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DEPARTMENT OF CROP AND SOIL SCIENCES

SCREENING SOYBEAN GERMPLASM FOR RESISTANCE TO RUST DISEASE AND POD SHATTERING

Thesis submitted to the Department of Crop and Soil Sciences, Faculty of Agriculture of the College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, in partial fulfilment of the requirements for the award of Master of Philosophy Degree in Agronomy (Plant Breeding)

BY

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DECLARATION

I hereby declare that this thesis has not been submitted for a degree to any other university. It is entirely my own work and all help and references have been duly acknowledged.

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DEDICATION

This work is dedicated to Prof. Harrison K. Dapaah.

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ABSTRACT

Soybean production has suffered yield losses from 10 to 80 % due to rust disease. Host plant resistance has been found to be the best control strategy. These experiments were conducted to determine the presence of the rust resistance gene(s) and evaluate them on the field for *P. pachyrhizi*, pod shattering, lodging and yield potential. Thirty four soybeans accessions consisting of 32 breeding lines and two local varieties from Ghana were used. Screening for presence of rust resistance gene(s) was done using simple sequence repeat (SSR) makers. The field experiment was laid out in randomized complete block design (RCBD) with three replications. Soybean genotypes were evaluated for rust resistance under natural epiphytotic condition. Lodging was scored using IITA descriptors. Pod shattering screening was done using oven dry method in the laboratory. Days to 50 % flowering, nodule count, plant height, days to maturity, seeds per 100 pods, 1000 seed weight and grain yield were recorded. SSR markers revealed genotypes SIT-E TGx1990-3F, SIT-M TGx1987-91F, SIT-M TGx1989-45F and SIT-E TGx1988-5F to have multiple resistance genes R*pp*1, R*pp*2 and R*pp*3, however genotype TGx1909-3F was identified not to have resistance gene. Out of the 34 soybean genotypes, SIT-M TGx1989-45F, SIT-M TGx1987-40F, SIT-E TGx19903F and SIT-M TGx1987-91F were found to be highly resistant to rust disease during phenotypic screening. It was revealed that 53 % of the genotypes showed erectness to lodging. Six genotypes, namely SIT-M TGx1904-6F, SIT-E TGx1835-10E, SIT-M TGx1987-40F, TGx1903-7F, SIT-E TGx1448-2E and ANIDASO were found to be moderately resistant to pod shattering. Genotype SIT-E TGx1989-45F was superior in terms of yield. Genotypes observed to have resistance gene(s) (R*pp*1, R*pp*2, R*pp*3, R*pp*4 and R*pp*5) to soybean rust could further be exploited and used in breeding programme. Further studies should be conducted to verify the genotype or varieties known to have some level of resistance to rust, shattering and lodging is stable/durable or not.

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CHAPTER ONE

INTRODUCTION

The first domestication of soybean (*Glycine max* (L.) Merrill) has been traced to North China, Asia and belongs to the family Leguminosae, in the subfamily Papilionideae. It is now widely grown due to its high protein and oil content, and adaptation to diverse conditions. It grows in the tropical, subtropical and temperate climate conditions, growing almost all over the world and comprises one of the major food crops of the world. Soybean is an annual small erect, semi-spread plant. Stems, leaves and pods may be hairy in some cultivars. They are self-pollinated species with less than 1% outcrossing (Norman *et al*., 1995).

According to FAO (2014), total world production of soybean in 2012 was 241.1 million metric tonnes. The three major world producing countries are USA (82 million metric tonnes), Brazil (65.8 million metric tonnes) and Argentina (40.1 million metric tonnes). Total production of soybean in Africa was 1.5 million metric tonnes with West Africa producing 437,115 metric tonnes. Soybean is cultivated in Sub-Saharan Africa to a very limited extent (Laswai *et al*., 2005; Shurtleff and Aoyagi, 2009).

Soybean production is concentrated in the three northern regions of Ghana, which falls within the Guinea savannah agro-ecological zones (Lawson *et al*., 2008), where the crop has been instrumental in social, economic and environmental benefits. In Ghana, Ministry of Food and Agriculture (MoFA) (2011) indicated the average yield potential of soybean as 1.9 t/ha.

There is the need for Africa to boost production by identifying high yielding genotypes, pest and disease resistant genotypes, shattering resistance, improved technology and adopting good agronomic practices.

Soybean research and production in Ghana are besieged with a lot of constraints. The numerous problems, arises from biotic and abiotic factors, preventing farmers from attaining the optimal yield potential. Seed viability, pod shattering, lodging, pests, diseases and narrow genetic base, among others, are examples of constraints affecting production of soybean Brink and Betay, 2006).

Pod shattering is regarded as one of the major problems for soybean growers since it reduces seed longevity and also can lead to serious yield losses. Delaying soybean harvest often promotes considerable yield loss for soybean growers (Philbrook and Oplinger, 1989; Tukamuhabwa *et al*., 2002) as percentage shattering increases. The extent of yield reduction attributed to pod shattering is determined by time of harvesting, environmental conditions and genetic attribute of the genotype and may vary from negligible to significant levels in the range of 1 to 100 % shattering (Agrawal *et al*., 2004). Breeders and growers find it of great interest to discover sources for resistance.

Soybean, like any other economically important crop, suffers from many diseases such as rust, frog-eye leaf spot, bacterial pustule, bacterial blight, and soybean mosaic virus (Hartman *et al*., 1999). Soybean rust (SBR) caused by *Phakopsora pachyrhizi* (H. Sydow and Sydow), an obligate biotrophic fungus is one of the most important foliar diseases affecting soybean worldwide (Hartman *et al*., 2005). SBR is an air-borne fungal pathogen. The features are most commonly recognised on the leaves and start in the lower canopy. Lesions also spread on the petioles, pods and stems and may be brown or reddish.

Development of the disease occurs rapidly as soon as the plant begins flowering. As the disease progresses, it causes defoliation leading to yield losses and also few seeds per pod (Harman *et al*., 2005).

SBR has been reported throughout the tropics of Asia for many decades (Hartman *et. al*., 1999), Africa (Levy, 2005) and Ghana (Bandyopadhyay *et al*., 2007). In tropical Africa, soybean rust is also reported in Uganda, Zambia, Sierra Leone, Nigeria,

Tanzania and Democratic Republic of Congo (Brink and Betay, 2006). Yorinori *et al*. (2005) reported that under excessive infestation; losses up to 75 % can be noticed in unprotected fields. Also Brink and Betay (2006) added that soybean rust disease is devastating and can lower yield by as much as 90 %. However, the rate of yield decrease may vary depending on the existing conditions and such conditions include: the genotype, environment and the time during the season when the rust becomes established (Hinson and Hartwig, 1977).

The occurrence of *P. pachyrhizi* in Africa is on the rise especially, in Ghana. This is due to low levels of resistance in soybean cultivars (Arias *et al*., 2008). Such a threat influences the net profit of the producers as well as jeopardising the livelihood and nutritional well-being of millions of people who rely on its oil and protein (Asafo-Adjei *et al*., 2005). The disease unfavourably influences the plant‟s general performance and yield. Therefore, managing SBR disease is an essential component of soybean production. A number of fungicides can control SBR but their effectiveness is highly determined by timely application and the prevailing environmental conditions (Mueller et al., 2009). An effort to reduce the SBR with fungicides application has led to; high cost of production, environmental pollution and development of *P. pachyrhizi* races tolerant to the fungicides (Caldwell *et al*., 2002). Even with fungicide application, there may still

be yield losses (Calvo *et al*., 2008). Therefore, genetic resistance is an economically and strategically important means of controlling soybean rust disease (Arias *et al*., 2008).

Five major sources of SBR resistance have been observed in soybean germplasm (Walker *et al*., 2011). The resistance is controlled mainly by a single dominant gene (Bromfield and Hartwig, 1980). However, other research has identified recessive genes controlling SBR resistance (Calvo *et al*., 2008). The resistance genes identified in soybean to *P. pachyrhizi* (R*pp*) are R*pp*1 (Mclean and Byth, 1980); R*pp*2 and R*pp*3 (Bromified and Hartwig 1980); R*pp*4 (Hartwig, 1986) and R*pp*5 (Garcia *et al*., 2008). Identification of these resistance genes were made possible with the assistance of biotechnology. Biotechnology tools such as Marker Assisted Selection (MAS) enhance the efficiency in the breeding process. These genetic markers have facilitated the development of plants resistant to diseases and pests. It is a diagnostic tool for tracing the presence of the target resistance (R) genes for which direct selection is difficult or not possible.

Host plant resistance is the strategy of most concern to plant breeders. It is prioritized over cultural, chemical, biological and regulatory control component of pest and disease management. Resistant crops enhance environmental compatibility that do not require specialized application, and apart from their preference based on agronomic desirability; they usually do not require extra input.

In Ghana, the only research done on soybean rust was to ascertain the presence of the pathogen, *P. pachyrhizi*, in October 2006 by Bandyopadhyay *et al*. (2007). The authors reported that, SBR is in Ghana with disease incidence ranging from 50 to 100 % and disease severity ranging from 3 to 40 % of the leaf area on infected plants. The fast

spreading nature of SBR, coupled with its potential for causing severe yield losses, makes it an important disease in soybean growing countries. Hence, there is the need to screen for resistant soybean genotypes for seed multiplication or breeding against rust. This will make it possible for plant breeders to make progress in developing cultivars resistant to soybean rust disease. Therefore, screening soybean genotypes to identify resistance to rust disease, pod shattering and lodging with high yielding potential will help to increase production to a greater extent.

The main objective of this work was to identify soybean genotypes/varieties resistant to rust disease. The specific objectives were to:

i. determine the presence of the rust resistance gene(s) in the soybean genotypes, ii. evaluate the genotypes resistant to *P. pachyrhizi*, and iii. assess soybean genotypes for their resistance to pod shattering, lodging and

yield potential.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin of Soybean

Soybean is native to China and it has been cultivated since the 11th century (Hymowitz and Shurtleff, 2005). Studies show that soybean may have been domesticated as far back as 3500 BCE in different parts of the world and were not exclusive to China (Barlow, 2011). According to the ancient Chinese, in 2853 BC, the legendary Emperor Shen Nung of China named five sacred plants – soybean, wheat, millet, rice and barley (History of Soybean, 2008). Soybean crop reached Africa through missionaries in the early 19th century (CGIAR, 2005). Soybean was introduced into Ghana in 1909 by Portuguese missionaries (Shurtleff and Aoyagi, 2009).

2.2 Botanical and Morphological Description of Soybean

Soybean belongs to the family Leguminoceae, subfamily Papilionodeae, and the genus *Glycine*. More than 500 genera and 12,000 species of soybean exist (Shurtleff and Aoyagi, 2009). The cultivated species is *G. max* whiles *G. Soja* is a wild form according to Fageria *et al*. (1997). The cultivated species (*G. max*) has never been found growing in the wild. It is believed that it is most probable progenitor is *G. soja* (Hymowitz and Newel, 1981). Both species are annual. The similarity between *G. soja* and *G. max* are about 92 % (Powell *et al*., 1996). The divergence from *G. soja* and *G. max* is less than 0.2 % and is based on their nucleotide sequence (Kollipara *et al*., 1997), but they share many of the same alleles. Soybeans are diploid, but some authors are of the view that they are allopolyploid species, where heterosis and gene redundancy are of advantage (Comai 2005; Gill *et al*., 2009). They are self-pollinated species with less than 1 % out-crossing (Norman *et al*., 1995; Chaturvedi *et al*., 2011).

Soybean grows in the tropical, subtropical and temperate climates.

Soybean is herbaceous, hairy annual dicot plant with an extensive taproot system. The taproot may grow as deep as 2 m and adventitious roots grow from the hypocotyls. It is erect and can reach a height of 1 m (Jin *et al*., 2010) but occasionally, prostrate and freely branching forms are also found (Chaturvedi *et al*., 2011). Majority of the cultivars have their pods, stems and leaves covered with hairs but glabrous types also exist. The primary leaves are opposite, ovate and unifoliate. The secondary leaves are trifoliate and alternate; the leaves are compound occasionally with four or more leaflets present.

The flowers may be either white or purple (Hartwig and Hinson, 1962) and are borne in axillary racemes on peduncles at the nodes. A large number of flowers are produced by the plant but only about two-thirds to three quarters of them produce pods (Acquaah, 2007). Flowering in soybean is determined by day length, with short day length being the trigger for flowering (Major *et al*., 1975). The plant can generally produce 100 to 150 pods. The pod may be straight or slightly curved, varying in length from 2 to 7 cm consisting of single carpel, which is joined by a dorsal and ventral suture. The normal colour of a mature pod can range from light-yellow to black depending on the genotype. The pods are also pubescent. The shape of the seed is usually oval but can differ among cultivars from spherical to elongate and flattened.

2.3 Growth, Development and Nodulation of Soybean

Soybean germination is epigeal. Nelson and Larson (1984) suggested that, in epigeal germination, the hypocotyl is active and pulls the cotyledons above the ground during its growth (Fig. 2.1). Seeds begin to emerge from three to five days after sowing under favourable environmental conditions.

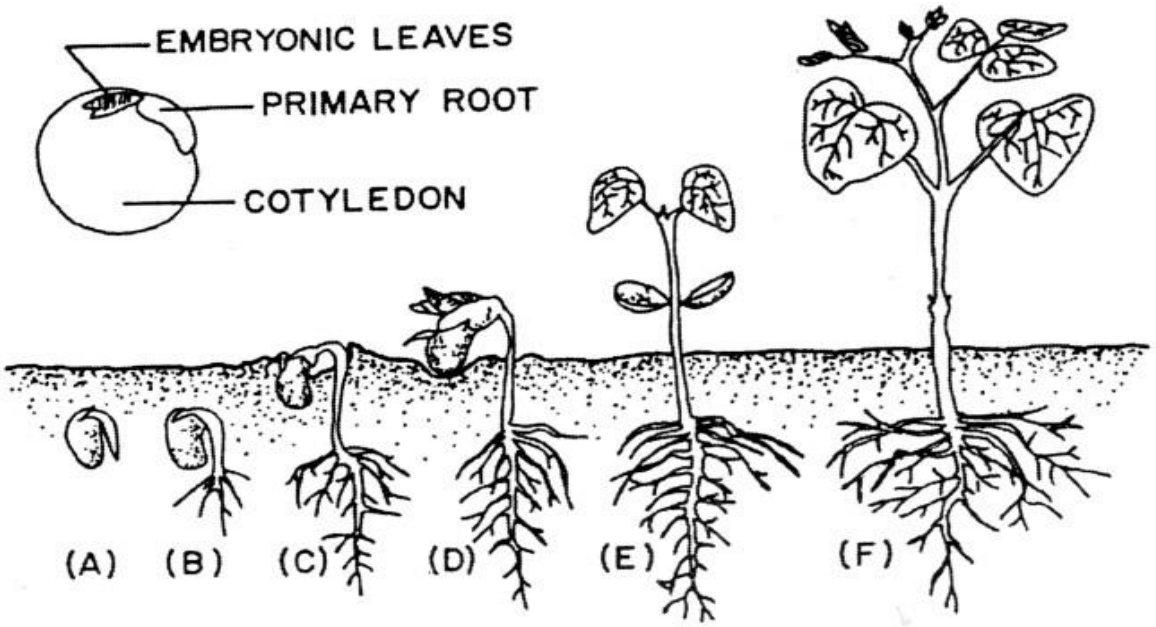

Fig. 2.1: Germination and seedling development of soybean

Emergence of the radicle to form the primary root (A), development of secondary roots (B), elongation of the active hypocotyl with the hypocotyl arch penetration through the soil surface (C), seedling becomes erect (D), with cotyledons attached to the first node (E), prior to drying and falling from the autotrophic seedling (F). Adapted from Nelson and Larson (1984).

Soybean cultivars are usually classified based upon their morphological growth habit. They exhibit growth pattern as determinate, indeterminate or semi-determinate (Bernard, 1972). The determinate types stop growing in height after flowering thereafter the stem continues to expand in width and the terminal bud usually becomes an inflorescence. Indeterminate cultivars continue to grow in height throughout the flowering and pod developmental stages. The semi-determinate types have indeterminate stems that terminate vegetative growth abruptly after the flowering period. Their growth habit and

flowering lie between the growth habits of determinate and indeterminate cultivars of soybean (Gary and Dale, 1997).

The knowledge of the developmental stages of soybean is crucial in evaluating its yield potential. The developmental stages in soybean are characterized by the standards established by Fehr and Caviness (1977). Soybean growth is characterized by two distinct growth phases. The first is the vegetative (V) stages which begin with the emergence of the young seedling from the soil surface and end with the start of flowering. It is characterized by the number of nodes on the main stem; beginning with the unifoliate node that has a completely unrolled leaf. The other is the reproductive (R) stages which cover growth from flowering through maturation. It is characterized by flowering, pod and seed development. Soybean growth and development are directly linked to day length and temperature. The plant does not change from vegetative to reproductive growth until a critical day length is met. Soybean is noted as a quantitative short-day plant (Gary and Dale, 1997) which means, flowering is promoted within a certain time range but is retarded and/or diminished outside this time frame (Loomis and Connor, 1992).

Soybean on an ideal soil is infected by *Rhizobium*. Soybean as a legume has developed a method to generate root nodules (Walter and Bien, 1989) through a symbiotic relationship with nitrogen fixing *Bradyrhizobium japonicum* (Kirchner) (SarkodieAddo *et al*., 2006; Crespi and Galvez 2000). During symbiotic association with *B. japonicum*, soybean plants have the ability to fix almost 200 kg N ha^{-1} yr^{-1} (Smith and Hume, 1987). The soybean crop then provides an added advantage for improving nitrogen status of soils through nitrogen fixation (Jaiswal *et al*., 2011), thus limiting the need for expensive and environmentally damaging nitrogen fertilizer. This is a major benefit in African farming systems, where soils have become exhausted and where fertilizers are expensive for

farmers. The nodule establishment occurs due to the sequence of multiple interactions between the leguminous plant and the bacteria

(Hopkins and Hüner, 2004). However, nodulation of soybean needs specific *Bradyrhizobium* species (Abaidoo *et al.,* 2007). Soybean breeders at International

Institute of Tropical Agriculture (IITA), Nigeria, developed new soybean genotypes for Africa, known as Tropical *Glycine* Cross (TGx), which nodulate with *Bradyrhizobium* spp. populations indigenous to African soils (Pulver *et al*., 1985). *Bradyrhizobia* are seldom available in soils where the leguminous crops (soybean, cowpea and groundnut) have not been grown previously (Abaidoo *et al*., 2007). Total N accumulation and N fixation are low during the early growth stage and then they increase rapidly at later stage. N fixation reaches maximum at R3/R4 stage and then drops (Sanginga *et al*., 1997).

2.4 Economic importance of soybean

The important role soybean is playing in the rural economy of Ghana cannot be overemphasized. Soybean is regarded as an element for increasing the food security and reducing poverty especially in rural communities in Ghana. Soybeans are used for vegetable oil, human food, livestock feed and industrial products (McKevith, 2005).

The seed is economically useful part of soybean plant. It contains approximately 40 - 42 % protein and 18 - 22 % oil and also good amount of nutrients such as carbohydrate, minerals, vitamins and dietary fibre (Antalina, 1999). Bewley and Black (1994) added that, the harvested seed of soybean has a high protein content of 380g/kg apart from carbohydrate of 380g/kg and oil of 200g/kg. In Ghana, there has being an expansion in soybean production due to promotion of its high nutritional and economic values by the Ministry of Food and Agriculture (MoFA) (Sarkodie-Addo *et al*., 2006). In terms of

production and utilization of soybean in Ghana, it is ranked third after groundnut and cowpea (MoFA, 2011).

Malnutrition, especially protein deficiency, which is abundant in many parts of Africa as animal protein is expensive for most citizens, is reducing due to soy protein. In Ghana, soy proteins are also being used in baby foods to avoid kwashiorkor (protein deficiency) in children (Asafo-Adjei *et al*., 2005). Soybean is aiding to reduce this effect as it is the only available crop that provides inexpensive and high quality protein source compared to eggs, meat and poultry. The role of soybean in making up protein deficiency in human diet cannot be underestimated (Khalid, 2000). Tweneboah (2000) also added that, the crop provides high quality protein for many resource poor inhabitants in Sub-Saharan Africa countries. The nutritional benefit for soybean consumers is well documented (Asalm *et al*., 1995; Asafo-Adjei *et al*., 2005). Most traditional foods in Ghana such as kenkey, banku, gari, sauces and stew are fortified with soybean to increase their nutritional levels without affecting the cooking time or taste (Asafo-Adjei *et al*., 2005; MoFA and CSIR, 2005). Soy protein contains all essential amino acids, which is vital for vegetarians (Rackis *et al*., 1961). The nutritional value of soybean makes it capable for eliminating if not reducing malnutrition problems in Ghana and has the capacity for developing the agriculture, health and industry sectors (Plahar, 2006).

Soybean oil can be used as source of power as well as cooking oil (Hayati *et al*., 2009; Moser, 2011). The biodiesel reduces particulate emissions; it is renewable, non-toxic and environmental friendly. Soybean oil is broadly used as edible oil, as it is almost cholesterol-free, which makes it the topmost choice of vegetable oil for domestic and industrial food processing (Addo- Quaye *et al.*, 1993; Mpepereki *et al*., 2000). The oil is used for human consumption as shortenings, margarine, and other fat and oil products, as

well as non-food applications. Soy oil is used in industries as paint, varnishes, printing inks, and other compounds. Lecithin, a product extracted from soybean oil, is a natural emulsifier and lubricant used in many foods, commercial, and industrial applications (Gibson and Benson, 2002).

Soybean cake left after the extraction of the oil is commonly used to feed animals. It is the most ordinary source of supplemental protein for poultry and is the standard to which all other protein sources are compared. Soybean meal protein digestibility in poultry of all ages and types is approximately 85 % (Woodworth *et al*., 2001). The amino acid profile of soybean meal is near to that of fishmeal, except methionine
(INRA, 2004).

Soybeans have the ability to generate root nodules (Walter and Bien, 1989) which initiate a symbiotic relationship with Rhizobiaceae in order to fix nitrogen (Crespi and Galvez, 2000). Thus, maintaining and sustaining soil fertility. Soybean involvement in rotation with cereals has the ability to reduce Striga seed bank in soils (Denwar and Ofori, 2003). It also has the potential of breaking pest and disease cycles when grown in rotation with cereals.

2.5 Yield determination in soybean

Yield of soybean crop is attributed to dry matter production, light interception, and the partition of dry matter into the plant‟s seed. Yield is a function of individual seed weight and seed number per area when the crop matures. Yield criteria are yield component, morphological factors such as seed per area, seed size and node number per area that

affect soybean yield formation; and growth dynamics parameters such as leaf area index, light interception and total dry matter (Khan *et al*., 2000; Sudarić *et al*., 2006).

Seed is the economically useful part of soybean. The yield component of soybean is determined by the number of seeds per unit area and individual seed weight (Kokubun *et al*., 2001). The yield components are established during the discrete periods of soybean development that is, flowering, pod formation and seed filling periods. The quantity of seeds per pod is genetically influenced (Shibles *et al*., 1975). The quantity of seeds is influenced by the number of pods, which is largely regulated by the number of floral buds that begin pods and attains maturity (Desclaux *et al*., 2000). Several floral buds are produced by soybean plants but large quantities of the ovaries are terminated prior to advancing into mature pods thus, pod quantity is principally determined during early stages of pod development (within five days after anthesis) (Dybing *et al*., 1986). The quantity of seeds produced in soybean is also affected by flowers per node, nodes per unit area, proportion of flowers that develop into mature pods, and seeds per pod (Khan *et al*., 2000; Liu *et al*., 2005; Arshad *et al*., 2006). The seed quantity per area is determined near the initiation of rapid seed filling, around 10 to 12 days after the beginning of seed filling (Board and Tan, 1995). The individual seed weight is the outcome of rate and the length of seed filling (Munier-Jolain *et al*., 1998), which is generally regulated during seed filling after the pod number had been fixed (Brevendan and Egli, 2003). Grain yield is controlled by genetic and environmental factors as well as their interactions (Yan and Rajcan, 2003; Vratarić *et al*., 2006; Sudarić *et al*., 2006)

2.6 Shattering in soybean

Shattering occurs in soybean when pods attain maturity. It happens along the dorsal or ventral sutures. This leads to dispersal of seed and is usually high under less humid

conditions. The degree of yield loss due to pod shattering in soybean may differ from 34 to 99 % (Tiwari and Bhatnagar, 1991). Shirota *et al*. (2001) reported yield losses due to shattering to be 422kg/ha. The shattering of soybean pods is one of the vital restrictions to mechanical harvesting as it minimizes crop yield and it is of great interest to breeders and farmers. This implies that breeding should have focal point on development of high yielding varieties with pod shattering resistance. Therefore, a high degree of resistance to pod shattering is significant in commercial soybean cultivation especially, in the advent of climate change and its associated high temperatures and prolonged drought (Tukamuhabwa *et al*., 2002). Identifying pod shattering-resistant cultivars will minimize yield loss at harvest and hence, increase yield.

There are several factors influencing pod shattering in soybean including, cultivar differences, anatomical structure of pod, environment, and genotype by environment interaction (Tiwari and Bhatnagar, 1991; Tukamuhabwa *et al*., 2002; Agrawal *et al*., 2004). The anatomical structure of pod of the crop can facilitate pod shattering before and during harvest as the result of natural movement of the canopy which result in pod knocking against each other, stem or branches. The loss is much affected by plant attributes such as pod length, width and angles (Thompson and Hughes, 1986). The environmental conditions contributing to pod shattering include: high temperatures, rapid changes in temperature, low humidity, wetting and drying (Tukamuhabwa *et al*., 2002).

There are established methods for evaluating pod shattering and have been tested, demonstrated and usable in breeding programmes. These include: the oven-dry method (AVRDC, 1979; Tiwari and Bhatnagar, 1997; Tukamuhabwa *et al*, 2002) in which pods are subjected to oven-drying for a specific period, field-screening method (Tiwari and

Bhatnagar, 1997; Helmes, 1994) which depends on visual observation in the field, mechanical cracking method (Kwon *et al.*, 1991; Timothy *et al.*, 2003) a laboratory procedure which involves testing and measuring individual pods for their mechanical properties to shattering resistance, and the desiccator method (Caviness, 1969) where pods are subjected to desiccation inside a desiccator.

2.7 Genetic diversity of Soybean

The success of soybean breeding programme depends on degree of variability in germplasm, choice of parents and selection procedure (Dong *et al*., 2004). Although soybean has a rich source of germplasm, narrow spectrum of variability is a problem to its breeding programme. This setback is worsened due to high level of self-pollination. Soybean germplasm evaluation is pivotal for gene bank managers, as it supports efficient sampling of genotypes/lines for crosses and removing duplication of planting material. Diversity in soybean serves as key for finding and incorporating new lines into elite soybean genotypes. Genetic distinction among genotypes are useful for planning future breeding efforts for yield, oil content, protein, pest and disease resistance improvement (Wang *et al*., 2006). Understanding the amount and distribution of genetic difference within and among soybean genotypes is a key for predicting the degree of inheritance, variation and extent of heterosis that are crucial for breeding.

Soybean genetic diversity can be evaluated by the differences in agronomic traits, morphological traits, pedigree information, isozymes and DNA markers (Sneller, 1994; Dong *et al*., 2004; Wang *et al*, 2010). The polymorphism can also be observed at morphological, molecular and biochemical levels. The accuracy of genetic variation is

determined by the method used. Compared with morphological variation, molecular polymorphism is generally considered to be independent of the environment (Gauthier *et al*., 2002).

2.8 Soybean rust (SBR)

Plant rusts, caused by Basidiomycetes of the order Uredinales, are most of the destructive diseases of plants. They cause damage to grain crops such as maize, oat, wheat and barley, and on ornamental plants, such as carnation (*Dianthus spp*.) and chrysanthemum (*Chrysanthemum spp*.), and also field crops such as cotton and soybean (Agrios, 2005). Soybean rust (SBR) or the Asian soybean rust (ASR) is caused by two related species of fungi namely, *Phakopsora pachyrhizi* (Sydow), the most aggressive and *Phakospora meibomiae* (Arthur), less virulent. The two species were thought to be the same but proved otherwise by Ono *et al*., (1992). They are differentiated based upon morphological characteristics of the telia. *P. pachyrhizi* contain one to seven layers of teliospores and *P. meibomiae* contain one to four layers of teliospores. In this work, SBR indicates either *P. pachyrhizi* or the disease caused by it.

P. meibomiae (Arthur) is the causal of „American" rust disease and has a host range of 66 species, including soybean (Sinclair and Shurtleff, 1975). The species are native to South America and is present on wild and cultivated legumes from Rico to Southern Brazil (Vakili and Bromfield, 1979). According to, Hartman *et al*., (1999), species which is found in limited areas in the Western Hemisphere and is not known to cause severe yield losses in soybean.

P. pachyrhizi (Sydow) is the causal agent of the „Asian" rust, native to traditional growing in the Orient. Asian soybean rust (ASR) or Soybean rust (SBR) is considered among the 25 of the 100 most dangerous exotic pests of the world (Ogle *et al*., 1979). It can infect and spread from many wild and cultivated hosts, including many garden legumes (Vakili and Bromfield, 1976). *P. pachyrhizi* is an aggressive foliar disease in many other soybean producing countries worldwide (Hartman *et al*., 2005). The pathogen can infect soybean any time after germination (Bromfield, 1984). The causal agent of SBR (*P. pachyrhizi*) is considered to be the most destructive foliar disease because of its ability to spread rapidly and its potential to severely reduce yields (Miles *et al*., 2003). *P. pachyrhizi* can infect over 95 plant species from more than 42 genera, including soybean and other *Glycine* spp according to Ono *et al*. (1992).

2.8.1 Geographic distribution of SBR

SBR was first discovered in Asia and now spread to major soybean producing regions of the world (Goellner *et al*., 2010). Also, according to Miles *et al*., (2003), SBR is considered as a global epidemic. It was identified in Japan since 1902. In 1934 it was identified in several Asian countries and in Australia (Bromfield and Hartwig, 1980) and also in India in 1951 (Sharma and Mehta, 1996). In Africa SBR was first confirmed and reported in Kenya, Rwanda and Uganda in 1996 (Levy, 2003). SBR was reported in Zambia and Zimbabwe in 1998, Nigeria in 1999 and Mozambique in 2000 (Akinsanmi *et al*., 2001) and Ghana in 2007 (Bandyopadhyay *et al*., 2007). *P. pachyrhizi* has been spotted on cowpea leaves from African countries such as Ghana,

Sierra Leone, Sudan, Tanzania and Uganda (Bromfield, 1977).

2.8.2 Disease cycle and epidemiology of rust

SBR epidemic is caused by the following environmental factors: moisture, temperature, wind and light (Mederick and Sachston, 1972; Yang *et al*., 1991; Kim *et al*., 2005). The optimal temperature range from 15 to 26 ºC is adequate to cause SBR epidemics (Bromfield, 1984). Maximum disease development occurs under a temperature regime of 17 - 27 ºC (Kochman, 1979). Adverse environmental conditions that negatively affect the host survival decrease the ability of the pathogen to reproduce. When such conditions happen the pathogens have an alternative host usually susceptible legumes, where it is protected from high temperatures by the plant foliage (Jurick *et al*., 2007). Winds help to increase spore dispersal as they pick up the urediospore easier. Direct impact of sunlight inhibits *P. pachyrhizi* urediospore germination, thus increasing mortality (Isard *et al*., 2006).

Once the soybean is infested, it produces asexual reproductive structures called uredia, which continue to reproduce for several days (Melching *et al*., 1979; Goellner *et al*., 2010) and mature in 6 to 7 days. Development of uredia and urediospore production are most often found on abaxial surfaces of leaves, which escape exposure to direct sunlight and ultraviolet radiation (Bromfield and Hartwig (1980), Isard *et al*., (2006), Goellner *et al*., (2010). Urediospore emerges from the uredia and are dispersed by wind. After settling on susceptible host, urediospore germinates in the presence of free moisture; leaves wet duration between 6 and 12 hours provides enough moisture for SBR to germinate to cause infection (Marchetti *et al*., 1976). The process of infection starts when urediospore on the leaf surface produces a germ tube, followed by formation of an appressorium (Ajay *et al*., 2010) and then penetrate directly into epidermal cells through the formation of hypha that emerges from the appressorium

(Koch *et al*., 1983). The hypha penetrates the host‟s epidermal cell wall to reach the mesophyll to where the fungal colonization begins. This process is usually completed 20 to 24 hours after inoculation (Koch *et al*., 1983). *P. pachyrhizi* develops haustoria structures that are responsible for nourishing the fungus and maintaining a parasitic relationship with the host cells (Agrios, 2005). The reproductive structures are formed and the life cycle begins 7 to 9 days after infection. A single disease leaf is sufficient to cause disease epidemic in a field (Bromfield, 1984).

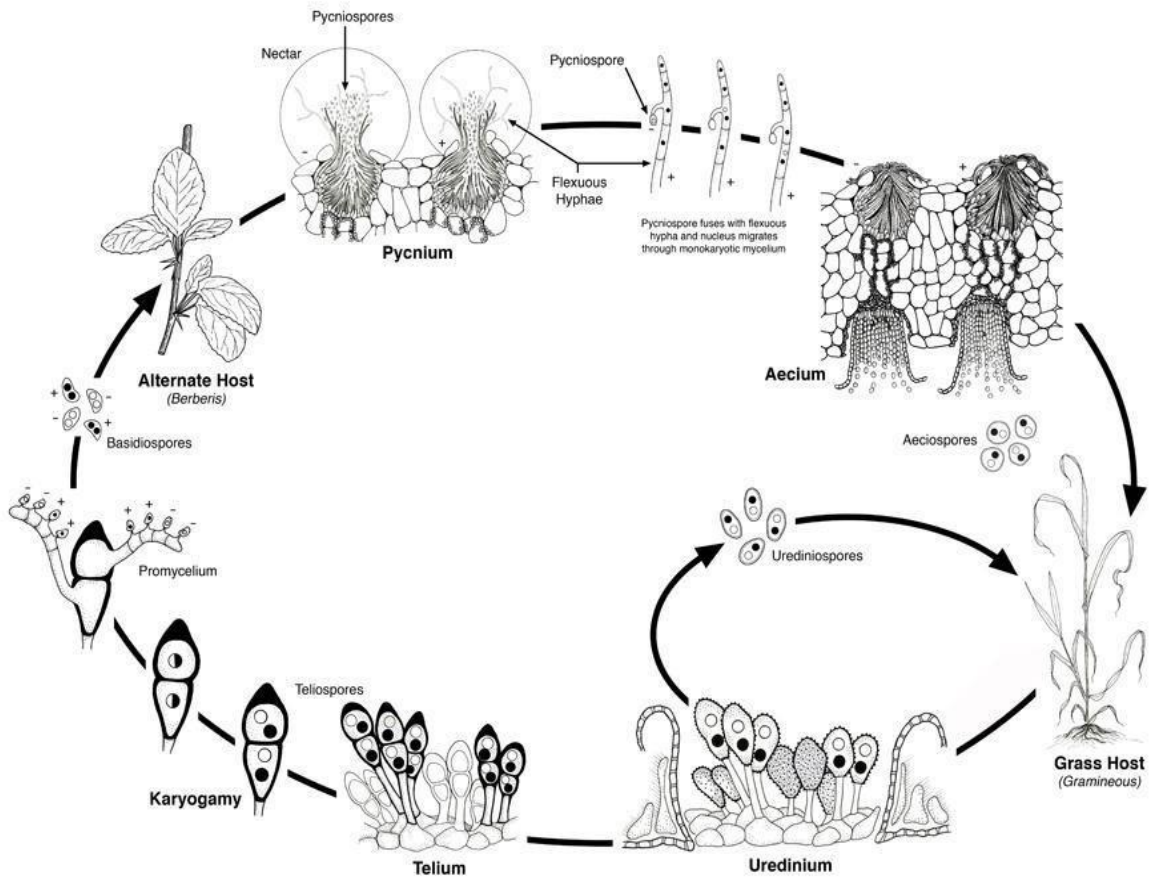

Fig 2.2: Life cycle of rust fungi (Leonard and Szabo, 2005)

2.8.3 Disease triangle of Soybean Rust

Plant pathologists were the first to coin the term “disease triangle” in 1960 (Mcnew, 1960). Plant disease occurs as a result of interaction between three elements: virulent

pathogens, susceptible hosts and the conducive environment (Scholthof, 2007). For successful infection of *P. pachyrhizi* on a soybean plant, these three basic conditions have to be met. These interactions are sometimes illustrated diagrammatically as the disease triangle (Fig. 2.3). Disease development cannot occur if any of the elements of the triangle are missing (Lucas, 1998).

The development of almost all plant pathogens is dependent on the environmental conditions which occur during the time that a crop is grown. Temperature, moisture, light and winds were all considered to be important environmental factors influencing disease epidemics (Mederick and Sachston, 1972; Yang *et al*., 1991; Kim *et al*., 2005). The most important environmental factors are temperature and humidity, followed by rainfall and wind. The temperature determines the rate of reproduction, the growth and dispersal processes that occurs in plant pathogens.

The more virulence and the inoculum level determine the degree of the rust disease. Pathogen effects are important but their effect is highly dependent on the existing environmental conditions, likely in a genotype specific manner (GpxE). Susceptibility to disease in terms of the host variations will influence the changes in infection, but will also depend on how different host genotypes/varieties respond to infection under different environments (GhxE). The presence of genetic variation in both host and pathogen (*P. pachyrhizi*) populations allows for genotype-specific patterns of infections (GpxGh). This shows that disease is the outcome of both genotypic interactions between hosts and pathogens, and how the environment modulates these interactions (GpxGhxE).

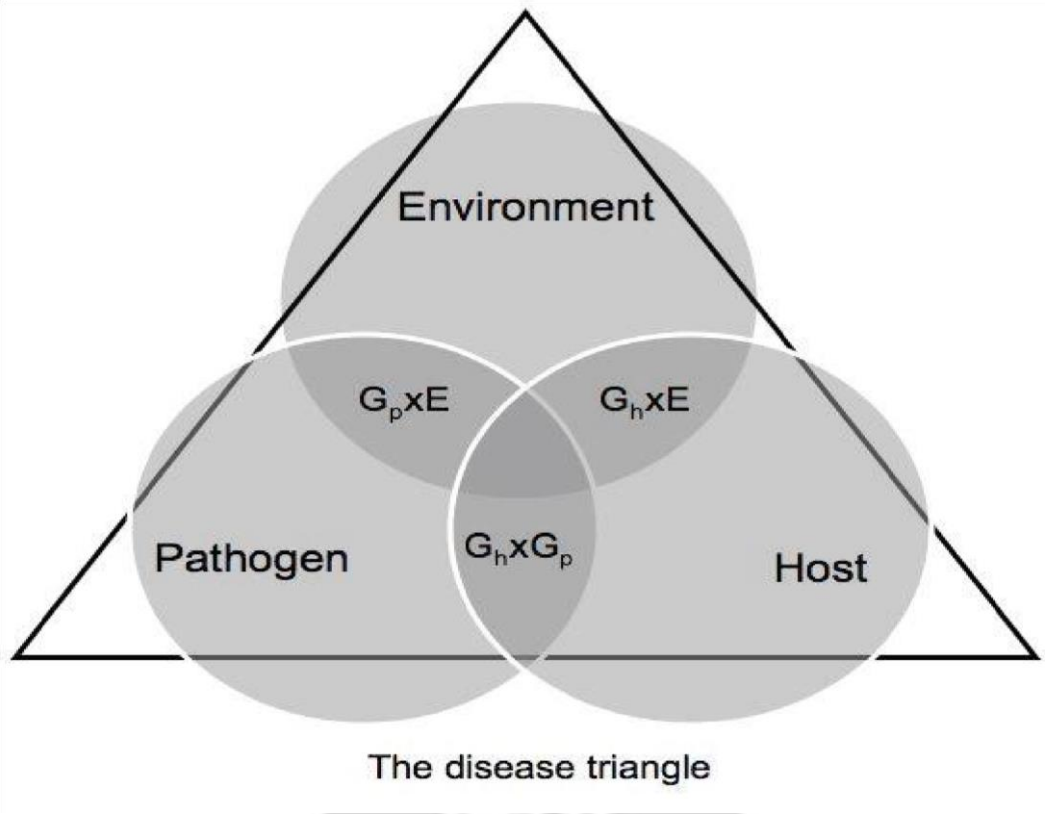

Fig 2.3: Disease triangle of Soybean Rust (Scholthof, 2007)

2.8.4 Host range of P. pachyrhizi

P. pachyrhizi is an obligate parasite and does not survive on dried or decayed tissues or in the soil and needs living hosts for survival and reproduction (Agrios, 2005). Urediospores survive (in resting or dormant stage) less than 2 days under ambient conditions (Yang, 1977). During unfavourable conditions it finds a host to survive (Agrios, 2005). It has the ability to penetrate directly into its host without finding an already existing opening caused by insects or through the stomata. This special feature enables the pathogen to have a wide range of host (Miles *et al*., 2003). The pathogen has the ability to survive on a wide range of crops worldwide. It infects large number of dicotyledonous plant such as common bean (*Phaseolus vulgaris*), wild soybean (*Glycine soja*), and yam bean (*Pachyrhizus erosus*), both on the field and in the laboratory (Yang, 1977). Ono *et al*., (1992) reported that 91 plant species in Papilionoideae serves as a host

for *P. pachyrhizi*. *P. pachyrhizi* has been spotted on cowpea leaves from African countries such as Ghana, Sudan, Sierra Leone, Tanzania and Uganda (Bromfield, 1977).

2.8.5 Symptoms and effects of rust on soybean yield and quality

Symptoms development occurs rapidly as soon as the plant start flowering. Rust is conspicuous on leaves. In severe cases of infection, lesions may be found on pods, stems and petioles (Caldwell *et al*., 2002). Small, angular, reddish-brown lesions develop on the under surface (abaxial) of the leaf. At this stage, the lesions appear similar to those of the bacterial pustule, *Xanthomonas campestris pv. Glycines* (Sinclair, 1982), but they have characteristic blister-like uredia with a central pore extruding urediospore on the abaxial side of the leaf (Yorinori, 1994). SBR usually infect older leaves at the base of the plant first and progresses upwards as the severity of the disease increases. Usually, initial lesions can be observed seven to nine days after infection. Lesions gradually increase in size, and they later turn from gray to tan, reddish-brown or dark brown.

Severity of the disease is reported by Devaraj *et al*., (2012) to cause yellowing, premature drying and defoliation and is favoured by continuous rainfall/high humidity with moderate temperatures and extended leaf wetness (Bromfield, 1984). Also severity of infection may be influenced by soybean cultivar, the pathogen strains and the environmental conditions (Sweets, 2002). SBR reduces leaf area index and photosynthetic efficiency (Goodwin, 1992; Kumudini *et al*., 2008). The canopy of the crop is affected due to defoliation, which limit photosynthesis and yield (Kumudini *et al*., 2008). Severely infected plants show early defoliation, compromising pod formation and filling and final grain weight (Yang *et al*., 1991).

The disease has effect on the yield components: pods per plants, seeds per pods and mean seed weight but it is also dependent on the variety, rust severity and time of first infection.

According to Hartman *et al*., (1991) yield and 100 seed weight was low when infection occurred at the beginning of flowering (R1) than at full seed (R6). The premature defoliation of leaves caused by the disease decreases the number of filled pods and the weight of seeds per plant thereby reducing yield. Yield losses occur due to increase in pod abortion and decreases in seed size and seed per pod, thus, reducing the net profit of the farmer.

2.8.6 Management of soybean rust disease

Strategies for managing plant diseases include: interruption of the disease cycles by crop rotation, fungicides applications, and crop/cultivar development (Krupinsky *et al*., 2002). The high yield losses associated with SBR needs an effective control strategy to mitigate its effect on the crop. The control measures involves: cultural, chemical and deployment of resistant/tolerant varieties.

2.8.6.1 Cultural Control

It involves modifying agronomic practices or adopting new practices to help prevent new incidences or progress of the disease (Bromfield, 1984). These practices include:

- planting early maturing cultivars, thereby reducing the time available for the pathogen to infect
- controlling weed hosts
- watering plants at noon to ensure that leaves have a chance to dry
- using soybean cultivars with short pod-filling stages and
- careful selection of planting sites, such as growing soybeans far away as possible from alternate hosts of SBR
- crop rotation with non-host plant to prevent pathogen build up.

These practices aid in reducing chances of SBR infection (Bromfield, 1984). Also, appropriate time of planting can be an immense value in preventing the disease. The early planting of early maturity cultivars reduce the period that the crop was exposed to environmental conditions favourable for SBR development. Yorinori *et al*., (2007), also suggested that early planting of early maturity cultivars reduced the impact of SBR on soybean. Few researches have been carried out on the use of cultural practices to manage SBR. Cultural practices as means of controlling SBR cannot provide ultimate solution.

2.8.6.2 Chemical control

Fungicide usage is recommended as a preventive measure or after early disease detection in the field (Godoy and Canteri, 2004). The application of fungicides to manage SBR involves three strategies; fungicides application in a predetermined calendar-based schedule (Levy, 2005), scouting and applying the fungicides after first detection of the disease, and utilization of forecast system that monitors disease progress in areas that are potential sources of inoculum. The calendar based program provides high level of yield protection because; the crop is protected from flowering till pod filling. It is unfortunate that, it leads to high cost of production and may have negative implications on the activities of non-target fungi. Moreover, the success of fungicides application depends on the time of application (Mueller *et al*., 2009) and the existing environmental conditions. The uses of fungicides in the control of SBR are affected by environmental differences (weather and farming systems) in soybean growing areas and economic factors (capital, cost of materials and availability of labour, fungicide and materials) (Bromfield, 1984). Application of fungicides such as benzimidazole and mancozeb has proven to suppress SBR progress when applied for three to five times (Hartman, 1996). They are not cost effective since multiple applications are needed to control the rust disease (Sinclair and Hartman, 1999). Additionally, there is concern for fungicide

residues on food crops and exposure to the consumers. Also over or under dosage may lead to resistance, which can be passed to other fungi (Caldwell *et al*., 2002). However, fungicide application is an option for managing SBR until disease-resistant varieties are developed (Shaner *et al*. 2005).

2.8.6.3 Resistant control / Host Plant Resistance

This is of great interest to the plant breeder and that is what he/she targets in cultivars. The ability of plant to resist disease can be achieved through traditional methods, mapping and genetic modification (Meksem *et al*., 2000). Resistance for disease is achieved through vertical or horizontal resistance. Vertical resistance is controlled by one gene and is non-durable whiles horizontal resistance is controlled by polygene and is more durable (Acquaah, 2007). A combination of the resistance types would be ideal, since horizontal resistance slows the rate by which a disease spreads through a field while vertical reduces the initial inoculating a field (Van Der Plank, 1965).

Breeding for resistance to *P. pachyrhizi* is of major concern to soybean breeders and has been conducted by classical germplasm screening based on three infection phenotypes: susceptible tan (TAN) lesions „Tan", reddish-brown „RB" lesions or incomplete resistance and an immune Response „IR" confirmed by Bromfield and Hartwig, (1980). A susceptible response occur when tan lesions develop, indicating fungal growth and development. Susceptible interactions (Tan) are characterized by tan-coloured lesions with sporulating uredia, whereas resistant cultivars generally develop reddish-brown lesions with little or no sporulation (RB). A resistant response leads to the formation of RB lesions indicating immune or hypersensitive reaction. The number of uredia on the lower leaf surface is generally two to five per Tan lesion and zero to two per RB lesion.

For both the RB and TAN lesion types, sporulation of uredinia has been reported to vary (Bonde *et al*., 2006). The „immune" phenotype is an incompatible interaction without any visible disease symptoms on host leaves (Bromfield, 1984; Pham *et al.*, 2009). Immunity to *P. pachyrhizi* occurs when no visual lesions are produced by the soybean plant. The IR has only been reported with R*pp*1, and only when inoculated with specific isolates (Bonde *et al*., 2006). Identification of lesion colour is not a reliable means to rate resistance or susceptibility of genotypes to rust severity. None of the soybean cultivars in present commercial production is resistant to all *P. pachyhrizi* isolates (Hartman *et al.*, 2005).

TAN = fully susceptible reaction, RB = resistant reddish brown lesions with defined margins, and immune reaction (IR) = no visible symptoms

Fig 2.4: Soybean rust reaction types (Miles *et al*., 2006)

SBR resistance is controlled by a single dominant gene (Hartwig, 1986). Recently there are six genes known to confer resistance to SBR in soybean. These genes are designated as R*pp*1 (Hyten *et al*., 2007), R*pp*2 (Silva *et al*., 2008), R*pp*3 (Bromified and Hartwig, 1980), R*pp*4 (Hartwig, 1986) and R*pp*5 (Garcia *et al*., 2008). There are also more recently discovered unnamed genes (Monteros *et al*., 2007). R*pp*1 confers an immune response for which there is no visible symptom on the plant (Miles *et al*., 2011). Plants with other

R*pp* genes identified to date produce dark reddish-brown (RB) lesions at the site of infection. Varieties/genotypes identified to have R*pp*1 are known to provide a higher level of resistance than *Rpp2*, *Rpp3* and *Rpp4* (Walker *et al*., 2011). SBR reduces the photosynthesis ability of the susceptible genotypes whiles resistant genotypes also showed lack of pathogen sporution lesion on the leaf thus, reducing the effect of the disease on photosynthesis and yield (Kumudini *et al*., 2010). Some aggressive isolates of *P. pachyrhizi* are able to overcome these sources of single gene resistance (Bonde *et al*., 2006). Currently, there is no commercial soybean cultivar containing all these genes or expressing resistance to all *P. pachyrhizi* races.

In conclusion, cultural practices may reduce the impact of SBR severity but cannot prevent yield losses. Fungicide application is expensive and affects negatively on nontarget living organisms. Therefore, the best strategy is breeding for resistant varieties.

2.9 Molecular markers

Traditional protocols for plant breeding are based on the phenotypic selection of plants with traits of interest. Conventional methods often encounter difficulties related to genotype by environment (G×E) interactions that can affect the effectiveness of the phenotypic selection. The screening protocols are time-consuming, expensive or often unreliable for particular traits such as disease resistance. Marker assisted selection (MAS) strategies can be used to reduce the time needed to select superior lines with resistance to SBR.

MAS facilitate selection of genotypes with desirable trait. This is achieved by selecting the marker linked to the gene of interest. MAS have become reality with the development and availability of genetic markers and genetic maps in crops. Markers play important

roles in plant breeding in three main ways. First, markers assist in identifying marker phenotypes at the seedling stage, thus reducing the needed time for maturation and reduction in population sizes (Yu *et al*., 2000). Second, markers are used to select for both qualitative and quantitative trait that are otherwise difficult to tag including variation within genotypes, resistance to pathogens and insects, tolerance to abiotic stresses, quantitative traits, recessive genes and also alleles that are not expressed in the selection environment. Third, markers can be used to select rare progeny that are as the result of recombination near the target gene, thus reducing the influence of linkage drag (Tanksley, 1993). Therefore, it is a diagnostic tool for tracing the presence of the target R*pp* gene for which direct selection is difficult or impossible.

Molecular markers work by highlighting polymorphisms within a nucleic sequence between different individuals. The different techniques employed are based either on restriction-hybridization of nucleic acids or on Polymerase Chain Reaction (PCR), or both. The examples of molecular markers include Random Amplified Polymorphic DNA (RAPD) (Welsh and McClelland, 1990), Variable Numbers of Tandem Repeats (VNTRs) markers (Vos *et al*., 1995), Single Nucleotide Polymorphism (SNP) (Choi *et al*., 2007), Restriction Fragment Length Polymorphism (RFLP) (Keim *et al*., 1997), Amplified Fragment Length Polymorphism (AFLP) (Vos *et al*., 1995) and Simple Sequence Repeats (SSR) or microsatellites (Akkaya *et al*., 1992). These different types of molecular markers are also different as to their potential to detect differences between individuals, their cost, facilities required, and consistency and replication of results (Schulman, 2007). A good molecular marker should have all or most of the following criteria: low cost, reproducible, automation, none epistatic, multiallelic, codominant, throughput and polymorphic (Cregan *et al*., 1999).

RFLP and Variable VNTR markers are examples of molecular markers based on restriction-hybridization techniques. The RFLP markers are relatively high polymorphic, co-dominant, and replicable, but this technique is not very widely used as it is time-consuming, involves expensive and radioactive/toxic reagents and requires large quantities of high quality genomic DNA (Welsh and McClelland, 1990). Moreover, the prerequisite of prior sequence information for probe construction contributes to the complexity of the methodology. These limitations led to the development of a new set of less technically complex methods known as PCR-based techniques. RAPDs were the first PCR-based molecular markers to be employed in genetic variation analyses (Welsh and McClelland, 1990). Most RAPD markers are dominant and therefore, heterozygous individuals cannot be distinguished from homozygotes. This contrasts with RFLP markers which are co-dominant and therefore, distinguish among the heterozygotes and homozygotes. Thus, relative to standard RFLP markers, and especially VNTR loci, RAPD markers generate less information per locus examined. One disadvantage of using RAPD technique is the reproducibility between different runs which is due to the short primer length and low annealing temperature. The AFLP technique is more laborious and time consuming than RAPD methods, but it has higher efficiency in detecting polymorphism than either RAPD or RFLP markers. Simple sequence repeats (SSR) markers are very informative, and reproducible (Vos *et al*., 1995) and has the ability to overcome the limitations associated with RFLP and RAPD.

2.10 Simple Sequence Repeat (SSR) Markers

Simple sequence repeat (SSR) DNA markers are also referred to as microsatellite markers. They are widely used for linkage map construction, quantitative trait loci (QTL) mapping and genetic diversity analysis. They are found in all eukaryotic genomes. They

have short tandem repeat motifs consisting of 1 to 6 base pair of nucleotides (Powell *et al*., 1996) with conserved flanking sequences, making it possible to design primers for their amplification by polymerase chain reaction (PCR).

Although, there are other types of molecular markers for mapping and QTL detection, SSRs are dominant in mapping studies because they are polymorphic, co-dominant, abundant and reproducible. Also, Cregan *et al*. (1999) stated that, SSR markers are low cost, automation, polymorphic, highly reproducible, co-dominant and low cost and easily applicable in soybean. Based on these qualities, significant progress has been made in the development of genetic maps for different plant species including soybean (Akkaya *et al*., 1992; Cregan *et al*., 1999). Roa *et al*., (2000), also confirmed that SSR markers are the marker of choice for molecular mapping of many crop species. SSR markers have been used in soybean for detecting specific genes that determine QTL of economic importance, and also aid in identifying agronomic traits, involved in genetic resistance to pests, diseases and yield, which are features of complex inheritance (Yuan *et al*., 2002). These qualities make SSR markers an ideal for crop improvement.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.0 Experiment 1: Screening for rust resistance gene in soybean genotypes using SSR molecular markers

This study was conducted at Council for Scientific and Industrial Research (CSIR) – Crops Research Institute (CRI) Molecular Biology Laboratory, Fumesua in Kumasi.

3.1.1 Planting Materials

Table 3.1: Soybean genotypes/varieties and their sources used for the study

Genotypes/Varieties	Source/Institution*	Country
TGx1909-3F	IITA	Nigeria
SIT-M TGx1990-67F	IITA	Nigeria
SIT-E TGx1987-11F	IITA	Nigeria
SIT-E TGx1988-3F	IITA	Nigeria
TGx1903-7F	IITA	Nigeria
SIT-E TGx1987-86F	IITA	Nigeria
SIT-M TGx1990-45F	IITA	Nigeria
NANGBAAR	CSIR-CRI	Ghana
SIT-E TGx1990-3F	IITA	Nigeria
SIT-E TGx1990-15F	IITA	Nigeria
SIT-E TGx1987-10F	IITA	Nigeria
SIT-E TGx1989-19F	IITA	Nigeria
SIT-M TGX1904-6F	IITA	Nigeria
SIT-E TGx1989-4F	IITA	Nigeria
SIT-M TGx1989-46F	IITA	Nigeria
SIT-E TGx1988-5F	IITA	Nigeria
ANIDASO	CSIR-CRI	Ghana
SIT-M TGx1987-91F	IITA	Nigeria

Table 3.1: Soybean genotypes/varieties and their sources used for the study

Cont'd

Genotypes/Varieties	Source/Institution*	Country
SIT-M TGx1989-42F	IITA	Nigeria
SIT-M TGx1987-14F	IITA	Nigeria
SIT-E TGx1740-2F	IITA	Nigeria
SIT-E TGx1989-21F	IITA	Nigeria
SIT-E TGx1987-62F	IITA	Nigeria
SIT-E TGx1990-97F	IITA	Nigeria
SIT-M TGx1989-45F	IITA	Nigeria
SIT-E TGx1989-20F	IITA	Nigeria

SIT-E TGx1990-2F	IITA	Nigeria
SIT-M TGx1448-2E	IITA	Nigeria
SIT-E TGX1835-10E	IITA	Nigeria
SIT-M TGx1987-96F	IITA	Nigeria
SIT-M TGx1987-40F	IITA	Nigeria
SIT- E TGx1990-8F	IITA	Nigeria
SIT-E TGx1990-5F	IITA	Nigeria
SIT-M TGx1440-1E	IITA	Nigeria

*****IITA: International Institute of Tropical Agriculture

CSIR-CRI: Council for Scientific and Industrial Research - Crop Research Institute

3.1.2 DNA Isolation

Genomic DNA was isolated from young leaves with DNeasy Plant Mini Kit according to the manufacturer‟s protocol (Qiagen sciences), Canada.

3.1.3 DNA quantity and quality estimation

DNA quality was checked on 0.8 % agarose gel in 1X TAE buffer by electrophoresis at 120 volts for 45 mins and stained with ethidium bromide visualized under ultraviolet transilluminator connected to a computer. Serial dilutions were carried out to get the desired quantity (concentration) of DNA for polymerase chain reaction (PCR).

3.1.4 SSR Primers

Five different SBR resistance genes have been identified and each has been mapped (Song *et al*., 2004). Simple sequence repeat (SSR) molecular markers were selected based on the reported genomic location of the known resistance to *Phakopsora pachyrhizi* (R*pp*) genes. SSR primers (Table 3.2) were obtained from Soybase

(http://soybase.org/resources/ssr.php). Nine markers associated with R*pp* genes were used for the molecular analysis to select for resistance genotypes.

Table 3.2: SSR markers and their primer sequences in relation to five soybean resistance loci on a soybean linkage map

SSR Markers	Primer sequence	Linkage Group	Position (cM)	Resistance gene	References
Sat_064	Fw: TAG CTT TAT AAT GAG TGT GAT AGA T	G	108.69	R*pp*1	Cregan *et al*. (1999)
	Rv: GTA TGC AAG GGA TTA ATT AAG				
Sat_165	Fw: GCG GAC AGG CAG CCA CAC ATC TTA	J	42.2	R*pp*1	Song *et al*. (2004)
	Rv: GCG GAT TAA ATC AGT TTG TAT CGA				
Satt620	Fw: GCG GGA CCG ATT AAA TCA ATG AAG TCA	J	53.71	R*pp*2	Silva *et al*. (2008)
	Rv: GCG CAT TTA ATA AGG TTT ACA AAT TAG T				
Satt708	Fw: GCG CAA TTT TAA GAG ATT TTC GGG ATA A	C2	115.48	R*pp*2	Song *et al*. (2004)
	Rv: GCG ACT CGG TTG ATT TTT TTT TCA ATT TTT T				
Staga001	Fw: GCG GAG GGG AGT TTG CAG ATT A	C2	119.84	R*pp*3	Song *et al*. (2004)
	Rv: GCG GCA AGG GCA ACT GAA AAA T				
Sat_307	Fw: GCG AAT TGG ACT AAA AGA ATA AGC ATC A	O	123.43	R*pp*3	Song *et al*. (2004)
	Rv: GCG TGT TTG GTA TAG AAA TGA GAA ATA AAA T				
AF162283	Fw: GCG AGT TCT GGA TGT AGG	G	87.94	R*pp*4	Yamanaka *et al*. (2008)
	Rv: GCG AGT TCT GGA TGT AGG				

Sat_166	Fw: GCG CTA ATT TAT CGG GAC CCA ACA TAT Rv: GCG GAA ATA GTG CAT TGA TGA AAA ACA	N	38.59	R*pp*4	Song *et al*. (2004)
Sat_280	Fw: GGC GGT GGA TAT GAA ACT TCA ATA ACT ACA A Rv: GGC GGG CTT CAA ATA ATT ACT ATA AAA CTA CGG	N	43.45	R*pp*5	Song *et al*. (2004)

3.1.5 Polymerase Chain Reaction

Polymerase chain reactions (PCR) were carried out in 10 µl volumes for nine markers. The components of the reaction mixture were PCR water 5.78 µl , 10x buffer 1 µl, $MgCl_2$ (25 mM) 0.9 µl, DNTPs (20 mM) 0.2 µl, forward and reverse primer 0.5 µl each, Taq polymerase 0.12 µl and template DNA 1µl all in 1x PCR buffer. The amplification was carried out in a thermocycler machine (Gene Amp® PCR system 9700 version 3.09, Applied Biosystems, California, USA) with the following conditions: the cycling consisted of 5 mins at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C; followed by 7 mins at 72 °C. Amplification products were left at 4 °C prior to electrophoresis. DNA loading dye (Fermentas) was added to the PCR amplification products and separated by electrophoresis in 2 % agarose gel.

3.1.6 DNA electrophoresis

The PCR products were run on 2 % agarose gel (7.5 µl ethidium bromide, 200 ml, 1X TBE, 4.0 g agarose) at 120 volts for 45 mins in electrophoretic setup. The DNA was visualized using an ultraviolet transilluminator connected to a computer.

3.1.7 Scoring of bands

The photographed gels were downloaded onto a computer and weighted bands were scored as presence (1) or absence (0) of band using DNA ladder as the reference (1 kb Invitrogen and 100 base pair Fermentas).

3.2.0 Experiment 2: Field screening of soybean genotypes for rust, shattering and lodging resistance and their yield potential

3.2.1 Experimental site

The experiment was conducted at Tampola, Navrongo in the Kassena Nankana District of the Upper East Region of Ghana. The area is located in the Sudan Savannah Agroecological Zone which experiences a unimodal rainfall pattern. The rain lasts for five to six months starting from April or early May and reaches its peak in August or early

September. The dry periods last for six to seven months starting from mid-November. The annual rainfall, temperature, relative humidity, wind speed, sunshine hours and solar radiation of the area are 885 mm, 28.6°C, 54%, 81 km day^{-1}, 7.9 h and 20.4 M J $m^{-2}day^{-1}$, respectively (Ghana Meteorological Agency, 2013). The research work was carried out between July and November, 2014. Planting materials are presented in Table 3.1

3.2.2 Soil sampling and Analysis

Five soil cores were taken at the depths of 20 cm using a soil augur from each replication and bulked to obtain three samples. The soil samples were air-dried and sieved using 2 mm mesh sieve to remove broken sticks and other debris before the following parameters below were determined.

3.2.2.1 Organic Carbon

The Walkley-Black wet combustion procedure (Nelson and Sommers, 1982) was used to determine organic carbon.

3.2.2.2 Organic Matter

Percent organic carbon was multiplied by 1.724 (Van Bemmelen factor) to obtain percent organic matter (Nelson and Sommers, 1982).

3.2.2.3 Soil pH

This was measured in 1:2.5 soil to water suspension by the use of a glass Electrocalomel electrode (Mclean, 1962) pH metre.

3.2.2.4 Total nitrogen

The Macro Kjeldahl method described by Bremmer and Mulvaney (1982) was used. A 10 g soil sample (< 2 mm in size) was digested with a mixture of 100 g potassium sulphate, 10 g copper sulphate and 1 g Selenium with 30 ml of concentrated sulphuric acid. This was followed by distillation with 10 ml boric acid (4 %) and four drops of indicator and 15 ml of 40 % NaOH. It was then titrated with Ammonium sulphate solution. Based on the relation that 14 g of nitrogen is contained in one equivalent weight of NH_3, the percentage of nitrogen in the soil was calculated as follows:

$$\text{Total N in the sample} = \frac{14\,(A-B) \times N \times 100}{1000 \times W}$$

Where,

A = Volume of standard acid used in the titration,

B = Volume of standard acid used in blank titration,

N = Normality of the standard acid, and

W = Weight of soil sample used.

3.2.2.5 Available phosphorous

The Bray^{-1} test method was used for the determination of phosphorus with dilute acid fluoride as the extractant (Jackson, 1958).

3.2.2.6 Exchangeable bases (Ca, Mg, K, Na)

The exchangeable base cations were extracted using ammonium acetate at pH of 7.0. Calcium and Magnesium were determined using the EDTA titration method (Moss, 1961) while potassium and sodium were determined using the flame photometer.

3.2.3 Land preparation, Layout, experimental design, and planting

The land was not ploughed but manually slashed with cutlass in order to maintain the stability of the pathogen community. It was also not burnt for the same reason. Stumping was done with mattocks and hoes. The debris was also manually collected. Linning and pegging were done at a planting distance of 75 cm between rows and 10 cm within rows. The experimental design used was randomized complete block design (RCBD) with three replications partitioned by two alleys of 1 m each. The two central rows were the test row from which data was taken. Each plot had four rows which was four meters long. Three seeds were planted per hill.

3.2.4 Cultural Practices

3.2.4.1 Thinning

Seedlings were thinned to two plants per hill, 20 days after sowing, when the soil was moist and seedlings well established.

3.2.4.2 Weeding

Weeding was done manually by hand using a hoe, on the third and sixth week after sowing to control weeds. Each weeding operation was completed on the same day for all the blocks on the day of weeding.

3.2.4.3 Pest Control

Spraying was done at 50 % flowering with Cypermetrin + Dimethoate 10 EC at the rate of 100 ml in 15 l of water using knapsack sprayer, at a recommended 14 days interval to control insects till the end of pod filling.

3.2.5 Fungus source

The soybean genotypes were screened for rust resistance under natural epiphytotic condition**.** When a hot spot of a disease is known, and natural epidemics are so frequent no artificial inoculations are needed (Tiwari *et al*., 1997). Bromfield (1984) also reported that, a single diseased leaf may be enough to initiate a disease epidemic in a field.

3.2.6 Evaluation of soybean genotypes for rust resistance

Rust severity was recorded using 0 - 9 disease rating scale (Table 3.3) by Mayee and Datar (1986). The scoring was done after flowering and before pod formation and their averages calculated. Evaluations were made during these reproductive stages of development because spore production and pustule development generally increase after plants begin to flower (Bromfield, 1984), and because variation in disease severity was typically high at these stages, while the most susceptible genotypes were not yet heavily defoliated. Based on disease rating, soybean test entries were grouped into 6 categories.

Table 3.3: Disease grade/score

Disease grade/score	% Leaf area affected	Disease reaction
0	Nil	Immune
1	<1	Highly resistant
3	1 - 5	Resistant
5	6 - 25	Moderately resistant
7	26 - 50	Susceptible
9	> 51	Highly susceptible

Source: Mayee and Datar (1986)

3.2.7 Agronomic characteristics of soybean genotypes

3.2.7.1 Days to 50 % flowering

This was recorded as a number of days after sowing until 50 % of the plants had one or more flowers.

3.2.7.2 Nodule count at 50 % flowering

At 50 % flowering, five plants were carefully dug from both ends of the two rows on each plot. The roots of the plants were carefully dug out, put in polythene bags, together with detached nodules collected from the soil. The roots were then put in a 1 mm mesh sieve and washed under running tap water to remove adhered soil. The nodules were gently removed, washed and counted.

3.2.7.3 Plant height at harvest

The heights (cm) of the plants were taken at maturity from the ground to the tip of the main stem for five sampled plants. This was done with the use of a rule. The average plant height (cm) was calculated for each treatment.

3.2.7.4 Lodging score

Lodging was scored using IITA descriptors. It was done at R8 (full maturity) when 95 % of the pods have reached mature pod colour. The rating system for lodging was scored using the scale 1 - 5 according to the scores: 1 = all plants erect, 2 = 25 % of plants lodged, 3 = 50 % of the plants lodged, 4 = 75 % of plants lodged and 5 = all plants lodged. The lodging score were described as 1 = all plant erect, 2 = slight lodging, 3 = plants lodged at 45 degree angle, 4 = severe lodging and 5 = all plants flat.

3.2.7.5 Pod shattering resistance

The genotypes used in the study were characterized for pod shattering to confirm their resistance level. The pod shattering was recorded at R8 when 95 % of the pod had attained maturity. The screening was done using the oven dry method in the laboratory. Twenty samples were collected from each genotype and were put in a paper bag (5 x 10 x 20 cm) for 10 days at room temperature for moisture content to equilibrate. The pods were then oven dried at 80 °C for 12 h. Pods that opened to release the seeds or opened but did not release seeds were considered shattered. The shattering percentage was calculated as the number of shattered pods per total number of pod expressed as percentage. The percentage pod shattering percentage was determined on a scale 1 - 5 recommended by Asian Vegetable Research and Development Centre (AVRDC, 1977).

The scale was described as 1 = very resistant, 2 = resistant, 3 = moderately resistant, 4 = moderately susceptible and 5 = very susceptible according to the scores as: 1 = 0 %, 2 = 1 - 10 %, 3 = 11 - 25 %, 4 = 26 – 50 % and 5 = ≥ 50 %.

3.2.7.6 Days to maturity

It was recorded as the date when 95 % of the pods had ripened, as indicated by their mature pod colour by changing from yellow to tan or grey.

3.2.7.7 Seeds per 100 pods

One hundred pods were sampled and their seeds counted.

3.2.7.8 One thousand (1000) seed weight

The 1000 seed weight was determined by counting 1000 seeds from the threshed and oven dried at 60 °C for 48 h for each plot and their weight determined in grammes (g) using an electronic scale.

3.2.7.9 Grain yield (tonnes per hectare)

Grain yield per hectare was determined by threshing the harvested plants from the two central rows of each plot. These were put in labelled envelopes and oven dried at 60 °C for 48 hrs to a constant weight, and then weighed. The resulting weights, in grammes (g) were then scaled up to tonnes per hectare to obtain the average grain yield per hectare (Okogun *et al*., 2005).

3.2.8 Data analysis

Data collected were analysed, using Statistix 9.0 statistical package. Analysis of Variance (ANOVA) table was computed and treatment differences were compared using the Least Significant Difference (LSD) procedure at 5 % level of probability.

CHAPTER FOUR

4.0 RESULTS

4.1.0 Experiment 1: Screening for rust resistance gene(s) in soybean genotypes using SSR molecular markers

4.1.1. Rust resistance alleles identified by SSR markers

Out of the nine primers used, Satt620 and Sat_166 were monomorphic. The remaining seven of the primers (Sat_064, Sat_165, Satt708, Staga001, Sat_307, AF162283 and Sat_280) produced polymorphism with significant differences. Therefore, screening of soybean genotypes for resistance gene presence was based on these seven markers. Expected alleles showing resistance or susceptibility were scored as present (1) or absence (0) (Table 4.1).

The banding pattern of primer Staga001 that was linked to rust disease resistance at 251 bp is presented in Plates 4.1. Staga001 identified 25 soybean genotypes to have resistance gene to SBR at the expected resistant allele (Table 4.1). Sat_064 identified nine soybean genotypes and Sat_165 identified five genotypes as having resistance gene to SBR. Also, primer Satt708 was able to identify nine genotypes, Sat_307 identified fifteen genotypes at 162 bp, 212 bp or 215 bp whiles AF162283 identified one genotype (SIT-M TGx1987-14F) at 200 bp as resistant (Table 4.1). SSR marker Sat_280 also identified six genotypes: SIT-M TGx1989-42F, SIT-E TGx1989-20F, SIT-E TGx1990-2F, SIT-E TGx1835-10E, SIT-M TGx1987-40F and SIT-E TGx19908F as having resistance to SBR at the expected 224 bp or 297 bp alleles. Genotypes,

SIT-E TGx1990-3F, SIT-M TGx1987-91F, SIT-M TGx1989-45F and SIT-E TGx19885F were found to have resistance gene to SBR by four different SSR markers, however genotype TGx1909-3F was not identified by any of the primers to have resistance gene.

Soybean Genotypes	Sat_064 143 bp	Sat_165 228/277 bp	Satt708 240 bp	Staga001 251 bp	Sat_307 212/162/215 bp	AF162283 200 bp	Sat_280 224/297 bp	Response
TGx1909-3F	0	0	0	0	0	0	0	S
SIT-M TGx1990-67F	0	0	0	1	0	0	0	R
SIT-M TGx1987-11F	0	0	0	1	0	0	0	R
SIT-E TGx1988-3F	1	0	0	1	0	0	0	R
TGx1903-7F	0	0	0	0	1	0	0	R
SIT-E TGx1987-86F	1	0	0	0	0	0	0	R
SIT-E TGx1990-45F	0	0	0	1	0	0	0	R
NANGBAAR	0	0	0	0	1	0	0	R
SIT-E TGx1990-3F	1	0	1	1	1	0	0	R
SIT-E TGx1990-15F	0	0	0	1	1	0	0	R
SIT-E TGx1987-10F	0	0	0	0	1	0	0	R

SIT-E TGx1989-19F	0	0	1	1	1	0	0	R

Table 4.1: Soybean genotypes and their resistance or susceptible alleles

Table 4.1: Soybean genotypes and their resistance or susceptible alleles Cont'd

Table 4.1: Soybean genotypes and their resistance or susceptible alleles Cont'd

Genotypes	Sat_064 Sat_064 143 bp	Sat_165 Sat_165 228/277 bp	Satt708 Satt708 240 bp	Staga001 Staga001 251 bp	Sat_307 Sat_307 212/162/215 bp	AF162283 AF162283 200 bp	Sat_280 Sat_280 224/297 bp	Response
Genotypes	143 bp	228/277 bp	240 bp	251 bp	212/162/215 bp	200 bp	224/297 bp	Response
SIT-M TGx1904-6F	0	0	1	0	0	0	0	R
SIT-M TGx1989-45F	1	0	1	1	1	0	0	R
SIT-E TGx1989-4F	1	0	1	1	0	0	0	R
SIT-E TGx1989-20F	1	0	0	1	0	0	1	R
SIT-E TGx1989-46F	1	0	0	0	1	0	0	R
SIT-E TGx1990-2E	0	0	0	1	0	0	1	R
SIT-E TGx1988-5F	1	0	1	1	1	0	0	R
SIT-M TGx1990-2E	0	0	0	1	0	0	0	R
ANIDASO	0	0	1	0	1	0	0	R
SIT-E TGx1835-10E	0	1	0	1	0	0	1	R
SIT-M TGx1987-91F	1	0	1	1	1	0	0	R
SIT-M TGx1987-96F	0	1	0	1	1	0	0	R
SIT-M TGx1989-42F	0	1	0	1	0	0	1	R
SIT-M TGx1987-40F	0	1	0	1	0	0	1	R
SIT-M TGx1987-14F	0	0	0	0	0	1	0	R
SIT-E TGx1990-8F	0	0	0	1	1	0	1	R
SIT-E TGx1740-2F	0	0	0	1	0	0	0	R
SIT-E TGx1990-5F	0	0	0	1	1	0	0	R
SIT-E TGx1898-21F	0	0	0	1	0	0	0	R
SIT-M TGx1440-1E	0	0	0	1	1	0	0	R
SIT-E TGx1987-62F	0	1	1	1	0	0	0	R
SIT-E TGx1990-97F	0	0	0	1	0	0	0	R

Allele associated with rust resistant or susceptible gene, 1 = indicates presence of the allele and 0 = indicates absence of the allele

R = Resistant and S = Susceptible

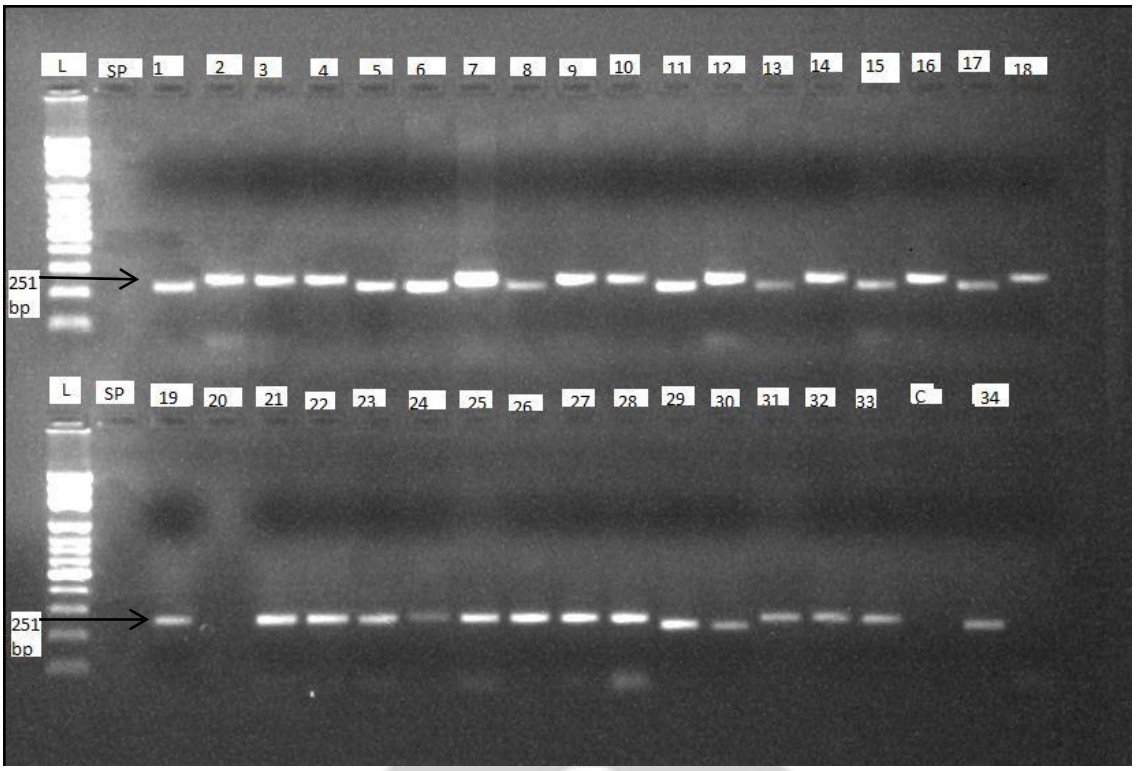

Plate 4.1: Primer Staga001 detected resistant genotypes at 251 bp.

L-100bp DNA ladder, SP-Space, 1- TGx1909-3F, 2- SIT-M TGx1990-67F, 3-SIT-E TGx1987-11F, 4-SIT-E TGx1988-3F, 5- TGx1903-7F, 6- SIT-E TGx1987-86F, 7- SITM TGx1990-45F, 8- NANGBAAR, 9- SIT-E TGx1990-3F, 10- SIT-E TGx1990-15F, 11- SIT-E TGx1987-10F, 12- SIT-E TGx1989-19F, 13- SIT-M TGX1904-6F, 14- SITE TGx1989-4F, 15- SIT-M TGx1989-46F, 16- SIT-E TGx1988-5F, 17- ANIDASO, 18- SIT-M TGx1987-91F, SP-Space, 19- SIT-M TGx1989-42F, 20- SIT-M TGx198714F, 21- SIT-E TGx1740-2F, 22- SIT-E TGx1989-21F, 23- SIT-E TGx1987-62F, 24- SIT-E TGx1990-97F, 25- SIT-M TGx1989-45F, 26- SIT-E TGx1989-20F, 27- SIT-E TGx1990-2F, 28- SIT-M TGx1448-2E, 29- SIT-E TGX1835- 10E, 30- SIT-M TGx1987-96F, 31- SIT-M TGx1987-40F, 32- SIT- E TGx1990-8F, 33- SIT-E TGx1990-5F, C - Control and 34- SIT-M TGx1440-1E.

4.1.2 Summary statistics about the SSR markers used

Allelic frequency, number of alleles, gene diversity, percentage heterozygosity and polymorphic information content (PIC) values of the nine primers used to screen the 34 soybean genotypes against rust disease is presented in Table 4.2.

Table 4.2: Summary statistics of nine SSR markers

Markers	Allele Frequency	Allele Number	Gene Diversity	Heterozygosity	PIC
Sat_064	0.25	13	0.84	0.69	0.84
Sat_165	0.82	5	0.30	0.21	0.30
Satt620	1.00	1	0.00	0.00	0.00
Satt708	0.58	9	0.61	0.69	0.59
Staga001	0.34	4	0.69	0.00	0.66
Sat_307	0.38	7	0.75	0.96	0.72
AF162283	0.75	3	0.38	0.50	0.35
Sat_166	1.00	1	0.00	0.00	0.00
Sat_280	0.26	6	0.76	0.76	0.75
Mean	**0.60**	**5**	**0.48**	**0.42**	**0.47**

The highest percentage heterozygosity was produced by primer Sat_307 whiles primers Satt620, Staga001 and Sat_166 produced the lowest. Polymorphic Information Content (PIC) values of the primers ranged from 0.00 to 0.84 with a mean of 0.47 with primers Satt620 and Satt_166 as the lowest and primer Sat_064 as the highest value.

4.2.0 Experiment 2: Field screening of soybean genotypes for rust, shattering and lodging resistance and their yield potential

4.2.1 Soil analysis of experimental site

The percentages of organic carbon, organic matter and total nitrogen were 0.48, 0.83 and 0.07 respectively. The exchangeable cations were recorded as 0.21, 2.6 and 0.80 cmol/kg potassium, calcium and magnesium respectively. The value for available phosphorus was 20.22 ppm. The soil pH was 6.16 which suggested an extremely weak acid soil condition. The properties of the soil used are shown in Table 4.3.

Table 4.3: Results on soil analysis of experimental site

Soil properties		**Values**	**Recommended /Critical values**	**References**
% Organic carbon		0.48	_	
% Organic matter		0.83	0.5 – 4.0	Adepetu and Corey (1976)
% Total nitrogen		0.07	0.15	Adepetu and Corey (1976)
Exchangeable Cations Cmol/kg	Potassium	0.21	0.16	Akindrinde and Obigbesan (2000)
	Sodium	0.22	_	
	Calcium	2.60	2.5	Akindrinde and Obigbesan (2000)
	Magnesium	0.80	0.20	Akindrinde and Obigbesan (2000)
Available phosphorus (ppm)		20.22	<12 ppm (Bray-1 test)	Ferguson *et al*. (2006)
pH		6.16	5.5 – 7.0	Ferguson *et al*. (2006)

4.2.3 Field evaluation of soybean genotypes for resistance to P. pachyrhizi

Results on genotypes to rust severity are shown in the Table 4.4. The Table shows that significant differences ($p < 0.05$) existed among the genotypes in their resistance to rust (*P. pachyrhizi*). Reactions of 34 genotypes to rust revealed that, none of the genotypes

showed immune reaction to rust. Genotypes SIT-E TGx1990-3F, SIT-M TGx198791F, SIT-M TGx1989-45F and SIT-M TGx1987-40F were highly resistant (<1% leaf area affected). Genotypes SIT-E TGx1988-3F, SIT-E TGx1987-10F, SIT-M TGx14401E and SIT-E TGx1835-10E (the check) were resistant (1 - 5% leaf area affected). Genotypes TGx1903-7F, NANGBAAR, SIT-M TGx1989-46F, SIT-E TGx1990-2F, SIT-E TGx1989-21F, SIT-E TGx1990-5F, SIT-E TGx1990-15F, SIT-E TGx1990-8F, SIT-M TGx1987-14F, ANIDASO, SIT-M TGx1989-42F, SIT-E TGx1989-19F, SIT-E TGx1989-4F, SIT-E TGx1988-5F, SIT-M TGX1904-6F and SIT-E TGx1987-62F showed moderately resistant (6 - 25% leaf area affected). Genotypes SIT-E TGx198786F, SIT-E TGx1989-20F, SIT-E TGx1448-2E and SIT-E TGx1987-96F were susceptible (26 - 50% leaf area affected) whilst Genotypes TGx1909-3F, SIT-M TGx1990-67F, SIT-E TGx1987-11F, SIT-M TGx1990-45F, SIT-E TGx1740-2F and SIT-M TGx1990-97F were highly susceptible (≥ 51% leaf area affected).

Table 4.4: Rust severity score

Genotypes	% Leaf area affected	Response*
SIT-E TGx1988-3F	4.0	R
TGx1903-7F	23.7	MR
NANGBAAR	23.3	MR
SIT-E TGx1990-3F	0.5	HR
SIT-E TGx1990-15F	20.3	MR
SIT-E TGx1987-10F	4.0	R
SIT-E TGx1989-19F	11.7	MR
SIT-M TGX1904-6F	9.7	MR
SIT-E TGx1989-4F	11.0	MR
SIT-M TGx1989-46F	22.7	MR
SIT-E TGx1988-5F	10.0	MR
ANIDASO	15.7	MR
SIT-M TGx1987-91F	0.7	HR
SIT-M TGx1989-42F	15.7	MR
SIT-M TGx1987-14F	18.0	MR
SIT-E TGx1989-21F	21.3	MR

SIT-E TGx1987-62F	8.3	MR
SIT-M TGx1989-45F	0.4	HR
SIT-E TGx1990-2F	21.3	MR
SIT-E TGx1835-10E (check)	1.3	R
SIT-M TGx1987-40F	0.6	HR
SIT-E TGx1990-8F	18.7	MR
SIT-E TGx1990-5F	20.3	MR
SIT-M TGx1440-1E	3.0	R
TGx1909-3F	69.0	HS
SIT-M TGx1990-67F	68.3	HS
SIT-E TGx1987-11F	50.7	S
SIT-E TGx1987-86F	46.7	HS
SIT-M TGx1990-45F	63.3	HS
SIT-E TGx1740-2F	61.0	HS
SIT-M TGx1990-97F	54.3	S
SIT-E TGx1989-20F	41.7	S
SIT-E TGx1448-2E	42.7	S
SIT-E TGx1987-96F	27.3	
Mean	23.9	
CV (%)	8.9	
LSD (P < 0.05)	3.4	

*HR = highly resistant, R = Resistant, MR = moderately resistant, S = Susceptible, HS = highly susceptible

4.2.4 Agronomic characteristics measured in soybean genotypes

Agronomic characters of the soybean genotypes used in this study are presented in Tables 4.5 to 4.9.

4.2.4.1 Nodule count at 50 % flowering

Table 4.5 gives the results of nodules count. There were significant differences ($p < 0.05$) in nodule count among the genotypes. The nodule count ranged from 0 to 36 with a mean of 16.87. Genotypes SIT-M TGx1987-14F and SIT-E TGx1988-5F had 36 nodule counts per plant and were significantly different compared to the other thirty two genotypes.

Genotypes SIT-E TGx1990-5F, SIT-E TGx1987-86F, SIT-E TGx1990-3F, TGx19037F and SIT-M TGx1990-97F had nodules count in the range of 17 - 22 which were not statistically different from each other. Genotypes TGx1903-7F, SIT-M TGx1990-97F, SIT-E TGx1987-96F, SIT-E TGx1989-4F, SIT-E TGx1990-8F, TGx1909-3F, SIT-E TGx1989-19F and SIT-M TGx1987-91F were statistically not difference in nodule count (18 - 13) per plant, but differed considerably among genotypes SIT-E TGx199015F, SIT-M TGx1989-46F, SIT-E TGx1990-2F, SIT-M TGx1990-67F, SIT-E TGx1740-2F and SIT-E TGx1989-20F with their nodule count ranging from 0 - 4. Also, no significant differences ($p > 0.05$) were observed among genotypes TGx19093F, SIT-E TGx1989-19F, SIT-M TGx1987-91F, SIT-E TGx1987-11F and SIT-M TGx1990-45F which had 15, 15, 13, 11 and 11 nodules per plant respectively, but differed significantly ($p < 0.05$) among genotypes NANGBAAR, SIT-E TGx1987-10F, SIT-M TGx1904-6F, ANIDASO and SIT-E TGx1989-21F which recorded 24 - 27 nodule count per plant.

4.2.4.2 Plant height at harvest

Results from Table 4.5 show the mean of soybean plant height (cm) at harvest. Plant height at harvest differed significantly ($p < 0.05$) among the genotypes. Soybean genotypes fell within plant height range of 38.0 - 84.7 cm. Genotype SIT-E TGx198796F recorded the highest height (84.7 cm) that was significantly different ($p < 0.05$) from the lowest height (38.0 cm) recorded by genotype SIT-E TGx1987-11F. Genotypes ANIDASO, SIT-E TGx1835-10E, SIT-M TGx1987-14F, SIT-M TGx14401E, SIT-E TGx1987-62F, SIT-E TGx1740-2F and SIT-E TGx1990-5F had no difference in plant height at harvest and fell within range of 57.4 - 61.2 cm, but differed greatly from genotypes SIT-M TGx1990-67F, SIT-M TGx1990-45F, SIT- E TGx1990-8F and TGx1903-7F with similar plant height (50.3 - 49.1 cm).

Table 4.5: Nodule count at 50 % flowering and plant height at harvest of soybean genotypes

Soybean genotypes	Number of Nodules	Plant height (cm)
SIT-E TGx1988-3F	5	72.3
TGx1903-7F	18	49.1
NANGBAAR	26	42.6
SIT-E TGx1990-3F	21	43.5
SIT-E TGx1990-15F	0	51.3
SIT-E TGx1987-10F	24	54.8
SIT-E TGx1989-19F	15	53.4
SIT-M TGX1904-6F	27	41.9
SIT-E TGx1989-4F	16	40.7
SIT-M TGx1989-46F	2	56.1
SIT-E TGx1988-5F	36	52.1
ANIDASO	24	61.2
SIT-M TGx1987-91F	13	53.2
SIT-M TGx1989-42F	30	52.5
SIT-M TGx1987-14F	36	60.4
SIT-E TGx1989-21F	27	78.1
SIT-E TGx1987-62F	9	60.3
SIT-M TGx1989-45F	23	53.7
SIT-E TGx1990-2F	0	42.3
SIT-E TGx1835- 10E	25	60.8
SIT-M TGx1987-40F	28	82.7
SIT- E TGx1990-8F	16	50.0
SIT-E TGx1990-5F	22	57.4
SIT-M TGx1440-1E	8	60.4
TGx1909-3F	15	40.0
SIT-M TGx1990-67F	4	50.3
SIT-E TGx1987-11F	11	38.0
SIT-E TGx1987-86F	21	42.3
SIT-M TGx1990-45F	11	50.0
SIT-E TGx1740-2F	2	59.3
SIT-M TGx1990-97F	17	52.7
SIT-E TGx1989-20F	3	42.3
SIT-E TGx1448-2E	24	63.3
SIT-E TGx1987-96F	16	84.7
Mean	16.9	54.5
CV (%)	18.5	6.8
LSD (P < 0.05)	5.0	6.0

4.2.4.3 Days to 50 % flowering

Days to 50 % flowering of soybean genotypes are shown in Table 4.6. Genotype SITM TGx1987-40F took maximum days (52) and the minimum (40 days) were genotypes SIT-M TGx1990-97F and SIT-E TGx1989-20F for days to 50 % flowering. The number of days to 50 % flowering for genotypes SIT-E TGx1990-3F, SIT-E TGx199015F, SIT-E TGx1989-19F, SIT-E TGx1988-5F and TGx1909-3F was 41 but differed significantly ($p < 0.05$) from genotypes SIT-M TGx1987-91F, SIT-M TGx1989-42F, SIT-M TGx1989-45F, SIT-M TGx1990-45F which attained days to 50 % flowering in 46 days. Genotypes SIT-E TGx1448-2E, SIT-E TGx1987-86F and SIT-M TGx1440-1E took 45 days to have 50 % of their plants flowered and were statistically higher (3 days) than genotypes SIT-E TGx1989-4F, SIT-E TGx1989-21F, SIT-E TGx1989-21F, SIT-E TGx1990-2F, SIT-E TGx1835- 10E, SIT-E TGx1835- 10E, SIT- E TGx1990-8F, SITM TGx1990-67F, SIT-E TGx1987-11F and SIT-E TGx1740-2F which took 42 days to 50 % of their plants flowered.

4.2.4.4 Days to maturity

The result indicated that the days to maturity was statistically significant ($P < 0.05$) (Table 4.6). The maturity days of the genotypes varied from 84 (SIT-E TGx1987-10F and SIT-E TGx1990-2F) to 103 (ANIDASO) days. Genotypes SIT-E TGx1988-3F, SIT-E TGx1990-15F, NANGBAAR, TGx1909-3F and SIT-E TGx1989-20F matured on 88 - 89 days after planting whilst, genotypes SIT-E TGx1990-3F, SIT-E TGx198921F, SIT-E TGX1835- 10E and SIT-E TGx1987-11F matured on either 85 or 86 days. Also, genotypes TGx1903-7F, SIT-M TGx1987-91F, SIT-M TGx1989-42F, SIT-M TGx1987-14F and SIT-M TGx1987-40F matured 95 - 96 days after planting and were not

statistically different ($p > 0.05$) but differed among genotypes SIT-E TGx1989-19F, SIT-E TGx1989-4F, SIT-E TGx1987-62F, SIT-E TGx1987-86F and SIT-M TGx1990-97F which matured 90 days after planting.

Tables 4.6: Days to 50% flowering and maturity of soybean genotypes

Soybean genotypes	Days to 50 % flowering	Days to maturity
SIT-E TGx1988-3F	43	88
TGx1903-7F	43	95
NANGBAAR	48	89
SIT-E TGx1990-3F	41	86
SIT-E TGx1990-15F	41	89
SIT-E TGx1987-10F	43	84
SIT-E TGx1989-19F	41	90
SIT-M TGX1904-6F	40	92
SIT-E TGx1989-4F	42	90
SIT-M TGx1989-46F	47	98
SIT-E TGx1988-5F	41	87
ANIDASO	50	103
SIT-M TGx1987-91F	46	95
SIT-M TGx1989-42F	46	95
SIT-M TGx1987-14F	44	96
SIT-E TGx1989-21F	42	86
SIT-E TGx1987-62F	48	90
SIT-M TGx1989-45F	46	98
SIT-E TGx1990-2F	42	84
SIT-E TGx1835- 10E	42	85
SIT-M TGx1987-40F	52	96
SIT- E TGx1990-8F	42	91
SIT-E TGx1990-5F	41	87
SIT-M TGx1440-1E	45	101
TGx1909-3F	41	88
SIT-M TGx1990-67F	42	92
SIT-E TGx1987-11F	42	86
SIT-E TGx1987-86F	45	90
SIT-M TGx1990-45F	46	100
SIT-E TGx1740-2F	42	87
SIT-M TGx1990-97F	40	90
SIT-E TGx1989-20F	40	88
SIT-E TGx1448-2E	45	100
SIT-E TGx1987-96F	51	97
Mean	44.8	91.5

CV (%)	2.7	1.0
LSD (P < 0.05)	1.9	1.5

4.2.4.5 Lodging characteristics of soybean genotypes

The soybean genotypes varied significantly ($p < 0.05$) to lodging (Table 4.7). Genotypes SIT-E TGx1989-21F, SIT-E TGx1835-10E, SIT-E TGx1987-86F and SITE TGx1990-3F were lodged at 45 degree angle (50 % of the plants lodged). Genotypes SIT-E TGx1987-62F, SIT-M TGx1440-1E, SIT-M TGx1990-97F, SIT-E TGx1990-5F, SIT-E TGx1988-3F, ANIDASO, SIT-M TGx1987-40F, SIT-E TGx1990-8F, TGx19093F, SIT-M TGx1990-67F, SIT-E TGx1987-11F, SIT-M TGx1904-6F, SIT-M TGx1987-91F and SIT-E TGx1987-96F had slight lodging (25% of plants lodged). Genotypes SIT-M TGx1989-46F, SIT-E TGx1988-5F, SIT-M TGx1989-42F, SIT-E TGx1990-2F, NANGBAAR (the check), SIT-E TGx1740-2F, SIT-E TGx1989-20F, SIT-E TGx1448-2E, SIT-E TGx1990-15F, SIT-E TGx1987-10F, SIT-E TGx1989-19F, SIT-E TGx1989-4F, SIT-M TGx1987-14F, SIT-M TGx1989-45F, TGx1903-7F and SIT-M TGx1990-45F had all plants erected.

Table 4.7: Lodging characteristics of soybean genotypes

Soybean genotypes	No. of plant lodged	Response
TGx1903-7F	1.0	Erect
SIT-M TGx1987-14F	1.0	Erect
SIT-M TGx1989-45F	1.0	Erect
SIT-M TGx1990-45F	1.0	Erect
NANGBAAR (the check)	1.3	Erect
SIT-E TGx1990-15F	1.3	Erect
SIT-E TGx1987-10F	1.3	Erect
SIT-E TGx1989-19F	1.3	Erect
SIT-M TGx1989-46F	1.3	Erect
SIT-E TGx1988-5F	1.3	Erect

SIT-E TGx1989-4F	1.3	Erect

Table 4.7: Lodging characteristics of soybean genotypes Cont'd

Soybean genotypes	No. of plants lodged	Response
SIT-M TGx1989-42F	1.3	Erect
SIT-E TGx1990-2F	1.3	Erect
SIT-E TGx1740-2F	1.3	Erect
SIT-E TGx1989-20F	1.3	Erect
SIT-E TGx1448-2E	1.3	Erect
SIT-E TGx1988-3F	1.6	Slight lodging
ANIDASO	1.6	Slight lodging
SIT-M TGx1987-40F	1.7	Slight lodging
SIT- E TGx1990-8F	1.7	Slight lodging
TGx1909-3F	1.7	Slight lodging
SIT-M TGx1990-67F	1.7	Slight lodging
SIT-E TGx1987-11F	1.7	Slight lodging
SIT-E TGx1987-96F	1.7	Slight lodging
SIT-M TGx1904-6F	1.7	Slight lodging
SIT-M TGx1987-91F	2.0	Slight lodging
SIT-E TGx1990-5F	2.0	Slight lodging
SIT-M TGx1440-1E	2.0	Slight lodging
SIT-M TGx1990-97F	2.0	Slight lodging
SIT-E TGx1987-62F	2.3	Slight lodging
SIT-E TGx1989-21F	2.7	Lodged at 45°
SIT-E TGx1835- 10E	2.7	Lodged at 45°
SIT-E TGx1987-86F	2.7	Lodged at 45°
SIT-E TGx1990-3F	2.7	Lodged at 45°
Mean	1.7	
CV (%)	32.7	
LSD (P < 0.05)	0.9	

4.2.4.6 Evaluation of soybean genotypes for shattering resistance

Shattering evaluation of soybean genotypes did vary significantly ($p < 0.05$) (Table 4.8).

Tables 4.8: Percentage (%) shattering of soybean genotypes

Soybean genotypes	% Shattering	Response*
TGx1903-7F	13.33	MR
SIT-E TGx1448-2E	13.33	MR
ANIDASO (check)	15.00	MR
SIT-E TGx1835- 10E	18.33	MR
SIT-M TGx1987-40F	18.33	MR
SIT-M TGx1904-6F	20.00	MR
NANGBAAR	31.67	MS
SIT-M TGx1990-45F	35.00	MS
SIT-E TGx1990-15F	35.00	MS
SIT-M TGx1987-91F	41.67	MS
SIT-E TGx1987-62F	43.33	MS
SIT-M TGx1987-14F	45.00	MS
TGx1909-3F	51.67	VS
SIT-E TGx1990-2F	51.67	VS
SIT-M TGx1440-1E	56.67	VS
SIT-E TGx1990-5F	61.67	VS
SIT-E TGx1990-3F	66.67	VS
SIT-M TGx1990-97F	66.67	VS
SIT-E TGx1740-2F	70.00	VS
SIT-M TGx1989-42F	71.67	VS
SIT-E TGx1987-86F	73.33	VS
SIT-E TGx1987-10F	76.67	VS
SIT-M TGx1989-46F	80.00	VS
SIT-E TGx1988-3F	80.00	VS
SIT-M TGx1990-67F	80.00	VS
SIT-M TGx1989-45F	81.67	VS
SIT-E TGx1989-19F	90.00	VS
SIT-E TGx1988-5F	91.67	VS
SIT-E TGx1987-11F	91.67	VS
SIT-E TGx1989-4F	93.33	VS
SIT-E TGx1989-20F	93.33	VS
SIT-E TGx1989-21F	95.00	VS
SIT- E TGx1990-8F	96.67	VS
SIT-E TGx1987-96F	96.67	VS
Mean	60.20	
CV (%)	8.48	

LSD (P < 0.05)	8.32

*MR=Moderately resistant, MS = Moderately susceptible, VS = Very susceptible

The data presented above revealed that, pod shattering percentage ranged from 13.33 (TGx1903-7F and SIT-E TGx1448-2E) to 96.67 per cent (SIT- E TGx1990-8F and SIT-E TGx1987-96F). Results indicated that none of the genotype was very resistant or resistant to pod shattering. However, genotypes SIT-M TGx1904-6F, SIT-E TGx183510E, SIT-M TGx1987-40F, TGx1903-7F, SIT-E TGx1448-2E and ANIDASO (the

check) were found to be moderately resistant to shattering (11 – 25 % pods shattered). Genotypes SIT-E TGx1990-8F, SIT-E TGx1987-96F, SIT-E TGx1989-21F, SIT-E TGx1989-20F, SIT-E TGx1989-4F, SIT-E TGx1988-5F, SIT-E TGx1987-11F, SIT-E TGx1989-19F, SIT-M TGx1989-45F, SIT-E TGx1988-3F, SIT-M TGx1989-46F, SITM TGx1990-67F, SIT-E TGx1987-10F, SIT-E TGx1987-86F, SIT-M TGx1989-42F, SIT-E TGx1740-2F, SIT-M TGx1990-97F, SIT-E TGx1990-3F, SIT-E TGx1990-5F, SIT-M TGx1440-1E, SIT-E TGx1990-2F and TGx1909-3F were very susceptible to shattering (≥ 50 % pods shattered). Genotypes SIT-M TGx1987-14F, SIT-E TGx198762F, SIT-M TGx1987-91F, SIT-M TGx1990-45F, SIT-E TGx1990-15F and NANGBAAR were moderately susceptible to shattering (26 – 50 % pods shattered).

4.2.5 Yield and yields components of soybean genotypes

4.2.5.1 Seeds per 100 pods of soybean genotypes

Table 4.9 gives the results of number of seeds per 100 pods. There were significant differences ($p < 0.05$) in number of seeds per 100 pods among the genotypes. Genotypes SIT-M TGx1989-45F had the highest number of seed per 100 pods (257), however, this was not significantly different ($p > 0.05$) from genotypes SIT-E TGx1990-2F (248 seeds),

SIT-E TGx1987-10F (246 seeds) but, differed greatly from genotype SIT-M TGx1990-45F which produced the least number (97 seeds).

Genotypes SIT-E TGx1990-8F, SIT-E TGx1990-3F, SIT-E TGx1990-15F, NANGBAAR and SIT-E TGx1988-5F recorded their number of seeds per 100 in the range of 198 - 184 and were statically indifferent from each other. Also, genotypes SITE TGx1989-4F, SIT-M TGX1904-6F, SIT-M TGx1989-42F, SIT-E TGx1990-5F, SITE TGx1987-62F and ANIDASO recorded similar number of seeds per 100 pods (215 - 206). Genotypes SIT-E TGx1989-19F had 220 seeds per 100 and TGx1903-7F recorded 218 seeds which were similar, but differed from genotypes SIT-E TGx14482E, SIT-E TGx1987-96F, SIT-E TGx1987-86F, SIT-E TGx1987-11F, TGx1909-3F, SIT-E TGx1740-2F, SIT-M TGx1990-97F and SIT-M TGx1990-67F which had

number of seeds per 100 pods ranged from 113 – 98.

4.2.5.2 1000 seeds weight of soybean genotypes

Results of 1000 seeds weight of the genotypes are present in Table 4.9. Genotype SITE TGx1990-2F had 172.3 g which was different the other 33 genotypes. Genotypes SIT-M TGx1989-45F had 158.7 g and SIT-E TGx1990-3F recorded 151.5 g for 1000 seeds weight and was not different. There was no difference in 1000 seeds weight (137.7 - 145 g) among genotypes SIT-E TGx1987-10F, SIT-E TGx1990-5F and SIT-E TGx1835-10E, however it differed from genotypes SIT-E TGx1990-8F, SIT-E TGx1989-21F, SIT-E TGx1988-5F and SIT-M TGx1904-6F which recorded similar 1000 seed weights (115.5 - 122.8 g). No differences were observed among genotypes SIT-E TGx1987-62F, ANIDASO, SIT-M TGx1440-1E and SIT-M TGx1987-14F with 1000 seed weight ranged 98.0 - 102.6 g. Genotypes SIT-E TGx1987-86F, SIT-E

TGx1989-20F, SIT-E TGx1987-96F, NANGBAAR, SIT-E TGx1448-2E, SIT-E TGx1987-11F, SIT-E TGx1740 2F, SIT-M TGx1990-97F, SIT-M TGx1990-45F, SIT-M TGx1990-67F and TGx1909-3F had 91.7 – 86 g and were not different.

4.2.5.3 Grain yield of the soybean genotypes

Grain yield (t/ha) of the genotypes did vary significantly ($p < 0.05$) (Table 4.9). The yield potential of the genotypes evaluated ranged from 0.9 - 2.6 t/ha with the mean yield as 1.62 t/ha with lowest yields recorded by genotypes TGx1909-3F, SIT-M TGx1990-67F, SIT-M TGx1990-97F, SIT-M TGx1990-45F; and the highest by genotypes SIT-M TGx1989-45F. Similar yields were recorded by genotypes SIT-M TGx1987-40F and SIT-M TGx1987-91F (2.5 and 2.4 t/ha respectively) which were significantly higher ($p < 0.05$) than genotypes SIT-E TGx1835-10E, TGx1903-7F, SITE TGx1990-8F, SIT-E TGx1987-10F, SIT-E TGx1989-21F, SIT-M TGx1989-46F, SIT-E TGx1987-62F and SIT-M TGx1440-1E with their yields varying from 2.0 - 1.8 t/ha. Also, similar yields (2.2 - 2.1 t/ha) were recorded by genotypes SIT-E TGx19883F, SIT-E TGx1990-3F, SIT-E TGx1988-5F and SIT-E TGx1990-2F. There were no significant differences ($p > 0.05$) among genotypes SIT-E TGx1990-8F, SIT-E TGx1987-10F, SIT-E TGx1989-21F, SIT-M TGx1989-46F, SIT-E TGx1987-62F, SITM TGx1440-1E, ANIDASO, SIT-E TGx1990-5F and SIT-M TGx1904-6F with their yield varying from 1.9 - 1.7 t/ha. Genotype NANGBAAR had 1.5 t/ha grains which were not different from genotypes SIT-E TGx1989-4F and SIT-E TGx1990-15F which recorded 1.6 t/ha.

Table 4.9: Seeds per 100 pods, 1000 seed weight and grain yield of soybean genotypes

Genotypes	Seeds/per 100 pods	1000 seeds weight (g)	Grain yield (t/ha)
SIT-E TGx1988-3F	234	133.7	2.2
TGx1903-7F	218	110.2	2.0
NANGBAAR	186	90.8	1.5
SIT-E TGx1990-3F	191	151.5	2.2
SIT-E TGx1990-15F	189	127.7	1.6
SIT-E TGx1987-10F	246	137.7	1.9
SIT-E TGx1989-19F	220	133.3	1.4
SIT-M TGX1904-6F	215	122.8	1.7
SIT-E TGx1989-4F	215	147.8	1.6
SIT-M TGx1989-46F	146	131.0	1.8
SIT-E TGx1988-5F	184	121.3	2.1
ANIDASO	206	100.2	1.7
SIT-M TGx1987-91F	234	145.2	2.4
SIT-M TGx1989-42F	213	124.3	1.3
SIT-M TGx1987-14F	126	102.7	1.3
SIT-E TGx1989-21F	190	120.3	1.9
SIT-E TGx1987-62F	206	98.0	1.8
SIT-M TGx1989-45F	257	158.7	2.6
SIT-E TGx1990-2F	248	172.3	2.1
SIT-E TGx1835- 10E	167	145.0	2.0
SIT-M TGx1987-40F	210	148.8	2.5
SIT- E TGx1990-8F	198	115.5	1.9
SIT-E TGx1990-5F	213	144.0	1.7
SIT-M TGx1440-1E	179	100.5	1.8
TGx1909-3F	106	86.0	0.9
SIT-M TGx1990-67F	98	86.3	0.9
SIT-E TGx1987-11F	107	89.3	1.1
SIT-E TGx1987-86F	112	91.7	1.2
SIT-M TGx1990-45F	97	87.3	0.9
SIT-E TGx1740-2F	104	89.2	1.0
SIT-M TGx1990-97F	103	87.8	0.9
SIT-E TGx1989-20F	137	91.3	1.2
SIT-E TGx1448-2E	113	90.3	1.0
SIT-E TGx1987-96F	113	91.3	1.1
Grand Mean	177.0	116.9	1.6
CV (%)	5.9	4.5	9.4
LSD (P < 0.05)	17.0	8.5	0.3

4.2.6 Correlation Matrix

The results of the correlation matrix for nodule score, rust severity score, days to maturity, plant height at harvest, shattering, lodging, seeds per 100 pods, 1000 seeds weight and grain yield are presented in Table 4.10. The results showed significant positive correlation between nodule score and seeds per 100 pods (r = 0.3101); plant height at harvest and days to maturity (r = 0.2640); plant height at harvest and seeds per 100 pods (r = 0.2458); plant height at harvest and grain yield (r = 0.2014); seeds per 100 pods and weight of 1000 seeds (r = 0.6450); seeds per 100 pods and grain yield (r = 0.7495); and weight of 1000 seeds and grain yield (r = 0.6205). There were significant negative correlation between days to maturity and lodging characteristics (r = -0.2418); and days to maturity and weight of 1000 seeds (r = -0.3759). Rust severity score significantly negative correlated with nodule score (r = -0.2740), plant height at harvest (r = -0.3138), seeds of 100 pods (r = -0.7912), 1000 seeds weight (r = -0.6047) and grain yield (r = -0.8119).

Table 4.10: Pearson correlation coefficient of selected agronomic parameters

	DF	NS	RS	DM	PHH	SH	LD	SEEDS	TS
NS	0.1483								
	0.1369								
RS	-0.2164*	-0.2740*							
	0.0289	0.0053							
DM	0.5892*	0.0804	-0.0145						
	0.0000	0.4221	0.8849						
PHH	0.4821*	0.1730	-0.2701	0.2640*					
	0.0000	0.0820	0.0060	0.0073					
SH	-0.3014*	-0.1769	0.0950	-0.3326*	-0.0362				
	0.0021	0.0752	0.3421	0.0006	0.7176				
LD	-0.0311	0.0402	-0.0917	-0.2418*	0.1016	0.0388			
	0.7566	0.6881	0.3592	0.0143	0.3097	0.6987			
SEEDS	0.0780	0.3101*	-0.8336*	-0.1711	0.2458*	-0.1413	0.1153		
	0.4357	0.0015	0.0000	0.0854	0.0128	0.1566	0.2487		
TS	-0.2712*	0.0061	-0.6205*	-0.3759*	-0.0093	0.1220	0.0806	0.6450*	
	0.0058	0.9517	0.0000	0.0001	0.9259	0.2218	0.4206	0.0000	
GY	0.1365	0.1818	-0.8207*	-0.0524	0.2014*	-0.0923	0.1119	0.7495*	0.6205
	0.1714	0.0675	0.0000	0.6008	0.0424	0.3562	0.2629	0.0000	0.0000

Footnote: NS = Nodule score; RS = Rust severity score; DM = Days to maturity; PHH = Plant height at harvest (cm); Sh = Shattering; LD = Lodging; Seeds = Seeds of 100 pods (g); TS = 1000 seeds weight (g); GY = Grain yield (t/ha). * means significant at $p < 0.05$

CHAPTER FIVE

5.0 DISCUSSION

5.1 Screening for rust resistance gene(s) in soybean genotypes using SSR molecular markers

The SSR markers used to characterise 34 soybean genotypes showed that molecular diversity existed among the genotypes used for the study. The findings confirmed that most of the genotypes were of different genetic background. Due to high heterozygosity values found in this study, the markers used have high ability to detect heterozygosity in soybean hybridization work.

Most of the soybean genotypes identified by the primers to have presence of the rust resistance gene(s) were also found to be either highly resistant, resistant or moderately resistant under natural epiphytotic condition. For instance, genotype SIT-E TGx19903F and SIT-M TGx1989-45F were discovered by four different SSR markers to have resistance genes and were also confirmed highly resistant during field screening. This agrees with the assertion that genetic composition of soybean variety/genotype dictates its resistance to disease (Song *et al.,* 2004). Also, all the genotypes detected by SSR marker Satt708 as resistant were also found to have level of resistance during field screening, making it the best marker identified in selection for resistance to SBR. The SSR markers indicated some potentially useful sources of resistance to SBR that may be valuable to soybean breeders. This correspond to the findings of the study by Tran *et al*. (2012), who successfully applied molecular markers to detect the presence of resistance (R*pp*5) in HL203, an elite Vietnamese soybean variety to SBR. These results have indicated the significance of marker assisted selection (MAS) in identifying a targeted gene. Again, MAS is proven as a diagnostic tool for tracing the presence of the target R*pp* gene for which direct selection is difficult. In addition, with MAS, the breeder can

carry out several rounds of selection in a year without depending on the natural occurrence of the pathogen.

From the study, none of the SSR markers used was able to identify all genotypes to be resistant. This could be due to the polygenic nature of the genes controlling the rust resistance. It has been indicated that rust disease resistance is controlled by many recessive genes (Calvo *et al*., 2008). It could also be suggested that genotypes used to identify the markers associated to rust disease resistance are of different genetic background from those used in this study. Besides, the markers might have been identified using genotypes reacting to different strains of the pathogen (Agrios, 2005).

None of the soybean genotypes was identified to carry all the five dominant major resistance genes (R*pp*1, R*pp*2, R*pp*3, R*pp*4 and R*pp*5). This indicated that most of the lines identified as resistant were associated with single gene resistance. This is in conformity with Bonde *et al*. (2006) that, cultivars have single gene resistance. It is also supported by Hartman *et al.* (2005) that, none of the soybean cultivars in present commercial production is resistant to all *P. pachyrhizi* isolates. Long term utilization of these race-specific genes can prompt the pathogen to mutate and overcome them. This makes the disease devastating and challenges Ghana soybean breeders to develop soybean cultivars that have the multiple resistance genes to provide resistance to different races of *P. pachyrhizi*. To establish suitable varieties, plant breeders should optimize the plant genotype by choosing the most promising resistance genes and combinations to ensure stability/durability of resistance. Marker-assisted backcrossing can be gainfully employed for adding new resistance genes into popular and elite soybean genotypes that have been grown by Ghanaian farmers over the years on account of their unique agronomical characters. Gene pyramiding has also, been suggested to be effective to

overcome resistance instability conferred by single gene resistance to SBR (Hartman *et al*., 2005; Garcia *et al*., 2008). Use of SSR markers for gene pyramid has been validated by three independent SBR resistance genes, R*pp*2,

R*pp*3 and R*pp*4 by Maphosa *et al*. (2012).

5.2 Soil analysis

According to Adepetu and Corey (1976), organic matter value of 0.8 % is within the critical value of 0.5 - 4.0 % but total nitrogen value of 0.07 % is less than the its critical value of 0.15 %. The soil was high in the exchangeable cations based on the critical levels of 0.21, 2.6 and 0.80 cmol/kg potassium, calcium and magnesium, respectively (Akinrinde and Obigbesan, 2000). The available phosphorus value of 20.22 ppm is above 12 ppm (Bray-1 test) and a pH value of 6.16 is within the recommended value of 5.5 - 7.0 for soybean production (Ferguson *et al*., 2006). The properties of the soil made the planting site relatively good for soybean production, since the most of the soil test results are within the standards for its production

(Ferguson *et al*., 2006; Akinrinde and Obigbesan, 2000; and Adepetu and Corey, 1976).

5.3 Field evaluation of soybean genotypes for resistance to P. pachyrhizi

In the study, 34 genotypes of soybean were screened for rust resistance under natural epiphytotic condition (Table 4.4). There was a differential response of the genotypes resistant to SBR. The field evaluation identified or confirmed 24 soybean genotypes as highly resistant, resistant or moderately resistant to *P. pachyrhizi* and 10 genotypes as either susceptible or highly susceptible. These research results agree with Kim *et al*. (2005) and Yang *et al*. (1991) who reported that, the responses to SBR depends on the existing environmental conditions, genotype, inoculum level and the evaluation method. Similar results were reported by Patil and Basavaraja (1997) who evaluated several

soybean genotypes under natural epiphytotic condition and reported some (EC392530, EC-392538, EC-392539, EC-392541, SL-423, RSC-1, RSC-2, JS-80-21 and PK-1029) of soybean genotypes as moderately resistant. Hundekar (1999) also evaluated soybean genotypes for rust resistance and reported S-22, WC-12 and C-92 as rust resistant during field germplasm screening. Verma *et al*. (2004) evaluated 242 germplasm lines/cultivars of soybean under natural epiphytotic conditions for resistance to rust and reported only one line (SJ-1) as highly resistant, three lines *viz*., JS-19, RPSP-728, PK-838 as resistant, 16 lines as moderately resistant and rest were either susceptible or highly susceptible.

None of the soybean genotypes evaluated on the field showed immune reaction but during the molecular screening some genotypes were identified as immune. Also some genotypes that were known to have resistance gene during molecular screening were found to be susceptible during field evaluation. This was probably due to virulent races of the pathogen and high inoculum build-up due to yearly planting of soybean and/or alternate host plants at the experimental site. According to Sweets (2002), severity of rust infection is influenced by quantity of inoculum, interaction among hosts, pathogen strains and existing environment conditions.

5.4 Evaluation of soybean genotypes for their resistance to pod shattering

Identification of genotypes with potential for lowest pod shattering is one of the most important aspects in the management of pod shattering. The pod shattering values ranged from 13.33 to 96.67 %. Six genotypes were found to be moderately resistant with the rest either as moderately susceptible or very susceptible to pod shattering. This revealed the existence of genotypic differences among the genotypes tested. This is in line with the observations of Tiwari and Bhatnagar (1991), Tukamuhabwa *et al*. (2002) and Agrawal *et al*., (2004) who reported that pod shattering in soybean could be linked to cultivar

differences, anatomical structure of pod and genotype by environment (GxE) interaction. Investigations have indicated that there were significant differences ($p < 0.05$) in shattering resistance among different varieties (Caviness, 1969; Misra *et al*., 1980) and the features of pod shattering is genetically determined (Saxe *et al*., 1996). The environmental conditions such as high temperatures, rapid changes in temperature, low humidity, wetting and drying have been identified to contribute to pod shattering (Tukamuhabwa *et al*., 2002). Hence, the variation in pod shattering could partly be due to environmental conditions. The varietal differences in terms of pod shattering observed could further be exploited for breeding programme to improve soybean against shattering.

5.5 Yield and yields components of soybean genotypes

A comparison of seed yield and yield contributing traits (seeds per 100 pods, 1000 seeds weight) showed that there were significant differences among the genotypes evaluated (Table 4.9). Genotype SIT-E TGx1989-45F recorded the highest grain yield (2.6 t/ha), highest number of seeds (257) per 100 pods and second highest 1000 seed weight. This indicated that, yield is a function of individual seed weight and number of seed per pods when the crop matures. There were positive correlation between grain yield and other yield components such as number of seeds per 100 pods and 1000 seeds weight and these collaborate with the findings of Kokubun *et al*. (2001) that grain yield of soybean is controlled by the number of seeds per plant and individual seed weight. These findings are in conformity to reports of Liu *et al*., (2005) and Arshad *et al*., (2006) that number of pods and seed weight are most important plant traits contributing to improved economic yield in soybean crop and hence, suggested that these traits should be given more importance when selecting superior soybean genotypes. Khan *et al*. (2000) studied heritability and correlation among yield determining components of 86 genotypes in

Pakistan and reported that seed yield had a significant positive relationship with yield components (number of seeds per pod and
1000 seeds weight).

In this study, grain yield significantly correlated with plant height. This indicates that plant height contribute positively to grain yield, thus confirming the assertion of Basavaraja *et al*. (2005) and Mukhekar *et al*. (2004). Grain yield was negatively correlated with days to maturity in this study. This contrasts the reports of Ramana *et al*. (2000) and Bangar *et al*. (2003) that grain yield correlate with days to maturity positively.

The differences observed among genotypes in relation to seed yield and its contributing traits could be attributed to the genetic make-up of the genotypes evaluated (Acquah, 2007). The variations in terms of seed yield could be attributed to differences in degree of resistance to rust exhibited by the genotypes as the grain yield negatively correlated with the rust severity score (Table 4.10). This is in line with the observation of Goodwin (1992) and Kumudini *et al*. (2008) that soybean rust (SBR) reduces leaf area index (LAI) and photosynthetic efficiency, which limit photosynthesis and yield. Hartman *et al*. (1991) also reported that SBR incidence causes yield and seed weight reduction.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Seven out of the nine primers were polymorphic with significant differences whiles two were monomorphic. Resistance gene(s) was identified in all the genotypes except TGx1909-3F by the SSR markers. Genotypes SIT-E TGx1990-3F, SIT-M TGx198791F, SIT-M TGx1989-45F and SIT-E TGx1988-5F were known to have resistance genes

R*pp*1, R*pp*2 and R*pp*3 to SBR by four different SSR markers (Sat_064, Satt708, Staga001 and Sat_307) and also detected as highly resistant during field screening. All the genotypes (SIT-E TGx1990-3F, SIT-E TGx1989-19F, SIT-M TGx1904-6F, SIT-E TGx1989-4F, SIT-E TGx1988-5F, ANIDASO, SIT-M TGx1987-91F, SIT-E TGx198762F and SIT-E TGx1989-45F) detected by SSR marker Satt708 as resistant (R*pp*2) were also found to have level of resistance during field screening, making it the best marker identified in selection for resistance to SBR. None of the SSR markers used was able to identify all genotypes to be resistant or susceptible.

The phenotypic screening revealed 24 soybean genotypes as highly resistant, resistant or moderately resistant to *P. pachyrhizi* and 10 genotypes as either susceptible or highly susceptible. Lodging score of the soybean genotypes evaluated revealed 53 % of the plant as all erect, no soybean genotype exhibited severe lodging or all plant flat with the rest as either slight lodging or lodged at 45°. Six genotypes namely SIT-M TGx1904-6F, SIT-E TGx1835-10E, SIT-M TGx1987-40F, TGx1903-7F, SIT-E TGx1448-2E and ANIDASO (check) were found to be moderately resistant with the rest either as moderately susceptible or very susceptible to pod shattering. Genotype SIT-E TGx1989-45F was superior in terms of yield (2.6 t/ha) and producing 257 seeds per 100 pods as the highest value and recorded the second highest value in terms of 1000 seed weight.

6.2 Recommendations

From the results of the experiments, the following recommendations have been made.

i. Soybean genotypes SIT-E TGx1990-3F, SIT-M TGx1987-91F and SIT-M TGx1989-45F were observed to have multiple resistance genes (R*pp*1, R*pp*2 and R*pp*3) during molecular the screening and also highly resistant during field

screening to soybean rust, these genotypes should be further be exploited and used in breeding programme.

ii. Further studies should be conducted to verify the stability/durability of the varieties or genotypes known to have some level of resistance.

iii. Studies should be conducted for multiple years and locations to ascertain the yield stability, lodging and shattering resistance of the resistant soybean genotypes.

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APPENDICES

Appendix 1: PCR Reagents (Biolabs) for Soybean SSR Amplification

PCR Component	1x Reaction Volume
PCR water (Nuclease free water)	5.78µl
10x buffer	1µl

MgCl2 (25mM)	0.9µl
DNTPs (20mM)	0.2µl
5 µM Primer (F/R)	0.5µl
5 U/µl Taq polymerase	0.12µl
30ng/µl genomic DNA	1µl

Appendix 2: Thermocycling profile for amplification of soybean SSRs for Rust resistance gene

94°C 5 min. Initial Denaturation

94°C 1min. Denaturation

50°C 1min. Annealing

72°C 1min. Extension

72°C 7min. Final extension

NB: 94°C 1min. Denaturation, 50°C 1min, annealing 72°C 1min and Extension consist of 33 cycles

Appendix 3: Summary ANOVA for rust severity score

Source of Variation	Degree of freedom (df)	Sum of Squares	Mean of Square	F-value	P-value
Replication	2	25.5	12.76		
Treatment	33	44665.8	1353.51	315.16	
Error	66	283.4	4.29		
Total	101	44974.8			
Mean	23.87				
CV (%)	8.68				
LSD 5(%)	3.38				

Appendix 4: Summary ANOVA for nodule count at 50 % flowering

Source of Variation	Degree of freedom (df)	Sum of Squares	Mean Square	F-value	P-value
Replication	2	29.0	14.480		
Treatment	33	10084.7	305.596	33.08	
Error	66	609.7	9.238		
Total	101	10723.3			
Mean	16.87				
CV (%)	18.52				
LSD (5%)	4.95				

Appendix 5: Summary ANOVA for plant height at harvest

Source of Variation	Degree of freedom (df)	Sum of Squares	Means Square	F-value	P-value
Replication	2	21.8	10.876		
Treatment	33	13603.0	412.211	29.99	
Error	66	907.1	13.745		
Total	101	14531.9			
Mean	54.52				
CV (%)	6.80				
LSD (5%)	6.04				

Appendix 6: Summary ANOVA for Days to 50 % flowering

Source of Variation	Degree of freedom (df)	Sum of Square	Mean of Square	F-value	P-value
Replication	2	0.73	0.3627		
Treatment	33	1005.92	30.4825	22.71	
Error	66	88.61	1.3425		
Total	101	1095.25			
Mean	43.78				
CV (%)	2.65				
LSD (5%)	1.89				

Appendix 7: Summary ANOVA for Days to maturity

Source of Variation	Degree of freedom (df)	Sum of Squares	Means of Square	F-value	P-value
Replication	2	0.55	0.2745		
Treatment	33	2775.49	84.1058	103.85	
Error	66	53.45	0.8099		
Total	101	2829.49			
Mean	91.51				
CV (%)	0.98				
LSD (5%)	1.47				

Appendix 8: Summary ANOVA for Lodging

Source of Variation	Degree of freedom (df)	Sum of Squares	Means of Square	F-value	P-value
Replication	2	0.2353	0.11765		
Treatment	33	23.9608	0.72608	2.51	0.0007
Error	66	19.0980	0.28936		
Total	101	43.2941			
Mean	1.65				
CV (%)	32.66				
LSD (5%)	0.88				

Appendix 9: Summary ANOVA for percentage (%) shattering

Source of Variation	Degree of freedom (df)	Sum of Squares	Means of Square	F-value	P-value
Replication	2	13.7	6.86		
Treatment	33	77962.7	2362.51	90.68	
Error	66	1719.6	26.05		
Total	101	26.05			
Mean	60.20				
CV (%)	8.48				
LSD (5%)	8.32				

Appendix 10: Summary ANOVA for Seeds per 100 pods

Source of Variation	Degree ofFreedom (df)	Sum of Squares	Means of Square	F-value	P-value
Replication	2	202	100.95		
Treatment	33	260176	7884.12	72.59	
Error	66	7168	108.61		
Total	101	267546			
Mean	175.98				
CV (%)	5.92				
LSD (5%)	16.99				

Appendix 11: Summary ANOVA for 1000 seed weight

Source of Variation	Degree ofFreedom (df)	Sum of Squares	Means of Square	F-value	P-value
Replication	2	144.8	72.42		
Treatment	33	64067.3	1941.43	71.38	
Error	66	1795.0	27.20		
Total	101	66007.1			
Mean	116.88				
CV (%)	4.46				
LSD (5%)	8.50				

Appendix 12: Summary ANOVA for grain yield

Source of Variation	Degree ofFreedom (df)	Sum of Squares	Means of Square	F-value	P-value
Replication	2	0.0165	0.00824		
Treatment	33	24.2734	0.73556	31.59	
Error	66	1.5369	0.02329		
Total	101	25.8268			
Mean	1.62				
CV (%)	9.42				

LSD (5%)	0.25