SCREENING OF KENYAN SOYBEAN CULTIVARS FOR RESISTANCE TO DIFFERENT RACES OF *PHAKOPSORA PACHYRHIZI* (SOYBEAN RUST) AND DETERMINATION OF MOLECULAR BASIS FOR RUST RESISTANCE

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DECLARATION

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

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This thesis is dedicated to my husband Wilys and daughters Mitchel and Grace

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ABBREVIATIONS AND ACRONYMS

AHC	Agglomerative hierarchical clustering
AUDPC	Area under disease progress curve
CEBIB	Center for Biotechnology and Bioinformatics
CIAT	International Center for Tropical Agriculture
CTAB	Cetyl Trimethylammonium Bromide
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization
FPR	Farmer participatory research
GAIN	Global Agricultural information Network
IITA	International Institute for Tropical Agriculture
JIRCAS	Japan International Research Center for Agricultural Sciences
KALRO	Kenya Agriculture and Livestock Research Organization
MT	Metric tonnes
PCA	Principal component analysis
PCR	Polymerase Chain reaction
PDI	Percent disease index
PPB	Participatory plant breeding
PRA	Participatory rural appraisal
PVS	Participatory variety selection
RH	Relative humidity
SSR	Simple Sequence Repeats

TGx Tropical Glycine Cross (as used by IITA)

TSBF Tropical Soil Biology and Fertility

USAID United States Agency for International Development

WHO World Health Organization

ABSTRACT

Soybean (*Glycine max (L.) Merrill*.) is a highly nutritious plant which plays an important role in the world's economy, however soybean rust disease caused by the fungus *Phakopsora pachyrhizi*, is a major challange to the soybean industry. The disease among other constraints has significatly affected crop yields in most soybean growing countries. High yield losses associated with *P.pachyrhizi*, have been reported worldwide. The first report of the pathogen in Kenya was made in 1996 and it has continued spreading agrressively and affecting soybean bean industry in the country. Studies to determine resistance of soybean to rust disease have only been done on commercial varieties but no such studies have been done on the local varieties of soybean. Similarly no significant data has been collected on soybean rust disease severity and incidence in the country. In this study, farmers' participatory research was used to collect data on soybean varieties preferred by farmers and the major constraint facing soybean farming in Western Kenya region. Soybean rust disease incidence and severity levels were also established in Khwisero, Butere, Mumias and Teso sub counties of Western Kenya. The presence of the rust fungi was confirmed through microscopy and polymerase chain reaction (PCR) analysis. Seven local varieties of soybean (Nyala, Bossier, SB19, Hill, SB8, Gazelle and TGx1987-32F) were tested in the green house for resistance to soybean rust. To determine presence of rust resistance genes, 12 Simple sequence repeat (SSR) markers previously mapped on linkage groups of soybean were chosen and tested on the resistant varieties. The study revealed that the farmers in Western Kenya region grow mainly the local varieties of soybean and the area under soybean cultivation is <30% of the total land available for crop production. It was further noted that soybean rust disease is present in all the sub-counties with the percent disease index (PDI) ranging from 40.37% to 74.8%. The disease severity level in all the four sub-counties did not vary significantly ($P<0.05$). The disease incidences per farm ranged from 22%-87% with Teso Sub-county recording the highest average disease incidence (50.55%). Microscopy and PCR analysis identified the pathogen causing soybean rust disease as *P. pachyrhizi* and not *P. meibomiae.* Screening for rust resistance in the green house showed that the varieties TGx1987- 32F and SB8 were resistant producing Red brown lesion with low level of severity, low lesion number, low sporulation level and low area under disease progress curve (AUDPC) value. The other five varieties; Nyala, Bossier, SB19, Hill and Gazelle were susceptible to rust producing Tan lesion with profuse sporulation and high disease severity level. Simple Sequence repeats (SSR) markers analysis of the resistant varieties showed that they both contained *Rpp1, Rpp2, Rpp3* and *Rpp4* genes while the susceptible varieties *Rpp1, Rpp2* and *Rpp4* genes. The results of this study clearly indicate that adequate preventive measures have not been put in place to deal with soybean rust disease and other constraints of soybean production. It has also been demonstrated that the local varieties contain the rust resistance genes, however the resistance can be overcome by certain rust pathotypes. The varieties that have shown resistance to diverse rust isolates are possible sources of rust resistance genes that could be used in breeding programs.

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CHAPTER ONE

1 INTRODUCTION

1.1 Background

Soybean (*Glycine max (L.) Merrill*.) is an edible oil crop that belongs to the legume family *Fabaceae* and the genus *Glycine*. It is a native of China, introduced to Africa in the early 1800s, and to Kenya in the early 20th century. In Kenya soybean is grown as a cash crop by small scale farmers in Western, Rift valley, Nyanza, Eastern and Central provinces (Nassiuma and Wasike, 2002). The main importance of soybean is that it is highly nutritious with 40% protein 20% oil and 30% carbohydrates (Tefera, 2007). The world's major source of edible oils is soybean (30%) and it also accounts for 60% of vegetable protein (FAO, 2007). Soybean contains a unique isoflavones compound called genistein, many studies have demostrated that genistain possess remarkable powers of healing and disease prevention (Yu *et al*., 2000). Consumption of soybean reduces attack by various types of cancer particularly breast cancer, prostrate and colon cancer, it also reduces the risk of coronary thrombosis and heart attack. Soybean also reduces menopausal symptoms and increases calcium density and prevent osteoporosis (Yu *et al*., 2000). It contains unique proteins called peptides which increase its nutritional values. Examples of the peptides in soybeans include defensins, glycinins, conglycinins and lunasins. These compounds provide health benefits, such as improvement of immune function, control of blood sugar and regulation of blood pressure (Anderson and Bush, 2011). In addition soybean produces high yield (650 kg per acre) compared to common bean (250 kg per acre) under pure stand especially in low rainfall. It is more tolerant to pests and diseases, it fixes atmospheric nitrogen thus improving soil fertility and increasing production of subsequent crops (CIAT, 2006).

Globally, 6% of all total arable land is under soybean production and soybean has the highest percentage increase in area under production than any other crop (Hartman *et al*., 2011). Currently North America is the largest producer of soybean (42% of world production) followed by South America (32.1%), Asia (22.9%), Europe 1.6%), Africa 1.22% (FAO, 2002). Despite the availability of suitable agroecological conditions for soybean production in Africa total production is still far below the demand this is because of various biological and socio economic constraints. However production in Africa can be improved if key production constraints are addressed.

The production of soybean in Kenya is affected by numerous biotic and abiotic factors. Some of the constraints include, low yielding varieties, lack of markets, poor agronomic practices, lack of awareness for its potential, competition with other legumes, drought, water logging, and pest and disease attacks (Hartman *et al.*, 2011). Other factors include lack of high yielding varieties which are tolerant to low phosphorus and midseason moisture stress (FAO, 2005). Among the biotic factors affecting soybean production diseases are of great concern because of their final impact on yield. There are a number of diseases that infect soybean worldwide the most common disease are Anthracnose, bacterial blight, bacterial pustule, soybean rust, bean pod mottle virus, brown stem rot, charcoal rot, frog eye leaf spot, soybean cyst nematode and soybean mosaic virus among others (Ploper,1997).

Soybean rust caused by *P. pachyrhizi* has been identified among other diseases as the major challenge to soybean production worldwide. The rapid spread of the disease in the continent of Africa has led to major decline in soybean yield (Levy, 2005, Oloka *et al.*, 2008).

Losses due to soybean rust can be significantly high. In South Africa losses of 10-80% have been reported and in areas under monocropping system the losses can be as high as 100%. India has experienced losses of 10-90%, Japan 40% and Taiwan has reported losses of 23-90% in (Hartman *et al.,* 1999). It is therefore important that the major production constraints be addressed so as to improve the crop yield to be able to meet the market demands and sustain the production industries.

1.2 Problem Statement

Soybean yield in tropical Africa countries is low (less than 1.0 tonne/hectare) compared to temperate countries. Low yields in the tropics are attributed to a number of biological and socio economic constraints. Insect pest, diseases, pod shattering, sensitivity to photoperiod and environment, and non-adoption of appropriate management practices are some of the key biological constraints. Lack of awareness of soybean utilization and markets are the major socio- economic limitations to soybean production in Africa (Kawuki *et al*., 2003). Among the biological constraints, diseases are by far the most important (Hartman *et al*., 1999). Crop losses caused by diseases contributes to food insecurity by reducing to a greater extent the amount of food available for human and animal consumption. They also affect national and international trade in agricultural products, thus reducing farmers' earnings and increasing poverty levels (FAO, 2005). Soybean rust caused by *P. pachyrhizi* has particularly been identified as the most destructive soybean disease in recent times. The rust disease has widespread distribution and potential of causing very high yield losses. The disease was reported in Kenya, Rwanda and Uganda for the first time in 1996. It later spread to Zambia and Zimbabwe in 1998, Mozambique in 2000 and South Africa in 2001 (Kawuki *et.al*., 2003).

Yield losses from 10% to 80% which have been reported in Argentina, Asia, Brazil, Paraguay, South Africa, and Zimbabwe (Schnepf, 2005)

In terms of control and management of soybean rust fungicide is the main control measure for now. This is mainly due to the absence of soybean rust resistant cultivars. The use fungicides to control the disease commercial plantings significantly increases production costs it is therefore not a feasible option in small scale soybean plantings especially in developing countries (Miles *et al*., 2003). The fungicides are expensive and are not very effective at preventing epidemics as Bonde *et al*., (2006) noted yield losses of up to 50% under severe rust epidemics with chemical control. Other legumes that also form an integral part of the cropping system such as cowpea, pigeon pea and common beans are functional alternative hosts of *P. pachyrhizi* which makes control a great challenge (Anon, 2007; Slaminko *et al*., 2008). Cultural practices like destruction of alternate hosts, timely irrigation, early planting and growing early maturing cultivars can also reduce the incidence of the disease (Akinsanmi *et al.,* 2001). However, the rapid spread by wind-borne urediniospores and the large number of host species increases chances of soybean rust survival making cultural practices relatively ineffective (Hartman *et al*., 2005).

1.3 Justification

Planting of disease resistant cultivars is the most viable way to manage soybean rust disease. However there are no rust resistant cultivats available in Kenya currently. To identify rust resistant cultivars soybean plants must be screened for resistance to diverse pathogen populations (Twizeyimana *et al*., 2007). It is also important to identify the disease resistant genes in the varieties that show resistance reaction to the rust pathogen. DNA markers have

been used to identify specific genes and for marker assisted selection in plant breeding (Yamanaka *et al*., 2008). Simple sequence repeat (SSR) markers is the most commonly used marker because of their advantage over other molecular markers. They are abundant in the genome, highly polymorphic, multiallelic and hyper variable (Kuroda *et al*., 2009). In addition they can be easily analysed polymerase chain reaction (PCR) and gel electrophoresis ((Hyten *et al., 2007*). It is possible to perform marker assisted selection to enhance soybean rust resistance genes due to the availability of soybean SSR map (Song *et al*., 2004).

A major requirement in breeding programs is to involve farmers in the variety selection proscess. In breeding experiments where the farmers and other stakeholders are not involved there is poor adoption and dissemination of the resulting technologies (Osiru *et al.,* 2010). Farmers' participatory research (FPR) approaches like participatory plant breeding (PPB), participatory rural appraisal (PRA), participatory variety selection (PVS) and other approaches are being used for variety or technology development and diffusion, with the overall goal of ensuring that farmers adopt the new cultivars (Doward *et al.,* 2007). Participatory variety selection has been used to assist breeders in identifying farmer-preferred varieties that match with their environmental conditions, available resources, quality traits, and consumers' needs (Pandit *et al.,* 2007). This study therefore aimed at identifying farmers' preferred varieties of soybean, the constraints facing soybean production, assessing the severity of soybean rust in Western Kenya, evaluating various soybean varieties for resistance to soybean rust and identifying the molecular basis for rust resistance using SSR markers.

1.4 Research objectives

1.4.1 Overall objective

To screen selected Kenyan soybean cultivars for resistance to different races of *Phakopsora pachyrhizi* (soybean rust) and to determine the molecular basis for rust resistance.

1.4.2 Specific objectives

1. To identify varieties of soybean preferred by farmers and constraints facing soybean production in Western Kenya
2. To determine the severity of soybean rust disease on farm among the local varieties of soybean in Western Kenya
3. To screen local soybean varieties for resistance to diverse rust isolates under green house conditions
4. To determine the molecular basis of resistance to soybean rust among the local varieties of soybean

1.5 Research hypotheses

1. Farmers in Western Kenya have no preference for specific varieties of soybean and the constraints facing soybean production in Western Kenya does not vary across the sub counties.
2. Soybean rust disease severity does not vary with the soybean variety grown and location of the soybean farms.

3. Resistance of local varieties of soybean to soybean rust under green house conditions is not dependent on the diversity and origin of the rust isolates.
4. The local varieties of soybean do not possess different types of soybean rust disease resistance genes.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Importance of soybean to world agriculture

Soybean is a versatile crop it is used as human food, for production of livestock feeds, for industrial purposes and as a source of biofuels (Myaka *et al*., 2005). Soybean improves soil fertility by fixing nitrogen from the atmosphere and enhancing moisture retention (Sanginga *et al*., 2003). Studies have shown that there are varieties of soybean which can fix 44 to 103 kg of nitrogen/hectare per year (Sanginga *et al*., 2003). Soybean is an ideal crop for use in cereal rotation programs since it improves soil fertility and breaks life cycle of pests and diseases (Waymark, 1997). In addition the use of soybean in crop rotation is beneficial to the subsequent crops due to availability of the extra nitrogen left in the soil after harvesting of soybean (Chianu *et al.*, 2009). Soybean also has the capacity to strengthen family nutrition and health. Soybean has 40% protein, 20% oil, contains no cholesterol and contain omega 3 fatty acids that reduces the risk of chronic diseases. Soybean is the major source of the world's edible oils (CIAT, 2006). The oils extracted from soybean are used for multiple purposes such as cooking, making of margarine and other industrial purposes (CIAT, 2006). When grown as a cash crop soybean provide farmers with income that can be used to purchase essential farm inputs meet the family's financial requirements and improve the sustainability of agricultural production units (CIAT, 2006, Sanginga *et al*., 2003). In countries like Brazil and Argentina where there is surplus soybean production it provides a major source foreign currency (Ploper, 1997)

2.2 World production of soybean

Soybean is currently the most important grain legume in the world in terms of total production and trade. It accounts for approximately 60% of vegetable protein and 30% oil supply in the world (FAO, 2007). Six years average data (2000-2005) from FAO indicated that 82.8 million hectares of land was used for soybean production worldwide with 188 million tons of grain harvested. The world's major producer of soybean is USA, followed by Brazil and Argentina in the second and third place respectively. Other countries include China, India, Paraguay and Canada. The average area under soybean production in USA, Brazil and Argentina were 29.4, 17.8 and 11.9 million hectares from the year 2000 to 2005. The corresponding production figures were 77.3 million tons for USA, 44.5 million tons for Brazil, and 30.3 million tons for Argentina. The production of soybean in the three leading countries was more double that of Africa. Biotechnological innovations have been used to boost soybean production in the major producing countries. Consequently most soybeans grown in these countries have undergone some biotechnological modification (Jagwe and Nyapendi, 2004). In 2006 it was estimated that of the total soybean area, 58.6 million hectares was under GMO (James, 2006). The use of biotechnologically modified planting materials leads increased tolerance to common pests and diseases and higher crop yields (Jagwe and Nyapendi, 2004). The increase in yield quantity increases the farmers' income from soybean farming especially in commercial plantings.

2.3 Soybean production in Africa

It is widely believed that soybean farming was introduced in Africa around the nineteenth century by Chinese traders along the East coast (Giller and Dashiell, 2006). Soybean

production is Africa is still low in comparison to USA, Latin America and Asia. The continent of Africa only accounts for 0.4 – 1% of total world's soybean production (Chianu *et. al.,* 2008). Nigeria is the leading producer of soybean within Africa followed by South Africa, Uganda and Zimbabwe (FAO 2011). Other countries producing soybean include: Ethiopia (2.7%), Kenya (2.5), Rwanda (2.0%), Egypt (1.7%) and Democratic republic of Congo (1.4%). Countries such Cameroon, Benin, Cote d'Ivoire, Liberia, Burkina Faso, Zambia, Gabon, Tanzania Morocco accounts for less than 1% of soybean production within the Africa context. (Chianu *et al.*, 2008).

2.4 Soybean production and utilization in Kenya

The cultivation and consumption of soybean at domestic and industrial levels in Kenya has grown rapidly. This may be attributed to the search for alternative sources of proteins and cooking oil (Nassiuma and Wasike, 2002). Most soybean production is by smallholder farmers (with 0.1 to 0.2 ha) as a cash crop; while a few large-scale farmers use it in rotation with cereals for sustainable production (Mahasi *et al.*, 2011). The major soybean growing areas in Kenya are; Western, Nyanza, Rift valley as well as Central and Eastern provinces (Mahasi *et al.*, 2011). In 2011 the average production of soybean in Kenya was 2,000-5,000 MT, however the industrial demand is at average of 120,000 MT in 2011 of (FAO, 2011).

The major sectors utilizing soybeans are the food aid sector, the livestock industry, and industries involved in the processing of human food, especially those inclined towards dietary habits and hospitals. Livestock industry utilizes about 70-80% (35,000 - 40,000 ton per year) of the soybean while human consumption accounts for about 20-30% (10,000 - 15,000 ton per

year). Soybean is also used in mixed farming in Kenya to improve soil fertility and fix atmospheric nitrogen which is a limiting nutrient in most Kenyan soils (Chianu *et al.,* 2008). Soybean cultivation has been adopted by many farmers as an alternative source of income since it matures faster than other crops (personal observation) .

The demand for soybean in Kenya is constantly increasing and it is anticipated to rise to about 150,000 tons per annum (Jagwe and Nyapendi, 2004). The current average yield is 0.8 t/ha however the potential yield 1.5 – 3.0 t/ha, depending on the location (Mahasi *et al*., 2011). The low yield is attributed to the fact that, most soybean varieties are highly susceptible to biotic and abiotic stresses (Mahasi *et al.,* 2011). With the use of improved varieties and good management practices it is possible to improve soybean yields up to 3000 –3600 kg ha^{-1} (Chianu *et al.,* 2008). Despite the increasing demand for soybean the area under soybean production, yields and production quantities have not significantly improved during the period of 2010 to 2016 (Table 2.1) (FAOSTAT, 2017).

Table 2.1: Soybean production in Kenya from 2010 to 2016

Year	Yield (tonnes/ha)	Area (ha)	Production quantity (tonnes)
2010	0.95	1621	1540
2011	1.2578	1734	2181
2012	1.4997	1911	2866
2013	1.2231	2042	2497
2014	1.1173	2204	2463
2015	0.9428	2761	2603
2016	0.9061	2215	2007

Source: UN Food and Agricultural and Organization statistics (FAOSTAT, 2017).

To meet the country's annual demand for soybean and soybean products, more than 100,000 metric tonnes of soybean flour and more than 150 metric tonnes of soy protein products are imported into Kenya from China annually (Table 2.2), (GAIN, 2009).

Table 2.2: Soybean and Soy Products Imported into Kenya in 2002-2007

Soybean Products	2002	2003	2004	2005	2006	2007
Soybeans	188,271*	301,507	1,971,976	2,742,418	2,754,713	12,312,297
Crude soybean oil	3,880,034	12,892,530	2,651,134	935,495	4,005,174	6,608,435
Refined soybean oil	1,263	1,771	3,589	199,244	624,591	2,756
Soya bean flour and meals	38,249	10,452	504,325	1,564,878	800,453	4,873,143
Soya bean oil-cake and other residues	2,127,989	1,786,005	1,388,407	1,545,833	1,211,548	1,552,172
Soy proteins (Textured and concentrate)	339,076	202,021	472,592	671,114	577,758	583,577

*Figures in US$ Source: Global Trade Atlas (2008)

2.5 Constraints facing soybean production in Kenya

Soybean production in Kenya is low despite the increasing demand for soybean and soy products. The low production can be attributed to a number of abiotic, biotic and socio-economic constraints (Kawuki *et.al.*, 2003). The abiotic constraints include weather related factors such as drought, flooding and extreme temperatures (Hartman *et al.*, 2011). Drought and extreme temperature have been recognized as the major damaging abiotic stresses. Yield loss dues to heat and drought has been estimated to range between 18 to 28% on soybean (CGIAR, 2012). Other factors include decline in soil fertility, salinity and response to photoperiod (Hartman *et al.*, 2011). Socio economic factors include poor agronomic practices

such as; intercropping two or more cereal crops, lack of knowledge on recommended soybean varieties, inadequate weed control, inappropriate pest management practices, and lack of knowledge on fertilizer use (Chianu *et. al.,* 2008). Other factors include lack of high yielding varieties, lack of awareness of soybean processing and utilization, lack of access to local and international markets and lack of inputs (Chianu *et. al.,* 2008). Biotic constraints such as pests, weeds and diseases also have great effect on the final yield of soybean (Hartman *et al.,* 2011). Even though weeds are considered as a major challenge due to resistance of some weed species to herbicides such as glyphosate (Powles, 2010), diseases especially Soybean rust still causes major economic losses in many parts of the world (Hartman *et al.*, 2011).

2.6 Soybean rust

Soybean rust disease is caused by the fungi *P. meibomiae* and *P. pachyrhizi*. It is not possible to distinguish the two species of fungi by observation of symptoms in an infested field. Accurate diagnosis can only be made through polymerase chain reaction (PCR) assay. The assay takes advantage of the difference in nucleotides in the ribosomal internal transcribed spacer (ITS) region of the DNA (Frederick *et al*., 2002). The fungi *P. pachyrhizi* belongs to the phylum Basidiomycota, class Urediniomycetes and order Uredinales, which produce uredinia, on "dome-like" structures that give rise to asexual urediniospores. Hair-like hyaline hyphae called paraphyses grow inside uredinia. Paraphyses and sporophores are base structures for urediniosopore production (Bromfield, 1984). *P. meibomiae* is less aggressive while *P. pachyrhizi* is more aggressive and it infects over 95 species of plants from more than 42 different genera, as well as soybean and other *Glycine* species (Bromfield, 1984). The most susceptible host of *P. pachyrhizi* is kudzu (*Pueraria lobata* (Wild.), a weed species that is commonly found in the United States of America. Other common hosts are medic

(*Medicago arborea* L.), lupine *(Lupinus hirsutus* L.), sweet clover *(Melilotus officinalis (L.) Lam*), vetch (*Vicia dasycarpa* Ten), common beans (*Phaseolus vulgaris* L.), lima and butter beans *(Phaseolus lunatus* L.), pigeonpea (*Cajanus cajan* (L.) Millsp), garden peas (*Pisum sativum* L.) and cowpeas (*Vigna unguiculata)* (Bromfield, 1984).

Ideal environmental conditions have caused soybean rust disease to become endemic in most soybean growing areas in tropical Africa (Kawuki *et al*., 2003; Twizeyimana *et al*., 2007). Growth and multiplication of soybean rust disease is commonly enhanced by extended period of leaf wetness of 6 to 12 hours and temperatures of 15°C to 28° C (Hartman *et al*., 1999). However, spores of some virulent strains have been observed to germinate at 41°C (Li, 2009a). Establishment is also inevitable where there is a high relative humidity (RH) of 75-80% (Takumahabwa *et al.,* 2011). The fungi are capable of producing five different spore stages in a life cycle. These stages are spermagonia bearing spermatia and receptive hyphae, aecia bearing aeciospores, uredinia bearing urediniospores, telia spores bearing teliospores and basidia bearing basidiospores (USDA, 2008). Unlike other imperfect fungi *P. pachyrhizi*, shows high level of genetic diversity attributed to parasexuality, heterokaryosis and high mutation rate (Freire *et al*., 2008). Under field conditions disease development is caused by the uredinal stage which produces urediniospores during the multicyclic infections that occur in the growing season (Miles *et al*., 2003). The spores can develop within 5 to 8 days after urediniospores germination and infect leaves. Urediniospores production may persist a period of 3 weeks (Hartman *et al*., 1999). Reinfection is dependent on availability of moistures and appropriate conditions that favours growth and establishment. Since *P. pachyrhizi* is an

obligate parasite, urediniospores are harboured by alternative hosts before moving to the soybean crop during the growing season (Goellner *et al.,* 2010).

2.7 Infection process and symptoms of soybean rust

Soybean rust infection process begins in the low to mid-canopy and moves up the plant. The infection starts with urediniospores germinating to produce a single germ tube that grows across the leaf surface, until an appressorium is formed. Penetration of epidermal cells is direct through the cuticle by an appressorial peg (Miles *et al.*, 2005). During the infection process intracellular invasion of the leaf occurs once hyphae are formed within the mesophyll layer. Within 5 to 7 days volcano shaped uredinia with round ostioles are produced which release urediniospores on the abaxial surface completing the asexual reproduction cycle (Goellner *et al.*, 2010).

The symptoms observed are usually small, grey spots on the undersides of leaves and along leaf veins. The spots increase in size overtime and change colour (Sinclair and Hartman, 1982). The disease is first visualized as small water-soaked lesions mostly on the abaxial surface of the leaves which then changes into either grey, reddish brown or tan lesions; but sometimes they may appear on the petioles, pods, cotyledons and stems (Li, 2009a). The lesion colour varies depending on the lesion age, pathogen aggressiveness, host plant, and the interaction between the pathogen and the host (Li, 2009b). The shapes of the lesions tend to be angular to somewhat circular and are often concentrated near leaf veins (Twizeyimana *et al*., 2011).

The symptoms are however not exclusive to the rust, in the early stages of development the symptoms of soybean rust may look like that of other diseases such as bacterial pustule caused by *Xanthomonas axopondis* pv *phaseoli*, bacterial blight caused by *Pseudomonas savastanoi* pv *glycinea*) and brown leaf spot caused by *Septoria glycines* (Ivancovich, 2008; Soares, 2008). In order to make accurate diagnosis hand held lenses with a magnification of X10–X20 is used to show the characteristic volcano-like postules, with several openings containing urediniospores. It is however not possible to distinguished individual urediniospores, using hand lenses (Tukamuhabwa and Maphosa, 2011).

In absence of proper disease control measures, the symptoms can lead to leaf chlorosis, early defoliation and maturity resulting in significant reduction in crop yields (Hartman *et al.*, 2005a). The extent of decline in crop yield depends on the level of resistance of the soybean variety grown to soybean rust, timing of the planting season and weather conditions during the season (USDA 2010). It is also dependent on crop growth stage at which the disease begins and the severity of the infection. The crops are more vulnerable between early flowering and mid- seed development stages (Twizeyimana *et al.*, 2009).

2.8 Geographical distribution of soybean rust

The first report of Asian soybean rust was made in Japan in 1902 and was initially restricted to the tropical and subtropical regions of Asia and Australia (Twizeyimana *et al.*, 2011) the disease later spread throughout the main soybean growing areas of Asia, Australia and India in 1951 (Miles *et al.*, 2003). It later spread to Africa probably through airborne urediniospores movements; but the date of first appearance on the continent is not well documented (Levy,

2005). However suggestions are that aerial urediniospores spread from India to Central Africa causing the first outbreaks in Africa (Isard *et al*., 2006). The first confirmed report Asian soybean rust in the continent of Africa was in Uganda 1996 where it was observed on the experimental plots at Namulonge Agriculture and Animal Production Research Institute (NAARI) and it later spread throughout the country and to other countries (Levy *et al.,* 2002, Kawuki *et al.,*2002). The disease also spread westwards into Nigeria in 1999 and Ghana in 2007 (Akinsanmi *et al.,* 2001, Bandyopadhyay *et al*., 2007). Soybean rust has also been reported in other African countries such as, Cameroon, Mozambique, South Africa and Democratic Republic of Congo (Bandyopadhyay *et al*., 2007; Ojiambo *et al*., 2007; Pretorius *et al*., 2007). The disease later moved from Africa to South America, where it was reported first in Paraguay in 2001, Brazil in 2002 and Argentina in 2003 (Bonde *et al.,* 2006). It was reported in the United States of America in 2004 (Garcia *et al.,* 2008)

2.9 Rust resistance

Identification of rust resistance genes is very important in breeding for new varieties and improvement of existing varieties of soybean. Previous studies have identified a number of single unlinked dominant rust genes. McLean and Byth (1980) identified *Rpp1* gene which associated with immune reaction characterized by no lesions in plant leaves when inoculated with *P. pachyrhizi*. Other genes identified are *Rpp2*, (Bromfield *et al.,* 1980), *Rpp3* (Bromfield and Hartwig, 1980; Bromfield *et al.,* 1980*)* and *Rpp4* (Hartwig, 1986) which are all known to give resistant reactions with limited fungal growth, dark red brown lesions and limited sporulation (Hartwig and Bromfield, 1983). Another resistant gene *Rpp5* has also been identified (Garcia *et al*., 2008; Michelle *et al.,* 2009). The soybean plants that lack the resistance

genes have a susceptible reaction to infection by *P. pachyrhizi*. The symptoms of susceptible reactions are production of copious amount of spores and discrete tan colored lesions (Bromfield and Hartwig, 1980). Several soybean genotypes have also been reported to show resistance reaction, for example three TGx breeding lines in Nigeria (Twizeyimana *et al*., 2008, Hartman, 2012). Soybean rust resistance genes have also been reported in other plants; for example in *Glycine* species, *Pueraria* species and other legume species (Hartman, 2012). Each of these five genes conditions resistance to a limited set of specific *P. pachyrhizi* isolates (Twizeyimana *et al*., 2008). Plants with *Rpp1* for instance show immune reactions when inoculated with a few isolates. It is however not clear how the same plants would react when inoculated with diverse *P. pachyrhizi* isolates (Hartman *et al*., 2005a).

2.10 Role of Simple Sequence Repeats (SSR) markers in molecular breeding

The location of disease resistance genes loci in crops have been successfully identified by use of molecular markers and marker-assisted selection (Concibido *et al.*, 2004). The molecular markers include; restriction-fragment length polymorphism (RFLP) markers, random amplified polymorphic DNA (RAPD) markers, simple sequence repeat (SSR) markers (microsatellites) and amplified-fragment length polymorphism (AFLP) markers. The most common marker used in marker-.assisted selection is SSR. This is due to the fact that they are highly abundant in the genome, polymorphic, multiallelic and evenly distributed throughout the eukaryotic genome (Kuroda *et al.,* 2009). The multiallelic markers with high level of polymorphism have been used for detection of allelic differences between numerous species of plants and animals (Hwang *et al.,* 2008). The high level of polymorphism is due to the occurrence of a numbers of repeats in the microsatellite regions which can be detected by use

PCR (Kalia *et al.*,). In soybean the most common motifs are: AT, ATT, TA, TAT, CT, CTT (Mohan *et al.* 1997). The SSR markers have been continuously developed and utilized in molecular mapping in soybean (Shultz *et al.,* 2007). Molecular genetics linkage maps of soybean containing several SSR markers have been constructed (Cregan *et al.,* 1999, Song *et al*., 2004). All the rust resistance genes, *Rpp1, Rpp2, Rpp3, Rpp4 and Rpp5* have been mapped and SSR markers designed to facilitate for their selection (Hyten *et al.,* 2007). These known SSR markers can be used to map the loci of rust resistant genes in different soybean plants. The identified gene can then be integrated into breeding lines using molecular techniques.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Description of the study area

This study was conducted in Western region of Kenya coordinates 0°30′N 34°35′E/0.500°N 34.583°E/ 0.500. The region accounts for more than 90% of Kenyan soybean production (Tinsley, 2009). Soybean rust surveillance study was done in Khwisero, Butere, Mumias and Teso sub-counties in 2012 and 2013 during the October/ November soybean growing season. The sub-counties are located within the lower middle agro ecological zone with altitude range of 800-1500m, mean temperature of approximately 21° C and mean annual rainfall ranging from 1400mm to 2000mm (Table 3.1) (Jaetzold *et al.*,2009). Screening for rust resistance and evaluation of virulence level of soybean rust pathogen was done in the green house located within the University of Nairobi, College of Biological and Physical Sciences. Laboratory analysis was done at the University of Nairobi Centre for Biotechnology and Bioinformatics.

Table 3.1: Agro-ecological characteristics of the sub-counties where Soybean rust surveillance study was conducted

Sub-county	AEZ	Altitude(m)	Average Temperature (°C)	Annual Rainfall (mm)
Butere	LM1	1488	13.9 –30.2	1685 –1882
Mumias	LM1	1268	14.0-30.0	1400- 2600
Khwisero	LM1	1488	14.1 –27.1	1730 –1929
Teso	LM3	1220	21.0-22.0	1800-2000

LM1= lower midland zone1, LM3= lower midland zone 3, AEZ= agro-ecological zones.
Source: Jaetzold *et al.*, 2009

3.2 Identification of farmers preferred varieties of soybean and constraints to soybean production

To identify the farmers' preferred soybean varieties a structured questionnaire was used to interview the farmers and collect data (Appendix I). The farmers were identified with the help of agricultural extension officers and soybean farmers' groups. Sample households were randomly drawn from the list of farmers within the organized groups of soybean farmers from each sub-county. A total of 120 households participated in the research which included 30 farmers from each sub-county. The information collected included the farmers' demographic characteristics, soybean varieties grown, sources of planting materials, size of the land available for crop production, size of land under soybean cultivation, cropping systems, farmers' preference for specific varieties, the desired attributes of the preferred varieties. Common pest and diseases encountered in the farms, the farmers' disease management practices and data on the constraints facing soybean production and marketing in the region was also recorded.

3.3 Disease incidence and severity assessment

From each sub-county, twenty farms were randomly chosen for the surveillance study. Sampling in each farm was done using a W-pattern whereby 20 plants from 5 different locations within the W- pattern were observed for presence of rust lesions. For each plant selected the top, middle and bottom canopy were assessed. The data recorded from each farm included date of sampling, plot number, variety grown and disease assessment (presence, incidence and severity) (Appendix II).

Disease incidence was estimated as a percentage of individual plants expressing symptoms of the disease within the farm (Sikora *et al.,* 2011). The number of infected soybean plants within the W pattern transect were counted and the incidence calculated using the formula;

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total Number of plants observed}} \times 100$$ (Madden *et al.,*2007).

The severity assessment was done on a scale of 1 to 9 as described by Miles *et al.,* (2005) (Table 3.2) where a rating of 1 means no soybean rust symptoms observed on any leaflet, and a rating of 9 means greater than 20% of the leaflet surface is infected by rust. The ratings were further converted into percent disease index (PDI) using the formula;

$$\text{PDI} = \frac{\text{Sum of individual disease rating}}{\text{Total No. of plants observed} \times \text{Maximum disease rating}} \times 100$$ (McKinney's Index)

The severity per plant was determined by calculating the average severity of the top, bottom and middle canopies.

Table 3.2: Soybean rust disease severity assessment scale (Miles *et al*., 2005)

Severity	**% area infected of leaf**	**Number of lesions**
1	0	0
2	0.10 - 0.25	1-30
3	0.26 - 0.50	31-75
4	0.51 – 1.0	76-750
5	1.1 – 2.5	151-300
6	0.26 -0.50	301 -750
7	0.51 – 10.0	751- 1500
8	10.1 -20.0	1501-3000
9	>20	> 3000

3.4 Identification of *P. pachyrhizi*

3.4.1 Microscopic identification of *P. pachyrhizi*

Twenty leaflets with symptoms were randomly collected from each farm and pressed in between paper towels to dry and then transported to the laboratory for rust identification. The leaf samples were observed under a dissecting microscope at X40 and the ones with sporulating lesions noted. The ones with non sporulating lesions were put in moist chamber and observed after 12 hours and after 24 hours for spores. Spores were then dislodge from the leaves surface and observed at X100 magnification by mounting in distilled water and in shear mounting media containing 50% potassium acetate (2% aq.), 20% glycerine and 30% ethyl alcohol (95%) (Hernández *et al.* 2002).

3.4.2 DNA extraction and polymerase chain reaction (PCR) identification of *Phakopsora pachyrhizi*

Diseased soybean leaves collected during the surveillance study were gently rubbed on a wax paper to dislodge the spore. The spores were then transferred into a 2ml vial and tightly capped (JIRCAS, 2016). DNA Extraction was done using the modified CTAB method (Villavicencio *et al.,* 2007). Approximately 5mg to 10mg uredinospores were crushed in 600µl of prewarmed (65°C) CTAB extraction buffer containing 2% CTAB, 0.1% Mercaptoethanol, 1.4M Nacl, 20mM EDTA and 100mM Tris HCl (pH 8). The Homogenate was then incubated for 15 minutes at 65°C in the same buffer then extracted twice with 400µl of chloroform Isoamyl alcohol (24:1). Freeze isopropanol was added up to 0.6 of the final precipitate volume and kept overnight at 4°C. After incubation the mixture was centrifuged at 12000 rpm for 10 minutes and the supernatant discarded. The pellets were washed twice in 70% ethanol. The DNA pellets were then air dried for 30 minutes then resuspended in 50 µl of nuclease free water. DNA was visualized and quantified by loading 5µl of total DNA solution in 1% Agarose gel stained with ethidium bromide and using 1 Kbp DNA ladder as DNA size markers.

PCR amplification of genomic DNA was performed using *P. pachyrhizi* specific primers Ppa1(5′-TAAGATCTTTGGGCAATGGT-3′/Ppa2(5′- GCAACACTCAAAATCCAACAAT-3′) and *P. meibomiae* primers Pme1 (5′-GAAGTTTTTGGGCAAATCAC-3′/Pme2 (5′-GCACTC AAAATCCAACATGC-3′ (Fredrick *et al.,* 2002). Each 25 µl reaction mixture contained 10 mM Tris-HCl; 50 mM KCl (pH 8.3); 1.5 mM MgCl2; 0.001% (wt/vol) gelatin; dATP, dGTP, dCTP, and dTTP, each at a concentration of 100 µM; each primer at a concentration of 1.0 µM and 0.5 units dreamTaq DNA polymerase (Thermo Scientific). The

negative control consisted of the same reaction mixtures but had no template DNA added. The PCR assays was be performed with the following cycling conditions: 94°C for 5 minutes (preincubation) followed by 35 cycles of denaturation at 94°C for 1 minute, primer annealing at 55°C for 1 minute and extension at 72°C for 1 minute, followed by a final extension of 72°C for 7 minutes. PCR products were analyzed by electrophoresis on 1% agarose gels in 0.5× Tris-borate-EDTA buffer stained with ethidium bromide. The gel was visualized under UV (ultraviolet) light using Molecular Imager Gel Doc (Bio-Rad., UK) and the image captured.

3.5 Multiplication of *Phakopsora pachyrhizi* uredinospores

To obtain soybean rust inoculum and maintain the spore cultures the spores collected from the four different locations were bulked and inoculated on susceptible soybean variety Namsoy 1 using detached-leaf method as described by Yamanaka *et al.,* (2010). Disease free mature leaves of the susceptible variety were detached from the plants grown in the screen house. The leaves were then washed with six changes of sterile distilled water and placed with the abaxial side up in Petri dishes containing sterile paper towel moistened with distilled water. Spore suspension of 10^5 ml^{-1} in 0.04% Tween 20 solution was sprayed on the leaves then incubated at 21 ºC for 12 hours in the dark and then incubated in a growth chamber at 21 ºC under a 12 hours light photoperiod. Distilled water was added to each petri dish as needed to keep the paper towel moist during the incubation period (9-14 days) to allow for sporulation. After incubation the spores were harvested and stored for subsequent experiments.

3.6 Screening of soybean varieties for rust resistance

3.6.1 Soybean seeds and growth conditions

Seeds of seven varieties of soybean commonly grown in Western Kenya were obtained from Kenya Agricultural and Livestock Research Organization (KALRO) and used in the study. The varieties were Nyala, Bossier, SB19, Hill, SB8, Gazelle and TGX1987-32F (Table 3.3). The seeds were pre-germinated in Petri dishes for 2 days then grown in 25 cm squared plastic planting pots. Six seeds per pot of each variety of soybean were planted in three different pots in the greenhouse. The pots were laid out in a completely randomized design and the experiments replicated 3 times. After establishment the seedlings were reduced to three plants per pot by thinning. Nitrogen, phosphorus and potassium (NPK) fertilizer was then applied at the rate of 4 g/pot during second trifoliate stage (V2) of growth.

Table 3.3: Description of soybean varieties screened for soybean rust resistance

Variety	**Origin**	**Seed helium colour**	**Testa colour**	**Days to physiological maturity**	**Yield Kgs/Ha**
Nyala	KALRO Njoro	Dark/brown	Cream	90-160	700-2500
Bossier	KALRO Njoro	Brown	Cream	90-115	1800- 2200
SB19	KALRO (improved)	Brown	Cream	120-140	950-1500
Hill	KALRO Njoro	Brown	Cream	125-155	950-1500
SB8	KARI (improved)	Brown	Cream	90-120	700-2500
Gazelle	KALRO Njoro	Cream	Cream	109-165	800-1600
TGx1987-32F	IITA	Brown	Cream	90-120	800-1500

3.6.2 Plant inoculations

The plants were innoculated at the V3 (third trifoliate) growth stage. Stored urediniospores *P. pachyrhizi* isolates were heat shocked at 40°C for 5 minutes then hydrated overnight by floating them in a small plastic weigh boat on sterile distilled water in a petri dish. Urediniospore viability was determined by spraying inoculum of each isolate onto the surface of sterile water agar in petri dishes and determining the percent germination after 24h of incubation at 20°C. Inoculum was prepared by suspending urediniospores in 0.1% Tween 20 solution then mixing vigorously and filtering through a 53-µm pore size screen. The final concentration of urediniospore was then adjusted to 5×10^5 urediniospores/ ml. The soybean plants were innoculated by applying the inoculum on the abaxial side of the leaves using a hand sprayer (Pham *et al.,* 2009). In order to maintain high relative humidity necessary for infection inoculated plants were covered with polythene bags for 24 hours and temperatures maintained at 22°C-24°C (Twizeyimana, *et al.,* 2007)

3.6.3 Disease detection and ratings

Soybean rust disease severity and resistant reactions were evaluated 14 days after inoculation. Disease severity was assessed on a scale of 1 to 5 based on percentage of leaf area affected, where; 1 = no visible lesions, 2 = 0.1 to 2.5% leaf area affected, 3 = 2.6 to 10% of leaf area affected, 4 = 10.1 to 30% of leaf area affected, and 5 = over 30% of leaf area affected (Miles *et al.,* 2011). Lesion colour, number of lesions per $1cm^2$ and number of spores per lesion was also recorded. Sporulation levels were determined by counting number of lesions with pustules and expressing as a percentage of the total number of lesions. Sporulations were then scored using a scale of 1-5 as described by Miles *et al.,* (2008) where: 1 = no sporulation; 2 =

Less than 25% of fully sporulating lesions; 3 = 26% to 50% of fully sporulating lesions; 4 = 51% to 75 % of fully sporulating lesions; 5 = fully sporulating tan coloured lesions. To obtain area under disease progress curve (AUDPC) the disease rating was done twice a day from day seven after inoculation up to day 21. Area under disease progress curve (AUDPC) values were calculated using the formula below as presented by Kumudini *et al*., (2008);

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

Where

X_i = the disease severity score at the ith observation;

t_i = the time (day) at the ith observation;

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

n = the number of assessments.

3.7 Determination of molecular basis for rust resistance

3.7.1 DNA Extraction

Ten seeds of each variety that showed resistant reaction in the green house screening were grown in pots, and leaf tissue from the 10 plants bulked and used for subsequent experiments. DNA extraction from the leaf samples was done using Qiagen DNeasy plant mini kit according to manufacturer's instructions. Approximately 100 mg of leaf tissue was ground using motor and pestle then transferred into a 2 ml micro tube, 400 µl of AP1 buffer and 4 µl of RNase was then added and vortexed vigorously. The tube was then incubated at 65°C for

10 minutes and mixed by inverting the tube 3 times during incubation to lyse the tissues. After incubation 130 µl of buffer AP2 was added to the lysate mixed and incubated on ice for 5 minutes then centrifuged at 14000 rpm for 5 minutes. The lysate was then pipetted into a mini spin column in a 2 ml collection tube and centrifuged at 14000 rpm for 2 minutes. The flow through fraction was then transferred into a new tube without disturbing the cell debris pellets and 1.5 volumes of AP3 buffer added to it and mixed with a pipette. 650 µl of the mixture was then transferred into DNeasy mini spin column placed in a 2 ml collection tube and centrifuged for 1 minute at 8000 rpm. The flow through was discarded and 500µl AW buffer added to the DNeasy mini spin column and centrifuged at 8000rpm for 1 minute. After centrifugation 500 µl of AW buffer was added again then centrifuge 14,000 rpm for 2 minutes. The mini spin column was then transferred into a 2 ml microcentrifuge tube, 100 µl AE buffer added onto the membrane and incubated at room temperature (15–25°C) for 5 minutes and then centrifuge for 1 minute at 8000 rpm to elute the DNA. The DNA was then visualized on 1% agarose gel in 0.5× Tris-borate-EDTA buffer stained with ethidium bromide.

3.7.2 Simple Sequence Repeat (SSR) analysis

A total of 12 SSR markers previously mapped on linkage groups of soybean were chosen (Song *et al.,* 2004). The primer sequence for each of the SSR markers used for the study was retrieved from SoyBase and Toolbox (2015) (Table 3.4). The PCR analysis was performed in PTC-100 Peltier Thermal cycler, each 25 µl reaction contained 12.5 µl PCR master mix (Thermo Scientific), 2.5 µl of each specific SSR primer, 5.5 nuclease free water and 2 µl of sample DNA. The cycling conditions were; 95°C for 4 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, primer annealing of 50°C -60 °C for 30 seconds

(temperature varied depending on the annealing temperature of each primer pair) (Table 3.4) and extension at 72°C for 30 seconds, followed by a final extension of 72°C for 7 minutes. The PCR product were visualized in 0.8% Agarose stained with ethidium bromide. Where the bands were not clear the samples were visualised in 6% denaturing polyacrylamide gel.

Table 3.4: SSR markers primer Sequence (SoyBase and Toolbox 2015)

SSR Locus	Gene	Primer sequence 5' – 3'	motif	Annealing Temp. (°C)
Satt612	*Rpp4*	Primer 1. GTCATACTGGGTGTTTCATTTATGAC Primer 2. GCGCCTTTTAGTCTCTGAAAGTATTT	(TTA)10	56
AF162283	*Rpp4*	Primer 1 GCGAGTTCTGGATGTAGG Primer 2 GCGTGGCGGCTTTGGTAG	(AG)11	54
Satt191	*Rpp4*	Primer 1. CGCGATCATGTCTCTG Primer 2 GGGAGTTGGTGTTTTCTTGTG	(TAT)19	51
Satt288	*Rpp4*	Primer 1. GCGGGGTGATTTAGTGTTTGACACCT Primer 2. GCGCTTATAATTAAGAGCAAAAGAAG	(TAA)17	60
Sat_280	*Rpp3*	Primer 1 GGCGGTGGATATGAAACTTCAATAACTACAA Primer 2 GGCGGGCTTCAAATAATTACTATAAAACTACGG	(TA)30	59
Sat_275	*Rpp3*	Primer 1 GCGCGCTGGCAATTATTCAAAACTTAACGAT Primer 2 GCGAAGGCTACGGTGAATAGAAAGGAC	(AT)24	60
Sct_001	*Rpp2*	Primer 1 TTAAGTTTCCCTCTCTCTCT Primer 2 CTTGTTCCTTCGCTCAC	(CT)14	50
Sat_366	*Rpp2*	Primer 1 GCGGCACAAGAACAGAGGAAACTATT Primer 2 GCGGACATGGTACATCTATATTACGAGTATT	(TA)8gtatcaataaaat(TTA)4	59
Satt361	*Rpp2*	Primer 1 GCGCGGTCAATGAATCGGGAGACAC Primer 2 GCGGTTTTCAGCGTTATTAAGTTTTG	(ATT)10 (TTA)7	56
Satt215	*Rpp2*	Primer 1 GCGCCTTCTTCTGCTAAATCA Primer 2 CCCATTCAATTGAGATCCAAAATTAC	(TTA)11	54
Sat_064	*Rpp1*	Primer 1 TAGCTTTATAATGAGTGTGATAGAT Primer 2 GTATGCAAGGGATTAATTAAG	(TA)32	50
Sct_187	*Rpp1*	Primer1 CATGCTCCCATTCTCT Primer2 AACATTGGCTTTTTACTTAG	(TC)10	55

3.8 Data analysis

Data from the questionnaires were extracted by number coding the responses and the information stored in Microsoft excel spreadsheets. The data was the analysed using descriptive statistics frequencies, means, percentages and standard deviations were determined. The data was analysed using SPSS (Statistical Analysis Package for Social Sciences) Version 23 (2016). Data on disease severity and incidence were subjected to analysis of variance (ANOVA) using statistical analysis program Excel Stat (2015). The mean PDI and disease incidence were compared at P = 0.05. To evaluate resistance of soybean varieties to *P. pachyrhizi* the means and standard error of the disease severity, lesion number and sporulation level were calculated and analyzed using ANOVA (Excel stat 2015).

The data on SSR analysis was scored by visually assessing the banding pattern for each SSR primer. Clear bands appearing without any ambiguity were given a score of 1 (present) and 0 (absent) to create a binary matrix which was analyzed using the computer programme ExcelStat 2015. Jaccards coefficient was calculated and a dendrogram constructed using Agglomerative hierarchical clustering (AHC).

CHAPTER FOUR

4 RESULTS

4.1 Identification of varieties of soybean preferred by farmers and constrains facing soybean production in Western Kenya

4.1.1 Demographic characteristics of the households

A total of 120 respondents were interviewed in all the four sub-counties. The results showed that there were 54% male and 46% female farmers. Most of the respondents were head of households (90%) while 10% were either children, relatives or spouses of the head of the household. Most of the respondents were married (90%), those widowed consisted of 7% while 3% were either single or divorced (Figure 4.1). The ages of the farmers ranged from 26 years to above 55 years, 40% of the household heads were between 36 to 45 years (Figure 4.2). In terms of education 62% of the respondents had secondary education, 6% postsecondary education 30% had primary level education while the 2% had no formal education. The heads of most households (90%) had no formal employment and therefore depend on farming as the sole source of the family income.

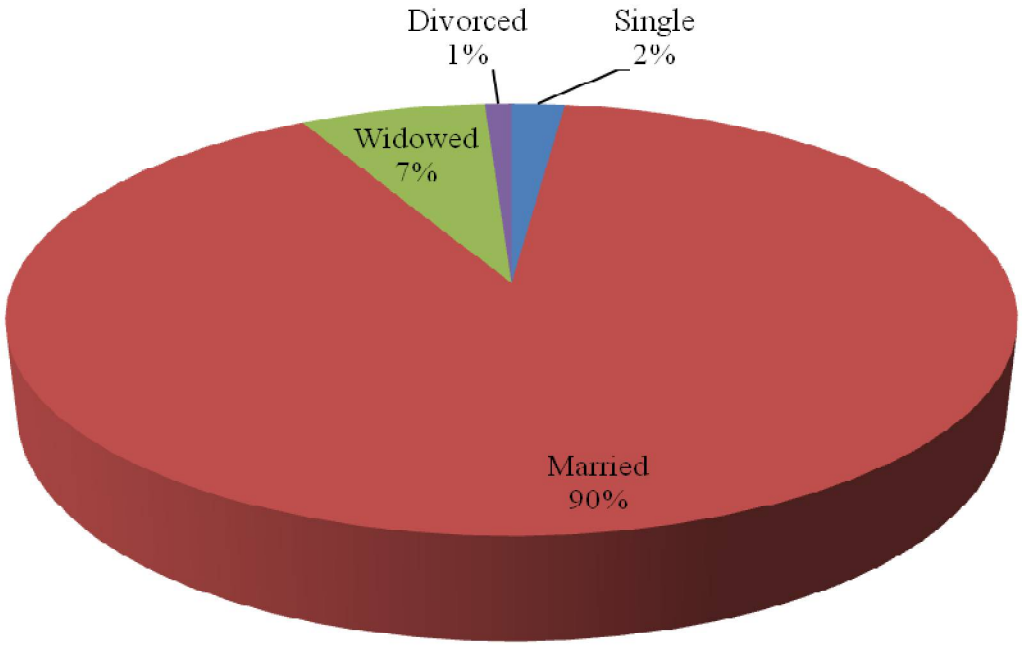

Figure 4.1: Marital status of soybean farmers in Western Kenya

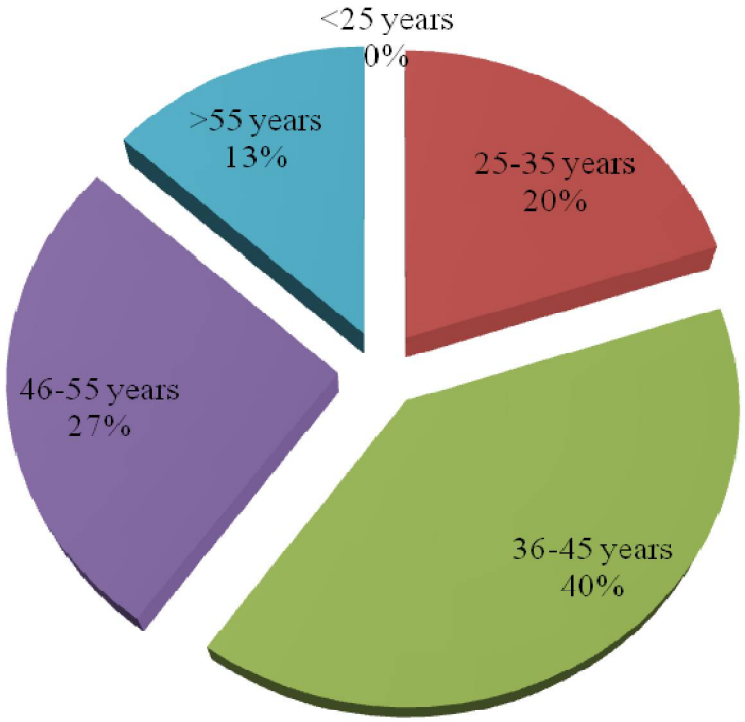

Figure 4.2: Ages of soybean farmers in Western Kenya

4.1.2 Land utilization and soybean production

The average size of land owned by each household is 4.27 acres out of which only 85% is available for cultivation. The land available for cultivation for the households is allocated to production of various crops with maize and sugarcane being the most cultivated 32% and 27% respectively while soybean is at 19% (Figure 4.3). The average land allocated to soybean production per sub counties was low compared to the total size of the land available. In Butere, Khwisero and Mumias sub-counties the average land allocated for soybean production was less than 2 acres per household. In Teso the average land ownership per household was greater compared to the other sub counties and the average land allocated to soybean production was 2 acres (27%) (Figure 4.4).

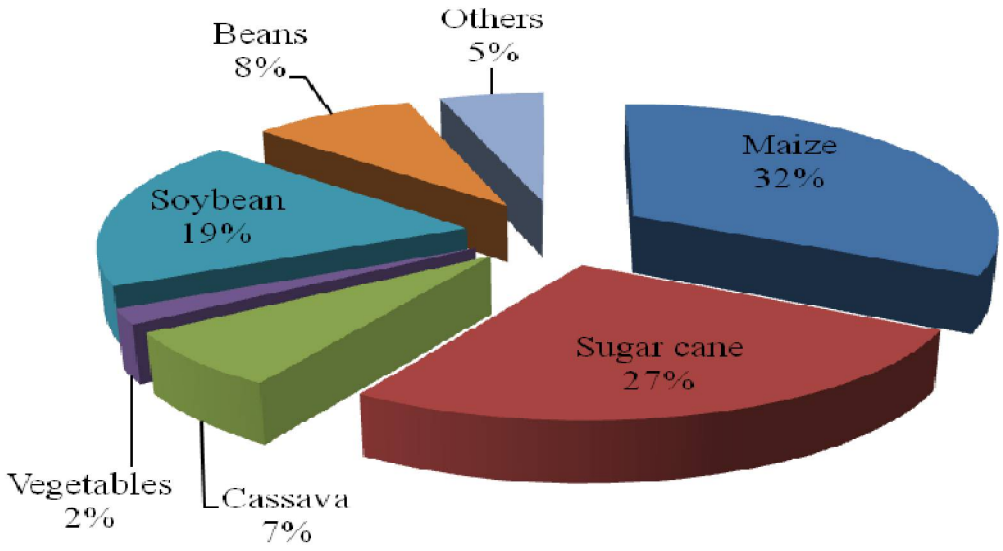

Figure 4.3: Proportion of land under cultivation of different crops by farmers in Western Kenya

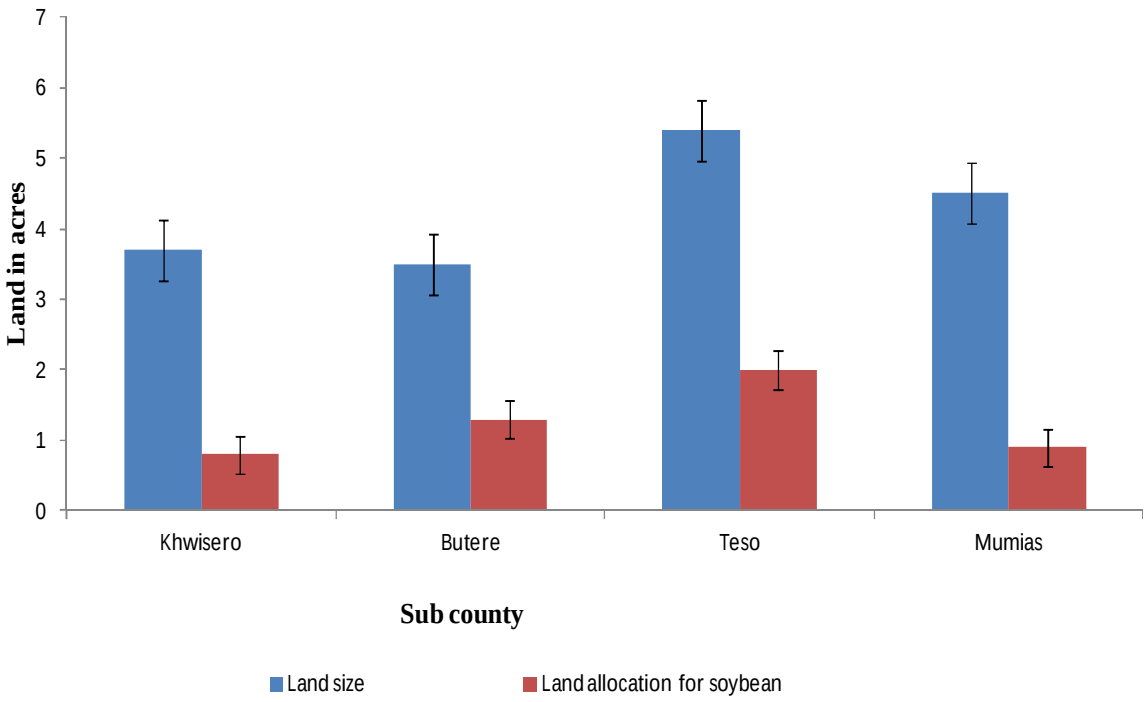

Figure 4.4: Land ownership per household in the four sub-counties of Western Kenya and proportion of land allocated for soybean production (Error bars represent standard errors of the mean)

The farmers interviewed had various reasons for growing soybean (Figure 4.5). The main reason for growing soybean was for income purposes in 40% of the households while 23% consumed soybeans as food or used it to make beverages. Some farmers (17%) used soybeans to process products such as; soy yoghurt, soymilk, soy chunks, soy flour and soy biscuits, the leftovers during processing are dried and used to fortify poultry feeds. Soybean is also grown for use as livestock feed soybean meals are added to the feeds and the stems and leaves are used as fodder. A few farmers (9%) grew soybean to increase soil fertility while 2% used the soybean leaves as source of green vegetable for family consumption. There were no farmers who processed soybean into cooking oils or biofuels.

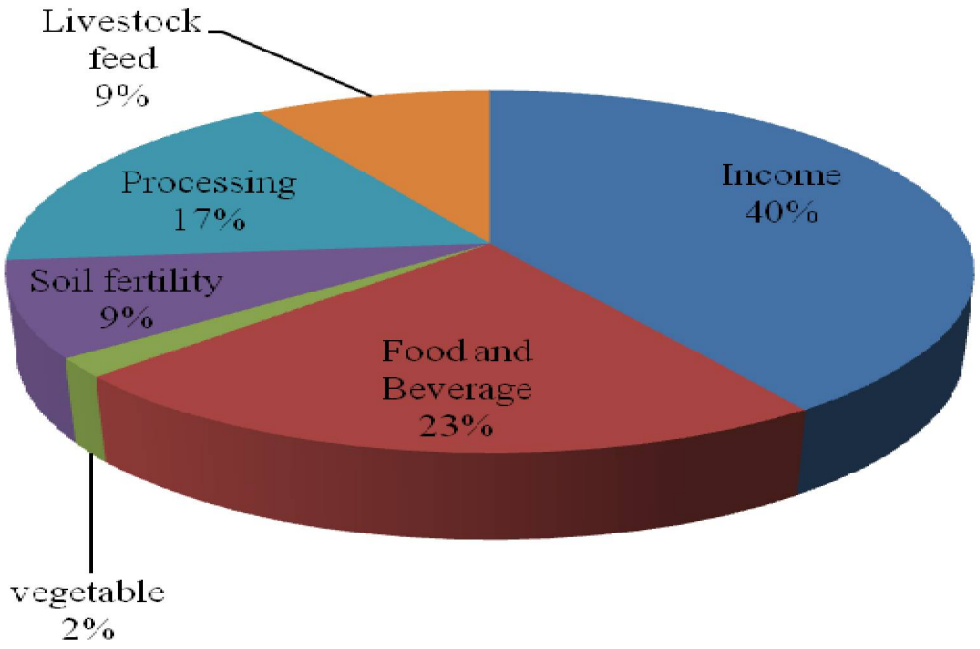

Figure 4.5: Utilization of soybean by the farmers in Western Kenya

4.1.3 Cropping System

Soybean is either grown as a mono crop or intercropped with other crops. Only 40% farmers grew soybean as a mono crop. In farms where intercropping was practiced 80% of soybean was intercropped with maize, the others crops intercropped with soybean included sorghum, sugarcane, beans, cassava and cowpea. Farmers gave varied reasons for intercropping, 54% cited food security as the main reason, while 27% intercropped because of the limited land size. Other reasons for intercropping were to increase soil fertility, to control pest and disease and to increase income by gaining from both crops (Figure 4.6). Soybean was planted in rows with varied spacing depending on whether it was a sole crop or intercropped however the farmers did not have any standard spacing. All the farms were weeded twice and only 20% of the farmers used organic fertilizers while 60% used farm yard manure and the others did not apply any form of fertilizer.

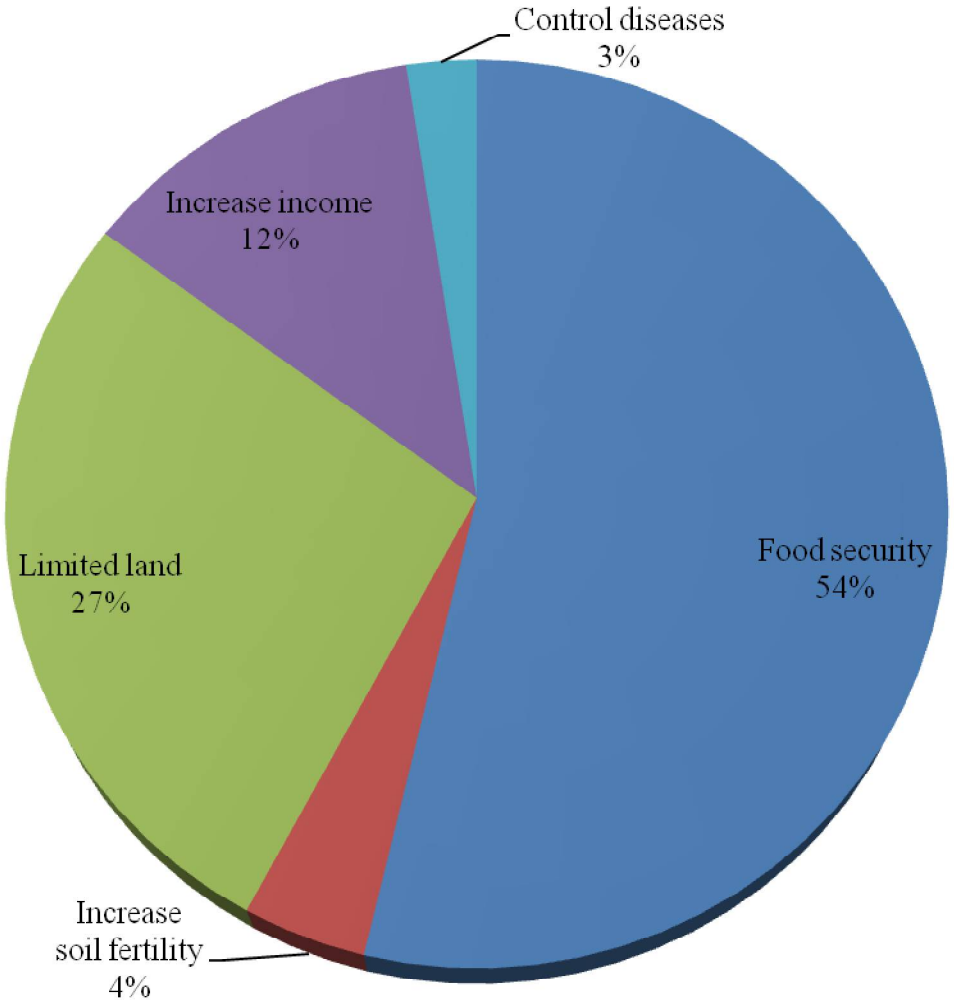

Figure 4.6: Farmers' reasons for intercropping soybean with other crops in Western Kenya

4.1.4 Sources of soybean seeds used by farmers

The majority of farmers interviewed (32%) obtained soybean seeds from the farmers' cooperative societies. The second major source of seeds was the open air markets (29%). Research institutions such as; CIAT-TSBF (Kenya) and Kenya Agriculture and Livestock Research Organization (KALRO) supplied seeds to 21% of the farmers. Only 2% of the

farmers obtained seeds from agrovet shops and no farmers obtained their seeds from the seed companies (Figure 4.7)

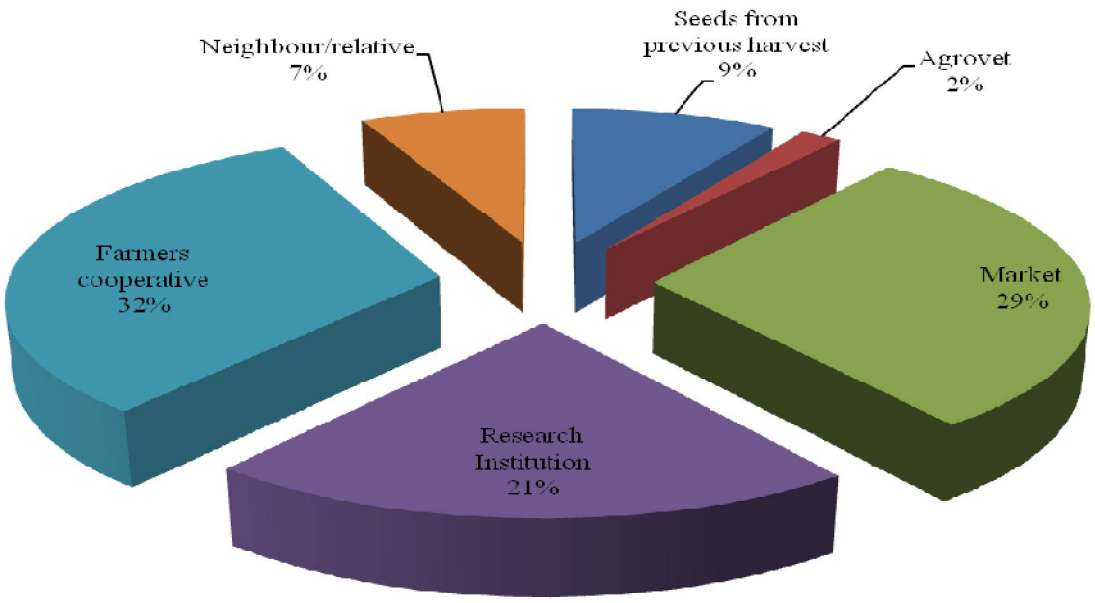

Figure 4.7: Sources of soybean seeds used by farmers in Western Kenya

4.1.5 Soybean varieties grown by farmers and the farmers' preferred varieties

The farmers were interviewed on their knowledge of soybean varieties; theirs preferred varieties, the desired attributes of the preferred varieties and the sources of seeds that they use. When asked about the varieties of soybean they grow, there were varied responses as recorded in Table 4.1. A total of 16 varieties were named with 8 being local varieties and 8 being improved varieties. The most widely grown variety was Nyala followed by Gazelle in Khwisero, Butere and Mumias Sub counties. However in Teso Sub-county the improved varieties Namsoy 4M and Maksoy1N were more widely grown as compared to the local varieties.

Table 4.1: Soybean varieties grown by farmers in Western

		Sub-county			
Variety	**Local/improved**	**Khwisero**	**Butere**	**Teso**	**Mumias**
Hill	Local	6.0	3.0	2.8	3.1
Bossier	Local	3.4	6.7	3.6	5.2
Gazelle	Local	27.8	25.3	15.4	24.7
Maksoy 1N	Improved	2.7	5.6	28.6	4.5
Namsoy 4 M	Improved	6.2	5.3	24.7	4.2
Nyala	Local	38.6	40.3	16.0	36.7
SB 8	Improved	2.1	1.3	0.0	4.7
SB 19	Improved	1.6	1.4	1.0	2.7
SB 25	Improved	3.2	1.5	2.5	3.5
TGX 1987	Improved	0.0	1.8	1.0	2.5
EAI 3600	Local	1.0	1.4	0.0	1.6
Duicker	Local	2.1	1.2	2.1	1.4
SB 15	Improved	0.0	1.0	0.0	2.4
SB 20	Improved	1.6	2.0	1.0	0.0
Sable	Local	2.4	1.2	1.3	2.8
SCS 1	Local	1.3	1.0	0.0	1.0
Total		100.00	100.00	100.00	100.00

The varieties of soybean preferred by farmers were ranked from the most preferred to the least preferred. The most preferred variety was Nyala (33%) followed by Gazelle then Maksoy 1N, Namsoy 4M and lastly SB 19 (6%) (Figure 4.8). The preference for specific differed across

the sub-counties (Table 4.2). Nyala was the most preferred variety in Butere, Mumias and Khwisero Sub-counties while in Teso sub-county the most preferred varieties were the improved varieties Maksoy N1 and Namsoy 4M.

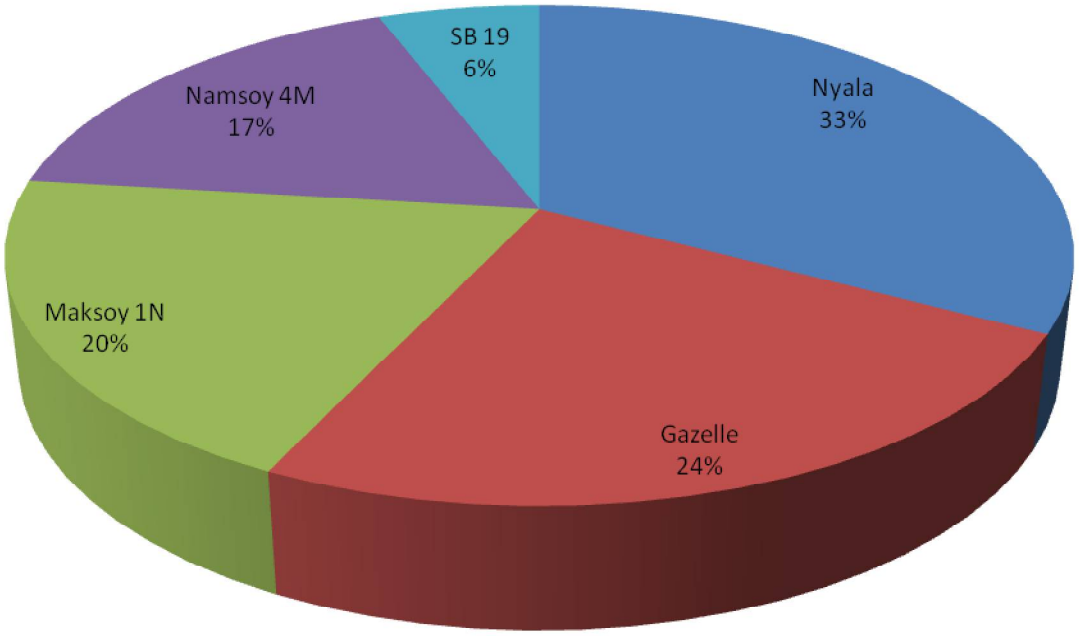

Figure 4.8: Farmers' preference for different soybean varieties in Western Kenya

Table 4.2: Farmers' preferred soybean variety in Western Kenya

	Sub-county			
Variety	**Butere**	**Mumias**	**Khwisero**	**Teso**
Nyala	43.8	32.7	35.8	19
Gazelle	23.7	29.8	27.6	15.8
Maksoy 1N	13.6	19.4	14.8	31.7
Namsoy 4M	13.7	11.4	13.5	30.3
SB 19	5.2	6.7	8.3	3.2
Total	100	100	100	100

The farmers' choice and preference for specific variety of soybean was based on the positive attributes of each variety. There were 16 positive attributes that the farmers mentioned and the attributes were ranked and a scale of 1-5, with 5 being the most desirable attribute by farmers. The mean ranking score of each of the traits in order of preference is as shown in Table 4.3. The farmers most preferred varieties were those with potential for high yield followed by pest and disease resistance, early maturity and drought tolerance. In some instances the preferences were based on availability of seeds and seed characteristics such as seed viability, seed color and size of the seed. The other positive attributes included improvement of soil fertility, high biomass production, the ease of cooking, low input, minimum pod shattering and a good adaptation to the environment.

Nyala was the most preferred variety because of early maturity, large grain size and the ease with which it can be intercropped. On the other hand Gazelle was recorded to be high

yielding, large grain size and attractive in color. The seeds for the local varieties were also noted to be easily available. However both Nyala and Gazelle were rated to be less resistance to pest and disease compared to the improved varieties. The improved varieties were scored highly in terms of resistance to pest and diseases, high biomass and the potential of improving soil fertility but there were rated poorly in terms of maturity, availability of seeds and yield potential.

Table 4.3: Rating of positive attributes of varieties soybean preferred by farmers from Western Kenya

	Soybean variety				
Attribute	Nyala	Gazelle	Maksoy 1N	Namsoy 4M	SB 19
High yield	4.3	4.8	3.4	3.4	3.2
Resistance to pest and disease	2.5	2.5	3.6	3.8	3.7
Drought tolerance	3.4	3.8	3.3	2.9	3
Early maturity	4.5	4.5	3.4	3.2	3.5
Large seed size	3.4	4.8	3.6	4.2	3.8
High biomass production	3.6	3.4	4.5	4.6	4.8
Less input required	3.1	3.5	3.4	3.4	3.6
Seed availability	4.8	4.7	3.0	3.0	3.0
Colour of seed	2.8	4.3	3.4	3.8	3.4
Environmental adaptation	3.6	3.8	3.2	3.3	2.8
Seed viability	2.8	2.8	3.5	3.5	3.5
Soil fertility	2.7	3.2	4.2	3.8	4
Minimum pod shattering	2.5	2.4	4.5	4.5	4.7
Ease of processing	2.4	2.3	2.6	2.8	3.1
Ease of cooking	2.4	2.8	2.8	.3.2	2.7
Overall rating	**3.25**	**3.57**	**3.49**	**3.59**	**3.52**

The farmer desired attributes of soybean were similar for the males and females in most case however when the farmers were asked to rank the varieties from the most preferred to less preferred the ranking varied with gender (Table 4.4). The male farmers preferred varieties that are early maturing, drought tolerant, resistant to diseases and pests and are high yielding. On the other hand female farmers had additional attributes such as large grain size, ease of cooking, attractive grain color and the easy of accessible of the seeds.

Table 4.4: Ranking of the Farmers' preferred varieties of soybean in western Kenya based on gender

MALE	**FEMALE**
Nyala	Gazzel
Gazzel	Nyala
Namsoy 4M	SB19
Maksoy 1N	Namsoy 4M
SB 19	Maksoy 1N

4.1.6 Soybean production and marketing constraints in Western Kenya

Soybean production and marketing in Western Kenya region faces several challenges (Table 4.5). The challenges vary in each sub-county but the most common challenge is pest and diseases (66.7%) in Khwisero sub-county 83.3% farmers mentioned pest and diseases as their greatest challenge. The next common challenge is the limited size of land (32.5%) it was established that the farmers had small pieces of land available for crop cultivation and the same land is used for production of soybean and other crops. Seed unavailability and lack of market were ranked third and fourth respectively. The low demand of soybean as compared

with demand for other legumes was also a challenge in all the sub counties. Even though low price was a challenge this was not considered to be so in Butere and Mumias. Changing weather patterns also affects soybean production, in the rainy seasons some areas get water logged and the crops performed poorly and in of the dry seasons the production was low. The cost of production which included labour during planting, weeding, harvesting, threshing and cost of inputs like fertilizers, pesticides, fungicides was considered high in all the sub counties. The other constraints mentioned by farmers included; lack of access to processing machines, lack of awareness on processing and utilization of soybean, low yielding soybean varieties, poor nodulation, pod shattering, weeds, rodents and vermin and postharvest losses.

Table 4.5: Soybean production and marketing constraints in Western Kenya region

	Sub-county				
Production and Marketing Constraints	**Khwisero**	**Teso**	**Butere**	**Mumias**	**Total**
Limited size of land	46.7	33.3	23.3	26.7	32.5
High cost of production	23.3	26.7	13.3	16.7	20.0
Seed unavailability	30.0	20.0	30.0	26.7	26.7
Changing weather conditions	6.7	20.0	16.7	13.3	14.2
Pests and Diseases	83.3	70.0	60.0	53.3	66.7
Lack of market	23.3	20.0	26.7	30.0	25.0
Weeds	20.0	13.3	3.3	6.7	10.8
Rodents and vermin	3.3	6.7	3.3	3.3	4.2
Low demand of soybean compared to other legumes	26.7	23.3	20.0	10.0	20.0
Lack of access to processing machines	26.7	23.3	10.0	10.0	17.5
Low prices	33.3	36.7	0.0	0.0	17.5
Lack of awareness on processing and utilization of soybeans	23.3	26.7	6.7	10.0	16.7
Low yielding soybean varieties	23.3	16.7	10.0	13.3	15.8
Poor nodulation	16.7	13.3	6.7	6.7	10.8
High pod shattering	3.3	3.3	6.7	10.0	5.8
Storage and post harvest losses	6.7	3.3	3.3	3.3	4.2

*Figures shown in percentage
*Percentage totals more than 100% because some constraints were mentioned more than once

4.1.7 Common pests and diseases and management practices

The farmers listed the most common pest and diseases that they have encountered in their farms. Among the pest and diseases listed soybean rust was the most common (52%), others were soybean mosaic virus, bacterial pustule, aphids, bacterial blight, white flies downy mildew, and termites (Figure 4.9).

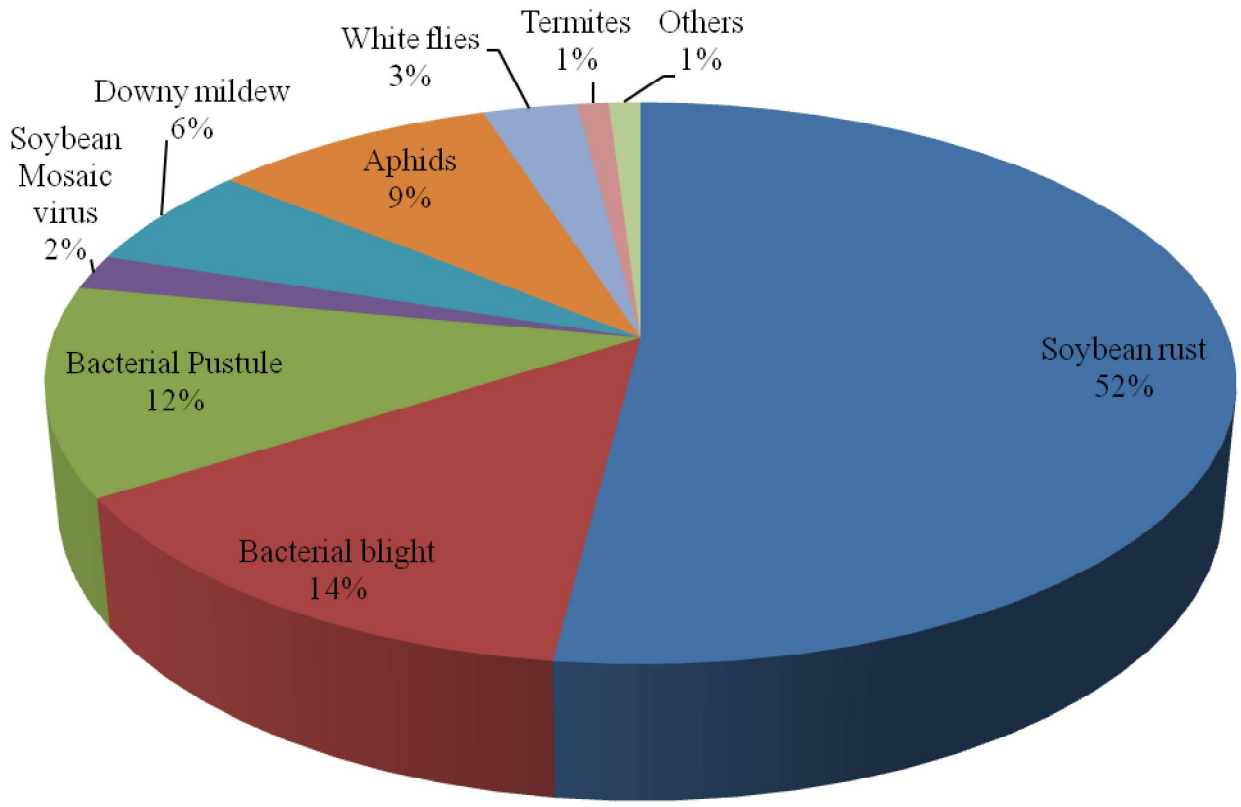

Figure 4.9: Common pests and diseases of soybean in Western Kenya

In terms management practices used to control pest and diseases most of the farmers (36%) did not use any method to control pests and diseases. About 24% of the farmers used pesticides, insecticides and fungicides to control various diseases. A few farmers applied the traditional methods of disease control like; uprooting of diseased plants, crop rotation, intercropping, weeding and planting of early maturating varieties (Figure 4.10).

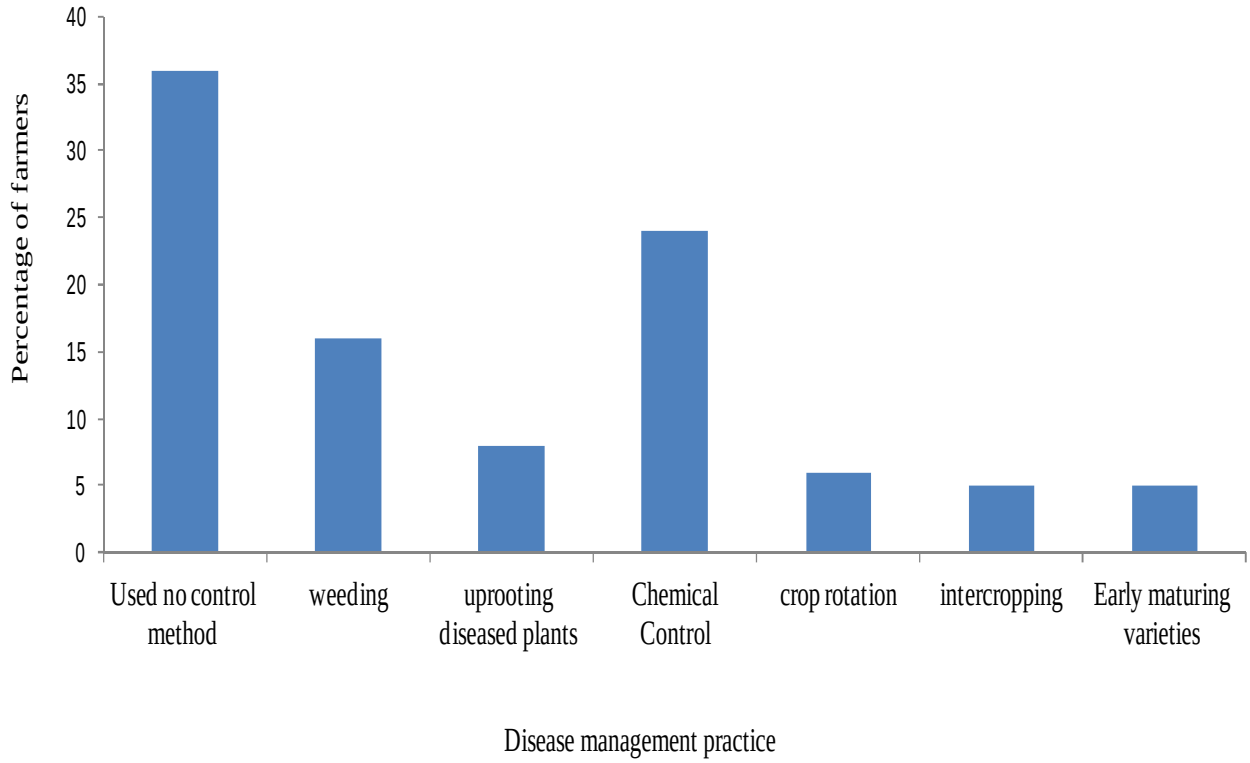

Figure 4.10: Soybean pest and diseases management practices used by farmers in Western Kenya

4.2 Soybean rust disease incidence and severity in Western Kenya

Field survey showed that all the farms selected for the study were infected with soybean rust. The symptoms rust observed included leaf yellowing, tan or reddish brown sporulating or none sporulating lesions on the underside of the leaves (Figure 4.11). Some plants had mixed reaction with both tan and reddish brown on the same leave of different plant of the same variety.

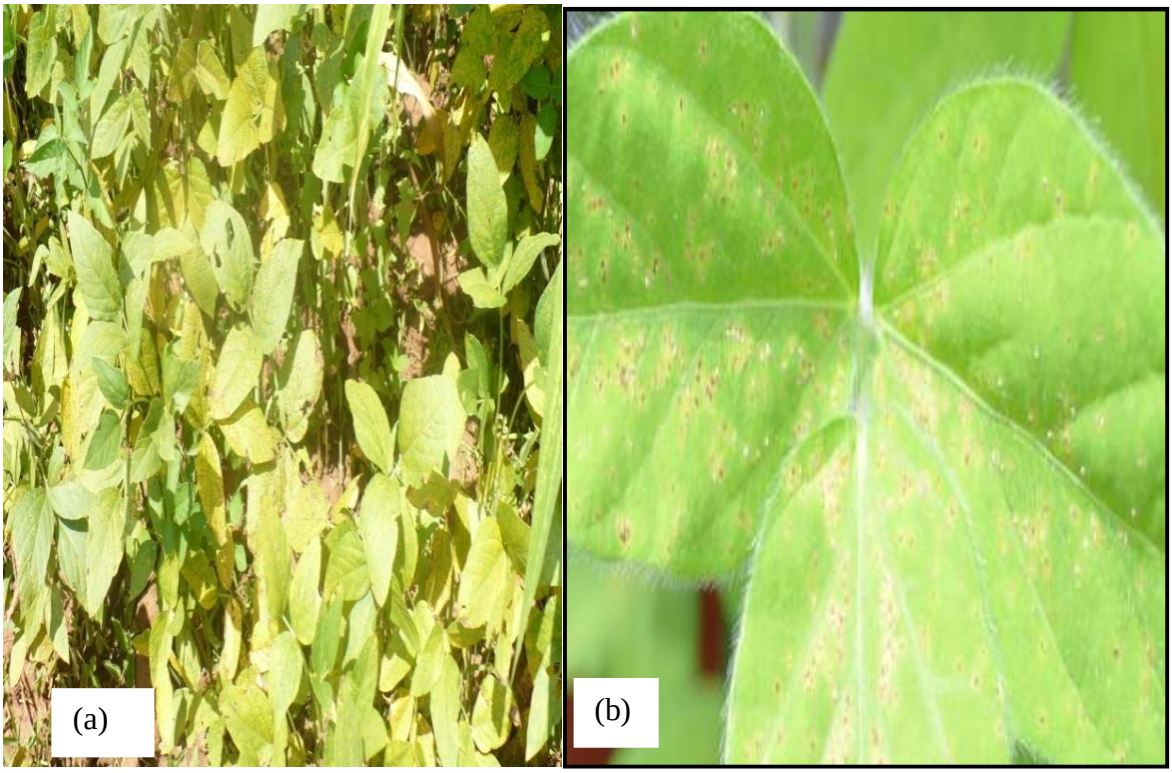

Figure 4.11: Symptoms of soybean rust disease as observed on soybean leaves in the farms surveyed (a) Infected soybean plants in the farm, (b) Infected soybean leaves with Tan lesions

The results of the survey showed that rust was prevalent in all the farms surveyed. The disease incidence per farm ranged 32% to 100% (Figure 4.12) the average incidence per sub-county was between 60% and 70.55% with Teso sub-county recording the highest incidence per farm (Figure 4.13).

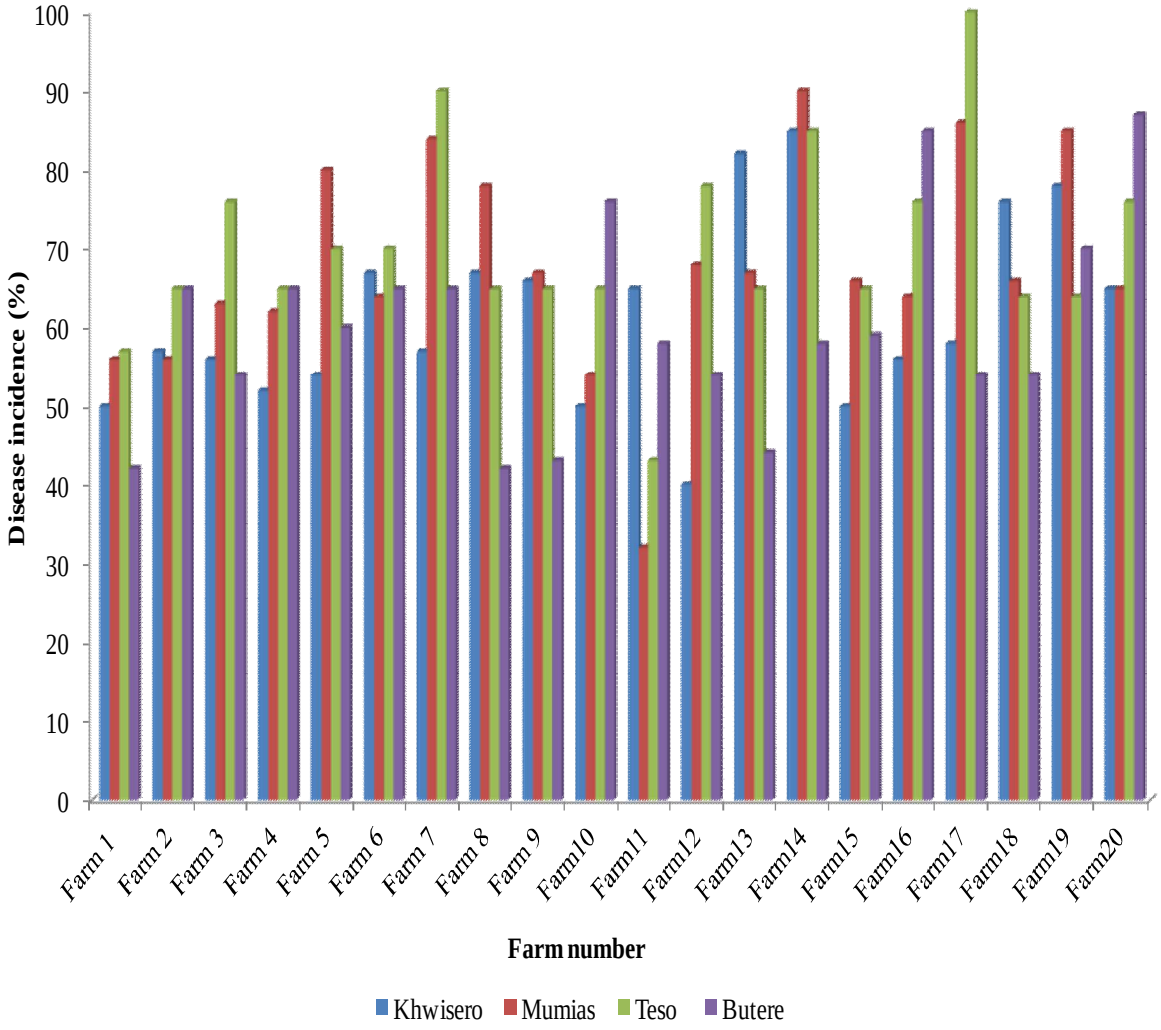

Figure 4.12: Soybean rust disease incidence in Western Kenya farms

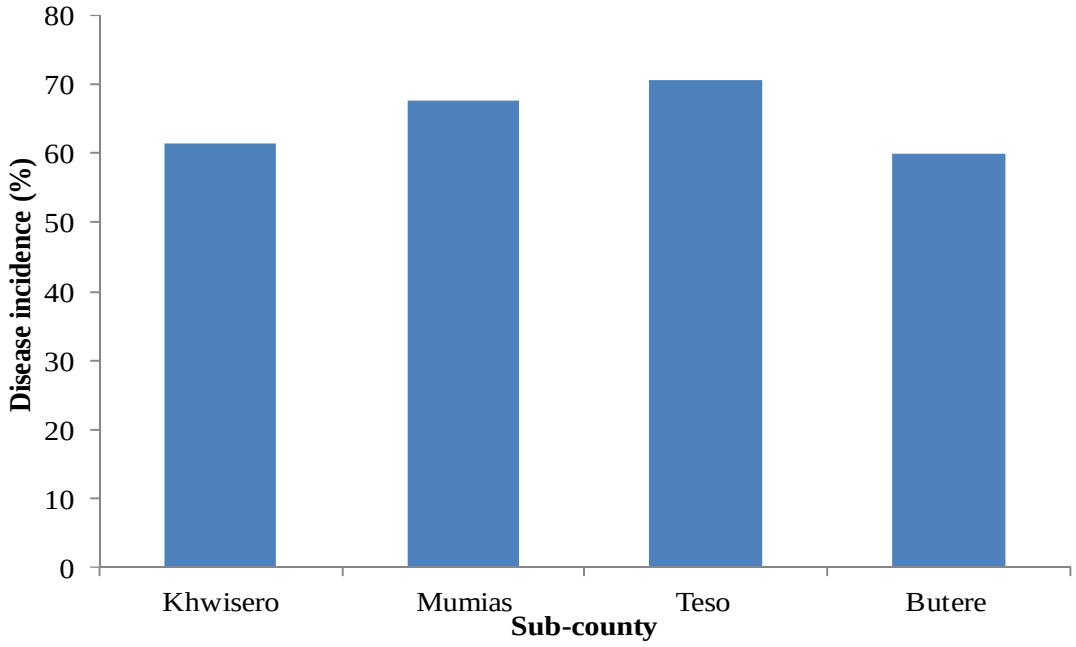

Figure 4.13: Soybean rust disease incidence in each sub-county of Western Kenya

The disease severity was high in all the sub-counties with the PDI for all the farms ranging from 40.37 to 74.81 (Table 4.6). The high severity was accompanied by heavy defoliation in physiologically immature soybean plants. The disease severity level within the four sub counties did not vary significantly (P = 0.088) (Table 4.7). Teso sub-county which is at the boarder of Kenya and Uganda had the highest disease PDI recorded (74.81) the mean PDI in Teso was 60.39. The sub-county with the least severity was Mumias sub-county with mean PDI of 55.05 (Figure 4.14). The sub-counties with the highest disease incidences also had highest disease severity (Figure 4.15).

Table 4.6: Severity of soybean rust caused *Phakopsora pachyrhizi* in Western Kenya

Farm	**Sub- County**			
	Khwisero	**Mumias**	**Teso**	**Butere**
Farm1	55.37	58.33	48.89	64.81
Farm2	64.81	56.48	53.33	57.78
Farm3	58.15	63.52	52.41	55.37
Farm4	58.52	57.81	46.49	58.27
Farm5	63.70	55.93	64.81	66.30
Farm6	69.26	57.41	47.96	40.74
Farm7	66.11	54.81	63.52	45.37
Farm8	54.63	51.30	53.70	52.96
Farm9	57.41	49.63	48.70	63.15
Farm10	53.89	60.00	50.37	66.48
Farm11	55.19	57.41	65.37	51.85
Farm12	70.18	57.05	58.57	41.43
Farm13	54.26	56.30	60.19	49.07
Farm14	52.41	57.04	64.91	62.59
Farm15	58.89	42.22	64.63	45.37
Farm16	58.52	50.37	82.96	56.85
Farm17	63.15	49.63	70.00	53.15
Farm18	52.04	48.52	68.15	52.78
Farm19	59.44	55.37	67.96	61.11
Farm20	40.37	61.85	74.81	59.07
Mean PDI	58.31	55.05	60.39	55.23

Table 4.7: ANOVA table comparing the disease severity in the four sub-counties

Groups	*Count*	*Sum*	*Average*	*Variance*
Khwisero	20	1166.287	58.31433	46.46425
Mumias	20	1100.965	55.04825	25.60025
Teso	20	1207.744	60.38718	99.92058
Butere	20	1104.522	55.22612	62.37528

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	398.3675	3	132.7892	2.26641	0.087542	2.724944
Within Groups	4452.847	76	58.59009			
Total	4851.215	79				

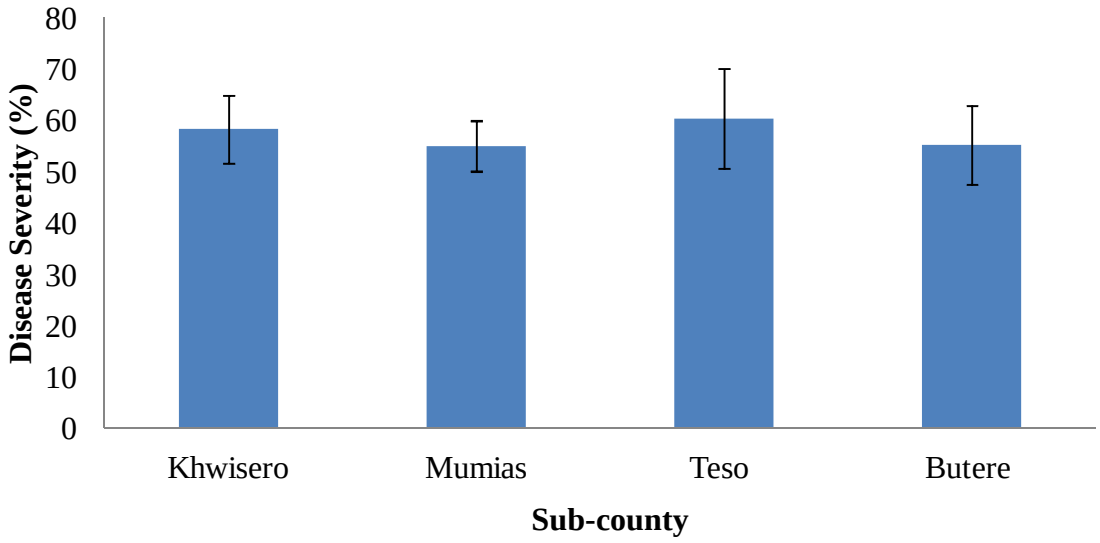

Figure 4.14: Soybean rust disease severity per sub-county of Western Kenya (Error bars shows the standard deviations of the average disease severity)

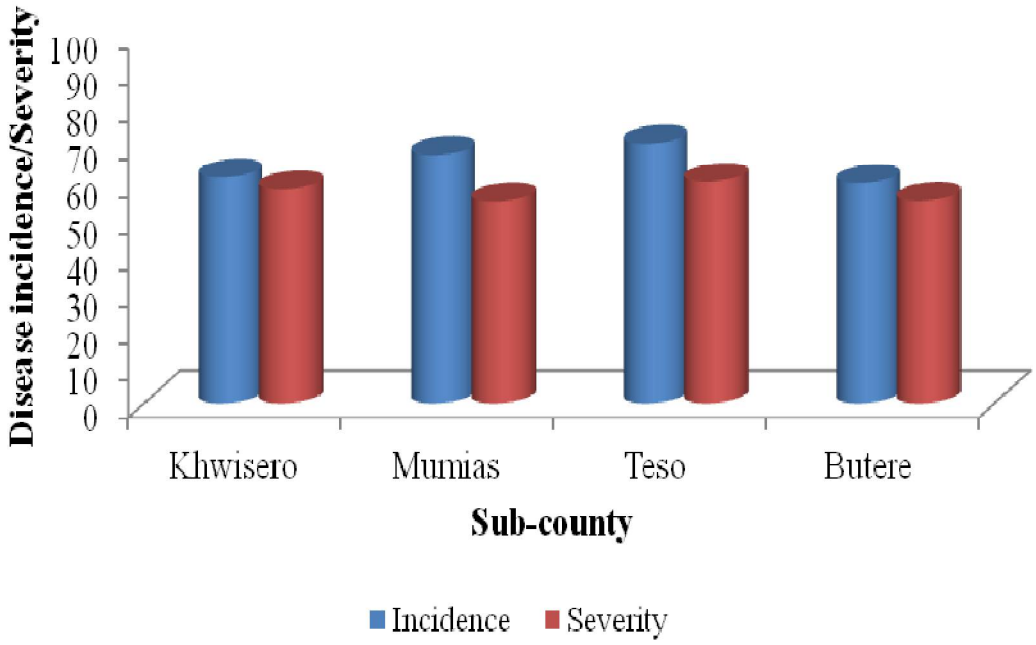

Figure 4.15: Comparison of disease incidence and severity in Western Kenya

The disease severity levels varied significantly dependng on the variety of soybean grown with farms having Gazelle showing high disease severity while farms where SB 8 was grown showed low level of disease severity (Figure 4.16) .

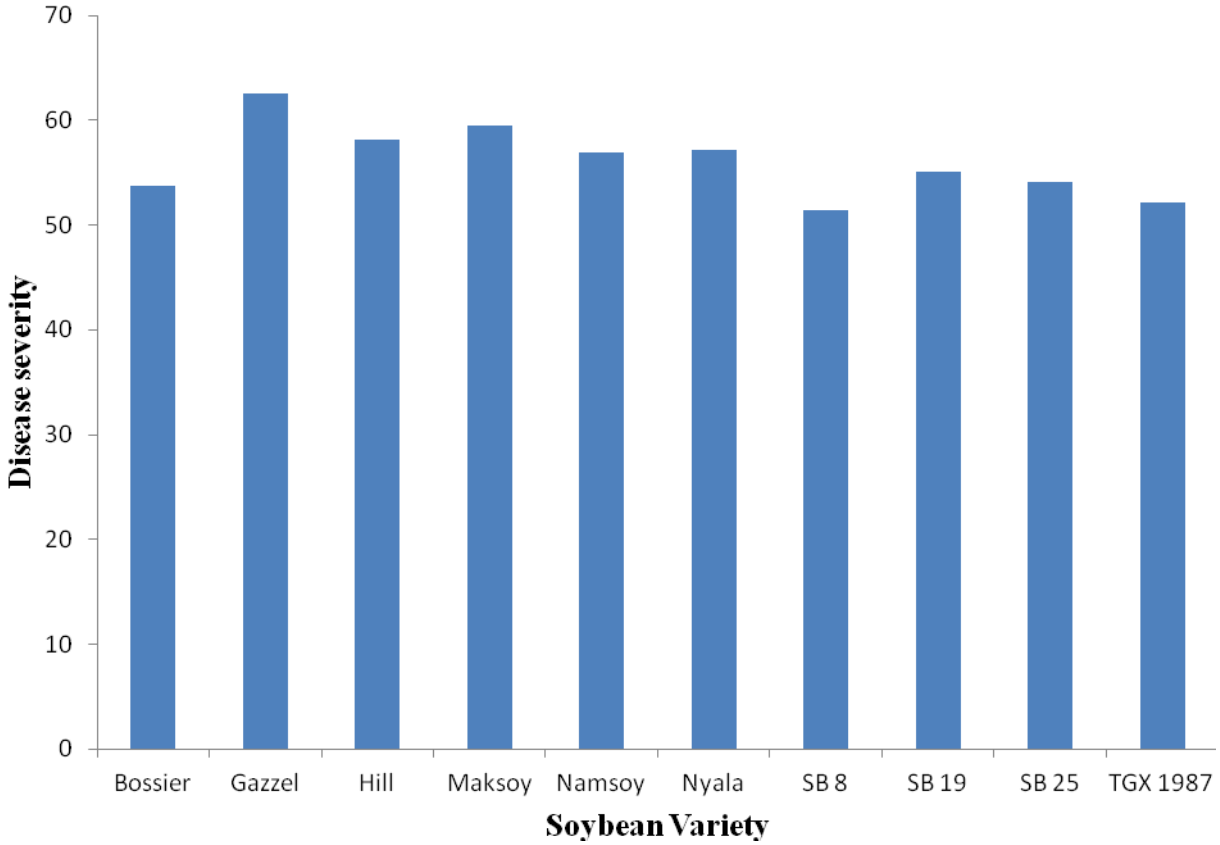

Figure 4.16: Soybean rust disease severity of different varieties of soybean grown by farmers in Western Kenya

4.3 Microscopic identification of *Phakopsora pachyrhizi* urediniospores

To confirm the causal agent of soybean rust in Western Kenya, microscopic observation of the leaves and dislodge spores was done. The leaves showed pustules with spores (Figure 4.17) the structures observed on the mounted spores were elliptical, hyaline colored, echinulate urediniospores approximately 14µm by 20µm in size (Figure 4.18) which are typical features observed in *P. pachyrhizi.*

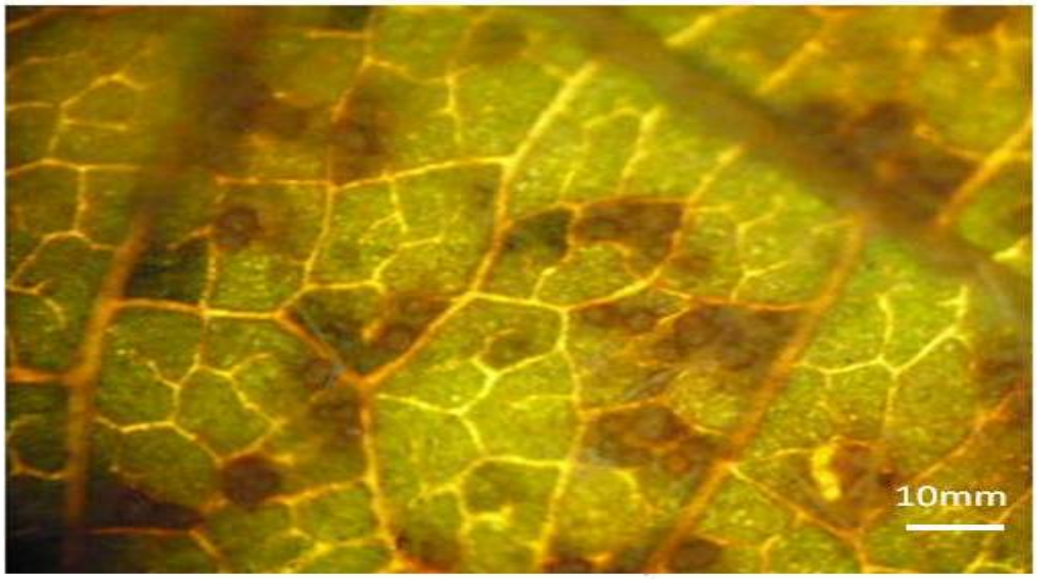

Figure 4.17: Soybean rust disease sporulating lesions on the lower side of the leaves, observed at X 40 magnification

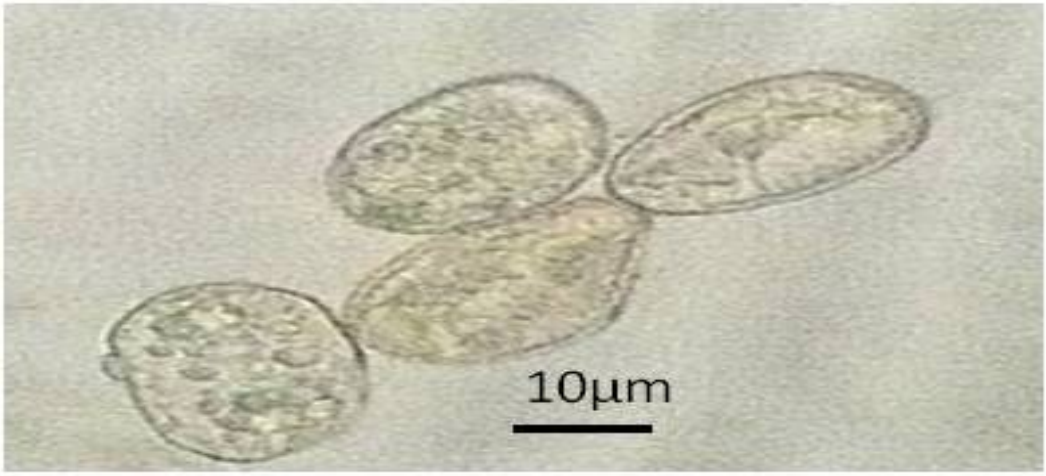

Figure 4.18: Urediniospores of *Phakopsora .pachyrhizi* observed under a microscope at X100 magnification

4.4 Identification of P. pachyrhizi using PCR

From the genomic DNA extracted from uredinospores of *P. pachyrhizi,* clear bands indicated the integrity of DNA being free from nuclease contamination (Figure 4.19). The extracted

DNA was used for PCR analysis using *P. Pachyrhizi* specific primers. Agarose gel analysis of the PCR product showed band of 147Kb (Figure 4.20) in all the 15 samples tested and there was no band in the negative control. There were no bands detected with *P. meibomiae* primers.

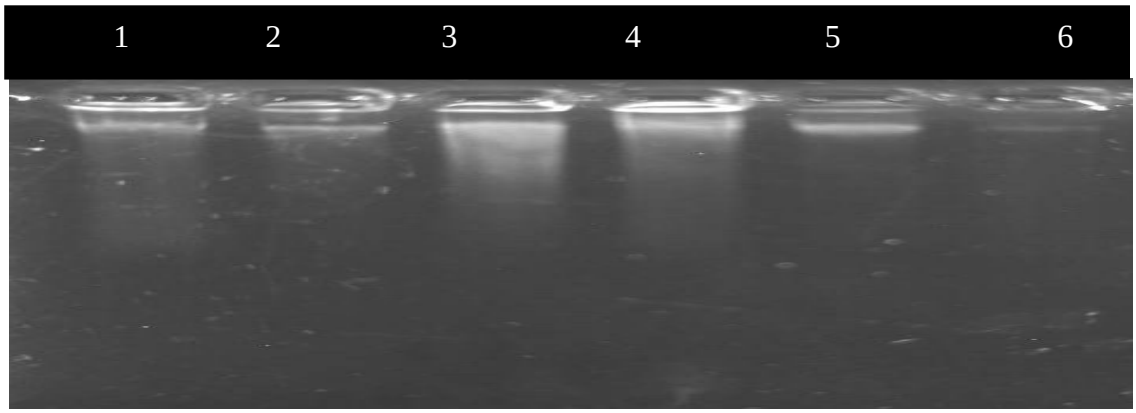

Figure 4.19: Soybean rust fungi genomic DNA, 1-6 DNA samples collected from different fields

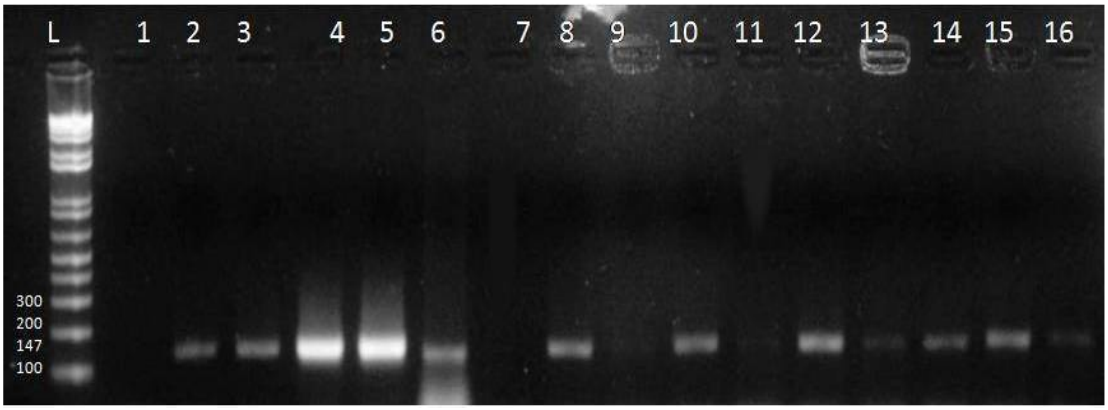

Figure 4.20: PCR results for the samples collected from Western Kenya (PCR performed using *Phakopsora .Pachyrhizi* specific primers Ppa_1/Ppa_2 (L- 1kb DNA ladder (thermo scientific), 1 -negative control 2-16 DNA samples collected from different farms)

4.5 Screening of selected varieties of soybean for resistance to soybean rust.

4.5.1 Disease Severity

Seven varieties of soybean were tested for resistance to soybean rust pathogen in the green house. The disease severity was recorded from day 7 after inoculation until day 21. The results showed there was a significant variation in the level of disease severity in all the seven varieties ($P<0.05$). The disease severity ranged from 2-5 with Nyala having the highest severity level and TGX1987-32F having the lowest (Figure 4.21). The severity levels increased significantly from day 7 to day 14 in all the varieties (Figure 4.22).

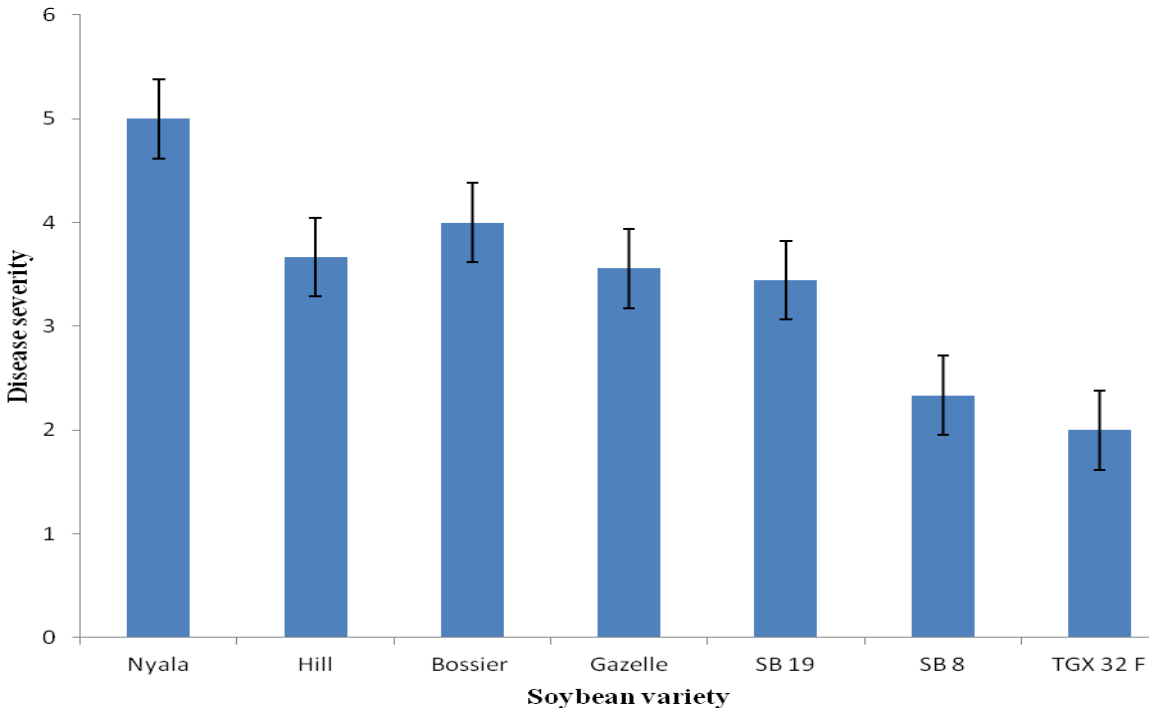

Figure 4.21: Soybean rust disease severity level in the soybean varieties screened in the green house for resistance to *Phakopsora pachyrhizi* (Error bars shows standard errors of the mean disease severity)

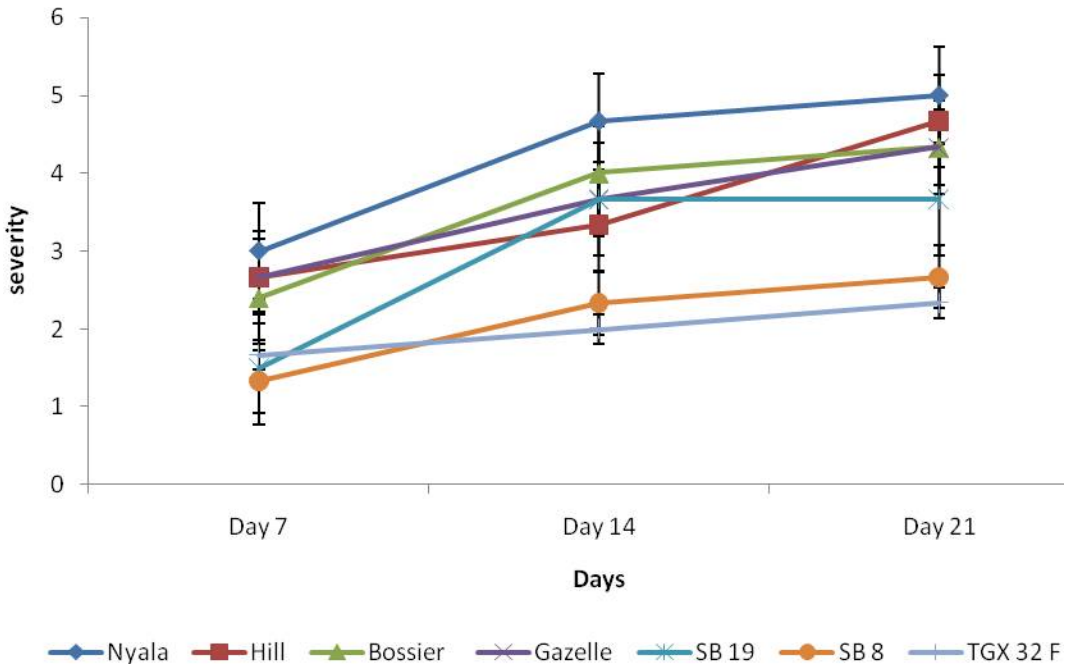

Figure 4.22: Average soybean rust disease severity levels recorded at 7 days intervals (Error bars represent standard errors of the mean disease severity)

4.5.2 Sporulation level and reaction types

Sporulation level, lesion type, lesion colour and disease progression was assessed. The soybean varieties showed varied reactions to soybean rust disease. The lesions colours were either red brown or tan, no variety showed immune or mixed reactions. Two varieties, SB8 and TGX1987-32F showed red brown lesions (Figure 4.23) which is a phenotypic characteristic of resistant variety. While Nyala, hill, bossier, SB19 and Gazelle showed Tan lesions (Figure 4.24) a characteristic of susceptible variety.

Figure 4.23: Soybean leaves with Red brown lesions (TGx1987-32F)

Figure 4.24: Soybean leaves with Tan sporulating lesion (Nyala)

The varieties with tan lesion had high level of sporulation compared to the varieties with red brown lesions. The sporulation level differed significantly between the varieties ($P< 0.01$) ranging from 1 to 3.75 (Table 4.8). The AUDPC values differed significantly among the varieties screened, Nyala which is more susceptible to rust pathogen had high AUDPC values, while TGx1987-32F which showed red brown lesions had the lowest AUDPC value. Soybean varieties with high rust severity level and high sporulation level had the highest AUDPC value (Table 4.8).

Table 4.8: Lesion color, AUDPC values and sporulation level of soybean varieties screened for resistance to soybean rust

Variety	Lesion color	Mean sporulation score	AUDPC
Nyala	Tan	3.25	71.19
Hill	Tan	2.75	58.29
Bossier	Tan	3.58	59.97
Gazelle	Tan	3.75	59.50
SB 19	Tan	2.60	49.00
SB 8	Red	1	34.98
TGx1987-32F	Red	1	33.83

4.6 Simple sequence repeat (SSR) analysis of selected soybean varieties for soybean rust resistance gene

The varieties that showed resistance reactions (SB8 and TGx 1987-32F) and the susceptible varieties Nyala and SB 19 were grown in the greenhouse and the leaves bulked and used for

SSR analysis. Primer Sat _064 associated with *Rpp1* rust resistance gene was amplified in all the four varieties and showed polymorphism only in TGx1987-32F. Three primers, Sct_001, Sat_366 and Satt361 associated with *Rpp2* gene were amplified in all the varieties while Satt215 also associated with *Rpp2* was only amplified in TGx1987-32F. For the primers associated with *Rpp3* gene Sat_280 and Sat_275 were amplified in all the varieties except for Nyala. Four primers Satt 288, Satt 612, Satt 191 and AF 1682283 associated with *Rpp4* gene were amplified in all the four varieties (Figure 4.25)

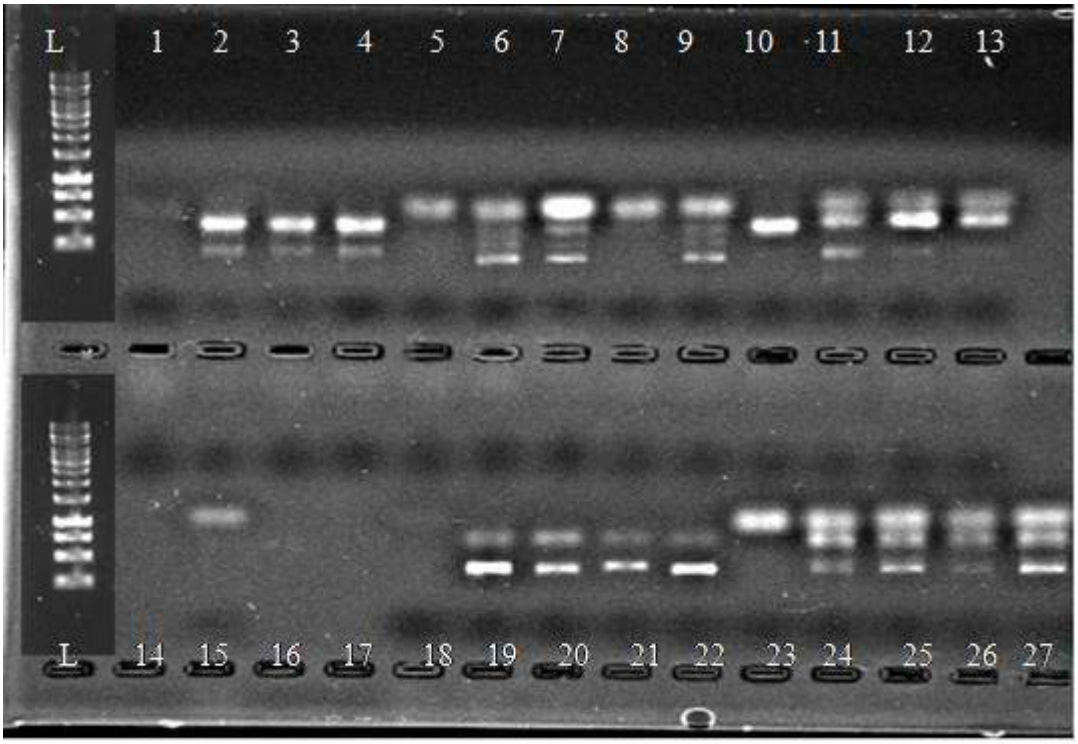

Figure 4.25: SSR based DNA profile of four varieties soybean tested for resistance to soybean rust

Lane ID	Variety	SSR	Lane ID	Variety	SSR
L	Ladder		14	SB 19	Satt 215
1	Nyala	Sat_275	15	TGx1987-32F	Satt 215
2	SB 8	Sat_ 275	16	SB 8	Satt 215
3	SB 19	Sat_275	17	Nyala	Satt 215
4	TGx1987-32F	Sat_ 275	18	SB 8	Satt 215
5	SB 8	Sat _064	19	SB 19	Satt 191
6	SB 19	Satt 288	20	SB 8	Satt 191
7	SB 8	Satt 288	21	Nyala	Satt 191
8	Nyala	Satt 288	22	TGx1987-32F	Satt 191
9	TGx1987-32F	Satt 288	23	SB19	Sat_064
10	SB 19	Satt 280	24	SB 19	Satt 612
11	SB 8	Satt 280	25	SB 8	Satt 612
12	Nyala	Satt 280	26	Nyala	Satt 612
13	TGx1987-32F	Satt 280	27	TGx1987-32F	Satt 612

Based on the SSR scores Jaccards coefficient was calculated to determine the relationship between the varieties. Dendrogram constructed using Agglomerative hierarchical clustering (AHC) (Figure 4.26). The similarity was also compared using Principal component analysis (PCA) and a biplot graph generated (Figure 4.27). The results showed that the varieties SB8 and SB19 are very similar and closely related to Nyala while TGx1987-32F did not cluster with any of the other varieties.

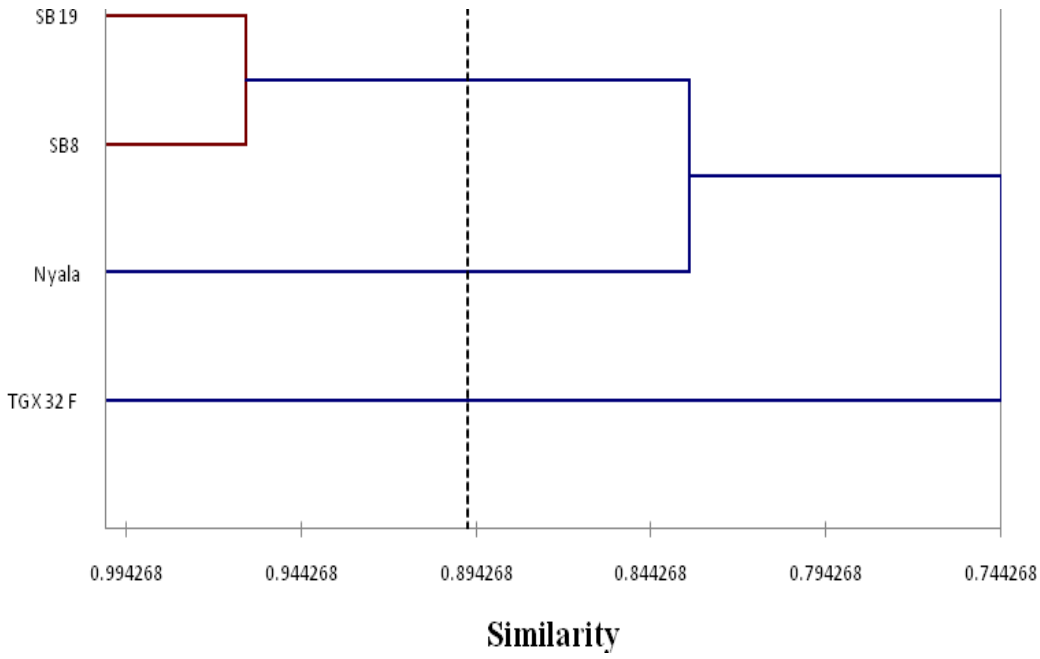

Figure 4.26: Dendrogram showing similarity coefficient of the varieties of soybean screened for soybean rust resistance

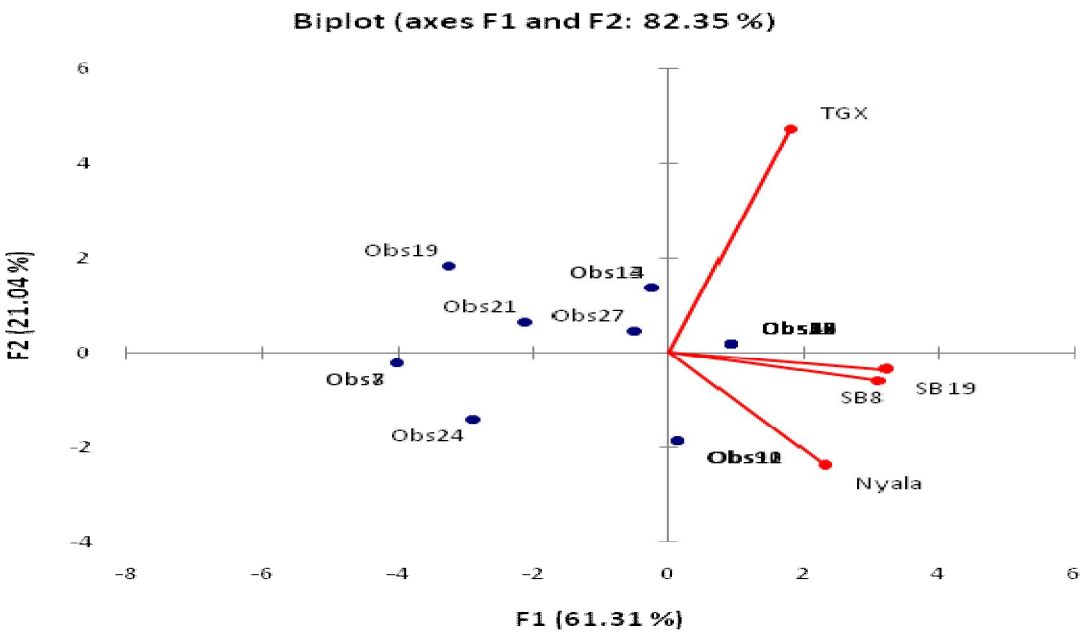

Figure 4.27: PCA analysis biplot showing similarity of the varieties based on SSR analysis

CHAPTER FIVE

5 DISCUSSION

5.1 Varieties of soybean preferred by farmers and constraints facing soybean production in Western Kenya

This study reveals that soybean farming in Western Kenya is continuously expanding and the farming has been embraced by both male and female farmers of different age groups, education and economic status. The existence of farmers' groups has made it easy to promote the crop and increase the number of farmers participating in soybean production within the region. In Western Kenya the crop is mainly grown as a source of income, for beverage production, animal feed, to improve soil fertility and for processing of various food products.

Soybean production is on a small scale basis in the Western Kenya region. This is due to the fact that the majority of farmers own small pieces of land. In addition the land allocated for soybean production is still small compared to the land available for production of other crops especially maize and sugarcane. Since the available land cannot be increased soybean production can only be enhanced if soybeans production is done as a substitution for crops already being grown (Tinsley, 2009). From this study 60% of the farmers used intercropping system to grow soybean. The major crop used for intercropping was maize (80%). These results are in agreement with the results reported by Chianu *et al.*, (2008), who reported that in Western Kenya region up to 70% of the soybean crops were either intercropped with maize or sugarcane. The local varieties can be easily intercropped this ensures maximum utilization of land and increase in food production. It also improves nutrition status, soil fertility and reduces pest and disease infestations (Yu *et al.*, 2009)

Consumption and utilization of soybean at household level is still low compared to other legume crops. It was established that majority of farmers grew soybean for commercial purposes. Lack of knowledge on utilization methods and the extra time required for cooking has been some of the reasons for low consumption. Soybean requires extra processing including the need for some cooking to deactivate the trypsin inhibitor enzyme. Soybeans will not soften when cooked like the other bean commonly grown and directly consumed in rural Kenya (Tinsley, 2009). The households who processed and used soybean regularly were roasting the beans then grinding to use as beverage, similar findings were reported by Chianu *et al.,* (2009). The limited immediate utilization of soybean at the household level compared to other legumes limits the expansion of its production area (Tinsley, 2009).

The farmers obtained seeds from varied sources, which includes farmers' cooperative societies, open air market, research institutions, agrovet shops and stored seeds or seeds from neighbours. The source of seed influences the farmers' choice of variety and quality of the seeds. The crop growth and final yield quality and quantity are always dependent on the variety and seed quality among other factors. Previous studies have revealed that the quality of seeds deteriorates every season because of the persistent use of stored seeds and seeds sourced from the markets (Gressel, 2006).

The farmers' in Western Kenya region ranked the local varieties (Nyala and Gazelle) as the most preferred over the commercial varieties. Despite the fact that commercial varieties of soybean are more tolerant to pests and diseases and have high biomass yields their cultivation and adoption is still low. The advantages of the local varieties were stated as having; large

grains, high yielding and early maturing. The early maturing varieties often escape off season dry spells, pod shattering and to some extent pest and disease infestation and give assurance that food insecurity is at least tackled before the season's harvest (Mahasi *et al.,* 2009, Idrisa *et al.,* 2010).

This study has identified a number of constraints affecting soybean production in Western Kenya region. The major constraint is pests and diseases and the diseases mentioned were soybean rust soybean mosaic virus, bacterial pustule, bacterial blight and downy mildew while the pests mentioned were white flies aphids, and termites. Similar findings were recorded by Wanderi (2012). Among the disease soybean rust was identified as the major challenge. It was noted that most of the farmers do not apply any disease management strategies a factor that may have lead to rapid spread of the rust fungi. There is great need for the government and stakeholders in soybean sector to market and promote soybean use since there is low demand for soybean as compared to other legumes was another major problem identified. The other constraints that needs to be addressed in order to improve production includes: unavailability of seeds, high cost of inputs, lack of access to processing machines

5.2 Soybean rust disease severity and incidences in Western Kenya region

The results of microscopic observation of the spores showed that the spores collected from the leaves of soybean with symptoms were urediospores of *P. pachyrhizi.* These are structures usually observed in the second stage of the reproductive cycle of the rust fungus. This shows that the farms are infected with *P. pachyrhizi* and not *P. meibomiae.* Similar structure and features were observed by Schneider *et al*.,(2005), Rodríguez *et al*.,(2006) and Murithi *et*

al.,(2014) who made the first reports of Asian soybean rust in the United States, Mexico and Tanzania respectively.

Molecular analysis is the most accurate method of diagnosis of *P. pachyrhizi* and to distinguish it from *P. meibomiae* and bacterial infections like brown spot and bacterial pustule which show similar symptoms (Hernández, 2004). Primers have been developed specifically for the two species of *Phakopsora* (Frederick *et al.*, 2000), this allows for quick and accurate identification of the species through PCR. The results of PCR analysis identified the isolates as *P.pachyrhizi* and confirmed that there were no incidences of *P. meibomiae.* This positively confirms the presence of soybean rust in the fields that were surveyed.

This study revealed that there is high soybean rust disease severity and incidence in the four sub-counties surveyed and there is no significant difference in the level of severity. This may be attributed to the close geographical location of the sub-counties which makes it possible for the spores to be transmitted easily across the sub-counties. Soybean rust pathogen is spread by wind therefore the rate of transmission is high (Schnepf, 2005).

The high disease severity in Western Kenya is also attributed to the farmers choice of varieties soybean most soybean farmers prefer Nyala and Gazelle varieties (Mahasi e*t al.*, 2009) despite the good attributes of the varieties they are more susceptible to rust disease. High severity is also linked to the ability of the fungal spores to live in the environment for long. The availability of alternative host like cowpea and other legumes plants grown alongside soybean also enable the spores to be dispersed further. Prevailing temperatures between 15°C

and 28°C and high moisture levels have also provided are ideal conditions for growth of the rust fungi. Disease incidence is favoured by a hot, humid environment which leads to reduction in leaf area available for photosynthesis and premature shedding of leaves (Hartman *et al.*, 1999). The high soybean rust disease severity in this region could have been due to the tropical climate experienced in the region with high rainfalls between April and June and moderate temperatures which favors the growth and spread of the rust fungi. Soybean is also grown in mixed cropping system in this region and this easily aids the dispersal of the rust fungi spores from alternative hosts.

In terms of control and management of soybean rust fungicide is the main control measure; this is mainly because of absence of rust resistant cultivars. Some farmers use foliar fungicides to manage the disease however this is not sustainable because of the high costs involved. The high cost of fungicides and lack of knowledge by farmers on the types of fungicides to use has also lead to increased severity of the disease. There is also little knowledge on the best timing for application of fungicides, therefore they may not offer effective control once the fungi has established itself on the crop. The farmers in Western Kenya region who cannot get access to fungicides use the traditional methods of diseases management like uprooting diseased plants, intercropping and planting of early maturing varieties.

5.3 Soybean rust resistance and molecular basis of resistance to soybean rust among local varieties of soybean

This study has revealed that the soybean varieties tested differed significantly in disease severity, lesion colour, sporulation levels and AUDPC. Lesion colour alone cannot be used to evaluate resistance (Yamanaka *et al.,* 2010), therefore this study combined several factors such as lesion colour sporulation level, disease severity and AUDPC to classify the varieties tested into resistant and susceptible lines. Reduction in size and number of urediniospores is also a desirable indicator of resistance when assessing single rust resistance genes (Bonde *et al*., 2006). Two varieties SB8 and TGx1987-32F were classified as resistant while Nyala, Hill, Bossier, Gazelle and SB19 were classified as susceptible. There were no immune reactions observed in any of the varieties. Nyala, Hill, Gazelle and Bossier showed high level of disease severity and sporulation as compared to the improved variety SB19 which showed similar susceptible reactions with less severity and sporulation level.

The variation in response to rust fungi can be due to the genetic diversity, physiological properties of the soybean varieties and the variation in virulence of different pathotypes of the rust fungi (Pham *et al.,* 2009, Twizeyimana *et al.,* 2009,). Variation can also be due the presence of different resistance genes among the soybean accessions which are known to react differently to *P. pachyrhizi* isolates (Garcia *et al.,* 2008). Farmers use yield and maturity as the most important criteria for selection of seeds. Nyala, Gazelle, Sable, SCS1 and Duicker that were introduced in Kenya from Zimbabwe in 1990s are highly susceptible to soybean rust (Mahasi *et al.,* 2009). Previous field and green house evaluations have identified Nyala to be susceptible with rust severity of 9 (1-9 scale) (Wanderi 2012). Despite the high level of

susceptibility to soybean rust Nyala and Gazelle are still recommended for growing in Kenya because of their high yield and short maturity period (Mahasi *et al.,* 2009, Njoroge *et al.,* 2015).

Previous research has shown that soybean genotypes that show the red brown reaction when inoculated with rust fungi have can be associated with single-gene resistance (Hartman *et al.*, 2005a). Identification such resistant genotype is a major factor that will ensure that useful sources of high resistance are selected for breeding programs (Sharma and Duveiller, 2007). The varieties SB8 and TGx1987-32F showed red brown lesions with low rust severity this type of genotypes with low rust severities may be sources of partial or rate reducing resistance to *P. pachyrhizi* (Miles *et al.*, 2006). Resistance mechanisms that have been identified against *P. pachyrhizi* are; specific resistance, partial resistance and tolerance (Hartman *et al.*, 2005a). Partial resistance characterized by reduced pustule number and increased length of latent period has not been widely used in breeding programs (Hartman *et al.,* 2005a). In breeding it is important to measure the latent period so as identify genotypes with a long latent period and hence a slower rate of rust development (Hartman *et al.,* 2005b).

Partial resistance is not widely used in breeding programs because of the complexity in its assessment. It is not be possible to compare plants or genotypes maturing at different times in the field because of the different environmental conditions that they are exposed to at similar growth stages (Jarvie, 2009). Partial resistance together with single gene resistance, or pyramids of several *Rpp* genes and partial resistance could be used in breeding soybean cultivars for resistance to *P. pachyrhizi* (Hartman *et al.,* 2005a, Pham *et al.,* 2009). Marker

assisted selection can be used to identify the resistances genes in SB8 and TGx1987-32F and this genes can then be integrated in other breeding lines.

SSR analysis for *Rpp1* gene in the test varieties using Sat 064 showed polymorphism in TGX1987-32F. *Rpp1* has been shown to map to soybean linkage group (LG) G between SSR markers BARC_Sct_187 and BARC_Sat_064 (Hyten *et al.,* 2*007*). The *Rpp1* gene is known to confer immune reaction when inoculated with few rust isolates however inoculation of some rust isolates on *Rpp1* or the other genes produces a resistant red-brown (RB) lesion with no or sparsely sporulating lesions (Hartman *et al.,* 2005a). The *Rpp2* gene for soybean rust resistance was identified in the resistant varieties TGx1987-32F and SB8 and susceptible varieties SB19 and Nyala using Sct_001, Sat_366 and Satt361 and Satt215 markers. It has been established that *Rpp2* maps to the linkage group J between SSR markers *BARC_Sat_255* and *BARC_Satt620 (*Silva *et al.,* 2008). The *Rpp3* was identified in SB19, SB8 *and* TGx1987-32F. *Rpp3 g*ene has been mapped to MLG C2 using single nucleotide polymorphism (SNP) in bulk segregant analysis (Hyten *et al.,* 2009). The *Rpp4* gene maps to Linkage group G between SSR markers BARC_Satt288 and BARC_AF162283 (Silva *et al.,* 2008). *Rpp4* has been found to be highly resistant and the resistance has never been overcomed under field conditions. However it is reported that under greenhouse conditions, resistance has been overcomed by other *P. pachyrhizi* isolates (Twizeyimana *et al.,* 2008).

In this study the susceptible varieties were positive for the *Rpp* genes with SB19 having *Rpp1* to *Rpp4* while Nyala has R*pp1,* Rpp2 and R*pp4.* The susceptible reactions (tan lesion) could have resulted due to different pathotypes of *P. pachyrhizi* or loss of resistance. In 2005 and

2006 Oloka *et al.,* (2008) identified four soybean accessions bearing *Rpp1* to *Rpp4* and were susceptible to rust populations originating from Uganda. In another study by Bonde *et al.,* (2006), *P. pachyrhizi* isolates collected from Zimbabwe produced TAN colour reaction on all soybean cultivars that had the resistance genes *Rpp1* to *Rpp4*. In the same study an isolate from South Africa produced RB infection types on soybean with know to posses *Rpp2, Rpp4* and *Rpp1*+ genes. These results suggested that there different rust genotypes of rust in Africa. Resistance to rust conferred mainly by the known *Rpp* genes have been identified to be specific to certain strains of *P. pachyrhizi* races of soybean rust (Bromfield and Hartwig, 1980; Hartwig, 1986, Garcia *et al.,* 2008, Bonde *et al.,* 2006). The use of these major resistance genes has been complicated due the high level of variation within *P. pachyrhizi* that causes rapid breakdown of resistance. There is no major gene that is resistant to all *P. pachyrhizi* isolates **(**Hartman *et al.,* 2005a**).** The varieties identified as susceptible in this study could be resistant to some races of *P. pachyrhizi* since they contain the resistant genes similarly the resistant varieties maybe susceptible to certain races of *P. pachyrhizi.*

CHAPTER SIX

6 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This current study has established that the farmer's in Western Kenya region prefer local varieties of soybean over the commercial varieties. The farmers' preference for specific variety is based on positive attributes such as size of grain, drought tolerance, early maturity and high yield. If these traits are taken into consideration during breeding programs then the production and yield of soybean in the region would be improved. There are a number of production constraints like pest and disease, seed unavailability, low demand for soybean as compared to other legumes that needs to be addressed by key stakeholders so as to improve production.

The study has also established that the soybean rust disease in Western Kenya region is caused by *P. pachyrhizi* and not *P. meibomiae.* Disease incidences and severity are high in all the soybean growing areas in Western Kenya region. The high incidence and severity of rust disease in Western Kenya shows that adequate control measures have not been employed to curb the rust disease. The results also implies that the rust population in this region is more virulent therefore proper disease management strategies needs to be adopted to avoid yield losses associated with the fungi. Susceptible varieties Nyala, Bossier, Gazelle, Hill, and SB19 recorded high disease severity, high sporulation levels, high lesion number and high values of AUDPC while TGx1987-32F 1987 32F and SB8 have been identified to be resistant to soybean rust and recorded low disease severity. The Soybean varieties with low lesion

densities, low disease severity and low sporulation level may be sources of partial resistance that may limit infection. It has also been shown that the soybean varieties grown in Kenya have the rust resistant genes. The genes can be in cooperated into other breeding lines with high yields through marker assisted selection resulting in improved yield quality and quantity and disease resistance of the existing breeding lines.

6.2 RECOMMENDATIONS

There is need to promote soybean production in Western Kenya region and address production constraints. It is therefore recommended that soybean breeders should work in partnership with the farmers to ensure that the seeds produced meet the farmers' expectations. There is need to identify more sources of rust resistance genes which can be used to enhance the resistance levels in susceptible varieties. This study used mixed races of soybean rust however it is important that the different pathotypes or races be identified so that it aids in improving the breeding strategies to increase soybean production in Kenya. The establishment of the rust fungi is affected by the environmental conditions therefore screening across different environmental conditions is recommended soybean rust has got alternative host legumes therefore studies should be done to determine severity under different cropping systems.

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Appendices

Appendix I Questionnaire on Farmers' preferred varieties of soybean and constraints affecting soybean production in Western Kenya

Questionnaire Number……..

District…….

Location ……

Village……..

Date……..

A Personal information

1. Name (optional)……..
2. Gender 1. Male 2. Female
3. Marrital Status:

   1. Single 2 Married 3 divorced 4 Widowed

4. Age

   1. less than 25 **2** Between 25-35 **3**. Between 36-45 **4** between 46-55 **5**. above 55

5) Education level

   1.No formal education 2.Primary 3. Secondary 4. Post Secondary

6. Are you the head of the household yes/ no
7. If you are not the head what is your relationship with the head of the household……..

B. Land Utilization

1. What is the size of your land in Acres? …….
2. What portion of the land is under cultivation? ……..
3. What is the size of land allocated to soybean production? .....
4. Which other crops are you growing? ..... (list all)

Crop	Acres

5. What are your reasons for growing soybean?

C. Soybeans production information

1. Which cropping system do you use for soybeans? 1. Mono crop (soybeans only) 2. Intercrop,

2. If intercropped, with which crops? 1. ----------

3. What are reasons for intercropping?

4. What is the line spacing used …….

5. How many times do you weed soybean

1. Once 2. Twice 3. Thrice 4. Four times

6. Which of the following inputs do you use in soybean production?

Manure

Fertilizer

Pesticides

Fungicides

Other (specify)

C. Soybean varieties grown and preferred varieties

1. What is the source of your planting material

1. Seeds from previous harvest 2. Agrovet 3. Market 4. Research institutions 5. Seed companies 6. Farmers Cooperative Society. 7. Neighbor/relative 8.Others(specify)

2. Which soybean varieties do you grow?

3. Which variety/varieties do you prefer what are their good and bad traits?

3. What is the source of your planting material?

1. Seeds from previous harvest 2. Agrovet 3. Market 4. Research institutions 5. 5. Farmers Cooperative Society. 6. Neighbor/relative 7.Others(specify)

D. Soybean Production constraints

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

2. Which pest and diseases have you encountered in your farm and which management strategy are you using?

3. Are the pest management strategies you are using effective? 1.Yes 2.no

Appendix II: Disease Severity Assessment form

FARMER FIELD DISEASE ASSESSMENTS

District……..

Date……..

Farm number	Farm location	Severity Score					Mean	Comments /Observations
		Plant 1	Plant 2	Plant 3	Plant 4	Plant 5		

Appendix III: Soybean Growth Stages (Smith, 1995).

Vegetative stages

Stage	Description
VE	Emergence - Cotyledons above the soil surface
VC	Cotyledon - Unifoliolate leaves unrolled sufficiently so that the leaf edges are not touching
V1	First-node - Fully developed leaves at unifoliolate node
V2	2 nodes- two nodes on the main stem with fully developed leaves
V(n)	n is the number of nodes on the main stem.

Reproductive stages

Stage	Description
R1	Beginning bloom with open flower at any node on the main stem
R2	Full flowering stage
R3	Beginning of pod formation
R4	Full pod formation
R5	Beginning of seed formation
R6	Seed fully formed - Pod containing a green seed that fills the pod cavity
R7	Beginning maturity
R8	Full maturity