives

The specific objectives were as follows:

1. To characterise adult and seedling plant resistance of selected elite soybean genotypes to five geographically diverse *P. pachyrhizi* field isolates;
2. To assess combining ability of 8 selected lines for *P. pachyrhizi* resistance over F_2 and F_3 generation across environments,

   and
3. To validate the use of microsatellite markers in pyramiding three soybean rust resistance genes and their effectiveness in breeding for disease resistance.

1.6.2 Hypotheses

The following hypotheses were tested in the study:

1. Resistance to soybean rust varies with seedling and adult plant stage and origin of *P. pachyrhizi* field isolate

2. Soybean rust resistance is heritable and can be transmitted to the progenies irrespective of generations and environments

3. Gene pyramiding regardless of gene combination and genetic background enhances soybean rust resistance

CHAPTER TWO

LITERATURE REVIEW

2.1 The Soybean Rust Pathogen

Soybean rust, *Phakopsora pachyrhizi* (Sydow and Sydow) also called Asian Soybean Rust (ASR) is the major constraint in soybean production. Soybean rust has a closely related species *Phakopsora meibomiae* which is a less aggressive form of rust found exclusively in Latin America (Goellner et al., 2010). These two species cannot be distinguished by direct observation of an infested field, but only through a polymerase chain reaction (PCR) assay that makes use of the 20% difference in nucleotides in the ribosomal internal transcribed region (Frederick *et al*., 2002). Worldwide, ASR is the greatest biotic threat capable of inflicting yield losses of up to 80%. The pathogen attacks the crop at any developmental stage causing premature defoliation that precludes grain filling which results in crop failure. Paradoxically, conditions that promote soybean cultivation favour soybean rust development (Miles *et al*., 2003).

2.1.1 Taxonomy and Symptomology of Soybean Rust

Phakopsora pachyrhizi is a plant pathogenic *Basidiomycete* of the Order *Uredinales* (Agrios, 2005; Goellner et al., 2010). The disease was first observed in Japan in 1902, and has since then been given several names including *Uredo sojae, Uromyces sojae, Phakopsora sojae* and *Phakopsora vignae* (Yang, 1991). It was in 1914 that the pathogen was isolated from Yam bean, *Pachyrhizus erosus* and termed *Phakopsora pachyrhizi* (Goellner *et al*., 2010). However, unlike other rust fungi with a complex growth cycles, ASR is capable of producing teliospores and urediniospores only. Similarly, unlike other imperfect fungi *Phakopsora pachyrhizi*, shows high level of genetic diversity attributed to parasexuality, heterokaryosis and high mutation rate (Freire *et al*., 2008). Under field

conditions disease development is caused by the uredinal stage which produces urediniospores during the multicyclic infections that occur in the growing season (Miles *et al*., 2003).

Early symptoms of ASR can often be confused with those of other pathogens, such as bacterial pustule (*Xanthomonas axopondis* pv *phaseoli*), bacterial blight (*Pseudomonas savastanoi* pv *glycinea*) and brown leaf spot (*Septoria glycines*) (Ivancovich, 2008; Soares, 2008). For accurate diagnosis hand held lens with a magnification of 10–20× shows the characteristic 'volcano'-shaped erumpent uredinia, with several openings (ostioles) which release urediniospores. Urediniospores, however, cannot be individually distinguished at these magnifications (Tukamuhabwa and Maphosa, 2011).

Soybean rust manifests mostly on the leaves, petioles and sometimes on the stems of soybean plants (Yang, 1991). During germination, rust pustules have been observed on the cotyledons. Soybean rust produces gray, tan to red-brown polygonal pustules (2–5 mm^2) on the under surface of leaves (abaxial surface), restricted by the vascular bundles, with urediniospores emerging from circular ostioles disseminated by wind. Symptoms can also manifest on the adaxial (upper) surface, in the advanced stages of disease development. Symptoms are first observed on the lower leaves as gray water-soaked lesions that change to small, chlorotic areas, which increase in size and change colour to either tan or red-brown. Under suitable conditions the disease progresses upward to spread throughout the canopy resulting in premature leaf yellowing and subsequently defoliation (Miles *et al*., 2003).

2.1.2 Soybean Rust Reaction Phenotypes

Based on the lesion colours, resistance or susceptibility to soybean rust of a line can be assessed (Bromfield and Hartwig, 1980). In general, genotypes with red-brown (RB) lesions have less sporulation, while the small and lighter tan (TAN) lesions are characterised by profuse sporulation (Figure 1). However, in some instances lesion colour has not been a reliable measure of resistance (Miles *et al*., 2011).

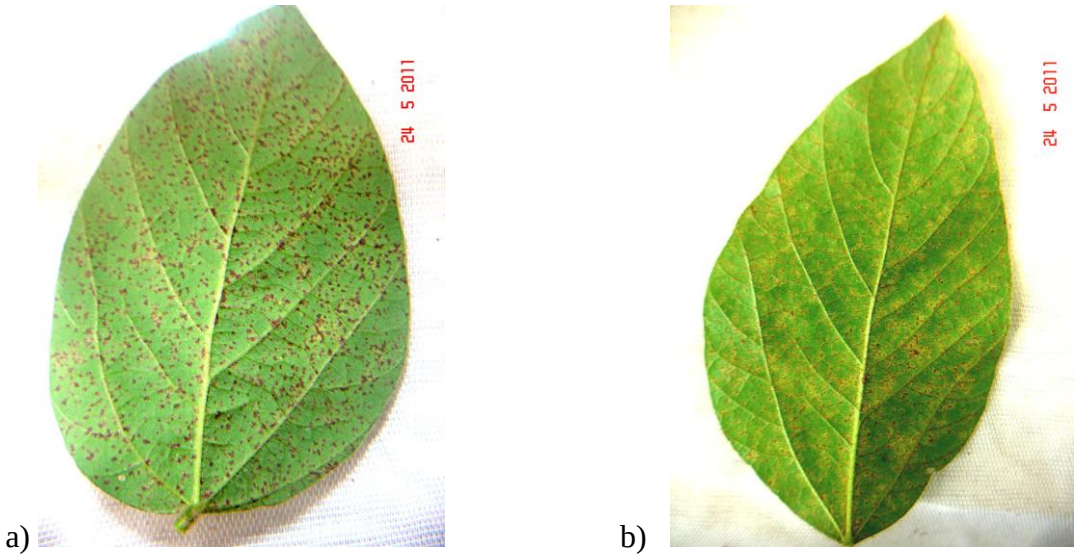

Figure 1 Characteristic soybean rust symptoms on the abaxial leaf surface a) red brown lesions b) tan lesions at Makerere University Agricultural Research Institute (MUARIK) (M. Maphosa, 2011)

During early disease development, lesions appear gray and water soaked when viewed against a light background. Immune genotypes have no visible signs of infection. Lesion colour, however, depends on the virulence of the pathogen, host genotype, the interaction of the host and pathogen and environmental conditions (Li, 2009; Yamanaka *et al*., 2010). Within lesion types a continuum was observed from red brown to tan leading to the development of a six colour category chart to facilitate the colour evaluation process (Kato and Yorinori, 2008). In addition, within each colour category sporulation varies from complete absence of spores to profuse highly sporulating uredinia. The red brown has

been classified into 5 different classes based on the percentage sporulation relative to a highly sporulating, susceptible cultivar (Pham *et al*., 2009).

2.2 Alternative Hosts of Asian Soybean Rust

Soybean rust is an obligate parasite with a range of multiple hosts. Unlike other rust pathogens with a narrow host range, ASR has more than 100 functional leguminous host plants (Formento, 2008; Slaminko *et al*., 2008; Soares, 2008). Such a broad host range is unusual for an obligate pathogen which suggests the occurrence of several different physiological races and complex virulence pattern (Burdon and Marshall, 1981; Hartman *et al*., 2005). Weeds and cultivated legumes which include common bean (*Phaseolus vulgaris*), scarlet runner bean (*P. coccineus*), lima bean (*P. lunatus*), cowpea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*), field pea (*Pisum sativum*) and lentil (*Lens culinaris*), act as inocula reservoirs and bridge hosts that habour spores in the uredinal stage.

Figure 2 shows a non-leguminous weed species *Euphorbia heterophylla* (in foreground) and soybean (in background) with typical ASR symptoms taken from a heavily infested field in Mubuku, Western Uganda. Given the widespread nature of weeds and importance of various leguminous species in African cropping systems such a wide host range makes management of soybean rust a challenge. The multiple host range of ASR is explicable by its unique ability to penetrate the cuticle and epidermal cells directly in comparison with most fungi. It also gains access to the plant through wounds and stomata (Marchetti *et al*., 1975), coupled with the non-specific induction of appressoria in the infection process of *P. pachyrhizi* (Miles *et al*., 2003). These factors imply that soybean rust has an accelerated invasion capability and can spread rapidly once the pathogen is established.

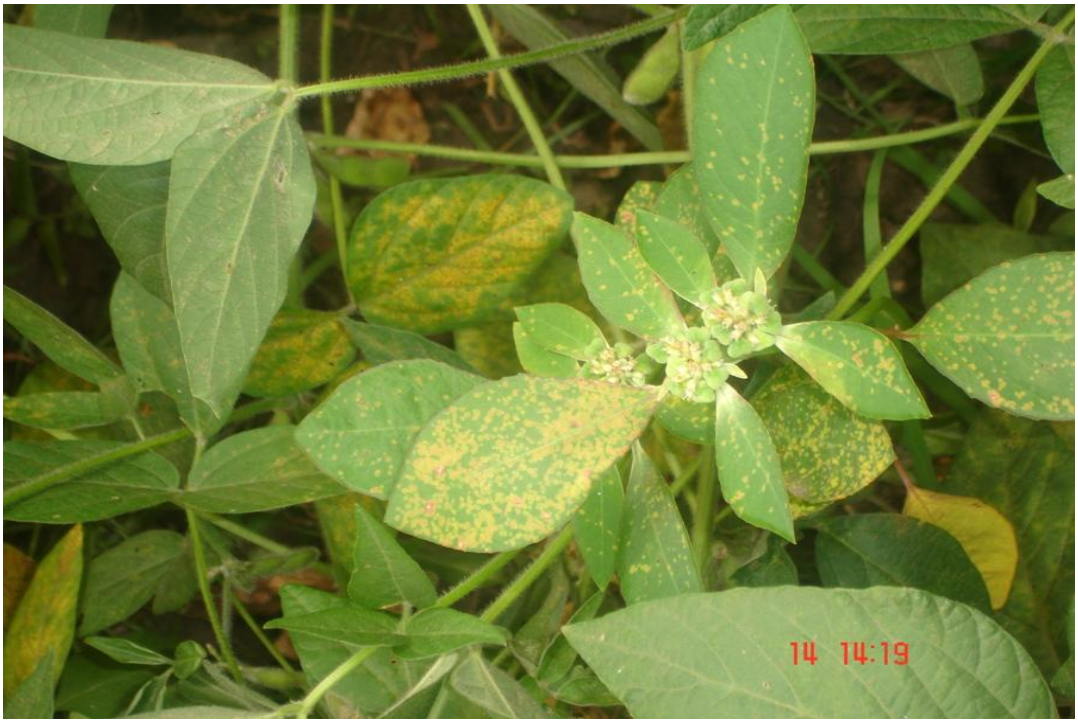

Figure 2. *Euphorbia heterophylla*, a weed at Mubuku, Western Uganda ASR symptoms with infected soybean leaves in the background (M. Maphosa, 2011).

2.3 The Infection Process of ASR

Urediniospores released from erumpent uredinia are transported by wind from alternative hosts to soybean. Infection can occur at any stage of phenological development under ideal environmental conditions. A continuous dew period of between 6-7 hours is required for the urediniospores to germinate and form a germ tube on a susceptible variety (Melching *et al*., 1989). Temperatures of between 17-25 ^{0}C and 70% relative humidity allow for optimum germination. However, spores of some virulent strains have been observed to germinate at 41^{0}C (Li, 2009). The spores cannot tolerate very low temperatures and tend to overwinter in warmer regions. In the case of Uganda, uniformly warm temperatures and high relative humidity imply the pathogen is always present due to ideal conditions, hence likely to pose a greater challenge to management.

During infection urediniospore penetration pegs emerge from the underside of the appressorium. Penetration occurs through the cuticle and epidermal cells resulting in necrosis of one epidermal cell (Marchetti *et al*., 1975). Intracellular invasion of the leaf occurs once hyphae are formed within the mesophyll layer. Within 5 to 7 days volcano shaped uredinia with round ostioles are produced which release urediniospores on the abaxial surface completing the asexual reproduction cycle (Goellner et al., 2010). Uredinia continue to develop for up to 4 weeks after the initial infection and urediniospores are produced for up to 3 weeks (Marchetti *et al*., 1975). Rainfall is the most important factor causing rapid increase in severity and disease spread. The major role that rainfall plays after infection is dislodging the urediniospore clumps through the splash effect and impact on lesions to facilitate their subsequent dispersal by the wind (Filho, 2008). This rainfall induced spread of ASR is necessary even in soybean under irrigation (Tschanz *et al*., 1986).

2.4 Influence of Crop Phenology on Rust Development

Asian soybean rust affects soybean at any developmental stage if virulent urediniospores, susceptible hosts and a conducive environment are available. Hence rapid screening of germplasm for resistance to rust has been done at the seedling stage under green house conditions (Miles *et al*., 2003; Li, 2009; Paul and Hartman, 2009). This is, however, a rapid screening method which does not guarantee adult plant resistance. Studies by Ribeiro *et al*. (2007) concluded that seedling evaluation and selection does not correlate with adult plant resistance because under field conditions the multiple cycles of re-infection promote greater pathogen virulence which is able to challenge the true resistance potential of a genotype. Consequently, several studies have evaluated ASR resistance under field conditions since it is more useful for practical plant breeding programmes

(Kawuki *et al*., 2003; Miles *et al*., 2008; Oloka *et al*., 2008; Pham *et al*., 2010; Walker *et al*., 2011).

In general, ASR symptoms and severity increases after flowering (R6-stages) under field conditions. This makes it ideal to evaluate genotypes during the reproductive stage as variation in severity is greatest during this period (Miles *et al*., 2006; Walker *et al*., 2011). Therefore, differences in maturity period should be considered during ASR resistance evaluation. Relative Life Time (RLT) technique which takes into account the life time the crop has undergone compared to its total life span (Wang and Hartman, 1992) corrects for difference in maturity allowing for accurate diagnosis of resistance. Relative life time is calculated using the formula:

$$\text{Relative Life Time} = \frac{\text{Days after Planting (DAP)}}{\text{Days to full maturity (DFM)}} \times 100$$

It is thus recommended to classify genotypes into maturity groups to evaluate lines with similar growth cycles for resistance to ASR (Tschanz and Wang, 1987; Wang and Hartman, 1992).

2.5 Race specificity considerations in evaluating for resistance

Race specific resistance to ASR has been well documented in several rust pathosystems through the use of monosporic purified isolates. Such pure isolates are obtained through a series of single spore isolation and inoculation to attain genetic uniformity (Li, 2009; Pham et al., 2009). Resistance to such monosporic pure isolates is, however, not the same as under field conditions where the pathogen occurs in mixtures, mostly with high virulence diversity. Moreover, single spore isolation may not represent the variation

present (Freire *et al*., 2008; Paul and Hartman, 2009). Under field conditions several races that have either homogeneous or heterogeneous virulence may occur. Consequently most studies have used such mixed isolates with either known or unknown composition. For example in Japan (Yamanaka *et al*., 2010), Nigeria, Paraguay (Miles *et al*., 2008), South Africa (McLaren, 2008), Uganda (Oloka *et al*., 2008), US and Vietnam (Pham *et al*., 2010; Walker *et al*., 2011) have used mixed isolates to determine the levels of ASR resistance to local populations. Lower levels of resistance may be observed when field isolates are used compared to single spore isolates. When using bulk isolates there are greater chances of mixed reaction phenotypes due to differences in virulence among the ASR isolates (Bonde *et al*., 2006). As such a mixture of RB and TAN lesions are observed there is ambiguity in the interpretation of the underlying genetics of resistance.

2.6 Soybean breeding for race specific resistance to ASR

Six dominant genes *Rpp1,* Rpp2, *Rpp3, Rpp4, Rpp5* and *Rpp? (Hyuuga)* that confer resistance to specific soybean rust races have been identified to be effective depending on the soybean rust-plant pathosystem (Kato *et al*., 2006; Hyten *et al*., 2007; Garcia *et al*., 2008). However, other effective major resistance genes and their source material do exist except they don't have specific names, for example PI 398507, FT2, PI 407912, PI 424473, UG5, TGx 1835-10E (Tukamuhabwa *et al*., 2001; Kiryowa, 2007; Arias *et al*., 2008). Additionally resistance to ASR can be conditioned by recessive genes (Calvo *et al*., 2008; Ray *et al*., 2011). However, the ability of soybean rust to develop races renders genes that individually condition resistance to a limited set of rust races inadequate (Laperuta *et al*., 2008). Given the high virulence diversity exhibited by soybean rust in different geographical locations there is need to identify comprehensive sources of resistance for manipulation and deployment.

Race-specific resistance genes manifest a hypersensitive response characterised by little or no necrotic lesions. This gives complete protection to the plant against the invading pathogen. Such an approach has been used soybean rust resistance breeding and in rust pathosystems of autogamous crops such as beans, wheat, barley (Stavely *et al*., 1989; Mauro *et al*., 1999; Lillemo *et al*., 2006). However, such resistance is ephemeral since it is overcome by genetic changes in the virulence patterns caused by shifts in pathogen populations (McDonald and Linde, 2002; Arias *et al*., 2008; Pfender, 2009). To ensure the enhanced trait performance of race specific resistance, research of establishing effective gene and background combinations through diallel analysis and through pyramiding three single resistance genes need to be undertaken.

The durability of race specific resistance is known to be short since it is overcome by genetic changes in the pathogen populations which are subject to selection pressure when a resistant cultivar is deployed at a large scale. Single gene resistance, was the first method of choice in breeding for resistance to ASR in the Eastern Hemisphere where the disease has been endemic for a longer period of time. In Africa and South America single gene resistance has also been the major control strategy of soybean rust. However, some of the resistance gene sources have become vulnerable to newer, more aggressive soybean rust races (Hartman *et al*., 2004; Morel *et al*., 2008; Pham *et al*., 2009). The genetic plasticity of *P. pachyrhizi* pathogenicity factors makes breeding for durable resistance a challenge especially in areas where the disease has become endemic (Hartman *et al*., 2004; Yorinori, 2008; Twizeyimana *et al*., 2009). In addition, the pathogen have been found to possess extra virulent genes which make it to easily overcome single gene resistance deployment. The presence of such multiple virulence genes in *P. pachyrhizi* is unusual since no soybean line is known to naturally possess more than one specific resistance gene

(Tschanz *et al*., 1986; Shanmugasundaram *et al*., 2004). This dilemma has presented a great challenge to soybean breeding particularly the use of race specific resistance genes.

Despite the presence of different pathogen races in some areas, specific resistance genes are still useful in controlling soybean rust particularly in Africa and South America where soybean rust is a relatively new disease. Pathogen racial diversity is expected to be still lower hence some specific sources of resistance could be effective for a longer period of time (Kato and Yorinori, 2008). For example, in South Africa where rust was first observed in 2001, resistance genes R*pp2,* R*pp3,* R*pp4* were still effective against rust (Pretorius *et al*., 2007). In Nigeria, Twizeyimana *et al*. (2009) reported that *Rpp1* and *Rpp4* are still moderately effective. In Brazil, R*pp2* and R*pp4* are still effective against most rust pathogen races six years after the appearance of ASR (Laperuta *et al*., 2008; Garcia *et al*., 2008). Some single genes such as *Rpp4* have shown a lot of promise in sustaining resistance, for example, up to 20 years in Asia (Hartman *et al*., 2005 as cited by Gracia *et al*., 2008).

In some rust pathosystems such as in wheat, some qualitative resistance genes present in IIA clones have been effective against pathotypes 101, 102, 103 and 104 (Pfender, 2009). Yet in most ASR breeding programmes limited information is available on germplasm with acceptable levels of resistance to several races found in different geographical areas. Given the high virulence diversity within ASR isolates and the great influence of weather on disease epidemiology, soybean rust resistance response to deployed genotypes may vary across locations and seasons. In order to understand the effectiveness of various genotypes to dominant local populations of rust it is important that potential genotypes are tested in their targeted areas (Pham *et al*., 2010; Yamanaka *et al*., 2010). Accordingly, some attempts have been made to identify lines with broad resistance to field isolates in

different geographical areas of some countries such as in Japan, Nigeria and US (Twizeyimana *et al*., 2008; Yamanaka *et al*., 2010; Walker *et al*., 2011). However, this needs to be done across seasons (years) to adequately capture the effects of season (year) and identify repeatable patterns of variation due to genetic resistance that are crucial for breeding purposes.

Single genes are easier to work with as they can be moved into elite breeding stock through a backcrossing scheme in a relatively short time. Dealing with such resistance enables efficient selection in the early generations of breeding programmes and increases cost effectiveness due to ease of evaluation. The prospects of employing marker assisted selection for single resistance genes is high and it expedites the introgression process of the resistance genes into various genetic backgrounds (Garcia *et al*., 2008).

2.7 Managing ASR through breeding for tolerance

Tolerance in ASR disease management relates to the ability of a genotype to yield despite the presence of disease symptoms. Rust tolerant genotypes have been researched extensively in environments with high soybean rust incidence such as in East Asia (Shanmugasundaram *et al*., 2004). Consequently, breeding effort has also focused on selecting for tolerance as a means of sustaining soybean productivity (Jarvie, 2008). To exploit tolerance in breeding programmes, it has been debatable whether genotypes for use should be homozygous or heterozygous. According to Jarvie (2008) segregating progeny are ideally useful for selecting rust tolerance through repeated exposure and selection, such as environments where the genotypes are adapted and there is heavy disease pressure. The observed variation among soybean cultivars for tolerance to ASR provides a strong basis for improvement of soybean (Kawuki *et al*., 2003). Furthermore, Arias *et al*. (2008)

have suggested the existence of additive genetic control of tolerance to ASR suggesting possibility of selecting new soybean varieties with improved tolerance to rust. Initial early generation selections can be based ASR tolerance and on desirable agronomic traits such as pod and seed development. In later generations such as $F_{5,}$ selection can be based on number of fully filled pods without any abnormalities despite high rust severity (Tschanz and Wang, 1987). Tolerance provides a durable means of managing rust but has remained elusive due to its polygenic nature and requirement for extensive evaluations. In seeking tolerant genotypes, breeders have to contend with genotype by environment interactions due to seasonal variation of severity and occurrence of rust epidemics which further complicates the process of evaluation (Jarvie, 2009). Despite these challenges tolerance remains a desirable attribute in mitigating the adverse effects of ASR in areas with high pathogen diversity.

Tolerance has great potential since it is durable and does not promote selective reproduction of soybean rust thus curtailing pathogen evolution (Arias *et al*., 2008). Selection of accessions for tolerance involves a number of approaches, but the materials have to be evaluated in paired soybean plots with and without fungicide application in different seasons and locations (Kawuki *et al*., 2003; Jarvie, 2009; Oloka *et al*., 2009). Several formulae can be used to assess tolerance of soybean to rust. The percentage yield loss is calculated using the formula:

$$\text{Tolerance} = \frac{\text{Yield of rust protected plot} - \text{yield of unprotected plot}}{\text{Yield of rust protected plot}} \times 100$$

Rust tolerance index (RTI) is another approach used to quantify tolerance which is computed as:

$$\text{RTI} = \frac{\text{Yield of unprotected plots}}{\text{Yield of rust protected plots}}$$

The superiority measure (P_i) is calculated based on sprayed and unsprayed yields (Lin and Binns, 1988).

$$P_i = \sum_{j=1}^{n} (X_{ij} - M_j)^2/(2n)$$

Where n is the number of seasons, X_{ij} is the *i*th genotype yield in the *j*th season and M_j is the maximum yield response in the *j*th season. A superior genotype has the lowest (P_i) value.

2.8 Partial Resistance to Soybean Rust

Partial resistance or rate reducing resistance has also been identified as a means of controlling soybean rust through production of 'slow rusting' cultivars. Soybean lines with rate reducing resistance are classified as moderately resistant (MR) in field evaluations with relatively fewer lesions during the growing season (Miles *et al*., 2006). Partial disease resistance (PDR) in breeding for soybean rust resistance is characterised by a reduced rate of epidemic development in a host plant population attributed to various components. Partial resistance to ASR is measured in terms of infection frequency, longer latent period, smaller sized lesions and less spore production per uredinia (Bonde *et al*., 2006; Arias *et al*., 2008).

When breeding for ASR resistance, a long latent period is desirable as the pathogen has a lower capacity to produce secondary infection. In case of ASR, the number of spores

resulting in reproducing infection measured per plant, per leaf or per cm^2 of tissue contributes to partial resistance of a genotype (Bonde *et al*., 2006). The major attributes of this form of resistance are its polygenic control, which is effective against a broad spectrum of pathogen races (Long *et al*., 2006; Ribeiro *et al*., 2009). It also acts by reducing the amount of secondary inoculum in development of a polycyclic disease like ASR (Marchetti *et al*., 1975). Though potentially beneficial, the utilisation of this form of resistance is not commonly applied in breeding for resistance to the long time and difficulty in its evaluation during the breeding process (Hartman *et al*., 2005; Bandyopadhyay *et al*., 2007).

2.9 Breeding for soybean rust resistance in Uganda: Successes, Challenges and Opportunities

Several studies on soybean resistance have been undertaken to find effective means of controlling ASR in Uganda. It was not until 2004 that two varieties Maksoy 1N and Namsoy 4M with specific gene resistance were released by Makerere University and National Crops Resources Research Institute (NaCRRI) (Tukamuhabwa *et al*., 2009). After their release significant grain losses to ASR were prevented. However, like in many cases where single gene resistance is deployed, Maksoy 1N and Namsoy 4M have shown increasing disease severity (Tukamuhabwa *et al*., 2009). This could be as a result of pathogen evolution as the area under soybean cultivation increases.

On the other hand, Maksoy 2N tolerant to ASR that was released in 2008 is still under cultivation and a resistant line Maksoy 3N released in 2010 and showing resistance stability in various geographical areas. Maksoy 1N is a breeding line TGX 1835-10E (TGx 1213-1D x TGx 1445-3E) from IITA Nigeria. Namsoy 4M is derived from NamII x GC00138-29 whereas Maksoy 2N and Maksoy 3N are derived from the cross TGx 1835-

10E x Duiker and Duiker x GC00138-29 respectively (Kiryowa, 2007; Tukamuhabwa *et al*., 2011). Despite these significant milestones reached by the soybean programme there is clearly a need to diversify the sources of resistance to effectively manage rust. Most of the resistant varieties released so far share common parentage suggesting that the entire germplasm is subject to genetic vulnerability (Tukamuhabwa *et al*., 2009). Other sources of resistance have been identified and there is need to evaluate them under local conditions to assess their effectiveness against existing soybean rust races.

In seeking for diversification of resistance genes, knowledge of the underlying genetics of potential sources of resistance needs to be deciphered and understood to facilitate rationalisation of the resistance breeding process and prevent over deployment of the same gene locus. Many avenues for breeding for resistance to ASR remain untapped by the local breeding programme. One example is the use of molecular breeding techniques such as Marker Assisted Selection (MAS) to pyramid several resistance genes into a single genetic background. Assembling multiple resistance genes into a single genotype has been suggested as a means of enhancing resistance and overcoming resistance instability due to single genes (Yamanaka *et al*., 2010). If suitable sources of resistance are identified and markers for the resistance gene are available, rapid introgression of the gene can be done through MAS. This can also facilitate pre-breeding which is increasingly becoming important in plant breeding with certain species of *Glycine* known to have resistance genes. Commercial soybean breeding deals with elite x elite crosses hence single seed descent is the most widely used breeding method (Jarvie, 2008). Most of the resistance gene sources have been identified in the Orient suggesting narrow adaptability. Thus it is important that soybean rust improvement programmes assess their breeding potential with adapted and farmer preferred varieties that are locally available.

Understanding the pathogen diversity has also been explored as a means of understanding ASR. During the period of 2003-2004 ASR was observed to consist of one dominant race and two minor races (Lamo, 2004). However, the scenario is likely to have changed as most resistant varieties show increasing disease severity. Identifying soybean lines resistant to all races of soybean rust is a great challenge. Nonetheless, the soybean breeding programme endeavours to identify sources of resistance with acceptable levels of resistance in the main soybean producing geographical areas given the great environmental diversity present in the country. Accordingly, these knowledge gaps and untapped areas presented an opportunity for this study to be undertaken with a view to enhancing genetic resistance to soybean rust.

2.10 Combining ability and G × E in soybean breeding

In any crop breeding programme the choice of parents for use in hybridisation is crucial if significant gains are to be realised. Genetic parameters such as combining ability are important to identify candidate parents for use in routine breeding to produce rewarding germplasm (Ribeiro *et al*., 2009; Iqbal *et al*., 2010). This is important since selection based on performance alone does not provide clear information on the breeding potential of a genotype. Diallel analysis is one of the most widely used methods for assessing parental combinations for use in a systematic breeding programme. General combining ability estimates are particularly important for a self pollinated crop (Nkalubo *et al*., 2009) like soybean where the final product is a pure-line and hybrid development is not economically viable. General combining ability is associated with additive gene effects whereas specific combining ability is indicative of genes having dominance and epistatic effects (non-additive gene effects).

In soybean hybridisation it is difficult to obtain sufficient F_1 seed hence most analyses use F_2 or later generation seed. Moreover, it has been reported that F_2/F_3 generations give better predictions on the combining ability of lines due to the dissipation of dominance gene effects (Bhullar *et al*., 1979). However, residual heterosis in the F_2 may inflate combining ability estimates and therefore falsify genetic gains that can be made in the later generations (Mukankusi-Mugisha, 2008). Therefore later generations can be used which give better estimates of the underlying genetic parameters.

Most studies undertaking combining ability analysis evaluate genotypes in one location which tends to underestimate the environmental variance component for the trait under investigation. This might result in misleading breeding results as it tends to imply low environmental and high genetic variance influence. When a diallel is evaluated across several environments consistent information on repeatability of the estimates over environments is obtained (Patil and Chopde, 1981; Ribeiro *et al*., 2009). This also gives a better estimate of the environmental contribution which is important in soybean rust whose expression is strongly influenced by the environment. The presence of genotype-by-environment interaction therefore necessitates evaluation of genotypes in many environments to determine their true genetic potential and broaden the scope of combining ability studies (Bhullar et al., 1979).

2.11 Gene pyramiding in plant breeding

Gene pyramiding in plants aims to assemble multiple favourable genes from multiple parents into a single genotype (Servin, 2004). There are several reasons why gene pyramiding is undertaken which include i) enhancing the performance of a given trait ii) broadening the genetic basis of released cultivars iii) introgressing genes into a deficient cultivar and iv) increasing durability of the resistance (Ye and Smith, 2008).

Sustenance of resistance is important during the breeding process for any particular pathogen. When single gene resistance is used against any pathogen it is highly vulnerable to changes in pathogen population (Sanghai-Maroof *et al*., 2008). However, given the relative ease of detection and introgression into susceptible backgrounds, single gene resistance will continue to play a role in soybean rust resistance breeding (Tukamuhabwa and Maphosa, 2011). Although these major genes cause resistance instability, strategies for enhancing durability and effectiveness of major genes have been suggested (Hittalmani *et al*., 2000). An option for extending the effectiveness of resistance genes is to pyramid genes conferring resistance to different races of the pathogen within the same genotype. In Taiwan and most parts of Asia where ASR has been endemic for a long time resistance genes such as *Rpp1* has been ineffective in the management of rust (Wang and Hartman, 1992). However, in some places other sources of resistance are still effective such as R*pp2* and R*pp4* in Brazil (Garcia *et al*., 2008). Given that each of the known resistance genes is effective against a given pathogen range, combining these genes into one background could broaden the resistance spectra.

The availability of molecular markers tightly linked to resistance genes R*pp1*, R*pp2*, R*pp3*, R*pp4* and R*pp5* implies that marker assisted gene introgression into different backgrounds is feasible. This is a desirable approach to gene pyramiding as conventional methods require extensive screening with several races which complicates the effort (Semagn *et al*., 2006; Sanghai-Maroof *et al*., 2008). Introgression of the resistance genes using MAS can be done even in the absence of the pathogen. Epistatic and masking gene effects make combining several genes without molecular markers to trace them a big challenge. In soybean such an approach of pyramiding resistance genes has been suggested as ASR displays high diversity (Hartman *et al*., 2005; Garcia *et al*., 2008; Yamanaka *et al*., 2010). Recently, Lemos *et al*. (2011) pyramided three resistance genes

R*pp2,* R*pp4* and R*pp5* into one genetic background using molecular markers and observed increased disease resistance based on various resistance parameters against a Brazilian soybean rust isolate 'BRP-2'. However, limited effort has been put in trying to explore this avenue of managing the devastating effects of ASR despite the availability of tightly dense molecular linkage map (Song *et al*., 2004). In other crop breeding programmes such as in rice breeding for *Xanthomonas oryzae* pv *oryzae*, barley breeding for stem rust, soybean breeding for soybean mosaic virus, MAS has been hailed as promising (Hittalmani *et al*., 2000; Singh *et al*., 2001; Sanghai-Maroof *et al*., 2008).

In view of the available information from the subject matter, ASR is a major soybean constraint that requires urgent attention. No research has been undertaken to understand how early generation segregating soybean populations respond to field isolates from different areas of Uganda. Understanding the response to ASR of early generation progeny will enable predictions to be made about parental breeding values thereby increasing efficiency of soybean breeding programmes. Prospects of enhancing genetic resistance of local soybean rust populations through marker assisted gene pyramiding have not been explored despite the availability of a dense molecular map for soybean. Such an effort could lead to more resistant genotypes and broaden the rust resistance genes in the available breeding lines. Ultimately, knowledge generated from these studies will stimulate further knowledge development and breeding towards the management of soybean rust disease both locally and internationally.

CHAPTER THREE

ASSESSMENT OF COMPARATIVE VIRULENCE AND RESISTANCE IN SOYBEAN USING FIELD ISOLATES OF SOYBEAN RUST

3.1 Introduction

Asian soybean rust (*Phakopsora pachyrhizi* Sydow and Sydow) is a major threat to soybean production worldwide. The pathogen is an obligate parasite that causes multi-cyclic infections during the growing season. The uredinal stage produces urediniospores responsible for disease development and subsequent yield losses are due to premature defoliation and reduction in photosynthesis that adversely affects grain filling (Miles *et al*., 2003). Since the disease was first detected in Japan, researchers have focused their efforts on several ways to manage it. Eradication or elimination efforts of the source of soybean rust inoculum are unlikely to succeed due to the wide host range including legumes which are an integral part of most cropping systems (Miles et al., 2007; Slaminko et al., 2008; Goellner et al., 2010; Hassanpanah, 2010). Chemical control, though effective, poses a greater challenge since its effectiveness depends on frequent symptom monitoring and timely routine fungicide application (Yorinori *et al*., 2005). Genetic resistance is currently the most economic and strategically important means of managing rust soybean being pursued by several soybean breeding programmes (Arias *et al*., 2008). Resistance to rust is conferred mainly by R genes (major genes) and is dependent on specific prevalent soybean rust isolates. Major resistance genes R*pp*1, R*pp2*, R*pp3*, R*pp4*, R*pp5* and R*pp?* (Hyuuga), have been identified to show resistance to specific races of soybean rust (Bromfield and Hartwig 1980; Garcia *et al*. 2008; Hartwig 1986, McLean and Byth 1980; Monteros *et al*. 2007).

Several studies investigating the effectiveness of single soybean rust resistance genes have used single spore isolates with evaluations done under controlled conditions at seedling

stage (Li, 2009; Paul and Hartman, 2009; Twizeyimana *et al*., 2009). However, in nature the soybean rust pathogens often exist as mixtures with either homogeneous or heterogeneous virulence among sub-populations with varying aggressiveness. Single spore isolation does not always capture the variability present within a field isolate (Freire *et al*., 2008; Paul and Hartman, 2009). Given the high virulence diversity within and among *P. pachyrhizi* isolates, use of single pure isolates in determining soybean rust resistance will not necessarily allow extrapolation to the field level. Moreover, differences have been observed in the capacity of soybean to express resistance depending on the stage of growth and environment. Seedling evaluation for soybean rust resistance does not always guarantee adult plant resistance (Ribeiro *et al*., 2007; Miles *et al*., 2008). Seedling resistance is, however, still important given that soybean rust attacks soybean at any phenological stage of development. Having both seedling and adult plant resistance guarantees complete protection of soybean irrespective of the time when the disease manifests.

In Uganda, previous race characterisation studies using race differentials and molecular analysis showed low racial diversity with three races found in most soybean growing areas (Lamo, 2004). However, the scenario is likely to have changed since two cultivars with specific resistance deployed in 2005 have shown increased susceptibility to soybean rust (Tukamuhabwa *et al*. 2009). In addition, no cultivar in Uganda utilises any of the available characterised classical resistance genes. The effective use of these exotic gene sources depends on knowledge of how they respond to local soybean rust populations. Differential response of the host genotypes to local rust populations can further help understand the virulence patterns of Asian soybean rust in Uganda. This information would help facilitate utilisation and development of the stable host resistance. Therefore the objectives of this research were to i) assess comparative soybean rust virulence patterns of natural

pathogen populations in the different major soybean growing areas and ii) identify soybean lines with stable seedling and adult stage resistance when challenged by geographically diverse field isolates.

3.2 Materials and Methods

3.2.1 Soybean lines

Twelve soybean lines including two susceptible checks (Wondersoya and Nam 2) were used in this study. Their pedigree, origin and source are presented in Table 1. Sources of resistance PI 230970 (R*pp2*), Ankur (R*pp3*), PI 459025 (R*pp4*), G00138-29, Maksoy 1N were previously characterised for rust resistance for three seasons at Namulonge (Oloka *et al*., 2008).

Table 1 A set of 12 putative differential soybean lines used to assess resistance to five field isolates using seedling and adult plants

No	Genotype	Pedigree	Reason for selection	Origin	Source
1	PI230970	G8586	Resistance gene (R*pp2*)	Japan	AVRDC, Taiwan
2	Ankur	G7955	Resistance gene (*Rpp3*)	India	AVRDC, Taiwan
3	PI459025	G10428	Resistance gene (*Rpp4*)	China	AVRDC, Taiwan
4	PI 459024	G10427	Resistant	China	AVRDC, Taiwan
5	G00138-29	(CH#1 x Anoka) x (Clarke 63 x 64.4)	Resistant	China	AVRDC, Taiwan
6	UG 5	-	Resistant	Uganda	MAK, Uganda
7	Maksoy 1N	TGx1835-10E	Resistant	Nigeria	IITA, Nigeria
8	Maksoy 2N	Duiker x GC00138-29	Resistant	Uganda	MAK, Uganda
9	Maksoy 3N	Duiker x TGx 1835-10E	Resistant	Uganda	MAK, Uganda
10	Namsoy 4M	Nam 2 x GC00138-29	Resistant	Uganda	NARO, Uganda
11	Nam 2	87D-668	Susceptible	Nigeria	NARO, Uganda
12	Wondersoya	-	Susceptible	Nigeria	IITA, Nigeria

NARO- National Agricultural Research Organisation- Uganda; MAK- Makerere University; IITA-International Institute of Tropical Agriculture; AVRDC- World Vegetable Centre

In addition, two high potential materials with resistance genes PI 459024, UG 5; three released cultivars Maksoy 2N, Namsoy 4M and Maksoy 3N were included. This set of soybean lines was also selected on the basis of a relatively similar growth cycle under the local field conditions to reduce the effect of crop phenology on disease severity during evaluation.

3.2.2 Field Procedures for assessment of Adult Plant Resistance

The layout for the field experiment was a randomised complete block design with three replications at the five sites of Makerere University Agricultural Research Institute-Kabanyolo (MUARIK), National Crops Resources Research Institute (NaCRRI), Iki-Iki (IKI), Nakabango (NAK) and Kasese (KAS) in 2010 and 2011 leading to 10 season-location environments. These sites represent areas of high soybean production in Uganda with endemic seasonal soybean rust epidemics. At each site each entry was sown at the same time with 25-30 seeds in 2 metre rows replicated three times. Entries were randomised across sites within a given year with a 60 cm x 5 cm inter- and intra-row spacing. Spreader rows of a susceptible cultivar Nam 2 were planted at the same time after every five rows around each replicate.

3.2.3 Field data collection and analyses

Reaction types and sporulation were evaluated at the R6 stage (Fehr *et al*., 1971) when symptoms were clearly seen on susceptible checks using three trifoliate leaves of the mid-canopy. Disease reaction types were recorded as Red Brown (RB), Tan (T) and Mixed (MX). Red brown lesion colour was considered a resistant reaction whereas tan lesions expressed susceptibility (Miles *et al*. 2003). Mixed reactions had both red brown and tan lesion of the same leaf or different plants of the same soybean line. Field sporulation levels were rated on a 1 to 5 scale (where 1= no sporulation, 2=sporulation less than 25%

of full sporulating lesion, 3=50% sporulation of a fully sporulating tan lesion, 4=sporulation 75% of a fully sporulating tan lesion and 5= sporulation comparable to fully sporulating tan lesion) (Miles et al., 2008) using the susceptible cultivar Wondersoya as a control. Severity scale was based on 1 to 9 scale where 1- no lesions; 2= 1-30; 3= 31-75; 4= 76-150; 5= 151-300; 6= 301-750; 7= 751-1500; 8= 1501-3000 and 9= >3000 lesions (Miles *et al*., 2008). Prior to analysis severity and sporulation scores were subjected to transformation by square root and arcsine methods respectively to normalise them. Back transformed data was presented as the final results. All analyses were done using GENSTAT software 13th Edition and means compared using standard error (Payne *et al*. 2010). Interactions between lines and isolates for sporulation and disease severity were analysed using ANOVA.

3.2.4 Screen house Procedures for assessment of Seedling Resistance

Two sets of three plants for each of the twelve genotypes were grown in wide trays in the screen house for inoculation in a split plot design. The main plot factor was the five isolates and subplot factor genotype. Using a handheld Liliput® vacuum composite soybean rust field isolates were harvested in June 2011 from random soybean leaves at the R6 stage at each of the five sites (used for the field trial). Isolates from NAK, MUARIK, IKI were inoculated on the same day and those from KAS and NaCRRI a week later. 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 millimetre using a Neubauer haemocytometer. Prior to inoculating each set of entries, germination ability of the spores was tested on water agar to ensure infectivity. In each set, three trifoliates one from each plant were artificially inoculated with 1.5 ml of spore suspension on the abaxial leaf surface using a Canyon® (Model 5A, England) hand sprayer. After inoculation plants were covered with polythene

bags for 24 hours at 22^0C-24^0C to maintain high relative humidity necessary for infection. Trays containing plants with each isolates were spatially separated within the screen house to avoid any possibility of cross contamination. After 24 hours polythene bags were removed for the duration of the experiment.

3.2.5 Screen house data collection and analysis

Using ×20 magnification lenses, the soybean lines were monitored for lesion colour, incubation period and latent period. Days to appearance of symptoms from inoculation day were recorded as incubation period and days to urediniospore production as latent period. Lesion density (cm^{-2}) and the frequency of sporulating lesions were recorded after 16 days from the middle leaflet and analysed using analysis of variance, means were compared using standard error. Starting at seven days after inoculation, data on lesion density were collected four times at three day intervals (up to 16 days) to plot the areas under disease progress curve (AUDPC) using the following formula:

$$\text{AUDPC} = \sum_{i=1}^{k} 1/2\,[(s_i + s_{i+1})(t_{i+1} - t_i)]$$

Where s_i is the rust severity at time *i*, t_i is the number of days after the first observation on assessment date *i* and *k* is the number of successive observations (Campbell and Madden 1990).

3.3 Results

3.3.1 Reaction type

Based on reaction types, soybean lines responded differentially to the five field isolates during screen house inoculation (Table 2). Soybean lines PI 230970 (R*pp2*), Nam 2 and Wondersoya produced consistently tan lesions typical of a susceptible reaction in all locations. However, the two classical resistance gene sources Ankur (R*pp3*) and PI 459025 (R*pp4*), local genotypes UG 5 and Maksoy 3N responded with a red brown

phenotype at all locations indicating presence of resistance genes. Mixed reaction responses were observed between lines PI 459024, GC00138-29, Maksoy 1N and Maksoy 2N in all the five field isolates tested. All field isolates showed at least one mixed reaction in the twelve soybean lines evaluated. Immune response was not observed in any genotype.

Table 2 Seedling reaction types shown by 12 soybean lines against Ugandan field rust pathogen populations

Source of Isolate	PI 230970 (Rpp2)	Ankur (Rpp3)	PI 459025 (Rpp4)	PI 459024	UG 5	GC00138-29	Namsoy 4M	Maksoy 1N	Maksoy 2N	Maksoy 3N	Nam 2	Wondersoya
MUARIK	T	RB	RB	MX	RB	MX	MX	MX	T	RB	T	T
NAK	T	RB	RB	RB	RB	RB	MX	T	T	RB	T	T
IKI	T	RB	RB	RB	RB	RB	MX	MX	T	RB	T	T
NaCRRI	T	RB	RB	RB	RB	MX	MX	MX	MX	RB	T	T
KAS	T	RB	RB	RB	RB	RB	MX	T	MX	RB	T	T

T-Tan, RB red brown, MX-mixed

3.3.2 Severity and sporulation

In the screen house experiment, analysis of variance indicated that there were significant isolate, genotype and isolate x genotype differences for severity and sporulation for seedling resistance. However, differences in the isolates had the strongest effects for disease severity whereas genotype had the strongest effects on sporulation rate (Table 3). In the field evaluations for adult plant resistance, all sources of variation were significant with isolate, genotype and isolate x year (season) contributing largely to the differences in severity and sporulation. The year (season) and isolate x year (season) effects were, however, more pronounced for severity than sporulation.

Table 3 Analysis of variance of soybean rust severity and sporulation of 12 soybean lines against five field isolates at the seedling and adult plant stage

Source of variance	df	Mean Squares		Mean Squares	
		Severity	Probability	Sporulation frequency	Probability
Seedling resistance					
Sets	1	1.37	0.130	0.04	0.351
Isolate	4	14.44	<.001	0.16	0.015
Isolate x Sets	4	0.54	0.456	0.03	0.685
Genotype	11	2.44	<.001	2.86	<.001
Isolate x Genotype	44	1.19	0.006	0.13	<.001
Error	59	0.58		0.05	
Adult plant resistance					
Year (season)	1	1.43	<.001	0.63	0.001
Isolate[a]	4	0.86	<.001	1.90	<.001
Isolate x Year (season)	4	2.18	<.001	1.11	<.001
Rep [Isolate x Year (season)]	20	0.02	0.569	0.05	0.930
Genotype	11	2.00	<.049	2.52	<.001
Isolate x Genotype	44	0.20	<.001	0.32	<.001
Genotype x Year (season)	11	0.35	0.968	0.32	0.977
Isolate x Year (season) x Genotype	44	0.19	<.001	0.15	<.001
Pooled Error	160	0.03		0.06	

[a] For adult plant resistance use of the term 'isolate' refers to the location where the field evaluation was done

In the field, the overall mean severity score was greater in 2010 with more soybean lines producing mixed reaction across test locations than in 2011. However, in 2011 IKI had the lowest mean severity score which was 3.2 less than previous year (Table 4). Susceptible lines Nam 2 and Wondersoya had severity scores consistently greater than the location means with predominantly tan and mixed reaction types.

Table 4. Severity of 12 soybean lines in the screen house for seedling resistance using Ugandan field isolates from five different sites

Location	Field, 2010					Field, 2011				
	IKI	KAS	MUARIK	NaCRRI	NAK	IKI	KAS	MUARIK	NaCRRI	NAK
Entry										
PI 230970 (R*pp2*)	6.0(RB)	5.3(RB)	3.3(RB)	3.3(RB)	3.3(RB)	2.0(RB)	3.6(RB)	3.3(MX)	3.6(T)	4.0(T)
Ankur (R*pp3*)	6.0(RB)	4.0(RB)	2.3(RB)	2.0(RB)	3.0(RB)	2.0(RB)	2.3(RB)	2.0(RB)	2.6(RB)	3.0(RB)
PI 459025 (R*pp4*)	5.6(RB)	4.0(RB)	2.3(RB)	1.0(RB)	2.0(RB)	3.0(RB)	4.0(RB)	3.3(RB)	2.0(RB)	2.0(RB)
PI 459024	4.0(RB)	5.3(MX)	3.0(RB)	3.3(RB)	3.3(RB)	4.0(MX)	5.3(RB)	2.0(RB)	2.0(RB)	4.0(MX)
GC00138-29	4.0(RB)	3.0(MX)	2.3(RB)	2.0(RB)	2.3(RB)	2.3(RB)	4.0(RB)	3.3(RB)	2.0(RB)	4.0(MX)
UG 5	5.0(RB)	2.6(MX)	2.0(RB)	1.0(RB)	2.3(RB)	2.0(RB)	2.6(RB)	2.6(RB)	4.0(RB)	2.0(RB)
Maksoy 1N	6.6(RB)	5.0(T)	6.0(RB)	5.6(MX)	5.0(T)	2.0(RB)	4.0(RB)	7.3(RB)	3.3(RB)	2.6(RB)
Maksoy 2N	5.6(RB)	6.6(MX)	3.3(RB)	3.6(MX)	4.6(RB)	2.6(MX)	2.0(RB)	6.6(RB)	4.3(T)	4.0(T)
Maksoy 3N	5.0(RB)	3.6(RB)	2.6(RB)	3.0(RB)	3.3(RB)	2.0(RB)	2.0(RB)	2.6(RB)	2.0(RB)	2.0(RB)
Namsoy4M	6.3(RB)	5.3(MX)	4.6(MX)	4.6(MX)	4.3(RB)	3.0(RB)	4.0(MX)	5.3(MX)	4.6(RB)	3.3(RB)
Nam 2	7.6(RB)	4.6(MX)	6.3(MX)	6.6(MX)	6.0(MX)	2.0(RB)	5.6(MX)	6.6(T)	6.6(T)	4.3(T)
Wondersoya	6.6(MX)	8.3(T)	6.3(MX)	5.6(MX)	6.0(MX)	5.0(MX)	7.3(T)	6.6(T)	4.6(T)	5.3(T)
Mean	5.7	4.8	3.7	3.5	3.8	2.7	3.9	4.3	3.5	3.4
SE±	0.29	0.45	0.48	0.53	0.40	0.28	0.46	0.58	0.41	0.29
LSD (0.05)	0.46	0.84	0.96	0.67	0.90	0.49	0.79	1.20	0.79	0.50

RB-red brown; MX-mixed; T-tan, SE±-standard error of the mean

Conversely, Ankur (R*pp3*), PI 459025 (R*pp4*) and Maksoy 3N had red brown lesions across locations with mean severity generally lower than the mean of the locations. There was no relationship between lesion colour and severity during the two seasons of evaluation in the five test locations.

In the screen house experiments, KAS isolate had the greatest mean sporulation frequency per square centimetre (Table 5), 11.1% more than the least sporulating isolate from NAK. Isolates from KAS and NaCRRI resulted in sporulation in all soybean lines whereas NAK did not show sporulation in three lines (Table 5).

Table 5 Mean sporulation frequency of 12 soybean lines in the screen house for seedling resistance experiment using Ugandan field isolates from five different sites

	Percentage sporulation frequency of isolate				
Genotype	IKI	KAS	MUARIK	NaCRRI	NAK
PI230970 (R*pp2*)	100.00	89.99	100	85.06	95.66
Ankur (R*pp3*)	73.58	38.46	0.00	1.31	13.39
PI 459025(R*pp4*)	31.50	30.76	10.87	39.53	10.19
PI 459024	83.97	47.86	80.38	11.31	35.47
GC00138-29	0.00	45.96	0.00	16.92	0.00
UG 5	0.00	13.21	42.48	18.45	0.00
Maksoy 1N	100	93.98	99.00	83.75	100
Maksoy 2N	100	94.49	100	98.98	100
Maksoy 3N	2.86	18.45	8.71	11.69	0.00
Namsoy4M	37.01	80.54	100	58.91	67.20
Nam 2	85.34	96.25	100.00	99.49	94.31
Wondersoya	100.00	99.49	100.00	97.94	100.00
Mean	59.52	62.45	61.78	51.94	51.35
SE±	12.18	9.55	13.04	11.38	13.04
LSD(0.05)	25.17	20.68	24.98	21.45	27.01

SE± standard error of the mean

Analysis of variance indicated significant ($P \leq 0.01$; ANOVA not shown) differences in incubation and latent period, lesion density, percentage frequency of sporulating lesions and AUDPC for lesion density of lines under screen house conditions. In general, most local cultivars showed longer latent periods that the exotic lines in response to the five

isolates tested (Table 6). Namsoy 4M had typically the longest latent period compared to PI 230970 (R*pp2*) and Maksoy 3N with the least. Despite the long latent period of Namsoy 4M, lesions density was the third highest in all the test lines. Contrary, Maksoy 3N had the shortest latent period and lowest number lesions per square centimetre, 6.4 lower than the overall mean. PI 230970 (R*pp2*) with a classical resistance gene and Maksoy 2N were equally highly sporulating and comparable to the susceptible check lines. Maksoy 3N, GC00138-29 and UG 5 had light sporulation with less than 15% of the uredinia sporulating per square centimetre. Maksoy 2N had the highest AUDPC followed by Wondersoya which differed significantly from all the exotic sources of resistance and cultivar Maksoy 3N (Table 6).

Table 6 Summary of soybean rust infection means across five Ugandan field isolates

Genotype	Incubation period (IP)	Latent Period (LP)	Number of lesions/square centimetre (LS)	Frequency of sporulating lesions (FS)	AUDPC
PI 230970 (R*pp2*)	5.2	7.2	22.8	94.1	57.7
Ankur (R*pp3*)	4.8	7.6	21.1	25.4	52.2
PI 459025 (R*pp4*)	4.6	7.5	21.9	24.6	55.7
PI 459024	5.1	9.1	19.8	51.8	44.3
GC00138-29	5.7	7.8	21.2	12.6	49.0
UG 5	5.2	8.2	21.7	14.8	56.3
Maksoy 1N	4.8	9.8	29.7	95.3	74.9
Maksoy 2N	5.1	10.2	37.0	98.9	85.8
Maksoy 3N	5.2	7.2	19.5	8.3	46.8
Namsoy4M	4.9	10.4	31.7	68.7	70.0
Nam 2	4.9	10.2	30.6	95.1	78.5
Wondersoya	5.1	9.9	33.7	99.5	81.4
Mean	4.98	8.75	25.9	57.4	62.7
SE±	0.08	0.37	1.78	11.1	4.21
LSD	0.32	0.81	3.38	24.01	9.10

SE± standard error of the mean; AUDPC-Area under disease progress curve

3.3.3 Correlations among soybean rust infection parameters

Using genotype averages across isolates a significant ($P \leq 0.001$) positive correlation was observed between percentage frequency of sporulating lesions and AUDPC (Table 7). Similarly, latent period and number of lesions density were significantly positively correlated with AUDPC. It was noted that incubation period had a negative non-significant correlation with other soybean rust resistance parameters evaluated. Mean disease severity of the adult soybean lines for the two years and seedling lesion density were positively correlated ($r=0.813$, $p<0.001$) (data not shown).

Table 7 Correlations among infection parameters evaluated during the seedling stage using five diverse field isolates

	IP	LP	LS	FS
LP	-0.224ns			
LS	-0.209ns	0.857***		
FS	-0.232ns	0.702**	0.799**	
AUDPC	-0.279ns	0.801**	0.972***	0.819***

***$P \leq 0.001$; **$P \leq 0.01$; ns-not significant; IP-Incubation Period; LP-Latent period; LS-Number of lesions per square centimetre; FS-F frequency of sporulation lesions; AUDPC-Area under disease progress curve

3.4 Discussion

The knowledge of rust virulence in soybean rust populations and how soybean lines react to field isolates in different regions is important for successful breeding and deployment of resistance genes (Miles *et al*., 2011). The reaction types obtained from the five isolates may indicate that each isolate is distinct which is suggestive of the existence of different race populations or virulence patterns (Yamanaka *et al*. 2010). It was, however, surprising that PI 230970 (R*pp2*) with a classical resistance produced tan lesions. This genotype was recommended for inclusion in the local germplasm after evaluations between 2005 and

2006 at NaCRRI in Uganda (Oloka *et al*., 2008). This could suggest resistance breakdown and underscores the importance of evaluating for resistance in the target geographic locations due to the differences in diversity and virulence of the rust pathogen.

Mixed lesions were observed on at least one genotype in all locations, which is indicative of a mixture of races with heterogeneous virulence (Miles *et al*., 2008). This was, however, more pronounced in MUARIK and NaCRRI which have the largest area of different experimental soybean lines every year. Increased virulence diversity could be an evolutionary consequence prompted by deployment of a wide assortment of lines in these two locations.

The significance of isolate-by-genotype interaction for severity and sporulation frequency implies that ranking of lines changes markedly with isolates. This presents a great challenge when breeding for resistance using specific gene resistance to manage soybean rust due to great differences exhibited by the lines. Furthermore, this underscores the importance of evaluating candidate lines using rust populations present in the target areas. In the field, the isolate x year (season) had substantial impact to disease severity greater than sporulation (Table 3). Though the two resistance indices are not completely independent of each other this could suggest greater effect of environment on severity.

This study purposefully used field isolates to understand comparative virulence and identify stable sources of soybean rust resistance. The greater preponderance of mixed reactions in the field attributed to heterogeneous race composition coupled with environmental factors that influenced the amount of inoculum and disease progress during the seasons (Miles *et al*. 2008; Miles *et al*. 2007). However, the significance of isolate, genotype and isolate x genotype interaction factors using severity and sporulation rate

indices (Table 3) and strong positive correlation of disease severity and lesion density both during seedling and adult plant stages suggests that these are related. Similarly, isolates from KAS, MUARIK and IKI were the most aggressive in both mean seedling and adult plant assessments compared to those from NaCRRI and NAK. The seedling resistance tests using field isolates can therefore be used to extrapolate resistance under field conditions with better accuracy. On the contrary, seedling and adult plant were observed not to be necessarily correlated during resistance evaluations in Brazil (Ribeiro *et al*., 2007). Differences obtained in seedling and adult plant resistance done in the US and Paraguay respectively were attributed to differences in virulence of the isolates used and longer, multiple cycles of exposure in the field (Miles *et al*., 2008). Our study, however, used the same field isolate of similar composition and virulence for both seedling and adult plant resistance hence the comparable results.

The positive correlation between AUDPC and sporulation relate to rapid advance of the disease caused by increased sporulation which results in more secondary infections. It was also observed that AUDPC was directly related to lesion number which is an important disease resistance index. A positive correlation between latent period and AUPDC suggested that lines that expressed disease urediniospores early have slower disease progress. This could imply that the resistance mechanism present in these lines responds rapidly once the rust pathogen is established in the host cells compared to those with a longer latent period.

3.5 Conclusions

Overall, the results indicate that soybean rust breeding programmes utilising specific resistance are challenged due to the restricted locations for which such resistance applies. The longevity of R*pp 2* was about 5 years, which is relatively short and further limits specific resistance. Great soybean rust diversity was observed in sites which grown several varieties every season. Latent period, lesion density and proportion of sporulating lesions are important disease resistance parameters that can be used to extrapolate disease progress. Maksoy 3N, UG5, G7955 and GC00138-29 are ideal sources resistance to soybean rust that can be used across the selected sites. Prospecting and exploration for other sources of resistance and strategies such as partial resistance and tolerance is highly recommended.

CHAPTER FOUR

COMBINING ABILITY FOR RESISTANCE TO SOYBEAN RUST IN F_2 AND F_3 SOYBEAN POPULATIONS

4.1 Introduction

Soybean rust (*Phakopsora pachyrhizi* Syd) is a very important disease in many soybean growing areas causing losses of up to 75% in unprotected fields under heavy infestation (Yorinori *et al*., 2005). In Africa, since the disease was detected in Uganda in 1996, it has become endemic and the most important threat to soybean production (Kawuki *et al*., 2003). Unlike other rust pathogens with a narrow host range, soybean rust (SBR) has more than 100 hosts including most cultivated legumes like cowpea (*Vigna unguiculata* L.), pigeon pea (*Cajunus cajan* L) and common bean (*Phaseolus vulgaris* L) (Slaminko *et al*., 2008). Most of these legumes form an integral part of soybean cropping systems, making rust management a great challenge.

Genetic resistance is the most economic and strategically important means of reducing yield losses due to soybean rust compared to chemical control. Several specific and partially resistant sources of soybean rust resistance have been identified in various germplasm collections. Genes R*pp1*, R*pp2*, R*pp3*, R*pp4*, R*pp5* and R*pp?(Hyuuga)* with resistance to specific rust races have been identified and mapped to different linkage groups (Hyten *et al*., 2007; Monteros *et al*., 2007; Garcia *et al*., 2008). The effectiveness of these resistance genes depends on the prevalent rust races in a particular location. For example, R*pp2* and R*pp4* were effective in Brazil (Kato and Yorinori, 2008); R*pp2*, R*pp3* and R*pp4* in South Africa (Pretorius *et al*., 2007); R*pp2* and R*pp3* in Uganda (Oloka *et al*., 2008) and R*pp1* and R*pp4* in Nigeria (Twizeyimana *et al*., 2009). This therefore implies that there is no universally acceptable resistant genotype.

Several other unnamed sources of specific gene resistance exist such as UG5, FT2, GC00138-29 are effective in various parts of the world (Kawuki *et al*., 2003; Laperuta *et al*., 2008). In addition other sources of germplasm utilise partial resistance characterised by a semi-compatible reaction with a range of red brown phenotypic manifestations with reduced sporulation and lesion density (Bonde *et al*., 2006; Jarvie, 2009; Walker *et al*., 2011). However, most of the resistant sources maybe un-adapted to the environment for which the disease resistance trait is sought. Thus prior to incorporating germplasm with any form of resistance into a soybean rust breeding programme it is important to evaluate their breeding potential with locally adapted, farmer preferred varieties.

Selection of parents for hybridisation requires critical consideration given that phenotypic performance alone does not always provide adequate information for breeding purposes. Combining ability studies provide a guide for selecting elite parents and desirable cross combinations for systematic breeding. More so, the expression of soybean rust resistance genes is influenced by the genetic backgrounds in which they are introgressed making it worthwhile to test the effectiveness of these resistance genes in different backgrounds (DeLucia *et al*., 2008; Yamanaka *et al*., 2010; Lemos *et al*., 2011). In diallel analysis for combining ability of a self pollinated crop like soybean F_2 and F_3 generations give better predictions on the performance of lines due to the decreased level of dominance gene effects (Bhullar *et al*., 1979) and availability of ample seed.

Most studies undertaking combining ability analysis, however, evaluate genotypes in one environment which tends to limit the scope of such studies. Evaluations in one environment result in misleading breeding results as it tends to imply low environmental and high genetic variance influence. When diallel crosses are evaluated across several environments, consistent information on repeatability of the estimates over environments

is obtained (Patil and Chopde, 1981; Ribeiro *et al*., 2009). This also gives a better estimate of the environmental contribution which is important in evaluating soybean rust whose expression is strongly influenced by the environment. The presence of genotype-by-environment interaction and pathogen variability therefore necessitates evaluation of early generation hybrids in many environments to determine their true genetic potential and broaden the scope of combining ability studies. Therefore, this study was aimed at i) determining soybean rust resistance combining ability parameter estimates across the F_2 and F_3 generations in one environment during different seasons ii) assessing the role of genotype x environment in combining ability for soybean rust severity and sporulation rate in five geographically diverse environments in the F_3 generation using Griffing's diallel analysis Method 2/Model 1 and iii) identifying parents and crosses with good combining ability for soybean rust resistance through estimates of general and specific combining abilities.

4.2 Materials and Methods

4.2.1 Experimental sites and Germplasm

The experiments were carried out at five sites; Makerere University Agricultural Research Institute-Kabanyolo (MUARIK), National Crops Resources Research Institute (NaCRRI), Nakabango (NAK), Iki-Iki (IKI) and Kasese (KAS). These sites represent the central, western, and eastern parts of the country where soybean is widely grown with severe seasonal soybean rust epidemics. Three SBR resistant lines G8586 (R*pp2*), G7955 (R*pp3*), UG 5; three moderately resistant cultivars Namsoy 4M, MNG 11.2, Maksoy 2N and two susceptible cultivars Wondersoya and Nam 2 (Table 8) were crossed in an 8 x 8 diallel mating scheme with no reciprocals sto generate 28 F_1 hybrids during 2008 and 2009. A have diallel was used since soybean rust is not influenced by maternal effects (Ribeiro *et*

al., 2007). All genotypes were rated for soybean rust based on similar maturity stage (R6) to reduce the impact of phenology on rust development.

Table 8 Description of the 8 parental lines used in the half diallel at MUARIK during 2008/2009

Genotype	Pedigree	Response to Rust	Source
G8586	PI230970	Resistant	AVRDC, Taiwan
G7955	Ankur	Resistant	AVRDC, Taiwan
UG 5	-	Resistant	MAK, Uganda
Namsoy 4M	Nam 2 x GC00138-29	Moderately resistant	NARO, Uganda
Maksoy 2N	Duiker x GC00138-29	Moderately resistant	MAK, Uganda
MNG 11.2	Duiker x TGx1835-10E	Moderately resistant	MAK, Uganda
Wondersoya	-	Susceptible	IITA, Nigeria
Nam 2	87D-668	Susceptible	NARO, Uganda

AVRDC-The World Vegetable Development Centre; NARO-National Agricultural Research Organisation, Uganda; MAK-Makerere University; IITA-International Institute for Tropical Agriculture

4.2.2 Progeny generation and experimental design

Morphological traits: days to flowering, hypocotyl colour, flower colour, pubescence and pod colour were used to eliminate selfed individuals. The 28 F_1 hybrids and their corresponding parents were grown in plastic pots in the screen house to generate adequate F_2 and parental seed for field evaluation. Subsequently, seeds from F_2 plants within a given cross were bulked to generate F_3 families. A randomised complete block experimental design with three replications was used for disease assessment in the field. Each 2 metre plot had at least 30 plants with a 60 cm x 5 cm inter- and intra-row spacing. Standard agronomic practices like weeding, fertilisation and pest control were done during the entire season (Tukamuhabwa, 2006). Weather data was collected for all the test sites from planting to the final disease severity and sporulation measurement (Table 9).

Table 9 Environmental characteristics of the selected locations during the evaluation period of 2010 and 2011

Location	Coordinates	Year	Rainfall (mm)	Mean Temperature ^{0}C Minimum	Maximum	Altitude (masl)
MUARIK	N00^{0}27'99	2010	261	18	27	1170
	E32^{0}36'41	2011	291	17	25	
NaCRRI	N00^{0}31'484	2010	273	16	28	1154
	E32^{0}36'932	2011	320	17	26	
NAK	N00^{0}31'418	2010	443	15	26	1178
	E33^{0}12'823	2011	413	14	20	
IKI	N01^{0}06'046	2010	201	15	28	1211
	E34^{0}00'119	2011	340	14	23	
KAS	N00^{0}31'484	2010	297	13	27	1006
	E30^{0}07'944	2011	363	15	28	

masl-metres above sea level

4.2.3 Progeny Disease Assessment and Management

Data on disease severity and sporulation were collected from the parents, F_2 and F_3 families separately at different times based on the final severity and sporulation measure. A susceptible variety Nam 2 was planted after every five rows at relatively higher plant density to ensure uniformity of natural inocula and high disease pressure. Genotypes were evaluated for soybean rust severity, sporulation and lesion colour during the reproductive growth stages (Fehr *et al*., 1971) starting from R5 until the severely infected leaves abscissed. Disease ratings were based on mean per plot. Severity scale was based on lesion density per leaflet on 1-9 scale 1- no lesions; 2= 1-30; 3= 31-75; 4= 76-150; 5= 151-300; 6= 301-750; 7= 751-1500; 8= 1501-3000 and 9= >3000 from three trifoliates of the mid-canopy (Miles *et al*., 2008) while the sporulation rating was based on a 1-5 scale (where 1= no sporulation, 2=sporulation less than 25% of full sporulating lesion, 3=50% sporulation of a fully sporulating tan lesion, 4=sporulation 75% of a fully sporulating tan lesion and 5= sporulation comparable to fully sporulating tan lesion). Reaction responses were assessed and grouped into three; red brown (RB), tan (T) and mixed reactions (MX).

4.3 Data Analyses

Analysis of variance for soybean rust severity and sporulation rate was applied to the parents, F_2 and F_3 data using the appropriate method for Randomised Complete Block Design. Data were transformed using the square root transformation prior to analysis of variance where necessary. Griffing's Method 2/Model 1 (Griffing, 1956) was used to estimate general combining ability (GCA) of parental lines and specific combining ability (SCA) for the F_2 and F_3 generation crosses. The Student's t-test was applied to examine the significance of the general and specific combining ability for severity and sporulation. Baker's ratio was used to compute the GCA/SCA components of variance based on the formula $2S^2_{gi} / (2S^2_{gi} + S^2_{ij})$ where g_i is the GCA effect of parent i and S_{ij} is the SCA effect of cross i x j. At MUARIK, F_2 and F_3 severity and sporulation estimates were compared to assess consistency of genetic contribution to soybean rust resistance. In the F_3, severity and sporulation rate assessment was done for the progenies in five separate locations (MUARIK, NaCRRI, NAK, IKI and KAS) to investigate the magnitude of genotype-by-environment on GCA and SCA effects for soybean rust resistance. Parental lines were evaluated in these five locations for two consecutive seasons to determine the relative contribution of genotype and environment in the two resistance parameters. A GGE biplot graphical display was generated to visualise the best performing F_3 progenies for soybean rust resistance compared to their parents across the five environments. All analyses were done using GENSTAT 13th Edition (Payne *et al*., 2010).

4.4 Results

4.4.1 GCA and SCA estimates for soybean rust resistance at MUARIK

Combined analysis of variance over two seasons revealed highly significant ($P \leq 0.01$) season and parent effects for both soybean rust severity and sporulation indicating the importance of environment and genotype in the control of these traits (Table 10).

However, only sporulation had a significant ($P \leq 0.05$) season x parent interaction. The high environmental contributions at MUARIK necessitated the study to be conducted across environments to broaden the scope of combining ability study findings.

Table 10 Analysis of variance combined over years for soybean rust severity and sporulation of parents at MUARIK in 2010 and 2011

Source of variation	df	Parents Severity Mean square	Parents Sporulation Mean Square
Season	1	94.5313***	22.7813***
Parents	7	8.9598***	3.3170***
Reps (Season)	2	0.4062	0.7812
Year X parents	7	1.1027	1.4241*
Pooled Error	14	0.9062	0.4241

***significant at $P \leq 0.001$, *significant at $P \leq 0.05$

The combining ability mean squares for resistance to soybean rust severity and sporulation rate at MUARIK are presented in Table 11. Highly significant ($P \leq 0.001$) differences were found for resistance and sporulation among the genotypes allowing further analysis for combining abilities. General combining ability (GCA) mean squares were significant ($P \leq 0.05$) for severity and sporulation across F_2 and F_3 generations (Table 11). On the contrary, specific combining ability was significant only for severity mean squares in the F_3 generation.

Table 11 Mean soybean rust severity and sporulation, general and specific combining ability of eight parental soybean lines evaluated at MUARIK in F2 and F3 during the 2010 and 2011 seasons respectively

Parents	Severity				Sporulation rate			
	Mean Score	GCA	Mean Score	GCA	Mean Score	GCA	Mean Score	GCA
	F_2		F_3		F_2		F_3	
1.G8586(R*pp2*)	3.0	-0.38***	3.5	-0.51*	2.0	-0.48**	3.3	-0.68***
2.G7955(R*pp3*)	2.0	-0.52***	2.5	-0.27	1.5	-0.90***	4.5	-0.54***
3.UG 5	2.5	-0.71***	4.0	-0.95**	1.5	-0.81***	4.5	-0.78***
4.Namsoy 4M	4.0	0.10	4.5	-0.16	3.5	0.11	5.0	0.41*
5.Maksoy 2N	2.5	-0.29**	4.5	-0.04	3.0	0.22***	5.0	0.17
6.MNG 11.2	3.0	-0.11	4.0	0.20	2.0	0.19	4.5	0.04
7.Nam 2	7.0	0.99***	7.0	0.66**	5.0	0.85***	5.0	0.42*
8.Wondersoya	5.5	0.93***	4.5	1.08***	5.0	0.83***	5.0	0.96***
Mean	3.7		4.3		2.9		4.6	
r	0.86**		0.53^{ns}		0.91**		0.72*	
df	Severity Mean squares				Sporulation Mean square			
	F_2		F_3		F_2		F_3	
GCA 7	4.1285***		4.141*		4.5508***		3.799***	
SCA 28	0.4653		3.664*		0.3688		1.6484	
Error 70	0.595		1.875		0.6025		1.054	
CV%	14.5		20.7		12.7		20.8	
GCA/SCA	8.872		1.13		12.33		2.25	
Baker's ratio	0.95		0.69		0.96		0.82	

GCA, general combining ability; SCA specific combining ability, r-correlation

***significant at $P \leq 0.001$, *significant at $P \leq 0.05$

Parental lines G8586 (R*pp2*), G7955 (R*pp3)* and UG 5 consistently showed high negative GCA for severity and sporulation with F_2 and F_3 generations indicating a high contribution of the parents to soybean rust resistance within the progenies. Genotypes Wondersoya and Nam 2 which had significant positive GCA effects contributed least to soybean rust resistance with confirming increased severity and sporulation observed on the plants. Interestingly, parent Maksoy 2N contributed to decreased severity but increased sporulation in the F_2 and F_3 generations respectively. In the F_2, both severity and sporulation had significant ($P \leq 0.01$) positive correlation of 0.86 and 0.91, respectively, with parental GCA performance. However, in the F_3 generation GCA and severity had a non-significant ($P \geq 0.05$) correlation whereas sporulation had a significant ($P \leq 0.05$)

correlation coefficient of 0.72 with GCA. Disease severity means ranged from 2.0 for G7955 (R*pp3)* to 7.0 for Nam 2 with an overall mean of 4.0 in F_2 and F_3 (Table 11). Genotype G8586 (R*pp2*), G7955 (R*pp3*), UG 5 and MNG 11.2 consistently had low severity. The two generations were planted in different seasons and sporulation was greater for all the genotypes in the F_3 with a mean difference of 1.7 with the F_2 generation.

4.4.2 GCA and SCA estimates in the F_3 over five locations

The analysis of variance combined over location for two consecutive seasons indicated highly significant ($P \leq 0.001$) parental (genotype) effects for soybean rust severity and sporulation. The relative contribution of each source of variation as a percentage of the total sum of squares is presented in Table 12. Parents averaged across environments contributed 75.68% and 60.00% to the variation observed in severity and sporulation. However, the season effects on sporulation and disease severity across the five environments were not significant. There was no change in the relative ranking of the parents with respect to severity and sporulation as season x parent interaction was not significant for the two seasons.

Table 12 Analysis of variance of the soybean parental lines combined over two years for soybean rust severity and sporulation in the five locations

Source of variation	Parents Severity		Parents Sporulation	
	Mean square	% contribution[a]	Mean Square	% contribution[a]
Season	2.500	6.85	0.876	23.44
Parent	27.596***	75.68	2.243***	60.00
Reps (Season)	2.413	6.62	0.269	7.22
Year X parent	1.214	3.33	0.197	5.28
Pooled Error	2.739	7.51	0.151	4.06

[a] relative percentage contribution of each source of variation to the total variance

***significant at $P \leq 0.001$

The F_3 progenies showed a similar pattern in general combining ability at all locations for severity and sporulation (Table 13). General combining ability and entries were significant ($P \leq 0.05$) in all the five locations for severity and sporulation. On the contrary, specific combining ability was significant ($P \leq 0.05$) only at MUARIK. The consistent significance of GCA effects suggested the stable contribution of additive gene effects across environments. Accordingly, Baker's ratio had high values with a minimum of 0.69 and maximum of 0.98 for both severity and sporulation.

Table 13 Analysis of variance for combining ability for soybean rust resistance severity and sporulation in the F3 generation at five locations

Source of variation	Location	df	Mean square	
			Severity	Sporulation
GCA	L1	7	4.141*	3.799***
	L2		3.052***	7.203***
	L3		2.465***	8.700***
	L4		2.280***	6.496***
	L5		8.854***	2.632***
SCA	L1	28	3.664*	1.014
	L2		1.166	1.040
	L3		0.515	0.675
	L4		0.449	1.697
	L5		0.400	1.295
Entry	L1	35	3.757*	2.107*
	L2		1.543*	2.480*
	L3		0.905*	2.384*
	L4		0.816*	2.657*
	L5		3.142*	1.562*

L1-MUARIK, L2-NaCRRI, L3-NAK, L4-IKI, L5-KAS

***significant at $P \leq 0.001$, *significant at $P \leq 0.05$

General combining ability effects combined over locations indicated that parents UG 5, G7955 (R*pp3*) and G8586 (R*pp2*) were good combiners for severity and sporulation (Table 13). In general, there was more variation at MUARIK and KAS for severity GCA effects, and NaCRRI and IKI for sporulation GCA effects due to the wide ranges obtained. IKI had the greatest number of parents contributing to resistance as shown by negative

contributions to severity and sporulation. Local varieties Nam 2, Wondersoya and breeding line MNG 11.2 did not contribute to resistance at NaCRRI. At all locations Wondersoya consistently contributed towards increased severity as indicated by a significantly positive GCA. On the contrary, UG 5 contributed towards increased resistance in all the test locations as shown by the positive and significant GCA effects (Table 14). Parent G8586 (R*pp2*) which has a classical resistance gene surprisingly contributed to increase sporulation at NaCRRI and NAK.

Table 14 Estimates of general combining ability (GCA) for soybean rust severity and sporulation in the F3 generation at five locations

Parents	L1	L2	L3	L4	L5	Combined
Severity						
G8586 R*pp2* (P1)	-0.51*	-0.28	0.01	-0.04	-0.86***	-0.31*
G7955 R*pp3* (P2)	-0.27	-0.49***	-0.54*	-0.33*	-0.59**	-0.50**
UG 5 (P3)	-0.95***	-0.60**	-0.84**	-0.46**	-1.16***	-0.79***
Namsoy 4M (P4)	-0.16	-0.38**	-0.16	-0.32*	-0.08	-0.14
Maksoy 2N (P5)	-0.04	0.02	0.50*	-0.10	-0.34	-0.02
MNG 11.2 (P6)	0.20	0.41*	0.10	0.24	-0.36	0.22
Nam 2 (P7)	0.66**	1.04***	0.37	-0.04	0.97***	0.58
Wondersoya (P8)	1.08***	0.26	0.58*	-1.05***	1.65***	0.96***
Sporulation						
G8586 R*pp2* (P1)	-0.68***	0.62**	0.38*	-1.07***	-0.52**	-0.06
G7955 R*pp3* (P2)	-0.54**	-0.95***	-1.27***	-1.36***	-0.27	-0.75***
UG 5 (P3)	-0.78***	-1.46***	-1.69***	-1.76***	-0.58**	-1.08***
Namsoy 4M (P4)	0.41*	-0.36*	0.39*	-1.51***	-0.32*	-0.09
Maksoy 2N (P5)	0.17	0.65**	0.57**	-0.86***	0.26	0.34*
MNG 11.2 (P6)	0.04	0.04	0.26	-0.5	0.35*	0.23
Nam 2 (P7)	0.42*	0.85***	0.62**	-0.62**	0.145	0.41*
Wondersoya (P8)	0.96***	0.61**	0.74***	0.754***	0.92**	1.00***

L1-MUARIK, L2-NaCRRI, L3-NAK, L4-IKI, L5-KAS

***significant at $P \leq 0.001$, **significant at $P \leq 0.01$*significant at $P \leq 0.05$

The performance of parents and their F_3 progenies based on predominant reaction phenotypes and sporulation scores is presented in Table 15. Parental lines responded

variably with tan, red brown and mixed reaction phenotypes. The general observation from the study was that mixed and tan lesion types were associated with high sporulation scores whereas red brown lesion types had lower sporulation scores. Most crosses with Red brown lesions involved parents with high negative GCA (Table 14 and 15) indicating involvement of complementary additive gene action. However, there were some exceptions such as crosses G7955 x Nam 2, UG5 x Namsoy 4M, UG5 x Maksoy 2N and UG5 x MNG 11.2 that had red brown lesions and sporulation scores lower than the overall mean of 3.5 (Table 15). These crosses indicated the involvement of additive gene action from parents UG 5 and G7955 (R*pp3*). KAS had the greatest mean sporulation (parents and F_3 progeny) level of 4.0 followed by NaCRRI (3.8), NAK (3.6), MUARIK (3.8) and IKI (2.6). KAS and MUARIK had the greatest incidences of mixed lesions with higher incidence of profuse sporulation among the progenies (Table 15). IKI had low sporulation levels which were 0.9 less than the overall mean.

Based on disease severity, parents showed high instability towards disease severity. A biplot for disease severity principal components for the parents and F_3 progenies is shown in Figure 3. Parent UG 5 had the least severity among eight parents across the five environments. Parent Maksoy 2N was the least stable genotype as shown by the greatest deviation from the Average Environment Axis of the comparison biplot (Figure 3). This study showed that F_3 crosses involving UG 5 produced progenies with the least severity such as G7955 x UG5, UG5 x Namsoy 4M, UG5 x Maksoy 2N, UG5 x MNG11.2. Similarly, a cross G8586 x G7955 possessing two resistance genes R*pp2* and R*pp3* had a lower soybean rust severity. Crosses G8586 x Namsoy 4M and UG5 x Wondersoya had stable severity across environments and they were derived from parents with relatively high severity stability.

Table 15 Lesion colour and mean soybean rust sporulation of parents and F3 progenies over five locations in Uganda

Entry	Location					
	MUARIK	NaCRRI	NAK	IKI	KAS	Combined and predominant colour
Parents						
G8586 (R*pp2*) (1)	MX(3.3)	T(5.0)	T(5.0)	RB(1.0)	RB(2.0)	RB(3.3)
G7955 (R*pp3*) (2)	RB(4.5)	RB(1.0)	RB(1.9)	RB(2.0)	RB(2.0)	RB(2.3)
UG 5(3)	MX(4.5)	RB(1.0)	RB(1.0)	RB(1.0)	RB(1.0)	RB(1.7)
Namsoy 4M (4)	T(5.0)	RB(1.5)	RB(2.9)	RB(1.9)	RB(2.0)	RB(2.7)
Maksoy 2N (5)	T(4.5)	T(5.0)	T(5.0)	MX(2.6)	RB(3.5)	T(4.1)
MNG 11.2 (6)	MX(4.5)	RB(1.9)	T(5.0)	MX(4.5)	T(3.9)	T(4.0)
Nam 2 (7)	T(5.0)	T(5.0)	T(5.0)	RB(1.0)	RB(2.9)	T(3.8)
Wonder (8)	T(5.0)	T(5.0)	T(5.0)	MX(5.0)	T(5.0)	T(5.0)
F_3 Progenies						
1 x 2	RB(2.5)	RB(2.5)	RB(1.9)	RB(1.5)	MX(2.6)	RB(2.2)
1 x 3	RB(2.9)	T(5.0)	RB(2.9)	RB(1.0)	RB(3.5)	RB(3.1)
1 x 4	RB(1.9)	MX(3.9)	MX(4.5)	RB(1.5)	RB(3.5)	RB(3.1)
1 x 5	RB(1.5)	MX(5.0)	RB(4.0)	RB(3.3)	MX(5.0)	RB(3.8)
1 x 6	RB(1.9)	RB(4.5)	RB(2.9)	MX(3.5)	RB(2.0)	RB(3.0)
1 x 7	RB(2.9)	T(5.0)	T(5.0)	T(5.0)	T(5.0)	T(4.6)
1 x 8	MX(2.6)	MX(5.0)	MX(5.0)	MX(4.5)	T(5.0)	MX(4.4)
2 x 3	RB(1.0)	RB(1.0)	RB(1.0)	RB(1.9)	MX(4.5)	RB(1.9)
2 x 4	RB(1.9)	T(5.0)	MX(5.0)	RB(1.5)	MX5.0)	T(3.7)
2 x 5	MX(1.0)	T(5.0)	RB(1.5)	RB(1.5)	MX(5.0	T(2.8)
2 x 6	MX(3.3)	RB(1.0)	MX(2.6)	RB(1.5)	MX(4.5)	MX(2.6)
2 x 7	RB(1.5)	MX(3.9)	RB(1.0)	RB(1.9)	RB(2.5)	RB(2.2)
2 x 8	MX(4.0)	T(5.0)	RB(2.9)	T(5.0)	T(5.0)	T(4.4)
3 x 4	RB(1.5)	RB(1.5)	RB(1.5)	RB(1.9)	RB(2.5)	RB(1.8)
3 x 5	RB(2.3)	RB(1.9)	RB(2.6)	RB(1.9)	RB(2.5)	RB(2.2)

3 x 6	RB(1.0)	RB(2.9)	RB(1.0)	T(2.6)	MX(5.0)	RB(2.5)
3 x 7	RB(1.5)	T(5.0)	T(2.6)	RB(1.5)	MX(5.0)	T(3.1)
3 x 8	MX(2.6)	RB(1.0)	RB1.9)	MX(2.6)	MX(5.0)	MX(2.6)
4 x 5	MX(5.0)	T(5.0)	T(5.0)	MX(3.3)	MX(3.9)	T(4.4)
4 x 6	MX(3.9)	RB(2.9)	RB(4.5)	MX(3.3)	T(5.0)	MX(3.9)
4 x 7	MX(4.5)	T(5.0)	T(5.0)	RB(1.0)	MX(3.9)	MX(3.9)
4 x 8	MX(5.0)	T(5.0)	T(5.0)	RB(1.5)	T(5.0)	T(4.3)
5 x 6	RB(2.0)	T(5.0)	T(5.0)	RB(1.0)	MX5.0)	T(3.6)
5 x 7	MX(4.5)	MX(4.5)	MX(5.0)	MX(3.3)	MX(5.0)	MX(4.5)
5 x 8	MX(5.0)	T(5.0)	T(5.0)	RB(3.9)	T(5.0)	T(4.8)
6 x 7	MX(3.9)	T(5.0)	T(5.0)	MX(4.5)	T(5.0)	T(4.7)
6 x 8	MX(5.0)	T(5.0)	T(5.0)	T(5.0)	T(5.0)	T(5.0)
7 x 8	MX(5.0)	T(5.0)	T(5.0)	MX(3.0)	T(5.0)	T(4.6)
Mean	3.3	3.8	3.6	2.6	4.0	3.5
SE±	0.238	0.267	0.258	0.227	0.207	

RB-RedBrown; T-tan; MX-Mixed Reaction; sporulation score based on a 1-5 scale in parentheses

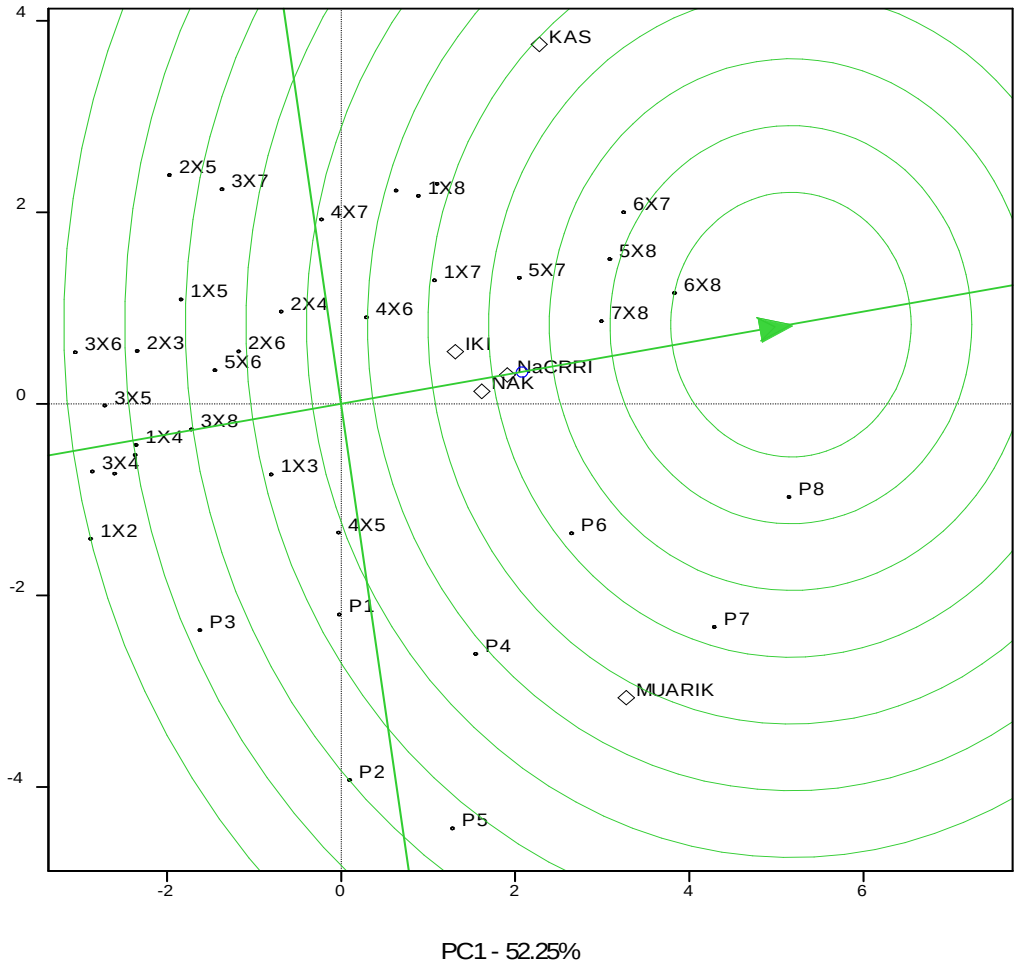

Figure 3 A GGE comparison biplot graphical display for soybean rust severity in the parents and F3 progenies evaluated in five locations. P1- G8586 (R*pp2*), P2-G7955 (R*pp 3*), P3-UG 5, P4- Namsoy 4M, P5-Maksoy 2N, P6- MNG11.2, P7-Nam 2, P8-Wondersoya. NB Some crosses were excluded for clarity

4.5 Discussion

4.5.1 Combining abilities for soybean rust resistance in the F_2 and F_3 progeny at MUARIK

Soybean rust presents a great challenge to soybean breeders due to high genetic diversity and strong environmental influence to disease epidemics. Therefore, it is important to evaluate progenies for resistance to local rust populations to obtain relevant information on high potential segregating material. In this study parental lines, F_2 and F_3 progenies were evaluated for resistance to soybean rust using disease severity and sporulation rate. The diversity among the parents indicated that hybridisation and selection might produce plants with lower severity and sporulation rate. During the evaluation of parental and

offspring performance across F_2 and F_3 at MUARIK, high environmental influence on severity and sporulation was observed. The F_2 evaluation was done during the second season of 2010 which had erratic rainfall of short duration and relatively higher temperature. On the contrary, F_3 evaluation was done during the first season of 2011 which received relatively high amounts of uniformly distributed rainfall and cooler temperatures (Table 9) which contributed directly to disease development through more pronounced secondary infection in the F_3 hence the relatively high mean severity and sporulation scores.

However, despite the strong contribution of the year (season) to severity and sporulation (Table 10), GCA effects for severity and sporulation were significant and unaltered across the two generations. A similar observation was made by Ribeiro *et al*. (2009) in Brazil that despite the high environmental contribution to soybean rust severity, general combining ability estimates are not affected. Although contrary to this study, their early generation segregating populations were evaluated for severity at the same time. GCA/SCA ratio was greater than one and Baker's ratio greater than 0.60 for the test environments significant importance of additive gene effects in contributing to the two traits. Accordingly, effective selection for severity and sporulation can be done within the segregating populations. However, these traits seem to be controlled by independent genetic systems as positive contribution to severity was not necessary associated positive contribution to sporulation as shown by the performance of Maksoy 2N at MUARIK. Specific combining ability was not consistent as it was significant only for severity in the F_3 generation at MUARIK suggesting that genotype-by-environment interaction has great impact in determining SCA effects.

4.5.2 Combining abilities of F3 progeny for soybean rust resistance in five environments

When combining ability studies are conducted in one environment the scope of the result is limited to that particular environment (Iqbal *et al*., 2010). The interactions between pathogen virulence, race composition and environment necessitated multi-location evaluation of the progenies. Therefore, this study evaluated F_3 generation progenies in five locations. Differences in parental genotypes had the strongest effects on the source of variance (Table 12). This high genetic contribution and significance of GCA implies that the selected parents can produce more resistant progenies across generations. Resistance based on sporulation rate is an equally important attribute that prevents rapid build up of inoculum during the growing season. The contribution of additive gene effects towards reduced sporulation suggests that breeding for low sporulation is possible. This has important implications for a polycylic disease like soybean rust where secondary inoculum has to be kept minimal during the season to minimise damage.

The consistency of GCA estimates over environments in the F_3 generation suggests that these populations are important in the identification of good combiners for soybean rust resistance. In addition, the F_3 population had decreased contribution of dominance deviations which gave better estimates of additive gene effects. In this study, GCA was considered to be more important since the desirable product of soybean is a pure line. For a self pollinated crop like soybean additive gene effects can be fixed. Parents UG 5 and G7955 (R*pp3*) were good combiners with high negative GCA for severity and sporulation across the test sites. The significant positive correlation between GCA estimates and mean parental performance for the combined data across sites (Figure 3) suggested that selection for soybean rust resistance can be done on the basis of parental performance.

Several crosses involving G8586 (R*pp2*), G7955 (R*pp3*), UG 5 and Namsoy 4M exhibited complementary additive components disease severity. Other crosses such as UG5 x MNG 11.2, UG5 x Maksoy 2N exhibited additive gene effects only (Figure 3). Therefore this suggests that genes for decreased severity can be fixed in the progenies of these crosses to maintain the good combination of resistance genes. It was noted that UG 5 (P3) contributed to lowest disease severity and sporulation by interacting in a positive way with different genetic backgrounds which explained the high negative GCA of this genotype. This makes UG 5 an ideal source of soybean rust resistance for soybean breeding programme. The cross G8585 x G7955 segregated for the two classic resistance genes R*pp2* and R*pp3*. The relatively low severity and sporulation in G8585 x G7955 implies that pyramiding these two resistance genes in one background could produce more resistant genotypes. This concurs with observations made by Lemos *et al*. (2011) that individuals with pyramided genes have enhanced resistance to soybean rust.

This study showed that red brown lesions were consistently associated with low sporulation which corroborates observations made by Bonde *et al*. (2006). Sporulation is an important attribute in breeding for soybean rust resistance as it ensures low secondary infection during the growing season. Each location, however, had a unique pathogen profile as evidenced by the distinct responses of the parental genotypes (Table 15). Future evaluations for resistance will require assessment of genotypes in these areas since each seems to have a different pathogen profile. Based on severity and sporulation scores, MUARIK and KAS tend to have mixed or aggressive pathogen races. This is due to predominance of mixed and tan lesions with profuse sporulation. Mixed responses are generally associated with a mixture of races and complex virulence patterns (Bonde *et al*., 2006; Miles *et al*., 2008). However, studies on the race composition of each location need to be undertaken for more accurate deductions to be made about predominant races. In this

study no consistent association between high severity and sporulation rating combining ability estimates was observed. This concurs with observations made by Walker *et al*. (2011) that high severity is not always associated with heavy sporulation suggesting that selection for the two traits has to be done independently to improve soybean rust resistance.

4.6 Conclusions

Overall, results from this study identified UG 5 a local genotype and accession G7955 (*Rpp*3) as useful sources of resistance to soybean rust that can be utilised by the local breeding programme. It also noted the importance of additive gene action in controlling soybean rust severity and sporulation across F_2 and F_3, and locations. However, the two genetic systems of severity and sporulation seem to act independently of each other. Based on the positive correlation between sporulation GCA estimates and parental rust severity means at MUARIK in the F_2 and F_3, and severity in F_3 selection of parents for good GCA for soybean rust resistance can be based on performance. Follow up studies need to be done to identify the resistance gene in UG 5 and undertake mapping studies on the gene as it has proved to be very valuable. Selection of F_3 progenies across environments for soybean rust severity and sporulation can reliably result in promising genotypes.

CHAPTER FIVE

ENHANCING SOYBEAN RUST RESISTANCE THROUGH *Rpp*2, *Rpp*3 and *Rpp*4 PAIRWISE GENE PYRAMIDING

5.1 Introduction

Soybean rust (*Phakopsora pachyrhizi*) is one of the most serious foliar diseases of soybean worldwide. Under heavy infestation losses of up to 75% have been observed in unprotected fields (Yorinori *et al*., 2005). The rapid spread of soybean rust together with the potential of causing severe yield losses makes it a very important disease of soybean (Miles *et al*., 2003). Several strategies for controlling soybean rust have been used such as fungicide application and genetic resistance. The effectiveness of fungicides, however, depends on timely application and use of appropriate spraying methods (Yorinori *et al*., 2005). More so, use of fungicides has cost implications and raises environmental concerns. Genetic resistance is therefore an economic and strategically important means of controlling soybean rust disease (Arias *et al*., 2008).

In soybean, resistance to rust is manifested phenotypically by red brown lesions (Bromfield, 1984; Bonde *et al*., 2006), and is conditioned by six major resistance genes R*pp*1, R*pp*2, R*pp*3, R*pp*4, R*pp*5, Rpp_{Hyuuga} which have been mapped to different linkage groups. R*pp1* linkage group (LG) G (Hyten *et al*., 2007), R*pp3* LGC2 (Hyten *et al*., 2009), R*pp2* and R*pp4* LGJ and G respectively (Silva *et al*., 2008; Yamanaka *et al*., 2008), *Hyuuga* LGC2 (Monteros *et al*., 2007) and R*pp5* LGN (Garcia *et al*., 2008). Long term utilisation of some of these race specific resistance genes has prompted the pathogen to mutate and overcome them. Empirical evidence in Africa, Orient and South America has shown that some of the once effective soybean rust resistance genes have been overcome by new rust races (Tschanz *et al*., 1986; Hartman *et al*., 2004; Laperuta *et al*., 2008). Despite resistance breakdown associated with race specific genes, they are still effective

against a broad range of pathotypes in wheat rust pathosystems (Pfender, 2009). The relative ease with which such monogenic resistance is detected and introgressed into susceptible backgrounds makes it a rapid mitigation strategy against soybean rust.

Gene pyramiding, which involves assembling multiple desirable genes into a single genotype has been suggested as one way to overcome resistance instability conferred by single gene resistance in many pathogens including soybean rust (Hartman *et al*., 2005; Garcia *et al*., 2008; Yamanaka *et al*., 2010; Lemos *et al*., 2011). The aim of this part of the study was to pyramid rust resistance so as to enhance soybean rust resistance to field isolates and broaden the genetic base available soybean breeding lines. However, incorporating such multiple gene resistance has remained a challenge using conventional methods due to the required extensive screening using gene specific pathogen races (Sanghai-Maroof *et al*., 2008). Conventional approaches are not always practically feasible in gene pyramiding given the fact that some genes were identified using foreign races whose access presents logistical and phyto-sanitary challenges. Accordingly, marker assisted selection was the most desirable alternative available for pyramiding resistance genes.

Several methods have been suggested for gene pyramiding when resistance genes are present in different parents such as production of F_2 and F_3, recombinant inbred lines (RILs) and double haploids (DH) (Servin *et al*., 2004). Utilisation of each population type depends on the availability of resources, objectives and germplasm available for the study. In soybean, the availability of a dense molecular map comprising of molecular markers such as SSR, RFLP, AFLP and isozymes (Song *et al*., 2004) makes marker-assisted selection for specific resistance genes in the early generations feasible. Moreover, several SSR markers tightly linked to known sources of resistance have been mapped making it

possible to trace them during hybridisation and facilitate their identification through marker assisted selection. However, no research has been done on the effect of pyramiding soybean resistance genes to enhance resistance trait performance against local Ugandan rust populations and increase the diversity of resistant gene sources. In rice, *Oryza sativum*, pyramiding has been done for three bacterial blight (*Xanthomonas oryzae pv. oryzae*) resistance genes using marker assisted selection, resulting in greater resistance (Singh *et al*., 2001). Saghai Maroof *et al*. (2008) pyramided three resistance genes for Soybean Mosaic Virus (SMV). Similarly, enhanced resistance to the fungus, *Magnaporthe grisea*, which causes rice blast was observed after genes *Pi*1, *Piz*-5 and *Pit*a were pyramided into one genotype (Hittalmani *et al*., 2000). The success of gene pyramiding strategies is largely facilitated by availability of molecular markers which are tightly linked to the gene of interest. Therefore the objectives of this study was to validate the use of marker assisted selection in F_2 and $F_{2:3}$ in pyramiding three resistance genes in pair-wise combinations thereby determining the most effective gene combinations for enhancing resistance to soybean rust.

5.2 Materials and Methods

5.2.1 Crosses and progeny development

Gene pyramiding was done through single crosses in a screen house using parental lines: PI 230970, Ankur and PI 459025 having three specific resistance genes R*pp2*, R*pp3* and R*pp4*, respectively. The crosses were done in pair wise combinations at Makerere University Agricultural Research Institute (MUARIK) during 2009 season and were implemented as follows; PI 230970 (R*pp2*) × Ankur (R*pp3*); PI 230970 (R*pp2*) × PI 459025 (R*pp4*) and Ankur (R*pp3*) × PI 459025 (R*pp4*).

Successful F_1 hybrid progeny were determined based on phenotypic marker traits like anthocyanin coloration of the seedlings, flower and pubescence colour to eliminate selfed individuals. F_1 plants were allowed to self to produce F_2 segregating populations which were screened to identify individuals possessing the two soybean rust resistance genes. Selected F_2 plants were scored for disease severity relative to other individuals with single resistance genes and advanced to obtain F_2-derived-F_3 ($F_{2:3}$) generation. Selected plants were harvested separately to ensure family identity. All individuals within the $F_{2:3}$ generation were screened for the two resistance genes and scored for disease resistance parameters.

5.2.2 Field experimental layout and phenotypic screening procedures

Field experimental plots of 2 metre rows with 30-35 plants for each parent and progeny were established, under natural ASR infection at MUARIK. Spreader rows of a highly susceptible variety Nam 2 were planted around the test material to ensure sufficient disease inoculum. Hybrids from R*pp2* × R*pp3*, R*pp2* × R*pp4*, R*pp3* × R*pp4* gene combinations were assessed for disease severity in addition to the at the R5 stage (Fehr *et al*., 1971). Rust severity was determined at weekly intervals, using a scale of (1-9) based on counting lesion density per leaflet from three trifoliates of the mid-canopy, where 1= no lesions; 2=1-30; 3=31-75; 4=76-150; 5= 151-300; 6= 301-750; 7= 751-500; 8= 1501-3000 and 9= >3000 lesions (Miles *et al*., 2008). Sporulation rate was evaluated based on a 1-to-5 scale (where 1 represents no-sporulation and 5 profuse sporulation). Using x20 magnification lenses soybean lines were evaluated for the number of lesions per square centimetre, proportion of sporulating lesions. Numbers of pustules per lesion were also assessed after vacuuming selected leaves with a hand held Liliput® vacuum to dislodge any urediniospores for easy counting.

5.2.3 DNA isolation and marker assisted selection

Genomic DNA was isolated from young soybean leaves using a Wizard Genomic DNA Purification Kit (Promega, USA). Simple Sequence Repeat (SSR) molecular markers (Table 16) flanking the resistance genes were synthesised by University of Cape Town and optimised according to their primer sequences for marker assisted selection. Parents were assayed for polymorphism using six SSR primers prior to F_2 and $F_{2:3}$ progeny screening. PCR was performed in a GeneAmp 9700 (Bio-Rad, USA) thermocycler in a 20 µl reaction volume containing 40 ng of template DNA, 0.5 µM of each primer, 0.2 mM dNTPs, 1 U of *Taq* polymerase, 2.5 mM $MgCl_2$ and 1 × PCR buffer. Amplification was done with an initial denaturing cycle of 94^0C for 2 minutes, followed by 32 cycles of 94^0C for 40 s; annealing at 48^0C for 40 s and 72^0C extension for 50 s and a final extension cycle at 72^0C for 5 minutes. The PCR amplicons were fractionated on 3-4% Metaphor (Lonza Bioscience, Singapore) agarose horizontal gel stained with GelRed™ Nucleic Acid Stain (Biotium, USA). Gel images were taken using a BioDoc-It™ Imaging System (Bio-Rad, USA).

Table 16 Simple sequence repeat based markers and their position in relation to three soybean resistance loci on a soybean linkage map

Marker	Linkage Group	Position[1] (cM)	Resistance Gene	Reference
Sat_255	J	43.85	R*pp2*	Silva *et al*. (2008)
Satt620	J	53.71	R*pp2*	Silva *et al*. (2008)
Satt460	C2	111.87	R*pp3*	Hyten *et al*. (2009)
Sat263	C2	118.78	R*pp3*	Hyten *et al*. (2009)
Satt288	G	76.77	R*pp4*	Yamanaka *et al*. (2008)
AF162283	G	87.94	R*pp4*	Yamanaka *et al*.(2008)

[1] Soybean SSR Map

5.3 Results

Out of the six SSR markers tested, two markers Satt460 and AF162283 produced polymorphism with significant differences in their amplicon sizes between the parents tested. Therefore subsequent screening of parents and segregating populations for

resistance gene presence was done based on these two markers. The results of SSR amplification of the parents and F_2 offspring segregating for the different genes are presented in Figure 4.

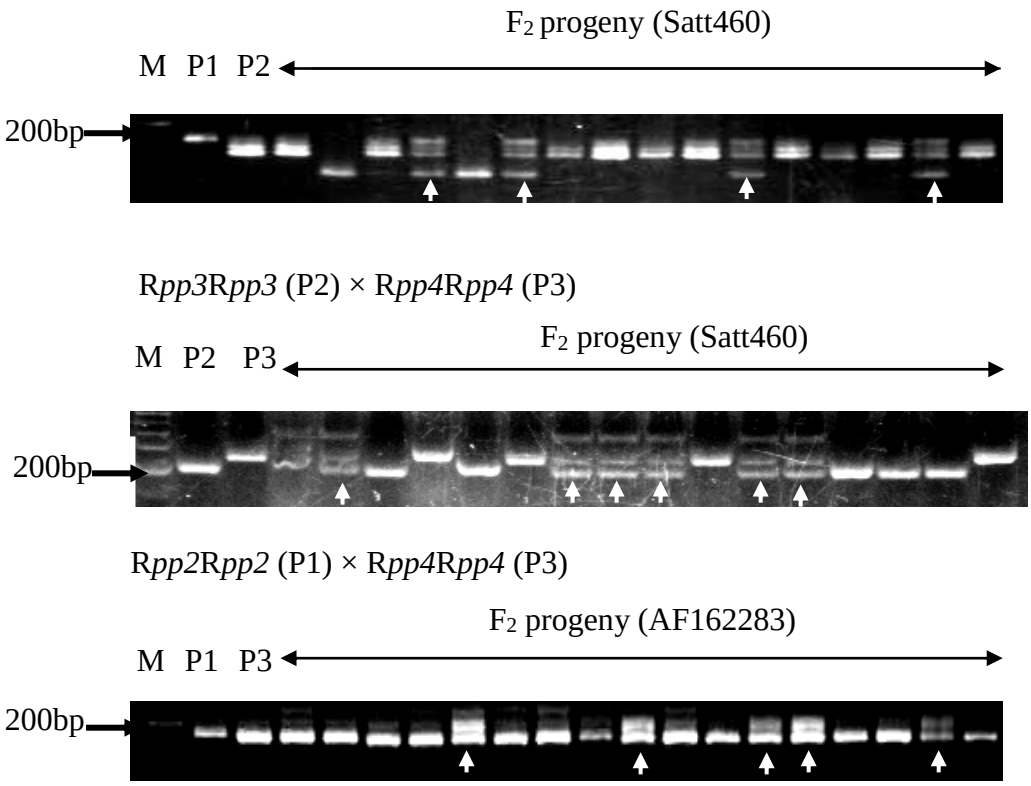

Figure 4. SSR markers for the different parents, crosses for the three resistance genes in pair-wise combinations. The arrows indicate individuals selected on the basis of possessing both parental alleles for further $F_{2:3}$ family molecular analysis. M- represents a 100 bp standard molecular weight marker

For each pair of gene combination 98 F_2 plants were assayed using the two polymorphic markers during the early stages (V1-R1) of soybean development. Selected individuals were tagged and assessed for disease severity and sporulation. Results from individual plant assays are presented in Table 17. In the F_2 generation, 27 plants were identified to be R*pp2_* × R*pp3_*, 19 plants; R*pp3_* × R*pp4_* and 11 plants R*pp2_* × R*pp4_*. Soybean rust severity was generally greater at time 2 compare to time 1 for parents and F_2 plants (Table 17).

Table 17 Severity and sporulation rate of genotyped F2 plants evaluated at two time intervals

Genotype	No of plants evaluated	Severity		Sporulation	
		T1	T2	T1	T2
Parents					
R*pp*2 R*pp*2	98	3.66±0.26	3.66±0.26	3.33±0.27	2.44±0.34
R*pp*3 R*pp*3	98	3.33±0.28	4.33±0.25	3.16±0.23	3.16±0.23
R*pp*4 R*pp*4	98	4.00±0.35	4.40±0.26	1.40±0.75	3.20±0.61
F_2 plants					
R*pp2_* × R*pp3_*	27	2.88±0.46	3.38±0.28	2.00±0.61	2.44±0.34
R*pp3_* × R*pp4_*	19	2.66±0.35	3.16±0.23	2.33±0.34	2.83±0.24
R*pp2_* × R*pp4_*	11	2.40±0.50	2.60±0.34	1.40±0.75	2.40±0.58
Mean		3.18±0.13	3.59±0.12	2.60±0.15	2.62±0.13

T-Time; at R5 and after one week later, ± standard error; notation R*pp_* implies the alternative allele was either dominant or recessive

The parental lines with the R*pp2* gene remained unchanged in severity during the two time intervals. All parents had higher severity compared to the selected plants with two gene combinations. Contrary to other genotypes, sporulation decreased from 3.3 to 2.4 for parent with the gene R*pp2*. The cross R*pp2_*× R*pp4_* had the lowest severity and sporulation rate followed by R*pp3_*× R*pp4_* for the two time intervals.

To ascertain the true genotypes, the selected F2 plants' seed was planted to produce $F_{2:3}$ families. Marker assisted selection of the parents and $F_{2:3}$ families were done using markers used in the F_2 generation. Ten plants were genotyped in each family to identify segregating families and infer their F_2 genotypes. For each family three individuals are presented in Figure 5. Molecular marker assay clearly distinguished segregating and non segregating families. In the $F_{2:3}$ generation for R*pp2*R*pp2* × R*pp3*R*pp3* four families were homozygous while R*pp2*R*pp2* × R*pp4*R*pp4* had two. All families from R*pp3*R*pp3* ×

R*pp4*R*pp4* were segregating and therefore in-depth phenotypic characterisation of disease parameters was not done for this family.

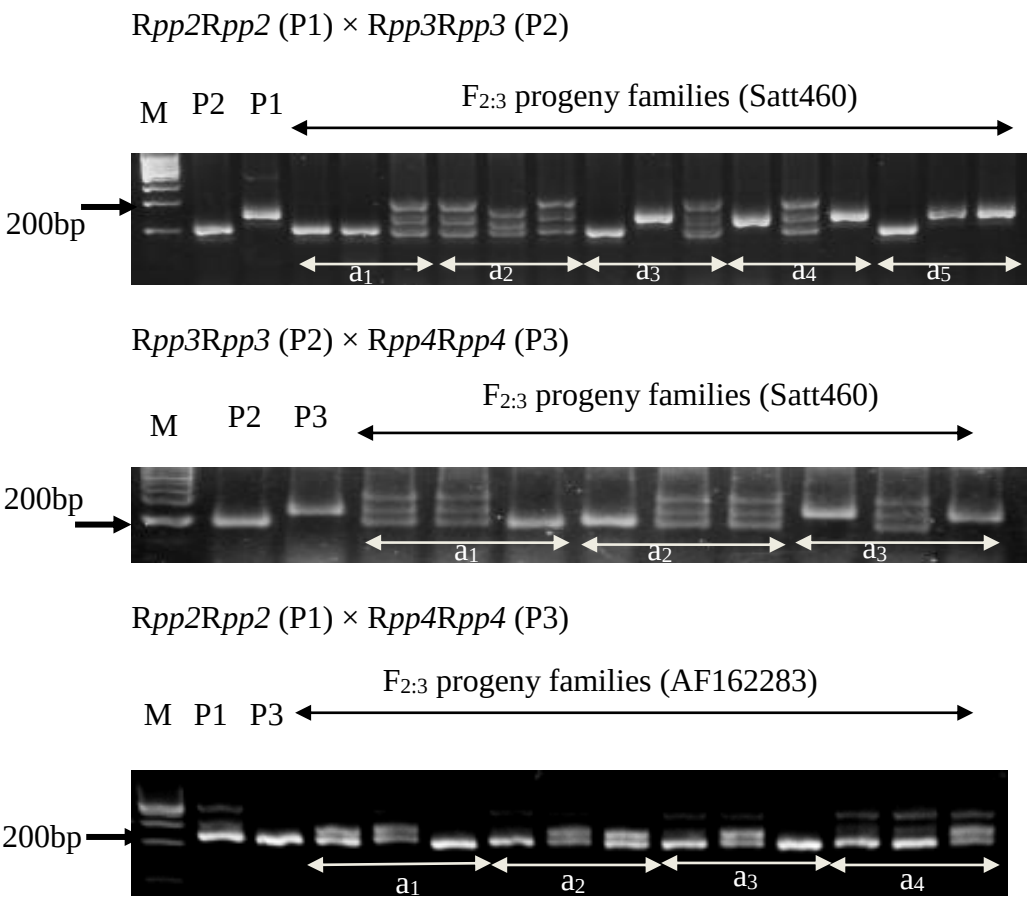

Figure 5 SSR markers for the different parents and F2:3 families with two gene combinations. The arrows indicate three individuals per family, a_1-a_5 shows the families selected for genotyping, families with equal number of amplified alleles were selected for all phenotypic characterisation of resistance. M- represents a 100bp standard molecular weight marker

The results of rust resistance evaluation of 10 plants per non-segregating $F_{2:3}$ family are presented in Table 18. Significant differences in disease severity, lesion per square centimetre and percentage sporulating lesions for parents and $F_{2:3}$ families were observed.

Table 18 Disease response parameters for the parents and ten homozygous dominant plants from F2:3 families

Genotype	Disease Severity	Lesions/cm^2	Reaction type	% Sporulating lesions	Pustules per lesion
Parents					
R*pp2*R*pp2*	4.91±0.48	45.39±5.68	RB	100	2.0
R*pp3*R*pp3*	3.28±0.39	24.52±4.56	RB	18	1.5
R*pp4*R*pp4*	3.20±0.33	24.93±3.90	RB	38	1.1
$F_{2:3}$ families					
R*pp2*R*pp2* × R*pp3*R*pp3*	2.62±0.48	18.41±3.16	RB	16	0.9
R*pp2*R*pp2* × R*pp4*R*pp4*	3.02±0.30	26.21±3.49	RB	15	1.9
Mean	3.14±0.19	24.95±2.62		24	1.2
F prob	≤0.05	≤0.05		≤0.01	ns

± standard error; ns- non-significant (P≥0.05)

Significant differences in disease severity, lesion per square centimetre and percentage uredinia with lesions for parents and $F_{2:3}$ families were observed. The $F_{2:3}$ family of R*pp2*R*pp2* × R*pp3*R*pp3* had the least lesions per square centimetre and frequency of lesions with uredinia. The family derived from R*pp2*R*pp2* × R*pp4*R*pp4* had a severity score lower than all the parents evaluated. However, its sporulation rate was higher than parents R*pp3*R*pp3* and R*pp4*R*pp4*. The numbers of pustules per lesion were not significantly different for all the genotypes evaluated.

5.4 Discussion

Based on the molecular data, resistance genes R*pp2*, R*pp3* and R*pp4* were successfully pyramided in pair-wise combinations in the F_2 generation. However, homozygous dominant and heterozygous individuals at both loci could not be readily distinguished. This could be attributed to the apparent dominant nature of the markers or similar sized alleles of the two genes which could not be resolved by the metaphor agarose used to fractionate the amplicons. Nonetheless, results from pyramiding of pair-wise gene combinations suggested occurrence of epistatic interactions among the independent

dominant genes R*pp2,* R*pp3* and R*pp4* since each gene is at a different locus. Complementary gene action resulted in additive increase of resistance in all instances across the F_2 and $F_{2:3}$ generations. Such complementary gene action for resistance was reported when resistance genes *Lr*9 and *Lr*24 were pyramided in wheat to enhance resistance to leaf rust (Moullet *et al*., 2008).

During the evaluation of F_2 generation, R*pp4*R*pp4* had the highest severity compared to other parents and genotypes. However, when it combined with R*pp3*R*pp3* and R*pp2*R*pp2* this resulted in low severity suggesting epistatic gene interaction of these genes. This corroborates observations by Yamanaka *et al*. (2010) and De Lucia *et al*. (2008) that the effectiveness of soybean rust resistance genes depends on the genetic background in which they are introgressed. This could further explain the continued utilisation of some classical resistance genes that have been overcome by certain soybean rust races. Parent R*pp4*R*pp4*, however, did not contribute to reduced sporulation in the combination R*pp3_* x R*pp4_* suggesting differences in genetic control of these resistance parameters (Maphosa *et al*., 2012).

In the $F_{2:3}$ generation some selected families were homozygous and resistance genes were fixed at both loci. Evaluation of $F_{2:3}$ generation for R*pp2*R*pp2* x R*pp3*R*pp3* had the lowest severity, lesions per square centimetre and pustules per lesion despite parent R*pp2*R*pp2* showing relatively high susceptibility, which supports further presence of complementary gene action for resistance to soybean rust. From the results, it can be deduced that the R*pp3* locus contributed to most of the resistance parameters compared to R*pp2* and R*pp4*. Such disproportionate contribution between resistance loci for resistance was observed by Lemos *et al*. (2011) during pyramiding of three soybean rust resistance loci. In their study, R*pp5* was the most important primary factor for resistance to soybean rust. Therefore, we

can infer that dominance and complementary epistasis exists among the independent soybean rust resistance genes. The numbers of pustules per lesion were not significantly different among the tested $F_{2:3}$ family genotypes. Thus, we conclude that pustules per lesion are not a good measure for selecting soybean rust resistant genotypes under field experimental conditions.

5.5 Conclusions

Though the presence of multiple virulence in soybean rust was seen as the main challenge to the efficacy of gene pyramiding (Shanmugasundaram *et al*., 2004), this study noted increased resistance in the two gene combinations. Furthermore, the results suggest that the utilisation of marker assisted selection in pyramiding soybean rust resistance genes is possible. Although the number of lines tested was small, the results from this study research demonstrate that pyramiding R*pp2* and R*pp3* in homozygous condition increases resistance. All the genes tested contributed complementarily to resistance, though, in a disproportionate manner. Introgression of these double resistance genotypes into farmer preferred cultivars is therefore recommended. This study did not focus on the durability aspect of the resistance genes which is crucial for any resistance breeding programme. Consequently, further research on evaluating soybean resistance genes for durability and using diverse pathogen populations is recommended as this is important for sustainable soybean production.

CHAPTER SIX

CONCLUSIONS AND FUTURE PERSPECTIVES

6.1 Introduction

Asian soybean rust remains a formidable challenge to sustainable soybean production worldwide. Resistance breeding against the pathogen is greatly compromised by the specificity of deployed genes given the high pathogen variability exhibited by the fungus. Moreover, there is no research that has been done on evaluating segregating progeny for resistance to soybean rust to evaluate the effects of the environment on resistance. This study on enhancing genetic resistance to soybean rust provides insights and potential solutions to the problem of soybean rust in the major soybean producing areas of Uganda. The aims of the study included evaluation of seedling and adult plants from putative race differentials against five field isolates to identify stable sources of resistance. The study also assessed F_2 and F_3 segregating populations of soybean in diverse environments to identify parents with good, consistent combining ability for soybean rust resistance. Lastly, marker assisted gene pyramiding of R*pp2*, R*pp3* and R*pp4* in pair-wise combinations was done to enhance and diversify the available sources of soybean rust resistance. A summary of the major research findings are as follows:

6.2 Seedling and adult plant resistance

Assessment of seedling and adult plant resistance for soybean rust using severity and sporulation indices resulted in consistent ratings for the tested genotypes. Reaction types were, however, strongly influence by the environment as seedling and adult plant response varied in the seedling and adult plant resistance done in the screen house and field respectively. Based on the reaction response of seedlings to field isolates each location had a different pathogen profile suggesting great diversity of the disease in Uganda. More

research needs to be undertaken on pathogen characterisation in each of these areas. Such research can be done using molecular marker technology that has high precision on organism genetic diversity. In addition sentinel plot can be laid out in each location to understand pathogen diversity seasonally. This can allow rational deployment of cultivars in given locations. Relationships between various disease resistance parameters showed significant, positive correlations between latent period, lesion number per square centimetre, frequency of sporulating lesions and area under disease progress curve. These findings based on the five field isolates imply that measurement of any of the aforementioned resistance parameters will give an accurate estimate of the others.

6.3 Combining ability for soybean rust resistance

The differences in local soybean rust populations in different locations necessitated the evaluation of segregating progeny in these areas to identify parents with good and consistent combining ability estimates. Rain fall was likely the major environmental contributor affecting disease severity and sporulation. Specific combining ability (non-additive gene effects) does not reliably contribute to soybean rust resistance. The populations selected can respond to selection for soybean rust resistance hence nore resistant genotypes are expected from these populations if the genetic material is adveanced. Among the parental soybean lines UG5, contributed to increased resistance reliably across the five locations tested.

6.4 Gene pyramiding for soybean rust resistance

Gene pyramiding is one of the strategies that were suggested as a means of enhancing single gene resistance and increasing diversity of available gene sources. Pair-wise combinations of resistance genes R*pp2*, R*pp3* and R*pp4* had greater resistance compared

to the individual single resistance genes in all cases. In this study several gene interactions were apparent. Complete dominance, complementary epistasis and additive gene action for the three independent genes were evident in controlling soybean rust resistance. Gene R*pp3* interacted and contributed positively to resistance with various genetic backgrounds for most parameters measured compared to R*pp2* and R*pp4* resistance genes. The genetic background in which a resistance gene is introgressed plays a crucial role in the manifestation of soybean rust resistance.

6.5 The way forward

The results show that it is important to evaluate soybean line for resistance to rust using target isolates. High potential genotypes UG5, Maksoy 3N, G7955 and GC00138-29 are ideal sources of soybean rust resistance in Uganda. These findings also imply that breeding for soybean rust has to focus on other strategies such as partial resistance and tolerance that have broad effectiveness for ASR management. Early generation progeny with fixable rust resistance genes need to be advanced to further to homozygosity and selection for other traits such as yield, plant architecture undertaken. It was also evident that gene pyramiding could enhance soybean rust resistance; however, there is need to check the effectiveness of these resistance genes against pathogens from the other parts of the country. In those areas were the pyramided genes are effective, they can be introgressed into farmer preferred soybean varieties.

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