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IDENTIFICATION OF SOURCES AND LOCI FOR DEVELOPMENT OF SOYABEAN [*Glycine max* (L.) Merrill] CULTIVARS THAT COMBINE BACTERIAL PUSTULE AND SEED DECAY RESISTANCE WITH HIGH POD CLEARANCE

BY

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DECLARATION

I do hereby declare that except for research works that have been duly cited and referenced, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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ABSTRACT

Globally, diseases constitute a major biotic constraint to soyabean production. Although, the list of diseases of soyabean in Ghana has been published, the incidence, severity, and distribution of listed diseases were not indicated. Secondly, the Savannah Agriculture Research Institute (SARI) has accumulated considerable number of soyabean genotypes most of which were sourced from the International Institute of Tropical Agriculture (IITA) and the United States Department of Agriculture (USDA). Efficient use of germplasm depends on knowledge of variation that exists among genotypes for traits of interest coupled with an understanding of genetic diversity. Therefore, it was imperative to morphologically and genetically characterise genotypes in SARI's germplasm to guide breeding efforts. Lastly, it is important to consider the architecture of released varieties in relation to farming systems including commercial farming. Most of the released soyabean varieties in Ghana belong to the early and medium maturing groups with low pod clearance. Low pod clearance is not a problem with subsistence farming systems where harvesting is manually done, but in commercial farms that use combine harvesters, low pod clearance could be the source of considerable losses. Detailed assessment of soyabean disease situation at production centres and detailed characterisation of genotypes in SARI's soyabean germplasm have been carried out to identify sources and loci of resistance to predominant diseases of soyabean in Ghana. The objective of this study were to: (1) carry out a survey of soyabean diseases across major production centres in Ghana and their incidence and severity, (2) evaluate SARI's soyabean germplasm for resistance to bacterial leaf pustule, (3) evaluate SARI's soyabean germplasm for resistance to soyabean seed decay, (4) assess diversity, population structure and identify lines with high pod clearance among genotypes in SARI's germplasm, (5) identify *Phomopsis* seed decay (PSD) and bacterial leaf pustule (BLP) resistant loci through genome-wide association mapping, (6) identify loci associated with pod clearance.

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Results of the survey revealed that some of the important diseases of soyabean in Ghana included Cercospora leaf blight (*Cercospora kikuuchi),* frog eye leaf spot (*Cercospora sojina*); target spot *Corynespora cassiicola),* bacterial leaf pustule (*Xanthomonas campestris* pv. *glycine),* downy mildew (*Peronospor manshurica),* leaf blight (R*hizoctonia solani),* brown spot (*Septoria glycine),* soyabean mosaic, alfafa mosaic, and soyabean seed decay due to *Diaporthe sp*. Bacterial pustule was the most wide spread with incidence ranging from 15.3% at Wenchi to 84.7% at Malzire in the Yendi municipality. Two viral diseases, soyabean mosaic virus and alfalfa mosaic virus were present at all 15 locations but mostly at very low incidence except for Ejura and Wenchi where incidence was very high. Cercospora leaf blight and Frog eye leaf spot both occurred at 13 of 15 locations surveyed. Generally, incidence and severity of identified diseases was higher at Malzire, Karaga and Gushegu than other locations with the exception of soyabean mosaic virus for which the highest incidence occurred at Ejura followed by Wenchi. Furthermore, this study identified soyabean seed decay disease, and molecularly identified the causal agent as *Diaporthe phaeolorum var. sojae* and tentatively designated the isolate as MTB-1. This is the first report of *Diaporthe phaeolorum var. sojae* occurrence on soyabean pods and seeds in Ghana. Twenty-eight lines were identified with immune response to *Xanthomonas axonopodis pv. glycines* which could be used to develop resistant varieties. Area under chlorophyll retention curve had a negative correlation with area under disease progress curve, which translates to a positive correlation with resistance to bacterial pustule. Area under chlorophyll retention curve could replace disease severity score in bacterial pustule screening trials. Though disease pressure was high, eleven lines, PI416806B, PI224271, PI417120 BRS 361, PI417013, TGX 1903-7F, PI594767B, PI423958, TGX 1485-1D, TGX 2006-3F and Liu Yuemang were highly resistant to PSD infection. Genotypes within SARI's germplasm differed widely for growth and reproductive parameters including plant height, number of branches, pod clearance, flowering and maturity periods, all of which were highly

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heritable except for number of branches. Likewise, high variation among genotypes for yield components, most of which were under genetic control was recorded. Principal component analyses identified number of pods per plants, number of seeds per plant, days to flowering and days to maturity as the main drivers of variation in SARI's germplasm. Genetic diversity analyses of a 96 representative panel showed high diversity among genotypes. Structure analyses revealed nine sub-populations within the panel. Genetic variation among sub-populations was high as indicated by high allele divergence frequency among sub-population. All genotypes in the 96 representative panel were distinct and most were almost fixed as reflected in high Fst values. Genome-wide association studies identified two SNP markers, S3_14585048 and S3_1991311 with large negative effect on pod clearance. SAUR like-auxin responsive protein known to positively regulate cell expansion to promote hypocotyl growth was identified at the physical position of the SNPs. Associations between genomic locations and resistance to bacterial leaf pustule were identified but the associations were not significant because of low statistical power due to small sample size. Nonetheless, three SNP markers, S16_34242886, S16_35202484 and S1_38672307 were found linked to three candidate genes, Glyma.01go93200, Glyma.g187500 and Glyma.g187400. All three candidate genes code for Leucine-rich repeat receptor like protein kinases known for eliciting immune response to pathogen infection. The SNP, S18_7792728 associated with PSD resistance occurred at a genomic region that harbours the candidate gene AT3G07040.1 (NBARC-domain-containing disease resistance protein) known to play critical roles in plants response to pathogens. This confirms the existence of resistant genes for PSD among the lines in the representative panel. The identified SNP could be validated to aid with marker-assisted selection.

This study presents a series of useful information on SARI's soyabean germplasm, prevailing diseases of soyabean and their distribution, sources of resistance and resistant loci, and the discovery of informative markers that can alter the architecture of future varieties.

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DEDICATION

This work is dedicated to the rock of my life, my mother, Mary Koryoe Barnor, and to uncle David Akornor and Maafio Marku for their generosity, and to my wife and son, Thamar and Nattey for their extreme patience and support.

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

Soyabean is one of the most important crops worldwide, as its seeds are used for the production of both protein meal and vegetable oil. It is one of few crops cultivated in the tropics, subtropics and temperate regions of the world (Asafo-Adjei, 2015). Soyabean oil is the world's most extensively utilized edible oil because of its low cholesterol level and a natural taste with odour that is nearly imperceptible (Van Ee, 2009; Gracia *et al.*,1997). Soyabean is produced more cheaply than other legume crops, and gives the highest oil yield per unit area among leguminous crops (Kawuki *et al.*, 2003).

Sub-Saharan Africa (SSA) contributed less than 1% to global soyabean production in the 2017 production season (FAOSTAT, 2018). Meanwhile, demand for soyabean and its products in Africa has been rising steadily due to growing livestock industry that uses soyabean as a major feed input (Joubert *et al.*, 2013). The consumption of soyabean products in the form of soy milk drinks, yoghurt, baked beans and tofu are also on the rise on the African continent (Dlamini *et al.*, 2014; Shannon and Kalala, 1994).

Globally, soyabean demand is projected to surge between 2010 and 2050 (Masuda and Goldsmith, 2009; Gasparri *et al.*, 2015). However, land availability for soyabean production will be generally limited by competing needs of land for urbanisation and land conservation (Goldsmith, 2008; Gasparri *et al.*, 2015). The area considered suitable for soyabean production in Sub-Saharan Africa (SSA) is in the range of 140 to 270 Mha (Shurtleff and Aoyagi, 2009; Gasparri *et al.*, 2015). Less than 3% of this area is currently under soyabean production and this makes SSA a likely frontier for future expansion in soyabean production to meet growing global demand (Hartman *et al.*, 2011; Gasparri *et al.*, 2015).

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Sinclair *et al.*, (2014) noted that much of West Africa could support soyabean production with the exception of pockets of locations in southern Ivory Coast and Ghana. However, Africa will have to adapt productive germplasm to prevailing abiotic and biotic constraints (Goldsmith, 2014) if it were to realise this potential.

Biotic stresses including pest, pathogens, and weeds constitute a major constraint to soyabean production globally (Hartman *et al*., 2011. Hartman *et al.*, (1999) reported that diseases are by far the most important biotic constraint to soyabean production globally. In Ghana, Offei *et al.* (2008) reported of bacterial leaf pustule, bud blight, charcoal rot, leaf spot, mosaic, purple seed stain, root rot, and web blight as the predominant diseases of soyabean. Bacterial leaf pustule listed among diseases of soyabean in Ghana is a known major disease in most soyabean producing countries including neighbouring Benin (Wrather *et al.*, 2001; Zinsou *et al.*, 2015).

Asante *et al*. (1998) reported the occurrence of *Phomopsis longicolla* and other *Phomopsis* species known for causing soyabean seed decay and as well as *Diaporthe phaseolorum* var. *meridionalis,* causal agent of southern stem canker of soyabean in Ghana.

Although, Phomopsis seed decay (PSD) caused by Diaporthe/Phomopsis complex of fungi was not listed in subsequent compilation of diseases of soyabean in Ghana (, Offei *et al.*, 2008), warm and wet condition that enhances the development of this disease (Wrather *et al.*, 2003) is common to the major soyabean production areas of the country. Soyabean seed decay is the primary cause of decreased soyabean seed quality in most production areas worldwide (Santos *et al.*, 2011; Li *et al.*, 2011). Additionally, early maturing varieties are said to be more susceptible to PSD infection than late maturing varieties (Suli *et al.*, (2013).

The infection and development of both bacterial leaf pustule and phomopsis seed decay is enhanced by warm and humid conditions (Wrather *et al.*, 2003). Unfortunately, Northern Ghana, which accounts for 77% of soyabean (MoFA-SRID, 2016) production, is humid and

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warm during the production season, a situation that predisposes the country's soyabean production to these diseases. Varieties developed for the Guinea Savannah agro-ecology are early to medium maturing types. However, early maturing soyabean varieties are generally susceptible to Phomopsis seed decay (Suli *et al.*, 2013). Therefore, developing early maturing varieties for the short rainfall duration that pertains to northern Ghana may inadvertently result in developing phomopsis seed decay susceptible lines.

Current soyabean breeding programmes at the Savannah Agriculture Research Institute (SARI) do not have gene deployment for disease control as an objective (Dr. Nicholas Denwar, personal communication). This leaves varieties vulnerable to possible outbreak of disease, especially bacterial leaf pustule and phomopsis seed decay, which the major production environment is suitable for the development of their causative pathogens. Considering the current push to expand soyabean production in Ghana coupled with projected climate change scenarios, it is important to begin to factor disease management, especially, the use of resistant genes to manage important diseases of soyabean into pending breeding programmes. Pre-emptive or anticipatory breeding strategies should be adapted to prepare for diseases that exist in Ghana but had not recorded an outbreak or diseases that thrive under conditions that are similar to those pertaining to soyabean production areas in Ghana.

Plant architecture is of major agronomic importance, as it strongly influences the suitability of a plant for cultivation, its yield and the efficiency with which it is harvested (Reinhardt & Kuhlemeier, 2002). Soyabean production in Ghana has largely been dominated by small holder farmers with very limited deployment of machines. Recent emergence of commercial farms which deploy combine harvesters for harvesting soyabean have complained of combined losses during harvest due to the low pod clearance of some released varieties. Farming system in the country is gradually changing. The use of selective herbicide for weed control on soyabean

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farm is gaining traction. It is therefore important that the architecture of future varieties reflect changing farming systems including mechanisation and herbicide control of weeds.

Germplasm is key for crop improvement. An understanding of genetic and morphological diversity within available germplasm ensures its effective use for crop improvement and conservation. The use of new sources of genetic variation to develop improved varieties is enhanced when the selection of parental genotypes is based on both phenotypic and genetic dissimilarity (Bisen *et al.*, 2014).

It is therefore important to evaluate SARI's soyabean germplasm for phenotypic and genetic diversity and identify lines that disease resistant to bacterial leaf pustule and phomopsis seed decay for development of resistant cultivars. It is important to identify lines with high pod clearance so that future cultivars can meet the needs of both peasant and commercial farmers. The goal of this study was to find sources of resistance to Phomopsis/Diaporthe seed decay (PSD) and bacterial leaf pustule leaf (BLP) spot diseases of soyabean towards the development of cultivars that combines high pod clearance with resistance to PSD and bacterial leaf pustule. The specific objectives of this study were to;

1. carry out a survey of soyabean diseases across major production centres in Ghana, and their incidence and severity
2. assess diversity, population structure and identify lines with high pod clearance among genotypes in Savannah Agriculture Research Institute's soyabean germplasm and identify loci associated with high pod clearance
3. evaluate Savannah Agriculture Research Institute's soyabean germplasm for resistance to bacterial leaf pustule
4. evaluate Savannah Agriculture Research Institute's soyabean germplasm for resistance to soyabean seed decay
5. identify loci associated high pod clearance, and PSD and BLP resistance through genome-wide association mapping

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and botany of cultivated soyabean

Soyabean [*glycine max* (L.) Merr.] (2n=2x= 40) is a leguminous plant belonging to the family, fabaceae (Singh, 2017). The crop originated from East Asia where its domestication is said to have occurred some 6000-9000 years ago (Kim *et al.,* 2012). The specific location in East Asia where soyabean is supposed to have originated has been the subject of debates. Whilst Guo *et al.* (2010) concluded on the Yangtze basin in southern China as the origin of cultivated soyabean, Han *et al.,* (2016) on the other hand pointed to Huang Haui valley in Central China as the origin of soyabean. Meanwhile earlier suggestions based on genetic diversity (Dong *et al.*, 2004; Li *et al*., 2010) and archaeological evidence (Lee *et al*., 2011) placed the origin of cultivated soyabean at Yellow River basin in China. The works of Guo *et al*. (2010); Zhou *et al*., (2015); and Han et *al*., (2016) concluded that cultivated soyabean originated from a single domestication event given rise to the single origin hypothesis (Gao *et al.*, 2010).

Archaeological evidence dating back to 5000–3000 years showed that Japanese and Korean soyabean seeds were distinct from that of China (Lee *et al*., 2011). This was supported by molecular analysis which showed that Japanese and Korean soyabean possess distinct gene pools in both their chloroplast and nuclear genome (Xu *et al*., 2002; Abe *et al*., 2003; Zhou *et al*., 2015). Considering the uniqueness of Japanese and Korean wild soyabean in addition to the long divergence time between *G. max* and *G. soja* (0.27 or 0.8 Ma), Sedivy *et al.* (2017) postulated a view on soyabean domestication in which a prolonged period of low-intensity management of wild soyabeans at multiple locations preceded the domestication event, which gave rise to cultivated soyabean. They therefore concluded that cultivated soyabean resulted

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from independent multiple domestication events at different locations in East Asia, given rise to the complex origin hypothesis about the origin of cultivated soyabean.

Cultivated soyabean belongs to the subgenus soja which contains *G. soja*, its wild progenitor and the cultigen, *G. max*, both of which contain 2n=40 chromosomes. Both species are cross-compatible with cultivated soyabean (Thseng *et al.*, 1999). Cultivated soyabean [*glycine max* (L.) Merr.] is an annual plant that exhibits epigeal germination with two cotyledons. Its root system is comprised of taproot and large number of secondary roots with which it establishes symbiotic relationship with nitrogen fixing bacteria. Stem growth may be determinate, semi-determinate or indeterminate with trifoliate leaves (Lersten and Carson, 2004).

2.2 Global distribution and production of soyabean

Soyabean is cultivated in various environments throughout the world. Production of soyabean occurs extensively in tropical environments as observed in Brazil, and under sub-tropical climate as pertains in Central America and southern USA. Soyabean production also occurs in temperate climate as seen in northern USA and Canada, and in the sub-arctic climate such as pertains in Sweeden and Siberia (Asafo-Adjei, 2015; USDA/FAS, 2018).

Global production of soyabean during the 2017/2018 cropping season stood at 346.919 Mmt (USDA/FAS, 2018). The three top soyabean producing countries, USA (119.518 Mmt), Brazil (112 Mmt), and Argentina (54 Mmt), accounted for 82% of the global soyabean output in the 2017/018 season (USDA/FAS, 2018). In Asia, China, the top producer of soyabean recorded a seasonal output of 14.2 Mmt, which was followed by India, the Asian second top producer with 9.5 Mmt (USDA/FAS, 2018). Globally, China and India ranked fourth and fifth, respectively with China doubling as the world's largest importer of soyabean (index Mundi, 2018).

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2.3 Soyabean production in sub-Saharan Africa

Sub-Saharan Africa (SSA) harbours 600 million hectares of arable land, 10% of which is currently under the cultivation of various crops including soyabean (Khojely *et al.,* 2018). Consequently, Sub-Saharan Africa is seen as the last frontier to expand soyabean production to meet the growing global demand. Khojely *et al.* (2018) described soyabean (*Glycine max* (L.) Merr.) as non-native and non-staple crop in sub-saharan Africa, a reflection of the origin and the limited role of the crop in traditional food preparation on the continent. South Africa, Nigeria, Zambia, and Uganda are the leading producers of soyabean in SSA (UDS/FAS, 2018). South led soyabean production in Africa with 1,575,000 mt in the 2017/2018 season (UDS/FAS, 2018). Nigeria, which obtained soyabean output of 600,000 mt during the same period, was second to South Africa in production.

Introduction and cultivation of soyabean in sub-Saharan is relatively recent with earliest records of cultivation dating back to 1903 in South Africa, 1907 in Tanzania, 1908 in Nigeria, and 1909 in Ghana (Plahar, 2005; Giller and Dashiell, 2006; Shurtleff & Aoyagi, 2009). Soyabean production in sub-Saharan Africa increased gradually, mainly, through increasing the area under production. Area under soyabean cultivation in sub-Saharan Africa rose from 20,000ha in 1978 to 1,500,000ha in 2016 (USDA/FAS, 2017). Correspondingly, soyabean production also rose from 13,000 mt in 1978 to 2,300,000 mt in 2016 (USDA/FASS, 2017). However, yield per unit area remained almost the same at 1.1 t/ha over the period (Khojely *et al.,* 2018). The current yield per unit area of 1.1 t obtained in sub-Saharan Africa in 2016 was far below the global average yield of 2.4 t/ha (USDA/FAS, 2018).

Among the top soyabean producing countries in sub-Saharan Africa, Nigeria recorded the largest area under production in 2016 (UDS/FAS, 2017) followed by South Africa. Increases in soyabean production in Nigeria was largely driven by increases in area under production as the country's yield per unit area remained at 1 t/ha between 2012 to 2104 and reduced to

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960kg/ha in the 2015 and 2016 production seasons (Khojely *et al.*, 2018). The third top producer of soyabean in SSA, Zambia was the second in terms of yield per unit area (1.94 t/ha) whilst.

Uganda, the fourth leading producer of soyabean in SSA has the least yield per unit area (600kg/ha) (Khojely *et al.*, 2018).

Despite the gradual increase in soyabean production over the years, SSA contributes less than one percent (1%) to global production and so is its share of global Farmer Revenue from soyabean FOASTAT, 2018). For instance, in 2012, Farmer Revenue from soyabean globally was USSD126 billion dollars of which Africa accounted for less than 1% of production earned a revenue of $1,012,500,000, representing 0.8% of the global revenue (Goldsmith, 2014).

Though, soyabean is rightly described as a non-staple crop in SSA, demand for the crop and its products has seen a steady growth over the years (Kolapo *et al.*, 2012). Demand for soyabean and its products in SSA is largely driven by a growing livestock industry (Joubert *et al.*, 2013) that largely relies on protein meal of which soyabean contributes about 67% of global consumption. Secondly, there is increasing consumption of soyabean oil and other products of soyabean (Dlamini *et al.*, 2014; Jobert & Jooste, 2013; Kolapo, *et al.*, 2012). In addition, the promotion and utilisation of soy-based food to fight malnourishment among children is widely pursued by many countries in SSA (Amagloh *et al.*, 2018). Gradually, soyabean is entering the food chain in sub-Saharan African countries whereby soyabeans enhance either traditional food preparations or original ingredient substituted with soyabean is gaining grounds across Africa. In Ghana, tubaani, an indigenous food originally prepared with cowpea is now prepared with soyabean (Khojelly *et al.*, 2018).

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2.4 Soyabean production in Ghana

Soyabean cultivation in Ghana started in 1909 during which soyabean was introduced to, and cultivated by farmers at Bimbila, Nakpanduri, Karaga, Tilli, and Bawku in the northern protectorate of then Gold coast (Quarshie and Nsowah, 1975; Plahar, 2006). The introduction of soyabean in Ghana was aimed at making soyabean a staple food, and an export commodity (Quarshie and Nsowah, 1975). Research conducted from 1909 to 1956 focused on understanding the agronomy of soyabean whilst promoting its consumption. The period saw seventeen annual trials conducted over 12 locations stretching from the Asuansi in the south to Tono in the Upper east region (Quarshie and Nsowah, 1975). Amidst initial disappointing results, understanding of the crops lead to improved results from 1956 onwards, and yield up to 1457 kg/ha was recorded for some of the trials. This initial effort at promoting cultivation and consumption of soyabean failed because farmers complained that it took too long a time to cook soyabean, in addition to having unappealing taste (Quarshie and Nsowah, 1975).

Scientific research from the 1960s to early 1970s by the Crop Research Institute focused on the utilisation of soyabean for feed meal, and vegetable oil (Mbanya, 2011). The absence of processing companies to absorb what was produced caused a glut. Consequently, there was no market for soyabean and as such, the farmers stopped soyabean production (Plahar, 2006).

A more sustained campaign to boost soyabean production began in the 1990s where the Ministry of Food and Agriculture joined efforts with non-governmental agencies (NGOs) including the Adventist Development and Relief Agency. Despite the failure of such programmes to make Ghana a soyabean production hub, soyabean production gradually gained traction as reflected in the adoption of released varieties and production technologies with production rising from 1,000 tons in 1979 to 10,000 mt in 1992 (CRI, 2010). Meanwhile, the

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country met its need for soyabean and soyabean products through imports, which stood at 198,000 mt in 2009 (MoFA, 2009).

Though, several areas in Ghana, including those located in the coastal Savannah, the forest savannah or transitional belt, Guinea and Sudan agro-ecologies have been described as suitable for soyabean production (Sinclair *et al.,* 2014), current production largely occurs in the Northern region which accounts for 70% of the production area, and 77% of the country's soyabean production (SRID, 2016). Soyabean production in Ghana has seen consistent increases from the 1990s, largely due to increases in area under cultivation supported by marginal increases in average yield. Area under soyabean cultivation rose form 47,000 ha in 2007 to 87, 000ha in 2016 and output rose from 50,000 mt in 2007 to 143, 000 mt in 2016 (MoFA-SRID, 2017). Maximum output of soyabean occurred in 2011 where production reached 165, 000 mt (MoFA-SRID, 2017). Increased soyabean production in Ghana is due to both increases in area under production, and in average yield. Average yield of soyabean in Ghana rose from 1.1t/ha in 2007 to 1.65 in 2016 (MoFA-SRID, 2017).

In Ghana, and most countries in sub-Saharan Africa, soyabean production is characterised by poor yielding varieties, limited application of fertilizers and non-inoculation of seed with *Bradyrhizobium japonicum* (Woomer *et al.,* 2012). Additionally, weak value chain that results in poor market access to farmers leading to very low producer price is reported to be a major hindrance to soyabean production in Ghana with similar reports from Nigeria (Aidoo *et al.,* 2014; Agada, 2015; Dogbe *et al.,* 2013; Mbanya, 2011). Most soyabean varieties released in Ghana were obtained from IITA and consequently, as noted by Khojely (2018), national contribution to soyabean varietal and production technology development, and dissemination has been limited in the past.

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2.5 Constraints to soyabean production

In Africa, constraints to soyabean are abiotic and biotic, as well as socio-economic challenges (Kawuki *et al.*, 2003). Abiotic factors including weather-related phenomena, soil nutrient, salinity and photoperiod are factors in the physical environment that limit crop production including soyabean (Hartman *et al.*, 2012). Global climate variability and climate change that is causing changes in temperature and rainfall is projected to affect agro-ecological zones (Nelson, *et al*., 2009) with consequent effects on crop productivity and production. Suggested changes due to climate change includes increased levels of CO_2, and extremes of temperature and rainfall. Whilst elevated CO_2 levels may increase photosynthetic rate in crops (Cure and Acock 1986; Mendelsohn *et al.*, 1994), temperature and rainfall extremes may have negative effects on soyabean production as observed in China by Zhang *et al.* (2009). Peralta and Wander (2008) showed that elevated CO2 caused a decrease in soil organic matter due to death of some microbial organisms resulting from elevated CO2 levels.

Ghana and many African countries have their soyabean fields dominated by cultivars from IITA which are short- day length, drought tolerance, and photoperiod insensitive cultivars, (Ibrahim, 2011) and so research programmes do not have photoperiodism as a major research goal. However, photoperiodism limits the introduction and use of superior cultivars from other continents. Tropical varieties from USA and Brazil have been found to be highly adapted to growing conditions in SSA but the temperate varieties flowered very early under the prevailing short-day conditions resulting in poor yields due to low vegetative growth (Ibrahim *et al.*,

2012).

Two abiotic factors known to dominantly influence soyabean phenology are photoperiod, and temperature (Cregan and Hartwig, 1984; Summerfield and Wilcox, 1978). In soyabean [*Glycine max* (L) Merr], transition from vegetative to reproductive development is largely

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influenced by changes in the length of light and dark phase within a day (Langewisch *et al*., 2017). The response to photoperiod is the major determinant of the maturity groups of soyabean cultivars (Hartman *et al*., 2012).

In the US, soyabean cultivars have been placed in 13 maturity groups ranging from MG00 for groups suitable for growth in northern US with longer day-length to MGX representing cultivars that are suitable for growth near the equator (short day length) (Zhang et al., 2007). Similarly, in China, soyabean cultivars have been placed in 12 maturity groups ranging from MG00 to MGIX (Wang *et al*., 2018). Photoperiod affects the distribution and performance of soyabean cultivars across the globe. When long day length cultivars are planted in shorter photoperiod areas as pertains in the tropics, they flower early, attaining very little vegetative growth resulting in fewer internodes, which translates into fewer number of pods per plants and lower yield (Hartman *et al*., 2012)

2.5.1 Drought as a production constraint of soyabean

Drought causes yield losses at varying degrees in soyabean when it occurs at germination, vegetative, and reproduction stages. Drought at germination causes uneven and spotty germination whilst drought at the vegetative stage reduces shoot growth and limit sink as reflected in reduced number of internodes at which pods are produced (Mannan *et al.*, 2016). Yield loss from drought stress in soyabean is exacerbated by the lack of nitrogen mineralization and nitrogen fixation that occurs under drought conditions (Kunert, 2016). In dry conditions, nodules cease nitrogen fixation because of a lack of soil moisture and lack of carbohydrate supply from the soyabean plant to *Rhizobia sp.* (Ku *et al.*, 2010). Argentina, the third largest producer of soyabean in the world and lead exporter of soymeal experienced drought during the 2017/2018 season which lead to a loss of a sixth of the projected yield for the season causing a rise in global soymeal prices (Terazono, 2018). The semi-arid and arid environments that

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include northern Ghana where most of the country's soyabean is produced are prone to drought (Hufstetler, 2007).

2.5.2 Salinity as constraint to soyabean production

Soil salinity is a term used to describe the salt content within soil water and it affects plant growth and development. The injurious effects of salinity on the growth and yield of crop plants arises from reducing water inside plant cells and the unfavourable effects of sodium (Na^+) and chloride (Cl^-) which negatively affect plant physiology and morphology (Miransari, 2016). Soyabean is described as being sensitive-to-moderately tolerant to salinity (Kao *et al.,* 2006) and therefore, suffers stress when grown in high saline soils. Different growth stages and different soyabean plant parts are adversely affected by salinity stress including plant height, plant biomass, leaf size, number of branches, internodes, pods and weight of 100 seeds, and plant weight (Miransari, 2016).

2.6 Important diseases limiting soyabean production

Biotic constraints including pests, diseases, and weeds pose a major challenge to soyabean production globally (Hartman *et al.*, 2011). Among the biological constraints to soyabean production, diseases are by far the most important (Hartman, 1999) and responsible for an estimated 11% of global yield loss in soyabean (Hartman, 1999). Soyabean diseases including Asian soyabean rust, bacterial leaf pustule, frogeye leaf spot, sudden death syndrome, phomopsis seed decay among others have been reported to cause significant losses to production. Rust caused by *phakospora pachyrihzi* Syd & P.Syd which causes the most significant yield losses in recent times (Akamatsu *et al.*, 2013). *phakospora pachyrihzi* is endemic in the eastern hemisphere and has caused significant economic annual yield losses in Africa, and South America **(**Sohrabi *et al.*, 2014**)**. Globally, soyabean rust has been the subject of research with many sources of resistance identified, and resistant loci mapped with linked

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markers made available for resistant variety development (Miles *et al.*,2008; Hyten *et al*, 2007; Ray *et al*, 2009; Kim *et al*, 2012; Gracia *et al*., 2011, and Yamanaka *et al*, 2015).

In Ghana, however, Asian soyabean rust has not been listed among the predominant diseases of soyabean (Offei *et al.,* 2008), despite the report of its incidence in the country in 2007 (Bandyopadhyay *et al.,* 2007). A disease survey carried out in Ghana in 2006 revealed that soyabean rust was wide spread (Bandyopadhyay *et al.,* 2007), thus officially confirming the presence of soyabean rust disease in Ghana. The survey which was carried out at locations in three regions covering three distinct agro-ecologies; forest, guinea savannah, and Sudan savannah, recorded disease incidence ranging from 50-100% in the farms surveyed (Bandyopadhyay *et al.,* 2007). Subsequently, 34 soyabean lines were screened for resistance to rust, five of which were highly resistant (Karikari and Amponsah-Antwi, 2017). The country has however not recorded soyabean rust outbreak at its major production areas including, Karaga, Gusehegu, and Chereponi in the Northern region where most of the country's soyabean is produced (MoFA-SRID, 2016).

Offei *et al.* (2008) reported bacterial leaf pustule, bud blight, charcoal rot, leaf spot, soyabean mosaic virus, purple seed stain, root rot, and web blight as diseases of soyabean in Ghana. The said publication did not make mention of rust. Also, there is no literature on the distribution and economic effects of the listed diseases on soyabean production in Ghana. However, considering the current push to expand soyabean production in Ghana coupled with projected climate change scenarios, it is important to begin to factor disease management, especially, the use of resistant genes to manage important diseases of soyabean. Pre-emptive or anticipatory breeding strategies could be adapted to prepare for diseases that exist in Ghana but had not recorded an outbreak or diseases that thrive under conditions that are similar to those pertaining to soyabean production areas in Ghana. To this end, soyabean seed decay due to Diaporthe/

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Phomopsis complex of fungi, and bacterial leaf pustules both of which thrives under warm and humid conditions were considered in this study.

2.6.1 Soyabean seed decay

Globally, many diseases, especially seed and seed borne diseases have been reported to cause significant yield loss, reduced grain quality, poor seed germination and high seedling mortality (Mengistu *et al.,* 2012). Many of the pathogens of economic importance to soyabean [*Glycine max* (L.) Merr] production are associated with its seed (Mengistu *et al.,* 2012) with *Diaporthe –Phomopsis* complex (DPC) of fungi, which causes seed decay, considered the most important.

Soyabean seed decay due to *Phomopsis/diaporthe* species of fungi is considered the most important pre-harvest disorder of soyabean seed worldwide (Sinclair, 1999).

Soyabean seed decay has been a serious problem in soyabean production areas with warm and wet weather for the past three decades including southern USA (Zhang *et al.,* 1998), Korea (Oh, 1998), Argentina (Pioli *et al.,* 2003), Italy (Riccioni *et al.,* 2003), Yugoslavia (Medic´-Pap *et al.,* 2007), Brazil (Costamilan *et al.,* 2008), and China (Cui *et al.,* 2009). Seed losses on global scale due to *Phomopsis longicolla* was approximated at 0.19 million metric tons in 1994 (Kulik & Sinclair, 1999). Effects of Phomopsis seed decay on yields in the United States from 1996 to 2007 ranged from 0.38 to 0.43 Mmt (Wrather and Koenning, 2009). In 2009, an estimated yield loss of 45 million bushels, 23 million of which was due to Phomopsis seed decay because of the hot and humid conditions that characterized pod fill to harvest periods of that season (Koenning and Wrather, 2012). Phomopsis seed infection causes pre- and post-emergence damping off, and in severe conditions, stands maybe affected to the point of reducing yield because of less plant population per unit area.

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In addition to directly decreasing yield, DPC infection causes seeds to shrivel, elongate, crack or split, mottle become moldy when there is sufficient moisture (Kulik and Sinclair, 1999). Diseased seeds including mottled and moldy seeds are graded lower (Federal Grain Inspection Service, 2004). DPC infection causes off-colour soyabean seeds but off-colour is one of the primary quality-relating factors that negatively influence the market grade of soyabean (Hepperly and Sinclair 1978; Sinclair 1982) and therefore, PDC infection generally reduces market grade and price of soyabean. Furthermore, DPC infected soyabean seeds have reduced germination whilst germinated infected seeds have reduced vigour (Smith *et al.,* 2008; Begum *et al.,* 2008; Li *et al.,* 2011; Gillen *et al.,* 2012; Mayhew and Caviness, 1994). (Mengistu *et al.,* 2009) reported a strong negative association between DPC seed infection rate and seed germination, vigour and seedling emergence.

DPC infection of soyabean seeds also affects the quality of products including soyabean flour, meal, and soyabean oil. Soyabean flour from infected seeds is reported to have unpleasant odour as well as being unpalatable (Hepperly and Sinclair, 1978). Similar characteristics are noted for oil obtained from infected seeds rancid seeds with higher peroxide values (Divilov, 2014).

Infected seeds also have altered fatty acid, and protein contents. Significant reduction in protein content, and decreased palmitic and oleic acid compositions were found to be significantly correlated with DPC infection (Wrather *et al.,* 2003; Fábrega *et al*., 2000**)**. Some isolates of *Phomopsis longicolla,* a member of the DPC of fungi produces the fungal metabolites, cytochalasin compounds that have the ability to bind to actin filaments and block polymerisation, and elongation of actin (Carter, 1967). Infected seeds with *Phomopsis longicolla* containing moderate to high levels of cytochalasin were found poisonous to sheep and rats (Allen *et al.,* 1992).

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2.6.2 Pathogens within the *Phomopsis- Diaporthe* complex (DPC) of fungi

Pathogens that constitute the complex includes *Phomopsis longicolla*; *Diaporthe phaseolorum* var. sojae; *Diaporthe phaseolorum* var. *caulivora; and D. phaseolorum* var. *meridionalis* (Sinclair, 1999; Rossman *et al.,* 2007). The members of Phomopsis/ Diaporthe complex of fungi belong to the order Diaporthales and Diaporthaceae. The members of the complex are responsible for a number of diseases on soyabean. *Phomopsis longicolla* is largely responsible for all reported cases of soyabean seed decay, accounting for about 85% of Diaporthe/Phomopis infection of soyabean seeds (Hobbs *et al.,* 1985; Zimmerman and Minor, 1993; Nevena *et al.,* 1997; Zhang *et al.,* 1999; and Suli *et al.,* 2013). Other diseases due to fungi in the complex include pod and stem blight, caused primarily by *D. phaseolorum* var. *sojae;* northern stem canker and top dieback, caused primarily by *D. phaseolorum* var. *caulivora*; and southern stem canker, caused primarily by *D. phaseolorum var. meridionalis* (Sinclair, 1999; Rossman *et al*., 2007).

However, these pathogens overlap in the type of diseases that they cause, often infecting several parts of the soyabean plants ranging from the roots to the seeds. *P. longicolla,* and *D. phaseolorum var. sojae* have been shown to cause stem cankers, and *P. longicolla* has been shown to cause pod and stem blight (Cui *et al*., 2009; Kmetz *et al*., 1979; Vidić *et al*., 1998). *P. longicolla, D. phaseolorum var. sojae, D. phaseolorum var. caulivora,* and *D. phaseolorum var. meridionalis* can all infect soyabean seed, and the first three of these fungi have been shown to cause seed rotting and molding with *P. longicolla* being the most virulent pathogen among the three (Asante *et al*., 1998; Kmetz et al. 1974; Kmetz *et al*., 1979; Sun *et al*., 2012).

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2.6.3 Management of Phomopsis seed decay of soyabean (PSD)

Many management strategies including cultural, biological, the use of chemicals, and resistant genes have been used to prevent, manage and control PSD.

2.6.3.1 Cultural approaches to PSD control

Planting of high-quality seeds that are free of PSD infection is the first step to ensuring reduced field infection and development of PSD. Therefore, the use of certified seeds is highly recommended. This may pose a challenge in Ghana where the use of farmers' own seed is more prevalent than the use of certified seed.

Field preparation is another cultural approach that could be used to reduce the incidence and severity of pod and stem blight, and PSD (Pathan *et al.,* 2009). Deep ploughing of previously infected fields to bury the debris or soyabean crop residue before the next planting helps to eliminate or reduce the source of inoculum and thereby reducing the risk of PSD infection (Roy *et al.,* 1994).

Another cultural approach to reducing the incidence and severity of PSD is crop rotation with non-legume crops. Continuous cropping of soyabean on the same piece of land enhances the build-up of Phomopsis/Diaporthe inoculum that in turns increases the incidence and severity of PSD. Rotating soyabean with cereals crops disrupts the build-up of inoculum and therefore, reduces the incidence and severity of pod and stem blight (*Sun et al*., 2013).

Soil nutrients especially potassium (K) and dolomite are said to hinder the development of PSD. Ito *et al*. (1994) reported of an inverse relationship between the concentration of potassium (K) in soil and leaves, and PSD incidence**.** Whereas Dolomitic lime which decreases the concentration of Potassium, increased the incidence of PSD whilst Jeffers *et al.,* (1982) observed that application of potassium (K) consistently lowered the incidence of mouldy seed but did not lower the incidence of PSD.

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2.6.3.2 Biological control of PSD

Biological control is environmentally sustainable and often, its inclusion in an integrated pest management strategy is promoted. Divilov, (2014) reported that the mycelial growth of IL12-DS-1, an isolate of *Diaporthe sojae* Lehman was inhibited by an Isolate of *Acremonium strictum*, and observed that *Acremonium strictum* parasited *Diaporthe sojae* isolate, L153. *Acremonium strictum* W. G though, a plant pathogen (Racedo *et al.*, 2013), has been reported to be an endophyte, a mycoparasite, and a saprobe (McGee *et al*., 1991; Domsch *et al.*, 2007; Rivera *et al.*, 2007).

2.6.3.3 Chemical control

Chemical control, mainly the application of fungicide can be used to control of PSD. Benomyl (Methyl (1-9butylamino) carbonyl)-1H-benzimidazol-2-yl can effectively control PSD when applied late pod developmental stage (Ellis *et al.*, 1974). Other fungicides including Dithane M-45, Zineb, Perrazole, Polyram combi, Mertect, and Afugan could be used to control PSD **(**Wu and Lee, 1985**).** Lee *et al.*, (1994) reported that spraying Benomyl at the R6 stage gave the highest reduction in *Phomopsis longicolla* and *Diaporthe Phaseolorum var. sojae* seed infection. Whilst Oh, (1998) reported that whole plant spraying is required to obtain significant seed infection reduction with benomyl.

2.6.3.4 The use of resistant genes

Finding resistance to pest and diseases is an important objective in breeding programmes and cultivar development. The use of resistant cultivars is considered the most effective means of disease, nematode and insect control (Fehr, 1991). The deployment of resistance to control diseases in plants is considered the most economically viable, and environmentally and ecologically friendly, and therefore, the most sustainable form of disease control.

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A number of sources of resistance to PSD have been identified from several resistant screening trials. These include Taekwamgkong (Sun *et al.,* 2012); PI417479 (Brown et al. 1987; Zimmerman and Minor, 1993); PI424324B (Smith and Nelson, 2011); Aksoy, PI209908, PI22787 and PI229358 (Minor *et al.*, 1995); PI82264 (Walters *et al.,* 1973); PI181550 (Athow, 1987); PI200501 (Ross, 1986); PI360841 (Brown *et al.,* 1987); PI42324B, PI458130 and PI 567381B (Smith *et al.*, 1993). Additionally, Li *et al*., (2015) reported 15 accessions including PI 189891, PI 398697, PI 417361, PI 504481, PI 504488, and PI 88490 in MG III; PI 158765, PI 235335, PI 346308, and PI 416779 in MG IV; and PI 381659, PI 381668, PI 407749, PI 417567, and PI 476920 in MG V to be resistant to PSD.

2.6.3.5 Markers linked to PSD resistant loci

A considerable number of genetic studies including mapping of resistant loci conferring resistance to PSD from a number of resistant sources have been carried out. The resistant loci of PI417479 was mapped to linkage group F using RLFPs markers (Berger and Minor, 1999). The mapping studies carried out using Random fragment length polymorphism (RFLP) markers had PI417479's resistance linked to the markers A708, and A162 on chromosomes 13 and 12, respectively (Berger and Minor 1999). Suli *et al.,* (2013) mapped the resistance of the Korean variety, Taekwang to two loci, PSD-6-1 and PSD-10-2 which occurred on chromosomes 6 and 10, respectively. The resistant loci, PSD-6-1 is flanked by the SSR markers Satt 100 and Satt 460 whilst the loci, *PSD-10-2* is flanked by the SSR markers, Sat_038 and Satt 243 (Suli *et al.*, 2013). It is noteworthy that the SSR marker, Satt460 is also linked to Asian soyabean rust resistant gene Rpp3 on the same linkage group, C (Monteros *et al.,* 2006).

Also, Suli *et al.,* (2013) proved that the linkage between PSD resistance and days to maturity where PSD resistance and days to maturity were mapped to the same chromosomal location flanked by the same markers. The quantitative trait loci (QTL), *Mat-10-3* associated with days

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to maturity was mapped to chromosome 10 and flanked by the SSR markers Sat_038 and Satt 243, the same markers that flanked the QTL, PSD-10-2, conferring resistance to PSD (Suli *et al.,* 2013). Similarly, Mat-6-2 also associated with days to maturity was mapped to chromosome 6, and flanked by Satt134 and Satt100. The same markers at the same chromosomal region harbouring the QTL *PSD-6-1* which confers resistance to PSD (Suli *et al.*, 2013). Based on qualitative analysis, the resistance in PI80837 was mapped to chromosome 14 flanked by the SSR markers, Sat_177 and Sat_342, whereas the PSD resistance loci in MO/PSD-0259 was mapped to chromosome 13, flanked by the SSR markers, Sat_317 and Sat_120 (Jackson *et al.,* 2009).

2.6.3.6 Inheritance of PSD

PSD is a highly heritable trait recording broad sense heritability of 0.44 and 0.83, and narrow sense heritability of 0.42 and 0.49 (Anderson and Buzell, 1985; Zimmerman and Minor, 1993). Resistance to PSD is conferred by a dominant gene with sources of resistance harbouring a single or two genes. Allelsim test showed that PI417479 habours two dominant complimentary genes which confers strong resistance to PSD (Zimmerman and Minor, 1993; Minor *et al.*, 1995). Studies carried out by Smith *et al.* (2008) confirmed that PI360841 also harbours two dominant complimentary genes. However, PSD resistant line, MO/P SD-259 harbours a single dominant gene (Jackson *et al.,* 2005).

2.6.4 Bacterial leaf pustule

Bacterial leaf pustule, caused by *Xanthomonas axonopodis* pv. *glycine,* is among the major diseases in several major soyabean (*Glycine max*L.) producing countries (Wrather *et al.*, 2001). It is one of eight documented diseases affecting soyabean in Ghana and the only bacterial disease among the eight (Offei *et al.,* 2008). On susceptible cultivars, bacterial leaf pustule is reported to cause yield loss in the range of 15-50% (Prathuangwong and Amnuaykit, 1987).

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Yield loss due to bacterial leaf pustule depends on the level of incidence and severity. Bacterial leaf pustule severity of 10.1-25, 25.1-50, and 50.1-75% could cause corresponding yield losses of 15-21, 38 and 53, respectively (Shukla, 1994).

Yield loss due to bacterial leaf pustule results from degradation of chlorophyll content of leaves as lesions (yellow pale spot) coalesces to form bigger spot and under severe infection may result in premature senesces and defoliation of leaves (Hartman and Murithi, 2017). When infection occurs at the early stages of plant growth, susceptible genotypes may suffer premature defoliation and death. In which case, seed weight, a key component of yield is reduced. The causal agent of bacterial leaf pustule, *Xanthomonas axonopodis pv. glycine* survives or overwinters on the debris; leaves, pods and stems, as well as the seeds of infected plants (Khaeruni *et al.*, 2007). These attributes have the propensity to make bacterial leaf pustule endemic in areas of continuous soyabean production on the same piece of land whilst spreading to new areas through the distribution of untreated seeds (Khaeruni *et al.*, 2007). On infected soyabean fields, the pathogen spreads from one plant to another or to nearby fields through rain splashes and wind (Goradia *et al.*, 2004).

2.6.4.1 Management of bacterial leaf pustule

Cultural practises including crop rotation through which the overwintered inoculum (pathogen) is avoided, and incorporating residue of infected plants through tillage into the soil to minimise the amount of inoculum available to infect new soyabean crops could be used to manage the bacterial leaf pustule disease. Chemicals have been extensively used to control bacterial leaf pustule. These include the chloromycitin, streptocycline and fytolan (Copper oxychloride) (Singh and Jain, 1988).

Induction of resistance to bacterial leaf pustule susceptible soyabean lines is another approach that has been developed for the control of bacterial leaf pustule (Khaeruni *et al.*, 2010) as an

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alternative to chemical control. Biological control is deemed ecologically and environmentally friendly as opposed to chemical control which causes damages to the environment and less sustainable because, the persistent use of chemicals leads to the emergence of resistant *Xanthomona axonopodis pv. glycine* strains (Khaeruni *et al.*, 2010). Khaeruni *et al.*, (2018) evaluated biological fertilizers (biofresh) composed of three rhizobacteria types, Bacillus ceresus ST21b, B. subbtilis ST2le, Serratia sp 2229A in different formulations and concluded that a solid formulation of biological fertilizer in combination of compost made from soyabean residue was effective at increasing the resistance of soyabean plants to bacterial leaf pustule.

Increased resistance to bacterial leaf pustule arose from increased salicylic acid and peroxidase content of plants treated with the biological fertilizer (Khaeruni *et al.*, 2018).

The use of resistant genes for the control of plant diseases is the most efficient approach and the control of bacterial leaf pustule is not an exception. Several sources of resistance to bacterial leaf pustule of soyabean has been reported. Some of which include Danbaekong, $SS_{2\text{-}2}$, and Pi96188 (Kim et al, 2010; Kim *et al.*, 2011) as well as CNS soy (Hartwig, 1951).

2.6.4.2 Molecular resources available for management of bacterial leaf pustule

A number of mapping studies have identified markers linked to the bacterial leaf pustule resistant gene that could be used for Marker Assisted Selection to facilitate bacterial leaf pustule resistant cultivar development. Deploying SSR markers, Narvel *et al.* (2001) mapped the bacterial leaf pustule resistant gene, Rxp to linkage group D2 flanked by the SSR markers, Satt372 and Satt014. On other hand, Kim *et al.* (2010) using RIL and NIL population fined mapped the Rxp gene resistant gene on chromosome 17 to 33 kb region flanked by the SSR markers, SNUSSR17_ and SNUSSR17_12. They found three genes within the 33 kb region and suggested two genes which codes for membrane protein and zinc finger protein as candidate genes offering resistance to bacterial leaf pustule (Kim *et al.*, 2010).

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In another study, a source (P196188) resistant to bacterial leaf pustule, had its resistant gene mapped to chromosome 10 linked to the SSR marker, Sat_108 (Kim et al. 2011). Yang *et al.*, (2011) used available genomic data on the bacterial leaf pustule resistant gene, Rxp to develop markers and identified the marker Rxp17-700 which was expected to be tightly linked to Rxp gene. This presents a list of markers that could be assessed, validated and used for the development of bacterial leaf pustule resistant cultivars.

Some level of the understanding of the nature of inheritance of the Rxp resistant gene has been reported. The nature of inheritance of resistance harboured by PI96188, and CNS soy were reported to be single recessive genes (Hartwig-1951; Kim *et al.,* 2011). It is note-worthy that with exception of Yang *et al.*, (2011) who developed Rxp markers using Rxp genomic sequence data, all other mapping studies stated here were carried out using bi-parental populations.

2.7 Genome-wide association studies (GWAS) and Genotype-by-sequencing (GBS)

Marker identification is key to marker-assisted selection which aids with the speed and accuracy of selection in breeding programmes. Conventionally, marker identification is achieved through quantitative trait loci (QTL) mapping based on populations. However, population- based mapping is time consuming, expensive and has limited resolution due to limited number of meiotic events accumulated over the period of population development (Jannink and Walsh, 2002). The limitations associated with population or interval-based QTL mapping are overcome with genome-wide association studies (GWAS) which utilises natural occurring variation in assembled germplasm to detect loci associated with desired traits was invented (Hall *et al.,* 2010). Assembled germplasm are often a collection of unrelated genotypes which come along with large number of meiotic (crossing over) events since their last common progenitor (Sonah *et al.,* 2013). Since development of a population is not a

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requirement for genome-wide association studies, time, and cost of developing population are saved (AlMarskri, 2012).

The advent of high-through put genotyping including genotyping-by-sequencing (GBS) provides high marker coverage on individuals in an assembled germplasm. Genome-wide association studies use the high maker coverage to scan the entire genome of individuals in search of genomic regions in linkage disequilibrium that are associated with traits of interests. In soyabean, many lines have been genotyped at higher marker coverage with the 50K SNP array and GBS (Song *et al.,* 2013; Sonah *et al.,* 2013). With GWAS, SNP markers linked to some important diseases of soyabean were identified including bacterial leaf pustule (Chang *et al.*, 2016); *Sclerotinia sclerotiorum* (Wei *et al.*, 2017), and sudden death syndrome (Wen *et al.*, 2014). Markers linked to other traits including 100-seed weight (Contreras-Soto *et al.,* 2017), seed oil content, and seed protein (Li *et al.,* 2018) were also identified through GWAS.

Genotyping-by-sequencing (GBS) offers a more flexible and an efficient approach to genome-wide level identification of genetic variation in crops (Fu and Peterson, 2011; Peterson *et al.,* 2014). GBS yields high-density genotypic information (Poland, 2012) on samples which is suitable for genome-wide association studies, and genomic selection (Poland and Rife, 2012; He *et al.,* 2014; Gore *et al.*, 2009; Yu and Buckler, 2006). GBS method provides the numerous generations of ancestral recombination events required for direct mapping of variation in natural populations without generating populations through crosses (Yu and Buckler, 2006).

Harnessing the advantages of GBS and GWAS for identification and use of markers will bring efficiency to soyabean breeding programmes in Ghana. This will translate into development and release of cultivars that combine high productivity with resistance to prevailing abiotic and biotic stresses.

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CHAPTER THREE

3.0 A survey of soyabean diseases across production centres in the Transition, Guinea and Sudan Savannah ecologies of Ghana

3.1 Introduction

Soyabean cultivation in Ghana has seen consistent rise largely due to increases in area under production (MoFA-SRID, 2016). Additionally, the ongoing "Feed the Future" programme in which soyabean is being targeted to alleviate poverty and eradicate malnutrition (https://www.feedthefuture.gov/country/ghana) is promoting the cultivation and utilisation of soyabean.

Globally, diseases are a major constraint to soyabean production (Hartman, 1999). Offei *et al*. (2008) published a catalogue of diseases of soyabean in Ghana. This compilation of diseases did not indicate distribution of the stated diseases across the soyabean production centres. Also, the incidence and severity of diseases catalogued were not indicated, making it difficult to assess their relative importance.

In the past, Ghana largely relied on IITA for soyabean germplasm. However, with the inception of the Soyabean Innovation Laboratory (SIL) project in Ghana, the USA has also become a direct source of soyabean germplasm to the country. Myriad of soyabean diseases have been reported in the USA that have not been observed in Ghana. The spread of soyabean production to new areas, receiving germplasm from IITA and the USDA, in addition to projected climate change scenario creates a ripe condition for the introduction of new pathogens in to the country whilst existing ones may become more virulent. It is therefore important to routinely survey soyabean production centres in the country for diseases, their incidence and severity.

Knowledge of crop diseases and their distribution in a country or across production zones is key to developing effective strategies for their control. Information on the distribution,

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incidence and intensity of crop diseases is adduced through disease surveys (Elizondo *et al.*, 2014; Kebede and Gidesa, 2017). Therefore, surveying soyabean production centres for diseases will aid in formulating appropriate disease management strategies including breeding for resistance.

The goal of this research activity was to identify dominant diseases of soyabean to inform the development of management strategies including the use of resistant genes.

The specific objectives were to;

1. identify diseases on cultivated soyabean fields in major production areas in Northern, Upper East, Upper West, Ashanti, and Brong Ahafo regions
2. estimate the incidence and severity of identified diseases using visual assessment; and
3. identify new and unidentified diseases under laboratory conditions.

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3.2 Materials and Method

3.2.1 Study area and time

A survey was undertaken in the major soyabean production areas in Ghana, most of which are in the Northern region. Also, production areas were selected to account for some of the different ecological zones in which soyabean is cultivated in Ghana. The study was carried out from the third week in September to the first week of October 2016. This period was chosen to ensure that plants were in advanced reproductive stage. Most soyabean fields in the three regions of northern Ghana are established between the last week of June and the first week of July. This sowing period ensures that maturity and harvesting occur in the months of October and November.

Districts of interest were pre-selected based on knowledge of soyabean production adduced from scientists at CSIR-SARI. However, Ejura and Wenchi were selected because they occur within a different agro-ecology. Both Wenchi and Ejura occur in the forest savannah transition belt and therefore have bimodal rainfall pattern whilst the remaining locations are found either in the Guinea savannah or the Sudan savannah, both of which have monomodal rainfall pattern. These two districts have higher annual rainfall and lower mean annual temperature than the remaining districts surveyed. In total, fifteen (15) locations in nine districts/ municipalities were visited for the selection of soyabean farms for disease survey. Most of the locations including Malzire, Sang, Karaga, Gushegu, Akukayili, Tingoli, Seripe, and Damango constituting eight out of a total of fifteen locations surveyed were located in the Northern region. Wa and Tumu were the only two locations surveyed in the Upper west.

Three locations, Navrongo, Binduri and Manga were surveyed for soyabean diseases in the Upper east region. In the Ashanti region, only one location, Ejura, was surveyed. Similarly, Wenchi was the only the location surveyed in the Brong Ahafo. The selection of Ejura and

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Wenchi was because they represent an entirely different agro-ecology. Also, scientists at SARI have begun multi-location evaluation trials in these areas with the hope of promoting soyabean production among farmers at Ejura and Wenchi

3.2.2 Field selection

Selection of locations and farms within districts were carried out with the assistance of staff of the Ministry of Food and Agriculture (MoFA) within the selected districts. Prior arrangements were made with district officers of MoFA who know soyabean farmers and their soyabean fields. Upon arrival at a location, soyabean farms were randomly selected with the assistance of MoFA officers. At every location, at three farms at 1 km or more apart were selected for the survey.

3.2.3 Identification of diseases on selected fields

Selected fields were first traversed to identify the prevailing disease(s). The compendium of soyabean diseases and pests (Hartman *et al.*, 2015), and a hand lens (Opticron, 23 mm doublet) were used to identify diseases on the field through visual assessment. Pictures displaying the symptoms and signs of various diseases in the compendium of soyabean diseases and pest were compared to those observed on the field. A hand lens of 10x magnification was used to magnify signs and symptoms for confirmation of diseases on the field. For diseases that could not be identified on the field, plant parts harbouring symptoms and or signs of such unidentified diseases were harvested into zip lock bags and placed in an ice chest for transportation to the pathology laboratory at SARI, Nyankpala, for identification.

3.2.4 Sampling method

Sampling units within farms was based on the method described by James (1971) and modified by Elizondo *et al.*, (2011). Two diagonal transects was made across each selected farm. Plants for disease assessment were selected at 5-10 m interval along the diagonal transects based on

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the size of the farm. At each interval, 20 plants were randomly sampled for estimation of disease incidence 5 of which were assessed for disease severity. Disease incidence was estimated by counting the number of diseased plants and expressed as a percentage of the 20 randomly selected plants at each 5-10 m interval along the diagonal. The estimation of disease incidence is mathematically written as indicated:

$$\text{Incidence (\%)} = \frac{number\ of\ diseased\ plants}{total\ number\ of\ plants\ (infected\ and\ un\ infected)}\ x\ 100$$

Severity of identified diseases were visually scored using standard diagram displaying severity on a1-9 scale (Subrahmanyam *et al*., 1982) where 1=no lesions; 2= few lesions in the lower canopy, 3= lesions on most leaves in the lower canopy; 4= lesions on most leaves on lower canopy and few lesions on upper canopy; 5= Both lower and upper canopy are completely covered by disease; 6= lesions cover leaves, petioles and pods with 5; 7= 25% defoliation; 8= 50% defoliation; 9=complete defoliation/ death of plant.

3.3 Confirmation of *Diaporthe sp.* causing soyabean seed decay in Ghana

3.3.1 Sampling of diseased tissues or organs

Blighted pods and stems showing pycnidia were harvested into Ziploc bags wherever they were encountered during the survey. Matured pods were manually threshed for visual assessment of seeds. The seeds were visually assessed for symptoms and signs of seed decay including wrinkling, shrivelling, molding, mottling, discolouration, cracking and splitting.

Seeds with any of the above symptoms were selected from the seed lot and cultured.

At SARI's experimental field in Nyakpala, soyabean fields were monitored until maturity. Upon physiological maturity, pods were sampled from plants in both experimental and non-experimental soyabean fields. Pods obtained from Nyankpala were also manually threshed and the resulting seeds visually assessed as those obtained from disease incidence survey. In all,

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150 pods yielding 282 seeds were visually assessed for signs and symptoms of seed decay including moldiness, mottling, distortion, shrinkage, and discolouration. Seeds and pods with signs and symptoms reminiscent of seed decay due to *Diaporthe sp*. were cultured for the development and isolation of pathogens for further analysis.

3.3.2 Isolation of disease-causing organism

Potato dextrose agar (HIMEDIA) was modified with lactic acid to inhibit bacteria growth during pathogen isolation from soyabean seeds. PDA of 15g was suspended in 1litre of distilled water. The resultant was heat sterilised by autoclaving at 121°C for 15 minutes. The prepared medium was acidified by amending it with 50% lactic acid (VWR International) to adjust the pH to 4.8. The acidified PDA (APDA) was poured into 9 cm- diameter petri dishes. In some cases, diseased seeds were first cultured on water agar HIMEDIA and fungal growth on water agar was sub-cultured on APDA. Symptomic seeds were surface disinfected with 70% ethanol, sterilised in 1 % sodium hypochlorite for 2 minutes and then rinsed in distilled water twice. Sterilised seeds were plated on the prepared APDA medium and incubated at a temperature range of 25-30 ℃ and 12 hr light/ 12hr dark. Cultures were monitored regularly for fungal growth. Mycelia emerging out of seeds was sub-cultured to obtain pure isolates of various fungi growing from the plated seeds. Pure isolates were incubated for 15 days with regular examination of morphology (colour, macrostructures/fruiting bodies) and microscopic features (spores).

3.3.3 Microscopy

Cultures which morphology conformed to fungi of interest were separated from other plates and further confirmed through microscopy for mycelia and spore architecture. PDA microslides were prepared with mycelia of isolated pathogen and incubated at 28 ± 2 ^{o}C for 7 days. Cover slides of microslides and media were examined for microscopic feature using

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Leica DM 1000 microscope at a magnification of 40 × and 60 ×.

3.3.4 Primer Design

The National Centre for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/nucleotide/) was searched for deposited sequences of members of the Diaporthe-Phomopsis complex of fungi. Partial sequences of 28S Ribosomal RNA genes of isolates of *Diaporthe phaseolorum var sojae*, *Diaporthe phaseolorum var caulivora* and *Phomopsis longicolla.* Nucleotide sequences were used to design primers for each tentatively identified fungi by loading obtained sequences into the software, primer3 (Untergasser *et al*., 2012). Parameters including annealing temperature range (57-60 ℃), desired number of nucleotides (18-25) and GC content (40-60 %) were set for the software for the generation of primers from nucleotide sequences of ribosomal DNA of suspected fungi. The designed primers were sent for synthesis at Inqababiotec in South Africa.

3.3.5 DNA extraction

Each tentatively identified pathogenic fungus was cultured in four petri dishes representing four replicates of each fungus. Matured fungi mycelium of 15 days old were used for the DNA extraction. The fungi mass was scraped off the media using a spatula. The scraped fungi mass was grounded in a mortar with a pestle and subjected to DNA extraction using the protocol by Doyle & Doyle, (1990) with slight modification.

3.3.6 DNA quality test

The quality of extracted DNA of each sample was quality checked by performing electrophoresis in a 2 % agarose gel. Samples with no bands, smears or very faint bands were discarded and extractions carried out on them again to obtain very good bands for further analysis.

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3.3.7 PCR amplification and gel electrophoresis

Designed primers synthesised by Inqaba biotec were used to amplify extracted DNA of fungi. PCR amplification was carried out in a 10µL reaction volume, consisting of 5µL of 2X one taq PCR master mix, 3µL of nuclease free water, 1 µL of forward and reverse primer and 1 µL of DNA. The PCR was carried out using the ABI thermal cycler (Applied Biosystems by life Technologies) with the conditions; initial denaturation at 94 °C for 3min, 35 cycles of denaturation at 94 °C for 30 secs, annealing at X °C for 30 secs (depending on primer) and extension at 72 °C for 30 secs. It was finally extended at 72 °C for 7 min and held at 4 °C. To test the fidelity of primers designed and synthesised for each fungus, soyabean DNA was taken through the amplification process using designed fungi primers. Additionally, primers designed for a specific fungus were tested on other suspected fungi as a way of determining if DNA amplification of primers were specific to the fungi to which they were designed.

3.3.8 Polyacrylamide gel electrophoreses of amplified DNA

Amplified products were electrophoresed using a 6 % polyacrylamide gel on a horizontal PAGE system (Cleaver Scientific). Band separation was carried out at a voltage of 120 V for three hours. A peristaltic pump (Williamson manufacturing company LTD, 201-AQUA-SC050) was used with the PAGE system to enhance even distribution of running buffer and to maintain equilibrium in conductivity of the buffer. After electrophoresis, the gel was stained with ethidium bromide for 30min and visualized using a UV trans-illuminator (UVP, Benchtop UV transilluminator). A photograph of the gel was then taken for further analysis.

3.3.9 Sequencing of amplified DNA products

Photographed gels were scored for bands and position of bands for each fungus. Fungi which DNA amplification by its primer was specific to it alone and yielded the same amplicon size for all its four replicates were selected as putatively confirmed. The PCR product (amplicon) and primer sequences of confirmed pathogenic fungi were sent to Inqaba biotec for sequencing

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of the amplicon in order to obtain partial sequences of the confirmed pathogens using the Sanger sequencing method.

Nucleotide sequences of the amplicon were loaded into the nucleotide Basic Alignment Search Tool (BLAST) within the online database, NCBI in search for similar sequences for the identification of the fungus isolated from infected soyabean seeds. Sequences of isolates, mainly, *Diaporthe* and *Phomopsi*s species obtained from NCBI were aligned with amplicon sequence and used to construct a phylogenic tree based on the weighted neighbour joining tree in the Darwin software (Perrier and Jacquemoud-Collet, 2006**).** Sequence dissimilarities were calculated at 1000 bootstraps or iterations.

3.3.10 Data collection and statistical analysis

Disease incidence and severity scores recorded for each farm were tabulated for each location. For each disease identified, the data from farms were used as replicates for location. The data was then subjected to analysis of variance (ANOVA) using the GenStat software (version 18). Where differences in diseases incidence, and severity differed significantly between locations, means were separated using Student-Newman-Keuls (SNK) method.

3.4 Results

3.4.1 Results of disease survey

A total of 60 fields were surveyed at 15 locations spread across five regions and three agroecologies. Ten diseases were identified during field survey (Fig 3.1). These diseases were cercospora leaf blight (A) due to *Cercospora kikuchii*; frog eye leaf spot due (F) cause by *Cercospora Sojina*; target spot (G) due to the pathogen, *Corynespora cassiicola*; bacterial leaf pustule (B) due to *Xanthomonas campestris* pv. *glycine;* downy mildew (H) due to *Peronospor manshurica;* leaf blight (D) due to *Rhizoctonia solani;* brown spot (E) due to *Septoria glycine;* soyabean mosaic (C) due to virus, soyabean seed decay(I) and alfafa mosaic virus (J).

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Additionally, there were evidence of sudden death syndrome, root rot, charcoal rot, and web blight diseases but they were localised on isolated fields at isolated locations.

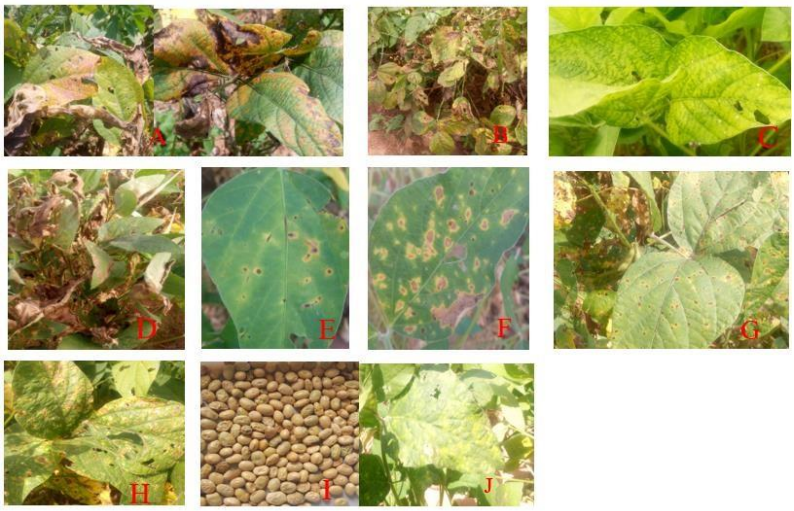

Fig 3. 1: Diseases identified during disease survey**:** A (Cercospora leaf blight); B (Bacterial leaf pustule); C (Soyabean mosaic virus); D (Rhizoctonia leaf blight); E (Septoria brown spot); F (Frog eye leaf spot); G (Target spot); H (Downy mildew); I (Seed decay); J (Alfalfa mosaic virus).

The distribution, incidence and severity of four diseases that were most consistent across locations are indicated in Table 3.1.

Incidence of bacterial leaf pustule due to *Xanthomonas campestriz pv. glycine* ranged from a locational average of 15% for Wenchi to 84% for Malzire in the Yendi Municipality. The incidence of bacterial leaf pustule in Malzire was significantly higher than that of Sang in the Yendi municipality; Akukayili in the Tolon district; SARI research station in the Wa municipality; Tumu in the Sissala west district, and Navrongo. However, the 84% incidence of bacterial leaf pustule recorded in Malzire was statistically comparable to that of Tingoli in the Tolon district; Gushegu and Karaga in the Gushegu-Karaga district; Seripe in the Bole-Bamboi district; Damongo in the Gonja west district, and Manga in the Bawku district. The severity of bacterial leaf pustule was highest in Malzire, and lowest in Wenchi. Malzire, Karaga, Gushegu, Tingoli and Akukayili, recorded statistically comparable levels of bacterial leaf pustule severity.

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The incidence of Cercospora leaf blight ranged from 22.5% to 80.1%. Karaga recorded Cercospora leaf blight incidence of 80.1% which was statistically similar to that of Gushegu, Akukayili and Navrongo but higher than the remaining locations. The least incidence of Cercospora leaf blight was 22.5% which occurred at Tingoli. Similarly, severity of Cercospora leaf blight was highest at Karaga which recorded severity was higher than the remaining 14 locations. The next location with high severity was Akukayili. Binduri, Gushegu Navrongo, and Manga were similar in Cercospora leaf blight severity. Cercospora leaf blight due to *Cercospora kikuuchi* did not occur at Wenchi and Ejura.

Karaga, Gushegu, Damongo, and Binduri recorded frog eye leaf spot disease incidence of 82%, 79.7%, 80%, and 72.3%, respectively. Although, Karaga recorded the highest incidence of Frog eye leaf spot, it was statistically comparable to that of Gushegu, Damongo and Binduri.
There was no occurrence of frog eye leaf spot at Seripe in the Bole-Bamboi district, and at Tingoli in the Tolon district. Damongo, and Gushegu recorded frog eye leaf spot severity of 4.4 and 4.2, respectively. The two locations were similar in frog eye leaf spot severity and statistically higher than all other locations surveyed in this study. The least incidence and severity of frog eye leaf spot occurred at Ejura and Wenchi.

Though it occurred at lower levels at most locations, viral diseases of soyabean occurred at all 15 locations that were surveyed. Ejura recorded the highest incidence of soyabean mosaic virus (8.40%). This was followed by Wenchi where average incidence of viral diseases of soyabean was 38.2%. The least incidence of soyabean mosaic virus occurred at Damongo.

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Table 3.1:Incidence and severity of diseases identified during survey

District	Location	Bacterial leaf pustule *(Xanthomonas campestris* pv. *Glycine)*		Cercospora leaf blight *(Cercospora kikuchii)*		Frog eye leaf spot (*Cercosporasojina)*		Mosaic virus
		DI (%)	DS	DI (%)	DS	DI (%)	DS	DI (%)
Yendi	Malzire	84.7^{a}	4.9^{a}	61.1e	3.5^{c}	50.1d	2.8cd	9.57
Yendi	Sang	46.27^{c}	2.0^{bc}	32.1g	1.7^{f}	50.8d	2.7cd	11.8
Karaga/Gushegu	Karaga	66.6^{abc}	3.4^{abc}	80.6a	5.3^{a}	82.3a	3.5b	5.4
Karaga/Gushegu	Gushegu	60.1^{abc}	3.9^{abc}	75.2c	3.4^{c}	79.7a	4.4a	10.4
Tolon	Akukayili	49.33^{bc}	3.9^{ab}	80.1ab	4.5^{b}	13.2f	1.4h	21.6
Tolon	Tingoli	65.8$_{abc}$	3.3^{abc}	22.5h	1.9^{f}	-	-	5.2
Bole Bamboi	Seripe	63.2^{abc}	2.9bc	67.9d	2.3^{e}	-	-	4.4
Gonja west	Damango	70.1^{abc}	1.83^{bc}	55.3f	1.87^{ef}	80.8a	4.2a	3.0
Wa Mun.	Wa	53.0^{bc}	2.3^{bc}	39.6g	1.6^{f}	42.3e	1.7gh	2.38
Sissala east	Tumu	43.3^{c}	1.9^{bc}	39.4g	1.5^{f}	38.8e	2.1fg	6.2
Binduri	Binduri	69.10 ^{abc}	2.1^{bc}	65.1e	3.3^{c}	72.3a	3.2bc	7.46
Kassana Nakana	Navrongo	52.0^{bc}	1.7^{c}	77.2ac	3.3^{c}	49.2d	2.4df	5.0
Bawku	Manga	74.7^{ab}	2.7^{bc}	74.3c	2.8^{c}	63.c	2.0fg	9.0
Ejura	Ejura	18.3^{d}	1.5^{c}	-	-	4^{h}	1.3^{h}	80.4
Wenchi	Wenchi	15.3^{d}	1.4^{c}	-	-	7^{h}	1.2^{h}	38.17

Note**:** numbers in the same column followed by the same alphabet are statistical similar at p=0.05
DI=disease incidence; DS=disease severity

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3.4.2 Results of pathogen confirmation study

3.4.2.1 Colony characteristics

From the 282 symptomatic seeds plated from the Nyakpala seed lot, 40% o had fungal growth by what was morphologically determined to be *Diaporthe sp.* [Lehman]. White aerial mycelium emerged from plated seeds as indicated in plates D&E in Fig 3.2. The growth was not fluffy, but slightly raised and yellowish reverse plate colour. The growth was slow, taking more than 15 days to completely cover the 9cm-diameter petri dish. A matured pure culture had a whitish mycelium with dark visible oblong fruiting structure forming after 10 days known as pycnidia. The pycnidia emerged from the aged culture with shot necks. Colony colour changes from white to gray which darkens with time as indicated in plate F of (Fig 3.1). the reverse plate however showed a yellowish colour in the middle of the culture. Microscopic examination showed that both alpha and beta conidia were produced as shown in plate B of (Fig 3.1). Puncturing pycnidia with a needle on a moist microslide under microscope showed oozing of abundant spores.

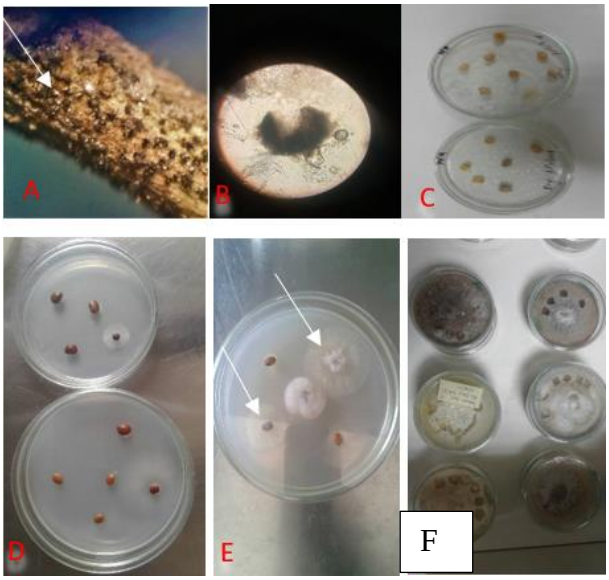

Fig 3.1: Pycnidia on soyabean stem(A); (B)Microscopic view of punctured pycnidia oozing spores; (C) culturing diseased seeds in water agar; (D) soyabean seeds plated on APDA with initial growth of *Diaporthe sp;* (E) A plate showing the distinction between diaporthe sp colony as indicated by the two arrows from *fusarium sp* colony which is the cotton like growth between the two *diaporthe sp* colonies (F) Displays the changes in colour of *diaporthe sp* as the culture ages (G) Pycnidia producing culture.

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3.4.2.2 Molecular identification of causal pathogen

SSR primer (MB-DpS1) with the pair, F-GGCCCGAGTTGTAATTTGCA; RCGGTCTCTGGCCGGTATTTA designed from the partial sequence of 28S Ribosomal RNA gene of *Diaporthe phaseolorum var sojae* isolate, PS/KR3 successfully amplified the DNA of the pathogen that infected 40% of the seeds plated with the same band size, an indication of the high fidelity of the primer, MB-DpS1. The Genebank ID of isolate PS/KR3 is JF704169.1(https://www.ncbi.nlm.nih.gov/nuccore/JF704169.1). The Primer pair of MBDpS1, amplified all 4 replications of the pathogen that was morphologically characterised as *Diaporthe sp.* but not the check DNA (Fig 3.3).

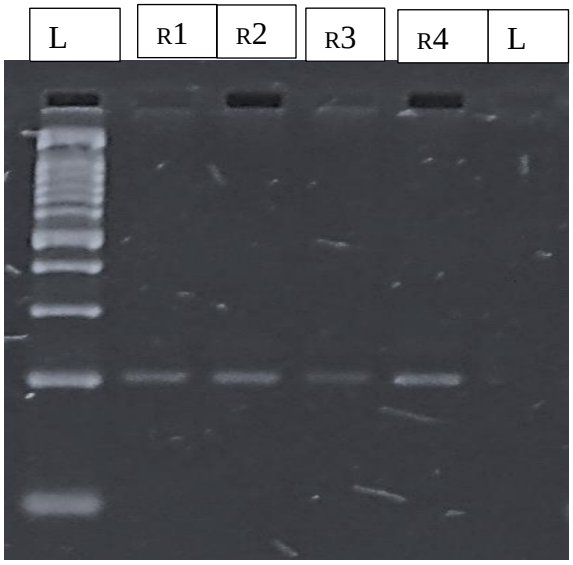

Fig 3. 2: Gel electrophoresis of 4 replicates of amplified 28S RNA gene of MTB-1 and the check (C).

The sequenced amplicon yielded 164 base pairs (bp) in size. At a query coverage of 99% and expected value (E-value) of 8e-74, the DNA sequence of the amplicon had a 99% match with more than 25 *Diaporthe/Phomopsis* species including *Diaporthe* isolates CBS.180.55; *Dioporthe sojae* isolate CBS.179.5; *Diaporthe Sp*.NVL.2017; *Diaporthe longicolla* isolate ER1679 and *Diaporthe sp*. HRP-2018b.

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Phylogenetic analysis placed the 15 isolates of *Diaporthe/Phomopsis* species into three major clades (Fig 3.4).

The constructed phylogenetic tree placed the Ghanaian isolate of *Diaporthe sp* (MTB-1) in major clade I, made up of three *P. longicolla* isolates, two of which were not genetically distinct and two *Diaporthe* species, one of which is a *Diaporthe sojae* (CBS 179.55) which occurred in the same subclade with MTB-1(Fig 3.4). The only other *Diaporthe sojae* isolate, CBS 180.55 occurred in major clade II with three isolates simply labelled as *Phomopsis* species, and one *Phomopsis longicolla* isolate (Fig 3.4).

The major clade III contains four isolates two of which were not genetically distinct and one *Diaporthe phaseoolorum var caulivor* isolate *(720*) which was the most genetically distant isolate in major clade III (Fig 3.4). In all, no major clade was made of isolates of a single species. Most major clades contain sub-clades contain both *Phomopsis/Diaporthe* species.

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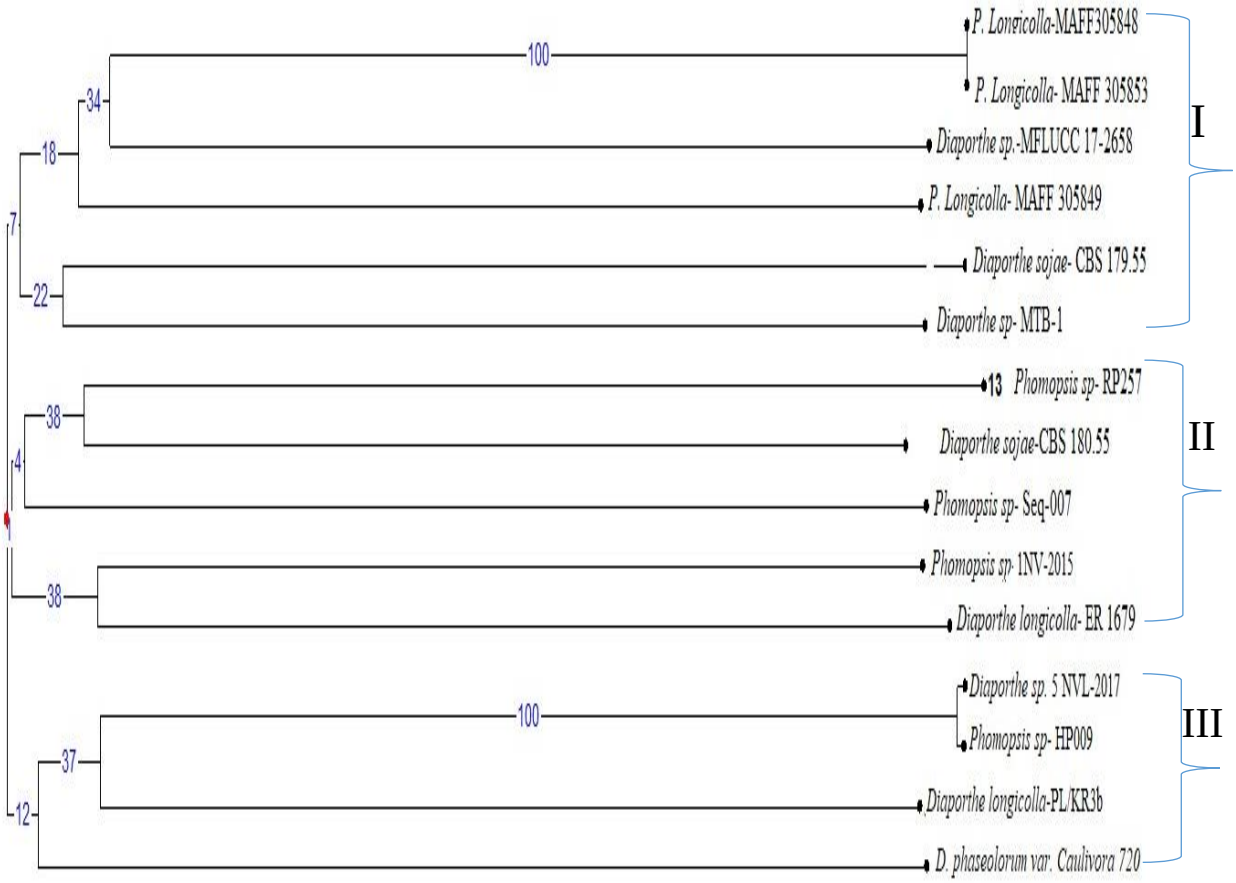

Fig 3. 3: A phylogenetic tree showing the relatedness of the isolated fungus (MTB-1) to 14 isolates of *Diaporthe* and *Phomopsis* species obtained from NCBI

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3.5 Discussion

Ten diseases of soyabean including, cercospora leaf blight (*Cercospora kikuuchi)*, frog eye leaf spot (*Cercospora sojina*); target spot *Corynespora cassiicola)*, bacterial leaf pustule (*Xanthomonas campestris* pv. *glycine)*, downy mildew (*Peronospor manshurica)*, leaf blight (R*hizoctonia solani)*, brown spot (*Septoria glycine)*, soyabean mosaic virus, alfafa mosaic, and soyabean seed decay due to *Diaporthe sp*. of fungi were revealed by the survey.

Among the diseases of soyabean identified, only Bacterial leaf pustule and soyabean mosaic virus occurred at all locations surveyed. The incidence of bacterial leaf pustule recorded in this study ranged from 15.3% to 84.7%. This result was slightly higher than what was reported by Zinsou *et al.,* (2015) who recorded bacterial leaf pustule incidence in the range of 15.8% to 70% during a survey of bacterial leaf pustule in the Guinea savannah agro-ecology of Benin. The occurrence and severity of bacterial leaf pustule at Malzire, Karaga, Gushegu, Tingoli and Akuyili were higher than all other locations surveyed. Although, Damongo, Binduri and Manga recorded high incidence of bacterial leaf pustule, the corresponding severity scores were lower.

All the locations with high bacterial leaf pustule severity occurred in the Guinea savannah agro-ecology whilst the locations that had high incidence but lower severity occurred in the Sudan savannah agro-ecology except Damongo, which also lies in the Guinea savannah zone.

The infection and development of *Xanthomonas campestris* pv. *glycine*, causal agent of bacterial leaf pustule is favoured by humid and warm environmental conditions (Bradbury, 1986; Heikmap *et al.,* 2014). Bacterial leaf pustule has therefore been described as an important disease to soyabean production in the tropics (Hokawat, 1993) especially in the Savannah regions where high humidity and temperature characterise the production season. For instance, rainfall, mean temperature and relative humidity of 846.8mm, 28.1 ºC and 78%,

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respectively were recorded for the 2016 cropping season (June to October) in the Tolon district which harbours Akukayili and Tingoli.

The high incidence but lower severity scores obtained at Damongo, Binduri and Manga may be due to late onset of the disease or absence of conducive environmental conditions arising from the different times of planting to support development of the pathogen. Resistant and tolerant cultivars could have equally been the reason for the high bacterial leaf pustule incidence but lower severity at Damongo, Binduri and Manga but most farmers across the surveyed locations claimed to be cultivating the same varieties obtained from the Savannah Agriculture Research with Jenguma being the most cultivated variety.

The least bacterial leaf pustule incidence and severity scores were recorded at Ejura (18.3% & 1.5) and Wenchi (15% &1.4) which occurs in the Forest transition zone of Ghana with bimodal rainfall, and therefore has two cropping seasons in a year. Most of the fields surveyed at Wenchi and Ejura were planted in the second cropping season, and therefore most plants were at the vegetative state or at the R2 stage. The stage of soyabean plants at Wenchi and Ejura stood in sharp contrast to stage of soyabean plants surveyed from remaining locations which ranged from R5-R6 growth stages. For instance, at Ejura, of the five soyabean fields surveyed, only two fields were at the R5 stage. The two farms at the R5 stage were Genotype-Environmental trial plot carried out by SARI'S soyabean unit at Ejura. The remaining three farms were ADRA soyabean –cereal intercropping demonstration trial in which soyabean plants were at yet to reach the flowering stage. Also, there were fewer soyabean farms at Wenchi and Ejura compared to the other locations surveyed.

Cercospora leaf blight caused by *Cercospora kikuuchi* occurred at 13 of 15 locations surveyed. There was no incidence of Cercospora leaf blight at farms surveyed in Wenchi and Ejura. The highest incidence and severity of Cercospora leaf blight occurred in farms at

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Karaga, Akukayili Gushegu and Malzire all in the Northern region. The wide variation between Malzire and Sang in Yendi municipality, and Tingoli and Akukayili in the Tolon district in terms of cercospora leaf blight incidence and severity is a demonstration of fluctuations among farms within locations for the occurrence of diseases. The three locations, Navrongo, Binduri and Manga in the Upper east region had similar level of cercospora leaf blight severity. *Cercospora kikuchii* was considered the fifth most important yield–depressing pathogen in the world (Sinclair and Hartman, 1999).

Another disease with wide distribution across the surveyed locations is the frog eye leaf spot disease due to *Cercospora sojina*, a pathogen that is closely related to *Cercospora kikuuchi.* Frog eye leafspot is an important constraint to soyabean production world-wide, especially in warm and humid production centres (Ma, 1994; Mian *et al.*, 2008; Pharm *et al*., 2015). The occurrence of frog eye leaf spot on soyabean in Ghana has been reported in previous publication on soyabean diseases (Offei *et al.*, 2008). Like bacterial leaf pustule, warm and humid conditions heighten the development of frog eye leaf spot. However, warm and humid conditions largely defines the soyabean production season in Ghana, which largely occurs in the Northern region where 77% of the country's soyabean is produced. The incidence and severity of frog eye leaf spot disease was high at Karaga, Gushegu and Damongo.

Among the three locations surveyed in the Upper east region, frog eye leaf spot incidence was high at Navrongo but not at Manga and Biduri. Although, Navrongo, Binduri and Manga are found in the Upper east region, they occur in different agro-ecological zones. Navrongo is located in the Guinea savannah ecology whilst Binduri and Manga are in the Sudan Savannah ecological zone. Wenchi and Ejura recorded the least incidence and severity of frog eye leaf spot disease among the 15 locations surveyed whilst Tingoli and Seripe had no incidence of frog eye leaf spot.

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Known sources of resistance to Frog eye leaf spot have been reported including PI594774 and PI594891 (Pham *et al.,* 2015). Also, the Brazillian cultivars, Bossier, Cristalina, Davis, Kent, Lincoln, Paraná and Uberaba are known sources of resistance to frog eye leaf spot (Gravina *et al.,* 2003). Pham *et al.,* (2015) fine mapped the resistance to frog eye leaf spot in PI594774 to 72kb on chromosome 13 and developed Kompetitive allele specific PCR(KASP) for detection of the frog eye leaf spot resistant allele on chromosome 13. These lines could be evaluated at the locations with high incidence and severity of frog eye leaf spot identified in this survey for their suitability for use as sources of resistance for cultivar development in Ghana.

Two types of viral diseases, soyabean mosaic and alfafa mosaic virus were present at all 15 locations surveyed. However, their incidences were very low at most locations except for Ejura and Wenchi. Ejura recorded the highest incidence of soyabean mosaic virus followed by Wenchi. Viral diseases are responsible of significant yield losses in major soyabean production centres such as the United States of America. For instance, an estimated 35 million United States dollars was lost to viral diseases in 2010 (Wrather and Koenining, 2010; USDA 2013). However, Hill and Whithman, (2014), suggested that losses due to viral diseases might be underestimated since viruses may be latent and still cause losses. Therefore, it is important to initiate management of virus caused diseases even if the incidence and severity are as low as recorded at most locations surveyed.

Fungi constitute the second largest group among eukaryotic organisms (Raja *et al.,* 2017) and responsible for most diseases of plants worldwide (Knogge, 1996). Three approaches including morphological characterisation, characterisation of secondary metabolite, and molecular characterisation are used separately, or in combinations for fungal identification (Raja *et al.,* 2017). In this study, I used to some limited extent morphology characteristics,

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and to a large extent, molecular characteristics to identify a fungal species causing seed decay of soyabean in Ghana.

Colony morphology of the fungus isolated in this study bear key characteristic known to members of the diaporthe/phomopsis complex of fungi which includes white areal mycelia that changes colour as the culture ages, and the presence of black pycnidia from which conidia is produced. The isolate produced both alpha and beta conidia which is characteristic of *Diaporthe phaseolorum var. sojae* and *Phomopsis longicolla* but not *D. phaseolorum var. caulivora.* Pyncnidia produced has short neck which is common to Diaporthe *phaseolorum var. sojae* but not to *Phomopsis longicolla* (Petrović *et al.,* 2016). Mengitsu *et al. (2014)* noted that morphological characteristics were not sufficient to make distinction between members of the diaporthe/ phomopsis complex of fungi.

Alignment of 28S RNA partial sequence of MTB-1 in BLAST yielded the same level of similarity to two isolates of *Diaporthe sojae* and five isolates of *Phomopsis longicolla.* Similarly, the phylogenetic tree based on 28S RNA gene sequence placed the newly isolated fungus in a major clade comprising of three isolates of *Phomopsis longicolla, and one isolate of Diaporthe sojae.* It is noteworthy that the Ghanaina isolate MTB-1 occurred in the same sub-clade with *Diaporthe sojae* isolate CBS 179.55, an indication that isolate MTB-1 is genetically closely related to *Diaporthe sojae* than other *Diapothe/Phomopsis* species used in this study.

The pairing of *Diaporthe/Phomopsis* isolates in the same major and sub-clades in some cases as indicated in the phylogenetic tree may be an indication of the confusion that dogged identification members of the *Diaporthe/ Phomopsis* complex of fungi (Santos *et al.,* 2011; Rensburg *et al.,* 2006). Alignment and clustering make it certain that newly identified pathogen is a member of the *Diaporthe sojae* species.

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3.6 Conclusions

Disease survey identified ten diseases seven of which were fungal diseases. Two viral diseases and one bacterial disease were identified in addition to the seven fungal diseases. Of ten diseases identified during the survey, bacterial leaf pustule, cercospora leaf blight, frog eye leaf spot and two viral diseases were present at varying levels of incidence and severity at almost all 15 locations surveyed. Bacterial leaf pustule and soyabean mosaic virus occurred at all 15 locations surveyed whilst frog eye leaf spot and cercospora leaf blight occurred at 13 of 15 locations surveyed.

The severity of identified diseases was higher at Malzire, Karaga and Gushegu than other locations with the exception of soyabean mosaic virus for which the highest incidence occurred at Ejura followed by Wenchi. Severity of bacterial leaf pustule, cercospora leaf blight and frog eye leaf spot was generally lower at the three locations surveyed in the Upper east region than locations surveyed in the Northern region. The two locations surveyed from the Transitional belt had the least prevalence and severity of diseases encountered during the survey except for soyabean mosaic virus for which incidence was highest at two locations occurring in the transitional belt.

Furthermore, this study identified soyabean seed decay disease, and successfully identified the causal agent as *Diaporthe sojae* and tentatively designated the isolate as MTB-1. This is the first report of soyabean seed decay due to *Diaporthe sojae* in Ghana. Asante *et al*. (1998) reported the occurrence of *Phomopsis longicolla* and other *Phomopsis* species known for causing soyabean seed decay and as well as *Diaporthe phaseolorum* var. *meridionalis*, causal agent of southern stem canker of soyabean in Ghana but not *Diaporthe sojae.*

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Diseases of soyabean are widespread at major production centres for which detail evaluations including assessment of economic damage is required for formulation of management strategies such as the use of resistant genes.

This research also provides knowledge of locations where specific diseases are prevalent. This will be useful for multi-location evaluation of sources of resistance. It could also serve as a guide for the extension service division of the Ministry for Food and Agriculture to direct resources at specific areas for famer education on management of specific diseases of soyabean.

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CHAPTER FOUR

4.0 Genetic diversity, population structure, and identification of key phenotypic traits driving variation in the assembled soyabean germplasm

4.1 Introduction

Genetic variability is useful for successful crop improvement programme. Thus, an understanding of prevailing genetic diversity within a population or a collection is key for planning an efficient plant breeding programme (Chandra *et al*. 2014). Soyabean production in Ghana has seen a steady rise from 50,000 MT in 2005 to 142, 000 MT in 2015 (MoFA-SRID, 2007 & 2016). However, the national average yield of 1.65 t/ha (MoFA-SRID, 2016) is far lower than the average of 3.38 t/ha for Brazil (Statista, 2018), and the 3.34 t/ha obtained in the US (USDA-NASS, 2018).

Production and utilization of soyabean in Ghana received a boost through the ongoing Soyabean Innovation Lab project funded by USAID - Feed the Future Programme. Under this project, the Council for Scientific and Industrial Research-Savanna Agricultural Research Institute (CIR- SARI) received soyabean germplasm as part of its collaboration with the United States Department of Agriculture and the University of Illinois as part of an effort to genetically improve the crop.

SARI, normally receives limited amounts of germplasm from only the International Institute of Tropical Agriculture (IITA), hence its gene pool was narrow with limited diversity. With the arrival of additional germplasm, it is important to re-evaluate the current germplasm holding for phenotypic and genetic diversity, and suitability for addressing abiotic and biotic challenges in major growing areas of the country (Buri *et al*., & Wakatsuki, 2010; Goldsmith, 2017).

The usage of new sources of genetic variation to develop improved varieties is enhanced when the selection of parental genotypes is based on both agronomic value and genetic dissimilarity

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(Bisen *et al.*, 2014). Therefore, estimation of variation and dissimilarity among soyabean germplasm assembled in Ghana will be required to effectively select parental lines for hybridization and development of improved varieties.

Conventionally, dissimilarity among genotypes was estimated based on their uniqueness in traits such as flower colour, seed shape, and colour, growth habit and so forth referred to as morphological markers (Holbrook & Stalker, 2003). However, the methods for evaluating some of these traits for differentiating genotypes are subjective, making the morphological approach to diversity study less efficient. More so, the influence of environmental factors on gene expression and its consequent effects on the stability of morpho-agronomic traits affects the repeatability of experimental results and thus, the inferences drawn from such experiments (Holbrook and Stalker, 2003).

The advances in molecular biology and biotechnology ushered in the era of molecular markers, especially DNA based markers which more efficient compared to morphological markers in the estimate of dissimilarity. According to Mullis & Faloona, (1987), the invention of the polymerase chain reaction (PCR) led to the development and utilization of PCR based markers such as simple sequence repeats (SSRs), single nucleotide polymorphism (SNP), random amplified polymorphic DNA (RAPDs) and amplified fragment length polymorphisms (AFLPs). SSR markers have been extensively used in genotyping and characterizing of soyabean (Bisen *et al.*, 2014; Guan *et al.*, 2010; Kuroda *et al.*, 2006; Li *et al.*, 2010).

More recently, the soySNP50K iSelect BeadChip that contains 50,000 SNP markers have been developed for characterization of soyabean genotypes, and quantitative mapping of desired loci (Song *et al.*, 2013). Increasingly, automated genotyping platforms and genotyping service providing companies are moving towards the use of SNP markers.

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Among SNP genotyping techniques, genotyping-by-sequencing offers an efficient method for genetic diversity studies, and estimation of population structure based on genomewide scale variation among test subjects (Poland and Rife, 2012). Bara *et al.,* (2018) used 45, 507 SNP markers generated with GBS to decipher the relatedness among crested wheatgrass lines.

In order to effectively utilize assembled germplasm in SARI's gene bank, understanding of the population structure, genetic diversity, and key phenotypic trait driving variation among the assembled germplasm is required.

The objectives of this study were to;

- examine the grouping of the 230 lines based on growth and agronomic parameters and identify lines with high pod clearance
- identify the most important traits based on contribution to total variation
- determine the structure and genetic diversity using SNP markers generated through genotype- by-sequencing (GBS)

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4.2 Materials and Methods

4.2.1 Plant materials

Two hundred thirty (230) soyabean lines comprising of 126 genotypes introduced from the United States Department of Agriculture (USDA); 56 advanced breeding lines sourced from the International Institute for Tropical Agriculture (IITA); 29 Zimbabwean accession; 13 Brazilian accessions; 3 Chinese soyabean lines obtained from the US, and 3 Ghanaian varieties.

4.2.2 Study area

The study was conducted at the experimental fields of the Savanna Agriculture Research Institute, Akukeyili in the Tolon district. The area is characterised by Ferric Luvisols of the Tingoli series which is well drained, devoid of concretions, and brown in colour (Atakora and Kwakey, 2016). The experiment was conducted in two seasons, 2016 and 2017. The year 2016 recorded a total of 846.8mm of rains spread across 54 days within the trial period which began in June and ended in October. Mean temperature of 28.1 ºC and mean relative humidity of 78% with average sunshine of 6 hours were also recorded for the same period. 2017 recorded 795.6mm of rainfall, mean temperature of 27.9, mean relative humidity of 75% and sunshine hours of 6.8 during the five months in which the trial took place.

4.2.3 Experimental design and field establishment

The trial was laid in an alpha lattice design in three replicates with each replication comprising 23 blocks of 10 lines per block laid out in two replications. Each line was planted in a single row of 5 m long with a plant spacing of 5cm. Rows were planted at 50 cm apart. Phosphorus in the form of Triple Superphosphate (TSP) was applied at a rate of 60 kg of P_2O_5 at emergence. Weed control was manually done with hoes.

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4.2.4 Data Collection and Analysis of morphological and phenotypic traits

- The number of days to flowering was recorded for the number of days from planting to when 50% of record plants for each line had at least one flower opened.
- The number of pods per plant was obtained by averaging the number pods counted on 20 plants for each line.
- The number of days to maturity was recorded at physiological maturity (R8).
- The number of seeds per pod was taken on whole plant bases where all filled pods of 20 plants were counted, manually threshed and seeds obtained.
- The number of seeds per plant was obtained from a sample of 20 plants of each line.

The values of yield component obtained were analysed using the R statistical software (version 3.5.1) (R Core Team, 2018). Genotypic effects wereestimated using a linear mixed-effect model (LMEM) with restricted maximum likelihood (REML) based on the equation below:

$y_{ijk} = g_i + r_j + b_k + \varepsilon_{ijk} \ldots eqn\ 4.1$

Where y_{ijk}, g_i, r_j, b_k and $\mathcal{E}_{ijk}$ denote observation on genotype i in block k nested within replication j, the effect of genotype i, effect of replication j, effect of block k nested within replication k and the effect of random error [$\sim N\ (\mu = 0, \sigma^2 = 1)$]. The combined results from year 1 and 2 was first subjected to analyses of variance which result showed that year to year variation was not significant. Also, interaction between year and lines was not significant. Therefore, the model was fitted using year as replication, given four replications to increase statistical power. The model was fitted using the lme4 package (Bates *et al.*, 2015) of R.

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To estimate the heritability of the various traits, *eqn 4.1* was refitted with all terms considered random. Based on the criteria proposed by Yan (2014), heritability was estimated using the equations below:

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} \ldots \text{eqn. 4.2}$$

$$4\sigma p2 = \sigma^2 + \frac{\sigma r^2}{\text{nj}} + \frac{\sigma b^2}{\text{nj}} + \frac{\sigma \varepsilon^2}{\text{nj}} \ldots \text{eqn. 4.3}$$

Where

H^2 is the heritability estimate,

σ_g^2 is the genotypic variance,

σ_p^2 is the phenotypic variance,

σ_r^2 is the replication variance,

σ_b^2 is the block nested within replication variance,

σ_ε^2 is the residual variance, and

n_j number of replications.

Percent quantile plot was performed in XLSTAT (version 2018.1). Likewise, multivariate analysis including factor analysis, principal component analysis, and cluster analysis were performed using the XLSTAT software (version 208.1).

4.3 Sampling of plant tissue for genotyping-by-sequencing

Ninty-six (96) lines were selected out of the 230 lines for genotyping. Selection was based on flowering and maturity groups, plant size, seed size and colour, and reaction to both bacterial leaf pustule and Phomopsis seed decay. Genotyping was done in 2018 after data from phenotypic and disease trials were used to select the 96 representative sample.

Sample collection kit comprised a collection box, a cutting mat, tubes with perforated strip caps, a cutting tool and a bag of silica gel (desiccant).

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For each of the 96 selected lines, leaf tissues were obtained by cutting 8 leaf discs with the cutter. These were placed in a labelled tube, covered with perforated strip cap and a desiccant-containing bag fastened to it. The collected tissues were sent to LGC genomics in Berlin, Germany for genotyping-by-sequencing.

4.3.1 Diversity, and population structure analysis

158,587 SNPs across all 96 samples were received from LGC-genomic. This was filtered in TASSEL (version 5.2.50) to obtain 32,638 SNP marker. These markers fully covered at least 66% of the 96 samples with allele frequency at or above 10% and a minimum read count of 8 and were used to examine the structure and diversity of the 96 representative samples. The genotypes were assigned population IDs (1-6) based on their origin. Bayesian distribution analysis that deploys Markov chain Monte Carlo (MCMC) simulation was carried out in the STRUCTURE programme (Pritchard *et al.*, 2000) to determine the number of subpopulation (structure) in the representative sample of 96 lines. Three independent runs were performed for each number of assumed subpopulations (K) ranging from 1 to 12, using the admixture model assuming that allele frequencies were correlated. A burn-in period of 50,000 and an MCMC run length of 100,000 for each run was performed according to the Evanno's method (Earl and vonHoldt, 2012; Evanno et al., 2005) to estimate the best number of subpopulations (ΔK) that explains the structure of the genotypes. The run with the maximum log likelihood was used to assign genotypes into subpopulations based on their membership probability. Genotypes with membership probability ≥ 0.25 were put into the same subpopulation. The STRUCTURE software was used to estimate the fixation index (Fst) for each subpopulation based on the estimated "K".

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4.3.2 Dendometric analyses

The relatedness of individuals within the 230 soyabean accessions was visualised in a neighbour joining hierarchical dendrogram in Darwin V6 (Perrier and Jacquemoud-Collet, 2006**)** based on molecular data. A phylogenetic tree was constructed using the neighbour-joining method based on a dissimilarity matrix generated using Euclidian distance. The analysis was performed at 2000 bootstraps with an average edge distance between bootstrapped trees being 0.6589.

4.4 Results

4.4.1 Distribution of quantitative traits

The number of days to flowering ranged from 23 to 51 days with a mean of 38 days (Fig 4.1). 25% of the 230 lines evaluated flowered within 34 days upon planting whilst 75% reached the reproductive stage within 42 days. It took 47 days for most (95%) of the soyabean lines to flower whilst the latter 5% took from 47 to 51 days to move from vegetative growth into the reproductive stage of development (Fig 4.1). The number of days to flowering was largely controlled by genetic effect as reflected in a broad sense heritability estimate of 0.9 (Table 4.1).

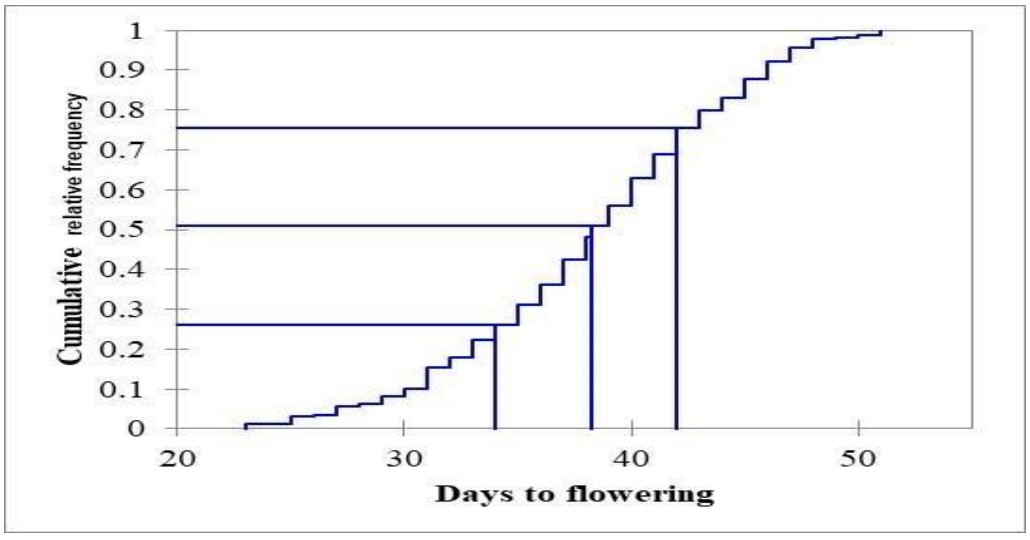

Fig 4.1**:** Distribution of 230 lines based on mean number of days to flowering

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The earliest maturing soyabean genotype attained physiological maturity in 82 days after planting whereas it took 138 days for the latest maturing genotype to attain physiological maturity (Fig 4.2). The mean number of days to maturity for the 230 genotypes was 108 days with a broad sense heritability estimate of 0.8 (Table 4.1). The lower 25% maturing lines matured between 82 to 100 days whilst 50% of soyabean genotypes matured in the range of 82 - 109 days. The upper 25% took between 115 to 138 days after planting to attain physiological maturity (Fig 4.2).

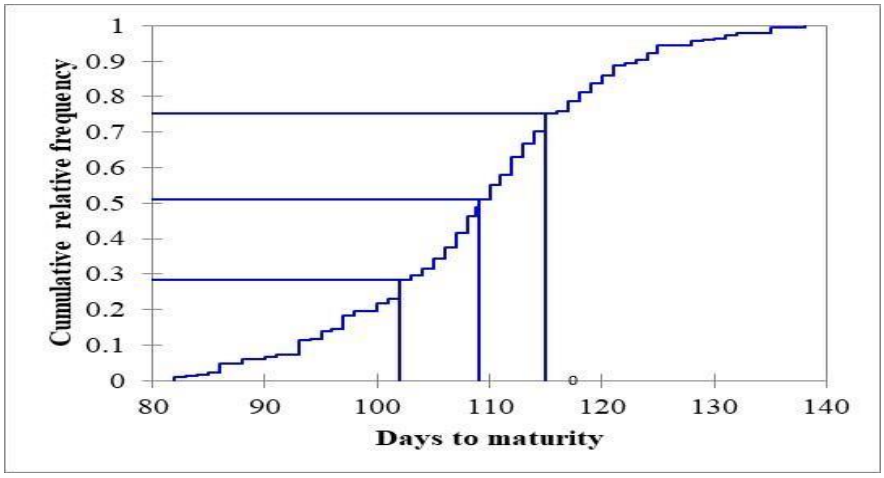

Fig 4. 2: Frequency distribution of number of days to maturity of 230 lines

The mean height at maturity of the 230 soyabean genotypes evaluated was 52.8cm. The shortest genotype had a height of 14cm whilst the tallest genotype was 96.8 cm (Fig 4.3). In all, the lower 25% of the genotypes had heights within the range of 14 to 37.1cm. The upper 25% of soyabean genotypes had heights between 64.8cm to 96cm (Fig 4.3). Broad-sense heritability estimate (H^2) for plant height at maturity was 0.49 (Table 4.1)

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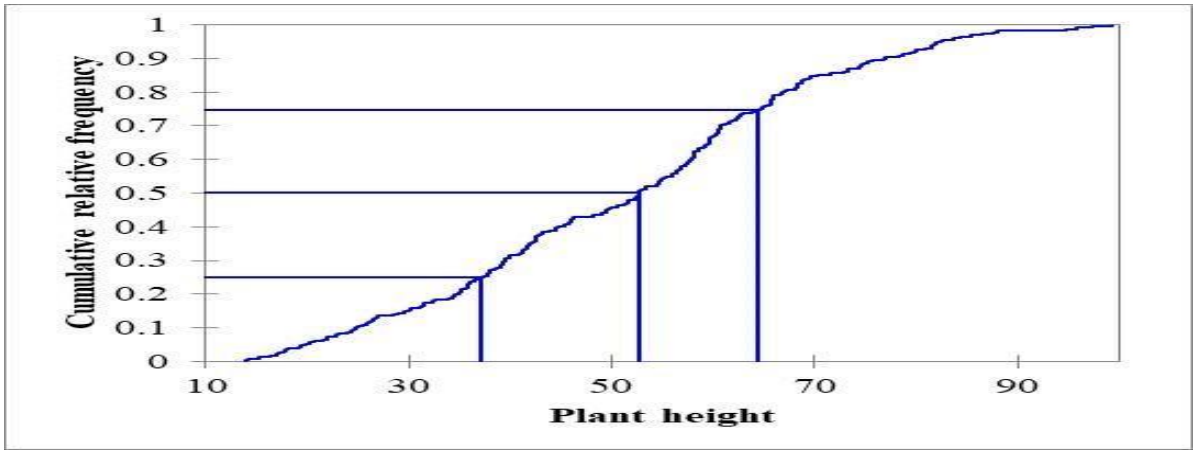

Fig 4.3: Distribution of plant height at maturity of 230 lines

The number of primary branches per plant ranged from 2-to- 21 with a mean of 7 branches per plant. 75% of the 230 soyabean genotypes studied had less than 8 primary branches per plant. Only 5% of the 230 lines had greater than 10 primary branches per plant (Fig 4.3). The number of primary branches per plant recorded a low broad sense heritability of 0.1 (Table 4.1).

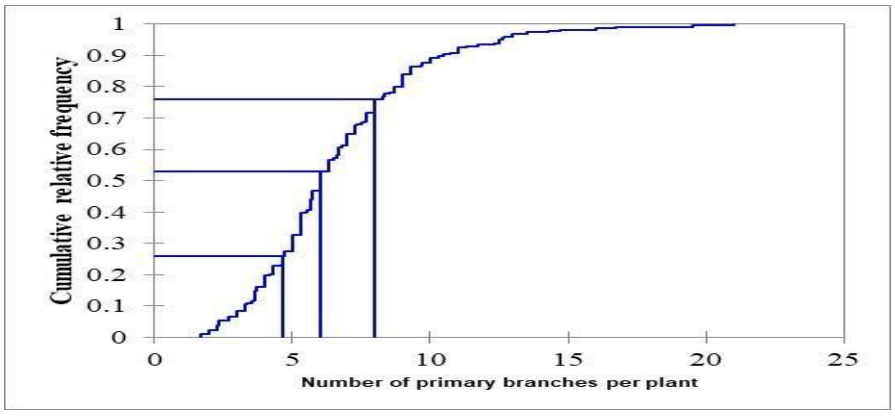

Fig 4: 4**:** Distribution **of** mean number of primary branches of 230 soyabean genotypes

Height at first pod was measured as the height of plant from the base to the first pod on the main stem. It ranged from 1.7 to 53 cm with a mean of 10.cm (Fig 4.5). 50% of the genotypes studied had a height at first pod of less than 10 cm. 25% recorded between 11 to 53cm in height from the base to the first pod on the main stem with 5 % of them recording a height of

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greater than 20cm from the base to the first pod on their main stem (Fig 4.5). The heritability estimate for plant height at first pod was 0.76 (Table 4.1).

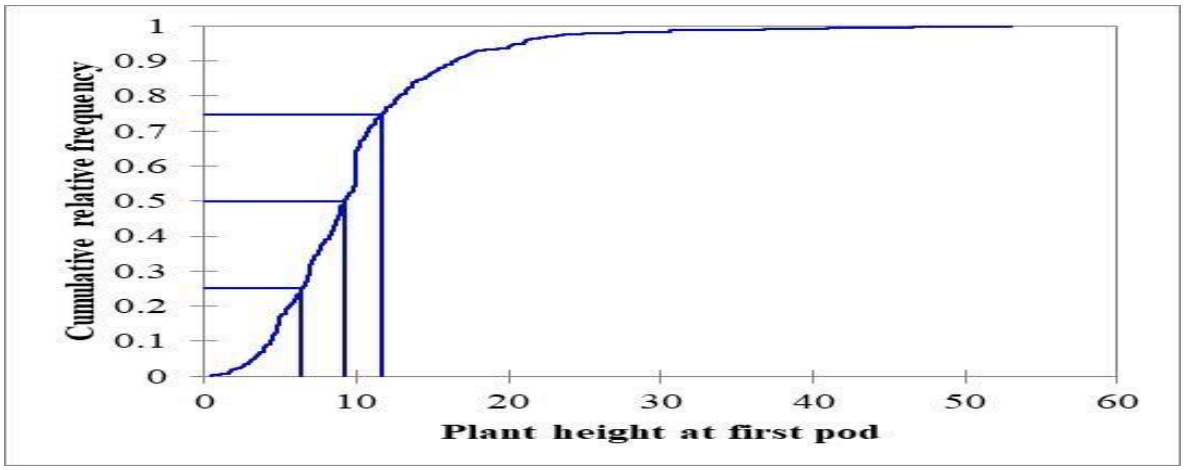

Fig 4.5**:** Distribution of plant height at first pod of 230 lines

The 230 soyabean genotypes investigated in this study produced pods per plant ranging from 17 to 290 pods, and a mean value of 72 pods (Fig 4.6). The lower 25% of the genotypes bore less than 50 pods per plant whereas the top 25 % produced more than 86 pods per plant. The top 10% recorded more than 120 pods per plants. On the other hand, the lower 10% of the 230 soyabean produced from 17 to 35 pods per plant (Fig 4.6). Number of pods per plant had a broad sense heritability of 0.7 (Table 4.1).

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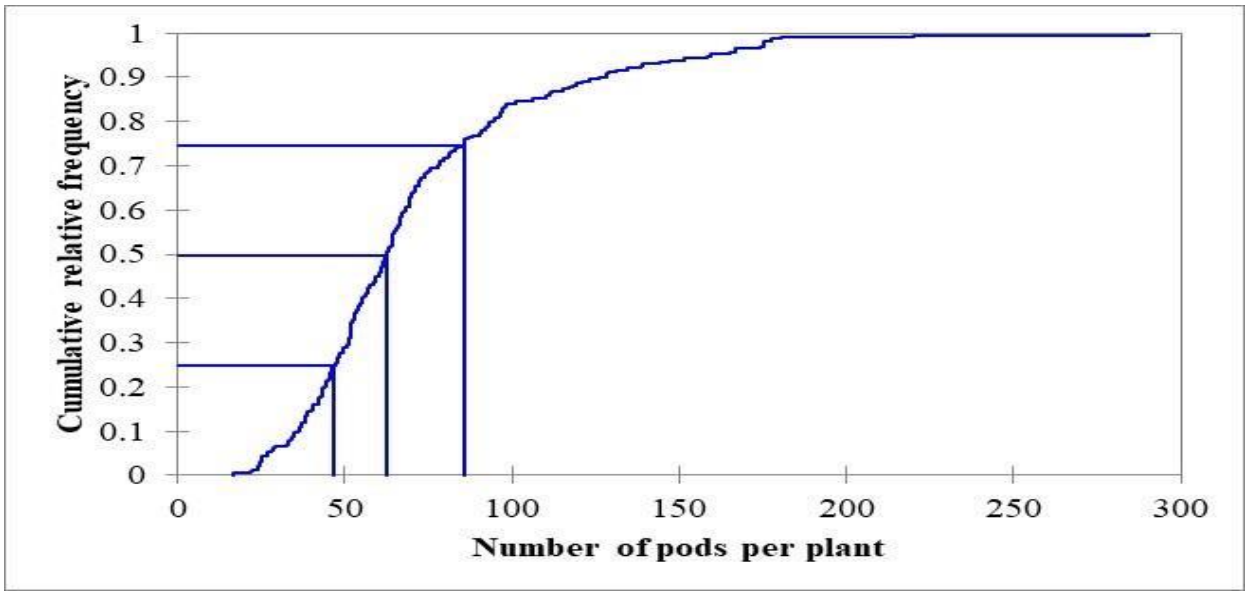

Fig 4.6: Distribution of mean number of pods per plant of 230 lines

The number of soyabean seeds in a pod ranged from 1.4 to 2.6 seed and a mean of 1.9 seeds in a pod with a broad sense heritability estimate of 0.6 (Fig 4. 7 & Table 4.1). The lower 25% of the genotypes recorded from 1.4 to 1.7 seeds in each pod whilst the upper 25% of the genotypes possessed from 2.1 to 2.6 seeds per pod. The top 10% in terms average number of seeds in a pod recorded from 2.2 to 2.7 seeds in a pod. On another hand, the bottom 10% recorded on the average 1.4 to 1.6 seeds per pod. 50% of genotypes evaluated had on the average 1.8 seeds per pod whereas 75% recorded an average of 2.1 seeds in a pod (Fig 4.7).

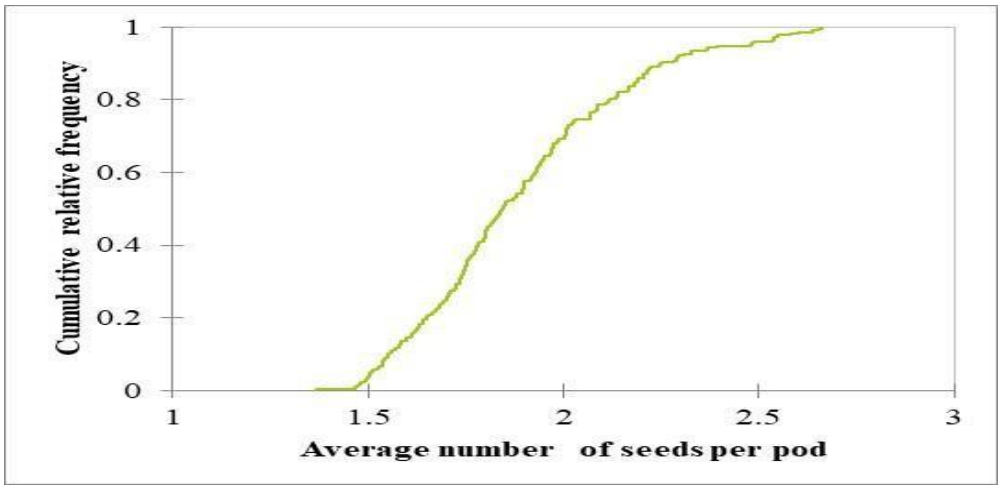

Fig 4: 7.Distribution of 230 lines based on mean number of seeds per pod

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The average number of seeds per plant ranged from 36 to 484 seeds with an average of 129 seeds for the 230 soyabean lines studied (Fig 4.8). The lower 10 percent of the lines, in terms of the number of seeds per plant, had from 36 to 59 seeds whereas the top 10% yielded between 200 and 484 seeds (Fig 4.8). The top 25% produced more than 140 seeds whilst the lower 25% recorded in a range of 36 to 84 seeds per plant (Fig 4.8). The number of seeds per plant was moderately heritable as reflected in the broad sense heritability estimate of 0.7(Table 4.1).

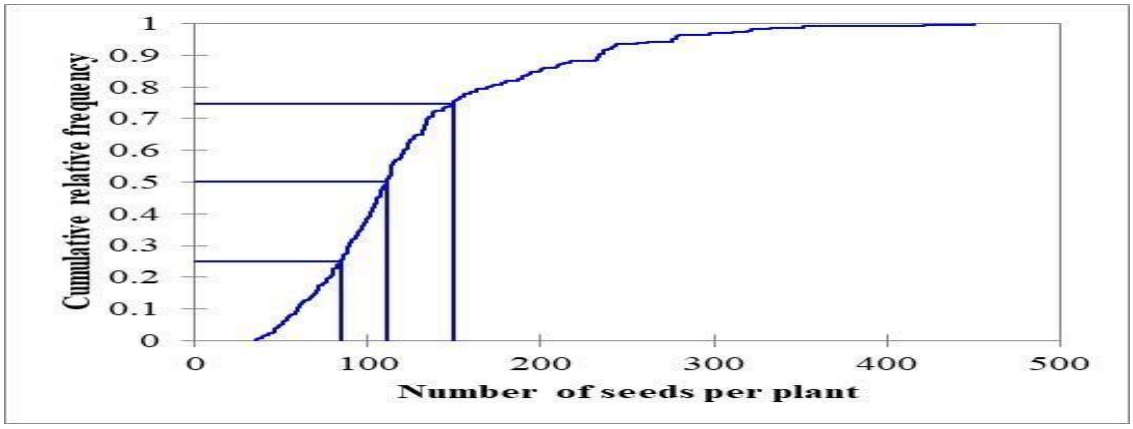

Fig 4. 8: Percentile plot showing distribution of number of seeds per plant of 230 lines

Seed weight of soyabean weighed in a lot of 100 seeds (100-seed weight) ranged from 6.7 to 24 g with a mean of 14.8 g for the 230 genotypes investigated (F4.9). 25, 50, and 75% of the materials studied had a seed weight of 12.8, 14. 8 and 16.6, respectively (F.9). The lower 10% of the genotypes had seed 100 seed weight of less than 12g whilst the top 10% had seeds weighing from 18.6 to 24.9 g. The lower 5% of the studied plant materials had 100-seed weight in the range of 6.7 to 10.5g whereas the upper 5% had the weight of 100 seeds in the range of 19.6g to 24.6g. (Fig 4.9). Estimated broad sense heritability for 100- seed weight was 0.8 (Table 4.1)

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Table 4.1: Variance of components and broad sense heritability estimates for eight quantitative traits

Trait	Line	Rep:Block	Rep	Residual	VG	VP	H2
DTF	36.4	2.2	0.0	19.6	36.4	41.9	0.9
DTM	112.6	14.6	0.0	90.4	112.6	138.9	0.8
100-SW	6	1	0.0	5.8	6.0	7.7	0.8
NPB/P	4.5	0	0.0	215.4	4.5	58.4	0.1
NPD/P	1157.8	103.8	37.2	1757.8	1157.8	1632.5	0.7
NS/P	3677.7	546.4	137.0	5220.8	3677.7	5153.8	0.7
NS/PD	0.1	0	0.0	0.2	0.1	0.15	0.7
PHT	275.9	3.2	0.0	1105.6	275.9	552.7	0.5
PLP	26	0.04	0.0	32.6	26.0	34.2	0.8

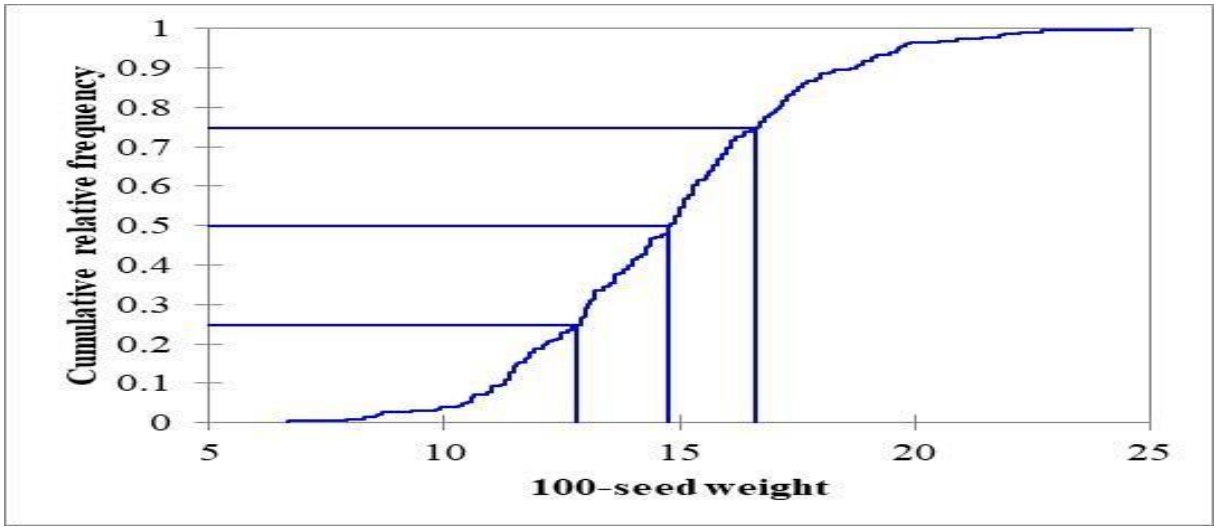

Fig 4: 9: Percentile plot showing the distribution of 100-seed weight of 230 lines

Number of days to flowering (DTF); number of days to maturity (DTM); hundred seed weight(100-SW); number of primary branches per plant (NPB/P), number of pod/ plant (NPD/P); number of seed per plant (NS/P); number of seed per pod (NSPD/P), and plant height at maturity (PHT).

4.4.2 Multivariate analysis of eight quantitative traits

Multivariate analyses based on principal components decomposed eight quantitative traits into eight factors (F) or components (Table 4.1). The first three components had eigenvalues of greater than one where PC1, PC2 and PC3 had eigenvalues of 2.69, 1.39 and 1.06, respectively (Table 4.1). The first component (PC1) accounted for 33.35% of the total variation whilst the second component (PC2) explained 17.32%, and cumulatively accounted for 50.67% of total variation. The third component (PC3) with an eigenvalue of 1.05, accounted for 13.210% of

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total variation and in addition to the first two components, 63.9% of total variation was explained. The cumulative of eight principal components explained 100 percent of the inherent variation in the data (Table 4.1).

Table 4.2: Eigenvalues, variability (%) and cumulative variability from PCA

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigenvalue	2.7	1.4	1.1	0.9	0.8	0.8	0.3	0.0
Variability (%)	33.4	17.3	13.2	11.7	10.4	9.7	4.2	0.2
Cumulative %	33.4	50.7	63.9	75.56	85.9	95.6	99.8	100.0

A loading plot of the first two components showed the association of the eight quantitative traits, number of days to flowering (DTF); number of days to maturity (DTM); number of pods per plant(NPD/P); plant height at maturity (PHT); number of primary branches per plant (NPB/P); number of seeds of per pod (NS/PD); number of seeds per plant (NSP); and hundred seed weight (100SW) and the first two components (Fig 4.10). The first principal component has large positive associations with the number of pods per plant (0.840), the number of seeds per plant (0.837), days to flowering (0.691) and days to maturity (0.650) (Table 4.2). Also, the number of primary branches per plant (0.42), plant height (0.39), and a hundred seed weight (0.18) positively associated positively with the first principal component (F1). Among the eight quantitative traits, only number of seeds per pod (-0.05) associated negatively with principal component one (F1).

Similarly, DTF, DTM, PHT, NPB/P and 100SW associated positively with principal component two (F2) whilst NPD/P, NS/PD, and NS/P negatively associated with principal component two (F2) (Fig 4.10 & Table 4.2). With regards to principal component three (F3), 100SW (0.726) had the largest positive association (Fig 4. 10). This was followed by NS/PD (0.623). DTF, DTM and NPB/P had a positive but low association with PC3. On the other

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hand, PHT (-0.201), NPD/P (-0.225), and NS/P (-0.017) associated negatively with PC3 (Fig 4.10 & Table 4.1)

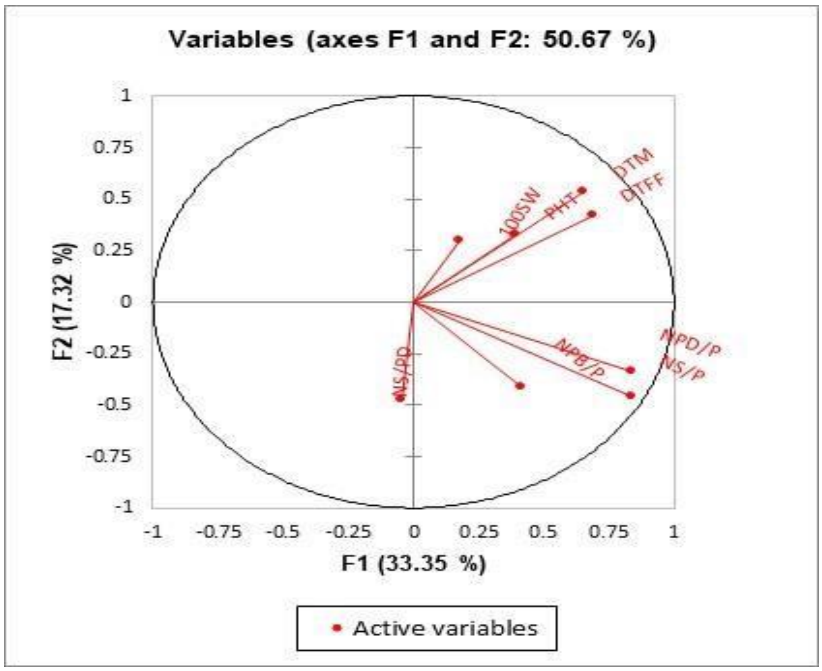

Fig 4. 10: Loading plot of the first two principal components (F1 &F2). Number of seed per pods (NS/PD), 100 seed weight (100SW), plant height (PHT), days to maturity (DTM), days to flowering (DTF), number of pod per plant (NPD/P), number primary branches per plant (NPB/P), and number of seeds per plant (NS)

The contribution of each variable in percentages to principal components is as indicated in (Table 4.3.). Principal component one (PC1) which accounted for 33.4% of observed variation had very high contributions from NPD/P, NS/P, DTF, and DTM which contributed 26.4%, 26.3%, 17.9&, and 15.8%, respectively. PHT, NPB/P and 100SW also contributed 5.8, 6.54 and 1.15% respectively, to observed variation in F1. NS/PD had the least contribution of 0.08 to the total variation in component one (PC1). With regards to principal component two (PC2), DTM (20.7%), NS/PD (16.3%), NS/P (15.31%), and DTF (12.8%) gave the most contribution to observed variation in PC2 which constitutes 17.3% of the total variation (Table 4.3). 100 SW (6.5) contributed the least to total variation in PC2. 100SW contributed 49.9% of the observed

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variation in F3. This was followed by NS/PD which contributed 36.8% to recorded variation in PC3.

Table 4. 3: Contribution of variable of variables (%) to observed variation in each component

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
DTF	17.90	12.81	0.04	7.25	8.08	6.81	47.08	0.02
DTM	15.84	20.67	3.30	0.18	6.48	5.09	48.36	0.07
PHT	5.80	7.75	3.87	26.03	32.39	23.71	0.45	0.00
NPB/P	6.54	12.38	1.29	2.37	35.24	41.99	0.13	0.06
NPD/P	26.43	8.25	4.79	8.87	3.84	0.89	0.02	46.90
NS/PD	0.08	16.32	36.76	30.84	2.94	8.94	0.21	3.91
NS/P	26.27	15.31	0.03	2.06	4.96	2.25	0.12	49.01
100SW	1.15	6.51	49.91	22.42	6.07	10.30	3.62	0.03

Table 4.4. showed the correlation between the eight studied quantitative traits. The number of days to flowering (DTF), a growth parameter had a significant positive association (0.64) with the number of days to maturity (DTM). Also, how long it took soyabean genotypes studied to flower (DTF) had a significant positive association with plant height (PHT), number of pods per plant (NPD/P), number of seeds per plant (NSP). There was also a weak but significant positive association between days to flowering (DTF) and the number of primary branches per plant (NPB/P) (Table 4.4).

How long it took soyabean genotypes to attain physiological maturity (DTM) positively and significantly correlated with the number of pods per plant (NPD/P), a number of seeds per plant (NS/P), and 100 seed weight (100SW) (Table 4.4). Primary branches produced per plant (NPB/P) significantly and positively associated (0.16) with the length of a juvenile phase of soyabean lines (DTF) evaluated. The number of primary branches per plant (NPB/P) had a significant positive association with the number of pod/ plant (NPD/P) and the number of seeds per plant (NS/P). There were also positive but non-significant association (0.10) between the

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number of primary branches per plant (NPB/P), and the number of seeds per plant (NS/P). A similar positive non- significant association (0.003) was recorded for the number of primary branches/plant and 100 seed weight (Table 4.4). The strongest association (0.94) occurred between the number of pods per plant and number of seeds per plant.

Number of pods per (NPD/P) plant had significant positive correlation with all quantitative traits evaluated except for number of seeds per pod (NS/PD) with which it recorded weak negative non-significant association (-0.12), and 100 seed weight with which it recorded a positive but weak association (0.01). The number of seeds per plant (NS/P) also recorded significant positive associations with all traits evaluated except for 100-seed weight (100SW) with which it had a positive but weak non-significant correlation (0.065). On the other hand, the number of seeds per pod (NS/PD) had a positive significant association with the number of seeds per plant (NS/P) but its association with other traits was not significant, mostly negative except for number of pod/plant (0.10) and 100 seed weight (0.01).

Table 4. 4:Pearson correlation matrix of eight quantitative traits

Variables	DTFF	DTM	PHT	NPB/P	NPD/P	NS/PD	NS/P	100SW
DTFF		**0.636**	**0.286**	**0.155**	**0.335**	-0.07	**0.318**	0.064
DTM	**0.636**		**0.224**	0.098	**0.283**	-0.119	**0.276**	**0.239**
PHT	**0.286**	**0.224**		0.045	**0.179**	-0.067	**0.162**	0.028
NPB/P	**0.155**	0.098	0.045		**0.278**	0.096	**0.333**	0.003
NPD/P	**0.335**	**0.283**	**0.179**	**0.278**		-0.121	**0.941**	0.031
NS/PD	-0.07	-0.119	-0.067	0.096	-0.121		**0.16**	0.014
NS/P	**0.318**	**0.276**	**0.162**	**0.333**	**0.941**	**0.16**		0.065
100SW	0.064	**0.239**	0.028	0.003	0.031	0.014	0.065	

Values in bold are different from 0 with a significance level alpha=0.05

The generated biplot from the first two principal components based on eight quantitative traits grouped the 230 soyabean lines into two major groups, I & II (Fig 4.11). Table 4.5 shows a sub-sample of the 230 as grouped together by principal component analyse.

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Most of the tropical glycine lines prefixed (TGX) obtained from IITA occurred in group I. Also, released varieties including Jenguma, Songdaa, Afayak, and TGX 1844-22E, recently released as "Favour" by SARI, all occurred in group I. The grouping of released varieties with other TGX lines obtained from IITA was not surprising. Most soyabean varieties released in Ghana were obtained from IITA as advanced breeding lines and subsequently adapted to Ghana's production conditions.

Group, I separated into two sub-groupings, IA and IB. Sub-group IB comprised of soyabean lines possessing a high number of pods, seeds, and primary branches per plant (Table 4.6). The number of seeds per plant had a significant positive association with the number of pods per plant (Table 4.4). Also, the number of seeds per plant had a weak but positive association with the number of primary branches per plant. Most of the lines in sub-group IB including the released varieties, Jenguma, Afayak, Songdaa, and TGX 1844-22E belonged to the medium maturity (100-to 120 days). Sub-group IA of group I is largely comprised TGX lines and the lines obtained from Brazil with the prefix BRS. Lines sub-group IA flowered and matured later than lines in the other groups. These lines were also taller with higher pod clearance (PLP) than lines in sub-group IB and other groups (Table 4.5).

Most lines in group II flowered and matured earlier than lines in group I. Also, most lines grouped in together in I had higher seed weight than most line grouped together to form group I. lines in sub-group IIA (Fig 4.11) possessed higher plant height with high pod clearance (PLP). They were early maturing but had high seed weight. They, however, had a lower number of pod and seed per plant. Lines that clustered to form sub-group IIB (Fig 4.11) were generally short with lower pod clearance values (PLP). The earliest flowering and maturing lines among the 230 lines evaluated occurred in this group.

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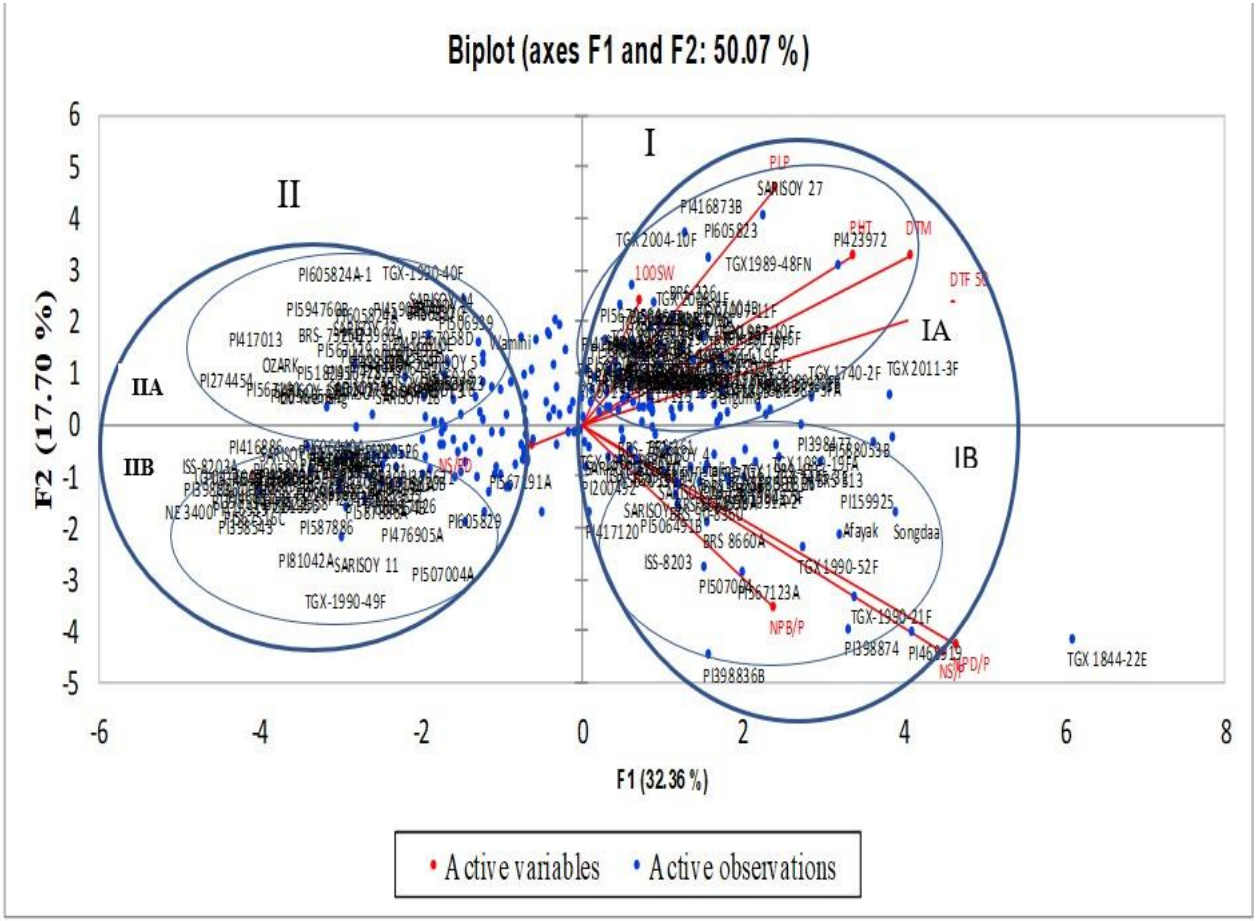

Fig 4 .11: Clustering of lines based on growth traits, and yield components

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Table 4.5: Growth traits, and yield components of lines grouped together by principal components

Line	CPA Group	PHT	PLP	PB/P	DTF	DTM	NPD/P	NS/PD	100-SW	NS/P
TGX 1740-2F	IA	95.2	19.2	11	43	120	110	1.7	14.1	185
TGX 1799-8F	IA	65.6	9.5	8	43	125	115	1.8	14.9	196
TGX 1805-8F	IA	65.3	12.9	7	44	120	73	2	13.5	143
SARISOY 26	IA	60.3	5.8	12	47	128	118	1.6	14	185
PI416873B	IA	41.6	39.5	3	44	117	45	2	17.5	92
PI605823	IA	82	28.7	5	44	138	53	1.7	11.8	91
TGX 2004-10F	IA	87.5	19.7	4	46	118	49	1.4	15.0	69
TGX1989-48FN	IA	52.6	12.7	4	43	121	46	1.8	15.6	82
BRS-326	IA	50.2	12	7	44	123	46	1.6	18.5	71
Afayak	IB	61.7	9.4	12	47	116	101	1.7	14.0	176
Songdaa	IB	49.5	11.3	16	4	119	165	2	19.2	332
TGX 1844-22E	IB	35.2	15.8	19	47	119	290	1.8	15.3	510
PI398836B	IB	35	3	7	31	105	96	1.8	11.5	173
PI567123A	IB	68.2	7.5	13	38	100	180	1.5	9.9	279
ISS-8203	IB	41.3	10.4	10	33	115	147	2	12.9	285
BRS 8660	IB	41.5	8.1	10	41	109	117	1.9	14.7	212
PI507004	IB	64.1	5.9	7	31	105	170	1.7	12.5	285
TGX 1990-52F	IB	61.1	4.3	9	41	116	175	1.9	18.2	321
PI468919	IB	66.1	8	13	36	114	248	2	12.9	484
TGX 1989-11F	IB	56	7	6	43	121	61	1.4	12.6	86
PI605824A-1	IIA	63.8	18.9	4	38	98	25	1.8	17.4	37
Wamini	IIA	75.8	10.3	4	37	101	64	2.1	14.0	127
PI594760B	IIA	45.2	14	3	37	106	47	1.8	16.2	81
OZARK	IIA	24.5	8.4	5	27	108	38	1.5	18.2	54
PI417013	IIA	66.5	11.2	2	28	98	24	2.3	15.8	55
PI274454	IIA	21.9	6.2	6	27	93	33	2.2	19.5	69
PI506938	IIA	83	11.2	7	38	93	67	1.7	14.8	107
PI594760B	IIA	45.2	14	3	37	106	47	1.8	16.2	81
PI605824A-1	IIA	63.8	18.9	4	38	98	25	1.8	17.4	37
ISS-8203A	IIB	17	6.2	3	29	93	27	1.7	13.6	36
PI605829	IIB	59	8.5	6	34	91	74	1.6	12.2	110
PI476905A	IIB	26	3.8	6	34	89	54	2.1	12.2	113
PI507004A	IIB	38	8.1	9	32	102	115	1.6	15.8	169
SARISOY 11	IIB	32.7	8.2	5	31	93	48	2.2	16.0	99
PI587886	IIB	29.5	7.1	3	31	108	41	2.1	16.1	87
PI587886A	IIB	24.1	1.6	9	33	103	53	2.4	16.1	122
PI81042A	IIB	47.3	3.6	8	32	86	81	1.8	12.0	130
NE 3400	IIB	26.4	2.9	2	23	83	33	2.3	13.2	75
PI398543	IIB	14.3	5.9	4	26	86	38	1.6	8.6	59
PI398512	IIB	31	4.8	5	85	33	1.8	40	12.1	49
	Mean	49.8	8.5	7.1	36	107	84	1.84	14.7	148
	S.E.M	1.3	3.2	0.6	1.3	2.2	9.9	0.04	0.4	18

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4.4.3 Genetic Diversity and Population Structure of 96 representative panel

The population structure of the 96 soyabean lines was inferred using Bayesian clustering analyses (Fig 4.12). The highest values of ΔK was recorded when 9 distinct sub-populations (clusters) were identified (Fig 4 .12) based on genetic variation. The nine inferred clusters did not correspond to the six origins (Table 4.4) with which the 96 lines were identified before performing structure analyses. However, lines from some origins were dominant in some clusters but lines from each of the six origins occurred in more than 1 cluster with the exception of cluster 9 (Table 4.6 & Fig 4.12).

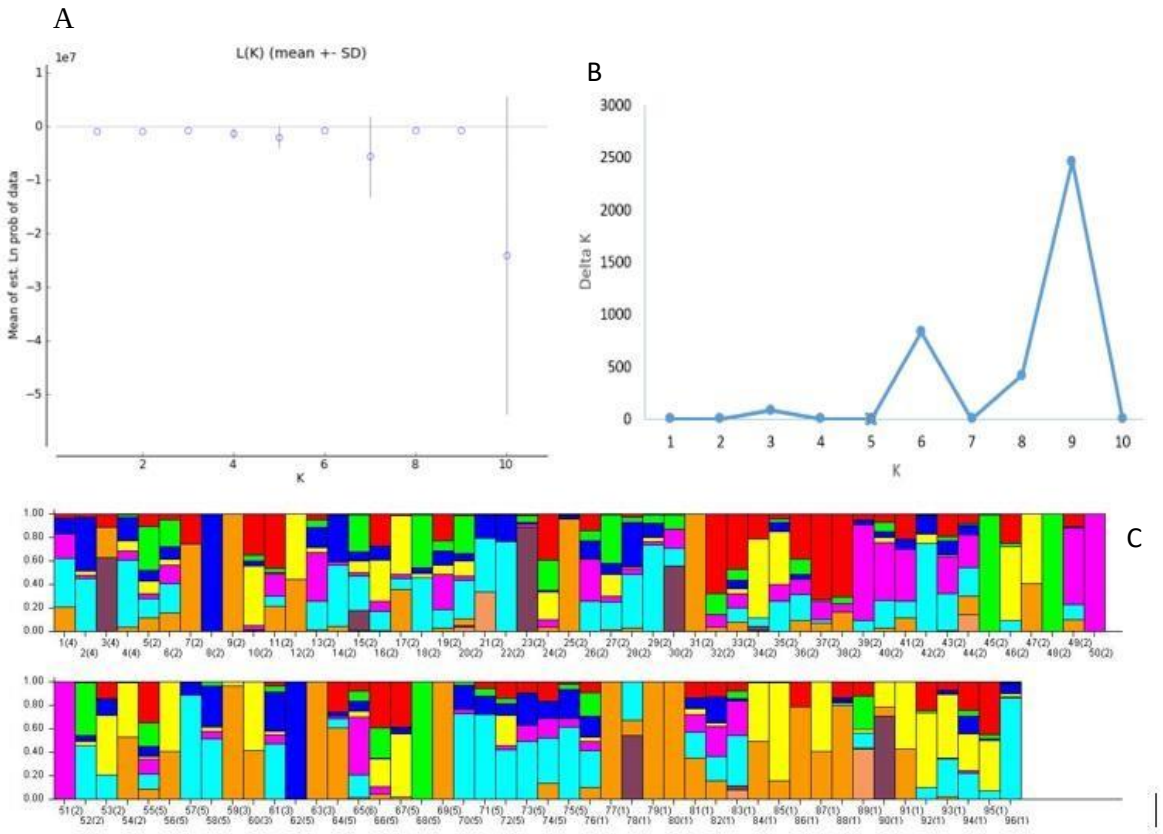

Fig 4 .12: Clustering of 96 soyabean lines based on Bayesian assignment. A (mean likelihood [L (k)± SD] over 12 runs assuming K= 1-10. B (ΔK), where the modal value of the distribution is considered as the highest level of structuring. C assignment of individual lines to the K= 9 where each individual is represented by a bar, with colours indicating the likelihood of assignment to the corresponding clusters(sub-population)

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The distributions of the 96 lines in 9 inferred clusters or sub-population are presented in Table 4.6. Cluster 1 consisted of 11 lines, 7 of which were plant introductions (PIs) obtained from the USDA through the University of Illinois. Three of the eleven lines that formed this cluster were sourced from Zimbabwe whilst one "TGX" prefixed line obtained from IITA joined the others to form cluster 1.

Cluster 2 comprised of six lines made up of 5 plant introductions (PIs) sourced from the USDA, and one Zimbabwean line whilst cluster 3 contains only two PIs. Cluster 4 is composed of 14 lines comprising 9 PIs, 7 IITA lines and one line each from Ghana and Zimbabwe. Cluster 5 made of 11 USDA lines (PIs), 2 IITA lines and the only line sourced from China.

Cluster 6 which was the largest cluster was also the most diverse cluster. Cluster 6 had lines from 5 of the six origins including 7 lines, 11lines, 3 lines, 2-line and1line from Zimbabwe, USA, Brazil, IITA and Ghana, respectively.

Six IITA lines, 4 PIs from the USDA, 4 varieties released in Ghana and one line sourced from Zimbabwe formed the 15 lines that occurred in Cluster 7. Cluster 8 consisted of 5 lines made up of 2 PIs from the USDA, 2 IITA lines and 1 Brazilian whilst cluster 9 had only one line, TGX1990-46F sourced from IITA. Lines from the 51 plant introductions obtained from the USDA occurred in 8 of the 9 inferred clusters with most occurring in cluster 4 (11 lines), and cluster 6 (11 lines).

Thirteen lines from Zimbabwe were used in this study, seven of them grouped together in Cluster 6.3 of 4 Brazilian lines evaluated grouped together in Cluster 7 whereas 4 of 5 Ghanaian varieties occurred in Cluster 7. The 21 IITA lines occurred in all clusters except cluster 2. The 21 IITA lines were widely distributed with the highest number (7) grouping in cluster 7.

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Table 4.6: Origin, and distribution of 96 lines in 9 inferred clusters based on Bayesian clustering analyses

Line	Origin	Cluster	Line	Origin	Cluster	Line	Origin	Cluster
SARI-S11	Zimb	1	TGX-1990-57	IITA	4	PI423960A	USDA	6
SARI-S-1	Zimb	1	TGX1990-40F	IITA	4	PI423958	USDA	6
SAR1-S4	Zimb	1	TGX1989-48F	IITA	4	PI417013	USDA	6
PI567123A	USDA	1	TGX-1989-45	IITA	4	PI399096	USDA	6
PI567104B	USDA	1	SARI-4DA	Ghana	4	PI200526	USDA	6
PI567099A	USDA	1	PI606397B-2	USDA	5	TGX-2011-3F	IITA	6
PI567068A	USDA	1	PI606397B	USDA	5	TGX-1834-5E	IITA	6
PI567053	USDA	1	PI605891B	USDA	5	SARI-5FK	Ghana	6
PI476905A	USDA	1	PI594767B	USDA	5	BRS-7980	Brazil	6
PI398512	USDA	1	PI594760B	USDA	5	BRS-360	Brazil	6
TGX2008-4F	IITA	1	PI588053A	USDA	5	BRS-326	Brazil	6
SARI-S-13	Zimb	2	PI567516C	USDA	5	SARI-S-14	Zimb	7
PI605891A	USDA	2	PI567129	USDA	5	PI224271	USDA	7
PI605791A	USDA	2	PI506938	USDA	5	PI398477	USDA	7
PI518295	USDA	2	PI417134	USDA	5	PI506491	USDA	7
PI416778	USDA	2	PI398836	USDA	5	PI567046A	USDA	7
PI200466	USDA	2	TGX1989-40F	IITA	5	TGX1835-10E	IITA	7
SARI-6FT	USDA	3	TGX-1988-3F	IITA	5	TGX-1844-22	IITA	7
PI274954	USDA	3	SARI-9LMG	China	5	TGX1845-10E	IITA	7
SARI-17	Zimb	4	SARI-S-9	Zimb	6	TGX1988-3F	IITA	7
PI657753	USDA	4	SARI-S-5	Zimb	6	TGX-1990-11	IITA	7
PI605854B	USDA	4	SARI-S30	Zimb	6	TGX1990-46F	IITA	7
PI605824A	USDA	4	SARI-S29	Zimb	6	Sal-1	Ghana	7
PI567076	USDA	4	SARI-S27	Zimb	6	SARI-3Jng	Ghana	7
PI567069A	USDA	4	SARI-S-19	Zimb	6	SARI-7QS	Ghana	7
PI416873B	USDA	4	SARI-18	Zimb	6	SARI-8SG	Ghana	7
PI416810A	USDA	4	SARI-2ZK	USDA	6	PI462312-2	U	8
PI398825	USDA	4	PI606440A	USDA	6	PI567034	USDA	8
PI3984-77A	USDA	4	PI588053B	USDA	6	TGX-1844-19	IITA	8
TGX2006-3F	IITA	4	PI561381	USDA	6	TGX1990-49F	IITA	8
TGX-2004-10	IITA	4	PI-534545	USDA	6	BRS-360-2	Brazil	8
TGX-1990-80	IITA	4	PI462312	USDA	6	TGX1990-46F	IITA	9

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Mean Fst was generally high for the all inferred clusters except cluster 8 and 9 (Fig 4.13.)

Mean Fst for lines in inferred clusters ranged from 0.007 to 0.9154. Most of the clusters had mean Fst above 0.5. Conversely, mean heterozygosity ranged from 0.048 to 0.446. Cluster 2, 3, 4, and 6 had heterozygosity of approximately 0.1. Heterozygosity was generally low except for cluster 8 and 9 which had heterozygosity of almost 0.5 (50%).

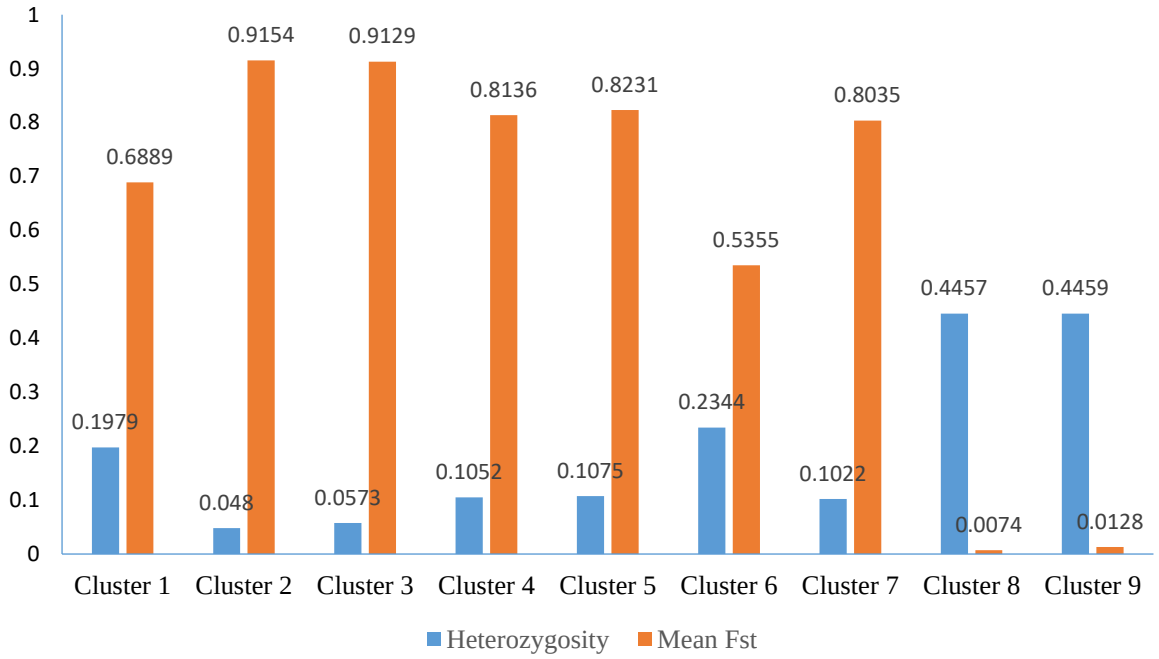

Fig 4 .13: A plot of mean Fst and Heterozygosity among soybean lines within clusters

Allele divergence frequency among clusters showed that soyabean lines in cluster 2 were more genetically distant from lines in other clusters with a value of 0.346 with cluster 3, 0.360 with cluster 7, 0.327 with cluster 5, 0.258 with cluster 4, 0.239 with cluster 7 (Table 4.7). It, however, had lower with cluster 8 (0.165) and Cluster 9 (0.159). The lines in cluster 1 also recorded pairwise values of (0.313) with those in cluster 2, 0.307 with cluster 3, 0.273 with cluster 4, 0.269 with cluster 5, 0.219 with cluster 6 and 0.269 with cluster 7, and could, therefore, be said to be more distant genetically to lines in these clusters. The genetic distance

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between lines in cluster 1 and those in cluster 8 and 9 was low. Also, lines in clusters 3, 4, 5 & 6 were relatively distant from lines in other clusters except for lines in cluster 8 and 9 which recorded lower pairwise values with lines in all other clusters due to their high heterozygosity. There were no genetic differences between the 5 soyabean lines that constituted cluster 8, and the line in cluster

9.

Table 4.7: Allele frequency divergence among sub-populations

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	-	-							
2	0.3126								
3	0.3071	0.3462	-						
4	0.2725	0.2579	0.3195	-					
5	0.2689	0.3276	0.317	0.3155	-				
6	0.2199	0.2389	0.2693	0.233	0.2682	-			
7	0.2685	0.3601	0.3305	0.3103	0.3064	0.2557	-		
8	0.1115	0.16	0.1645	0.1324	0.1505	0.0823	0.1438	-	
9	0.1112	0.1593	0.1639	0.1321	0.1499	0.0816	0.1439	0	-

Hierarchical clustering was largely influenced by the source of germplasm. The 96 lines were placed in two major groups both of which separated into several sub-clusters. Of 52 plant introductions obtained from the USDA, 38 were grouped in major cluster I which separated into many sub-clusters. The sub-cluster IA consisted of 19 lines, all of which were PIs.

The sub-cluster IB separated into sub-cluster IC & ID. ID is solely made up of PI. In IC, the four Brazilian lines grouped with PIs, three which occurred in a single clade whilst the remaining one (BRS-7980) occurred in another clade, closely related to PI-534545. All 21 lines obtained from IITA occurred in sub-cluster IIA. Sub-cluster IIB further separated into two sub-clusters, Bi & Bii. Bi consisted of only plant introductions whilst Bii further into clusters, one of which is made up of only PI lines whilst the other is made up of three lines from Zimbabwe and one PI. Most of the lines obtained from Zimbabwe occurred in Cluster IIC,

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though, they occurred in several subclusters. The varieties released in Ghana, SARI-3Jng, SARI-8SG, SAL-1, SARI-5FK, and SARI-7QS all occurred in IIC. SAR-7QS and SARI-8SG were most related to SAL-1 being the most distant among the five released varieties. SARI-3jng and SARI-FK also occurred in the same sub-clade.

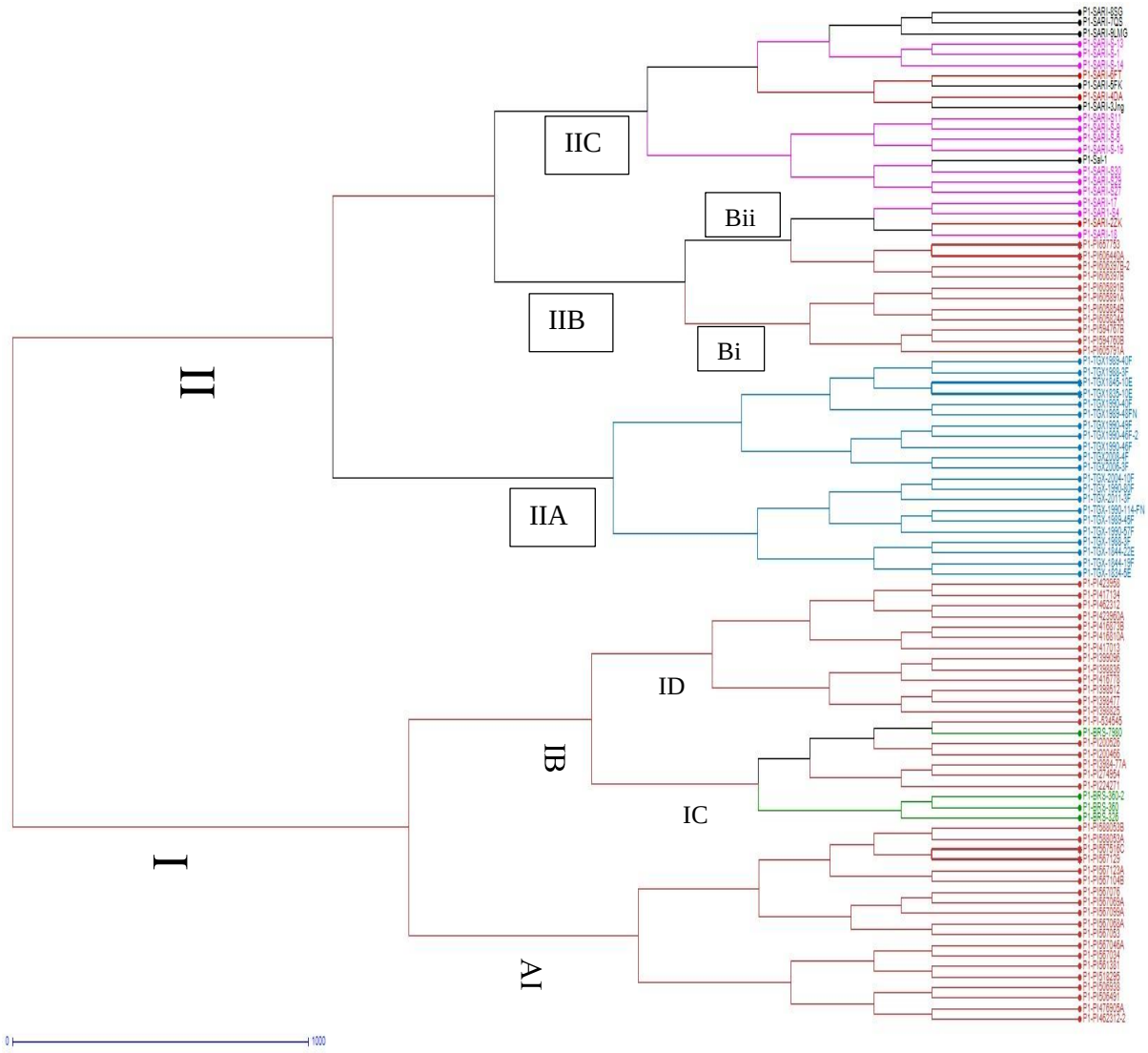

Fig 4. 14: Clustering of 96 soyabean lines using mean euclidian distance based on 3200 SNP markers. Red (Plant introduction, PIs sourced from the USDA), Blue (Lines from IITA, Nigeria), Green (lines from Brazil), Black (Released varieties in Ghana), Pink (lines from Zimbabwe)

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4.5 Discussion

Germplasm collections remain valuable tools for crop improvement. This could either be through direct cultivation of genotypes adapted to farmers' conditions in multi-location tests or hybridization to generate new varieties. To fully utilize such introductions, detailed information is required about the amount and nature of the genetic diversity available within each source of introduced germplasm. As noted by Ali *et al*. (2017), breeding for changes in phenology and stress tolerance to meet challenges of climate change require access to elite germplasm from other regions, multi-location testing systems that adequately sample the target population of environments and continually adjusting allele frequencies among a set of selected breeding lines

Flowering and maturity periods, the two key determinants of soyabean productivity (Jiang *et al.*, 2013; Zhai *et al.*, 2014) varied widely among the 230 soyabean lines evaluated. The number of days to flowering of lines in the germplasm assembly ranged from 23-51 days. Some of the lines obtained from the USDA were photoperiod sensitive and flowered in the third week of planting. They contributed to the wide variations. Broad-sense heritability estimate of 0.9 recorded for the number of days flowering was an indication that differences among 230 lines were largely due to genetic effect. Preetak *et al*. (2018) recorded broad-sense heritability of 0.93, which was comparable to the 0.9 recorded in this study. However, Desissa (2017) recorded a broad sense heritability of 0.59 for days to flowering which was relatively lower than what was recorded in this trial. The number of days to maturity was as low as 83 days for NE3400 and as high as 138 days for PI605823. The number of days to maturity recorded a moderately high heritability estimate of 0.8 in the broad sense which was lower than 89.5% reported by Preetak (2018). The heritability estimates of 0.8 recorded in this study were also lower than the 91.9% recorded by Preetak (2017). Heritability estimates are population specific

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and therefore differences between what was recorded in this study and that of other studies could be due to nature of population used for the study

The number of days to flowering and maturity are key drivers of differentiation in cultivated soyabean leading to the creation of maturity groups (Zhang *et al.*, 2007; Wang *et al.*, 2018) which is largely influenced by photoperiodism. Photoperiod sensitivity of soyabean lines has limited the use of productive temperate varieties in tropics. Of the 13 maturity groups of soyabeans, only members of the group (MG X) are suitable for cultivation in the tropics.
The high variation, and as well as high genetic variance recorded for days to flowering and maturity can be exploited to develop varieties for the different agro-ecologies suitable for soyabean production in Ghana and other African countries. For instance, the line Paranagoina reported to harbouring the long juvenile trait which delays vegetative growth in short-day genotypes (Lu *et al.*, 2017) was part of the lines evaluated. It could be used to adapt some of the productive but photoperiod sensitive temperate lines to the short-day period that characterises our production system as suggested by Miranda (2018).

Growth parameters, such as plant height at physiological maturity, and plant height at first pod (pod clearance) varied among the lines studied. Of the 230 genotypes studied, 50% (H^2=0.5) of the observed variation in heights among the lines was due to the genetic effect. In soyabean, height is closely related to yield (Zhang *et al.*, 2018). Early maturing and short plants are said to have reduced vegetative growth leading to fewer nodes, few pods and lower yield (Ibrahim *et al.*, 2012). Height at plant first pod (pod clearance) was highly heritable with 80% of observed variation accounted for by the genetic variance. Height at first pod or pod clearance is very important when combine harvester is being deployed for harvesting. Lines that pods closer to ground are difficult to feed into the combine which may result in yield loss (https://www.cornandsoyabeandigest.com/soyabeans/11-combine-adjustments-soyabeanharv

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est). For instance, SARI has a combine harvester for soyabean but it cannot be used for harvesting because of the generally lower pod clearance of current soyabean varieties (Dr. Nicholas Denwar, 2017, personal communication). The 230 lines possessed wide variation for pod clearance most of which (80%) was due to genetic variance. This could be used to develop cultivars with higher pod clearance for commercial farmers who normally deploys machinery including combine harvesters. lines in the sub-groups IA and IIA based on PCA both have relatively higher clearance. Lines in IIA were late maturing with higher clearance whilst lines in the sub-group IA were early maturing with relatively high pod clearance. Parents could be selected from these groups for cultivar development that combines high yield, earliness and high pod clearance since early to medium maturing lines are most desired in Ghana (Denwar and Wohor, 2011).

Heritability estimates of 0.7 for NPD/P recorded in this study was slightly lower than the 89.4 and 86.90 reported by Desissa (2017) and Prateek (2014) but comparable to 74% reported by Ali *et al.,* (2014). The 230 lines varied widely for 100 seed weight most of which was due to genetic effect and culminated in high heritability estimates. This result agreed with the observation by Zhang *et al*. (2013) that 100 seed weight was less affected by the environment. NS/P and NPD/P were strongly correlated (r=0.94) and had the same level of heritability. Silva *et al.,* (2014) reported that the number of pods per plant had a strong and positive correlation with productivity. NS/PD was moderately heritable but it was negatively correlated with all the growth parameters, and yield components except NS/P with which it had a significant positive association, and NPB/P with which it had a positive but very weak association.

Principal component analyses grouped the 230 lines into four sub-groups, mainly driven by the number of seeds per plant and number of pods per plant which accounted for 26.3 and 26.4, respectively and days to flowering and days to maturity also contributed to 17% and 15%,

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respectively. Number pods per plant which had the highest contribution to observed variation are the key determinant of soyabean productivity (Silva *et al.,* (2014), an indication that the assembled germplasm varied highly in the most important yield determinant.

Nine sub-populations (clusters) were inferred from a 96-representative sample from the 230 lines genotyped with 32000 SNP markers. Lines obtained from USDA were most diverse as they occurred in 8 of nine sub-populations. Mean Fst values were high most sub-population with the exception of sub-population 8 and 9. Hipparagi *et al*. 2018 recorded mean Fst values in the range of 0.464 to 0.688 for 5 sub-populations inferred from 75 soyabean accessions based on SSR marker. This study recorded mean Fst values in the range of 0.0074 to 0.915 for nine clusters based SNP markers. Generally, mean Fst was for most clusters were high, an indication of high inbreeding coefficient for lines evaluated. High differentiation or inbreeding is expected for a self-pollinated crop like soyabean. However, two clusters recorded very high heterozygosity values which showed that lines in those clusters were still segregating. SARI normally receives advanced breeding lines from IITA. Some of which may still be segregating Also, cluster 8 and 9 with high heterozygosity could be containing early breeding lines which mistaken got mixed up with the advanced breeding lines and therefore few generations of advancement would be required before they are placed in the germplasm pool for utilisation.

Hierarchical clustering grouped the 96 accessions into two major clusters with accession sourced from the USDA occurring in both clusters. Plant introductions obtained from the USDA distributed into all subpopulations except two IITA lines were closely related and grouped into two sole sub-populations. Lines from Zimbabwe occurred in two populations mixed with Ghanaian released varieties and few plant introductions from the USDA. Brazilian accessions were close to USDA accessions. This is a reflection of the extent of germplasm sharing between the two countries. IITA lines and USDA accessions were very distinct, and so

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the two sources could serve as different pools from which materials could be assembled for varietal development.

Although they occurred in the same major clusters which were similar to the pattern inferred from cluster analysis, the Ghanaian varieties were closely related to Zimbabwe accessions than to IITA accessions as revealed by hierarchical clustering.

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4.5 Conclusion

The assembled germplasm possessed high variation in most of the morphological traits evaluated. Heritability estimates in the broad sense revealed that variation between individuals for these traits were largely due to genetic effect. Most of Ghana's soyabean production occurs in the Guinea Savannah ecology with monomodal rainfall pattern. Early to medium maturing lines are more suited for this ecology for which reason, varietal development has been targeted at combining high productivity with earliness. However, a larger area of the country, spanning the Transition belt and Coastal savannah ecologies are deemed suitable for soyabean production and these areas with bimodal rainfall pattern can support different crop growth duration as compared to Guinea and Sudan ecologies. Fortunately, days to flowering and days to maturity, key determinants of growth duration, both varied widely and were highly heritable. This could be exploited to develop cultivars for the different agro-ecologies with regards to maturity period

The number of pods per plant, number of seeds per plant, and seed weight, which are the main determinants of soyabean productivity were also very variable and mainly under genetic control. The number of pods per plant, number of seeds per plant, and days to flowering were the main drivers of variation among the assembled germplasm. This understanding will not only guide the formulation of utilisation and conservation strategies, but also the decision on what characteristics to consider for future collections. Principal component analysis clustering showed that some accessions combined earliness with high pod and seed production. This could be exploited to develop early maturing but high-yielding cultivars for the Guinea and Sudan savannah ecologies.

Structure and diversity analyses proved that all the lines were distinct individuals and subsequently placed them into sub-populations which were genetically distant as revealed by

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allele frequency divergence and mean Fst values. Grouping by PCA based on phenotypic traits could be combined with grouping based on SNP markers to aid in parental selection, and as well conservation efforts. The distinctiveness of IITA accessions from the USDA collection proved the need to maintain and characterise these lines that SARI has accumulated over the years. More so, the IITA accessions are TGX lines which are adapted to the short-day length and high temperature in the tropics. Their utilisation for varietal development comes with little or no linkage drag as compared to the USDA collection in which some accessions were photoperiod sensitive, high pod shattering and had green, black and brown seed coat colours.

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CHAPTER FIVE

5.0 Field evaluation of soyabean lines for identification of sources of resistance to bacterial leaf pustule

5.1 Introduction

Bacterial leaf pustule, caused by *Xanthomonas axonopodis* pv. *Glycines* is among the major diseases of soyabean in several major soyabean producing countries (Wrather *et al.,* 2001; Kim *et al.,* 2008; Kime *et al.,* 2011). It was the only bacterial disease among eight documented diseases affecting soyabean in Ghana (Offei *et al.,* 2008). Bacterial leaf pustule is reported to cause yield loss in the range of 15-50% (Rahayu, 2007).

The main characteristic of bacterial leaf pustule disease is brown lesions with yellow halos on adaxial side of leaves with raised pustules at the abaxial side of leaves (Sinclair, 1999a). The yellow-brown lesions on the adaxial side of the leaves coalesces into bigger spots, depleting the chlorophyll content of leaves, and under severe conditions this may results in premature leave senescence, defoliation and death of plants (Hartman and Murithi, 2017).

Xanthomona axonopodis pv. glycine the causal agent of bacterial pustule, survives or overwinters on the debris; leaves, pods, and stems, as well as the seeds of infected plants (Sinclair, 1999b). On infected soyabean fields, the pathogen spread from one plant to another or to nearby fields through rain splashes and wind (Goradia *et al.,* 2004). Infected seed is one of the important means of spreading bacterial leaf pustule disease (Khaeruni *et al.,* 2007). The use of farmers' own seed, and sharing of such seeds among farmers is a common practise in Ghana like in many countries in sub-Sahara Africa. This practise facilitates the spread of seed borne diseases including bacterial pustule.

Cultural control including crop rotation, tillage and altering planting dates are some of the strategies used to controlling bacterial leaf pustule (Houng *et al.,* 2012). For instance, Houng

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et al., (2012), observed that delaying of planting for 15-30 days relative to the traditional planting date at Suwon and Naju in Korea reduces incidence of a bacterial pustule significantly for susceptible and moderately susceptible cultivar. The brevity of the cropping season in the Guinea and Sudan savannah ecologies, where most of Ghana's soyabean is produced leaves very little room for delaying the planting date. The use of agro-chemicals is the predominant approach to controlling bacterial pustule but excessive use of chemicals often gives rise to resistant strains in addition to ecological and environmental damages (Acuna *et al.*, 2009). Therefore, much emphasis is being placed on the need for a more sustainable, and ecological friendly approaches (Khaeruni *et al.*, 2010).

The use of biological agents to control pests and diseases including bacterial leaf pustule has been suggested as a more sustainable and environmentally friendly than agro-chemicals (Bale *et al.*, 2007*;* Khaeruni *et al.*, 2010; Bommarco, *et al.*, 2013). Khaeruni *et al.*, (2018) evaluated biological fertilizers (biofresh) composed of three rhizobacteria types, *Bacillus ceresus* ST21b, *B. subbtilis* ST2le, *Serratia sp* 2229A in different formulations and concluded that a solid formulation of biological fertilizer in combination of compost made from soyabean residue was effective at increasing the resistance of soyabean plants to bacterial leaf pustule. Increased resistance to bacterial leaf pustule arose from increased salicylic acid and peroxidase content of plants treated with the biological fertilizer (Khaeruni *et al.*, 2018). Biological control, though very sustainable, its application is not popular due to lack of investment (Bale, 2007). Although cultural and biological control of plant diseases are known to be sustainable compared to agro-chemicals, the use of resistant genes remains the most effective approach to controlling pests and diseases. The use of resistant genes for the control of plant diseases is cost effective and environmentally friendly (Matsuo, 2014). Identification of sources of resistance presents the first step towards deploying resistant genes for disease control (Lovato, 2011). Identification of sources of resistance to pests and diseases is achieved through disease screening. Screening

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of crops for resistance to pest and diseases is performed under natural infestation, mostly on the field or through artificial inoculation. As noted by Abawi (2010), field screening for resistance provides for evaluation under a variety of interacting pressures including pathogenic and non-pathogenic organisms. Additionally, screening crops for diseases provides useful information on released varieties needed by farmers to minimise losses by choosing varieties suitable for a given area based on knowledge of prevailing pests and diseases (Lovato 2011). Several sources of resistance to bacterial leaf pustule have been reported including Danbaekong, SS_{2-2}, and Pi96188 (Kim *et al.*, 2011), and CNS soy (Hartwig, 1951). However, race specificity of resistant genes and environmental effects on gene expression often results in the breakdown of resistance with the emergence of new race of the pathogen (Brun *et al.*, 2010). Therefore, it is important that sources of resistant are evaluated in a target environment to determine their effectiveness before deployment in breeding programmes. Additionally, there is the need to avoid linkage drag in order to speed up varietal development and release. Most soyabean cultivars released in Ghana and other African countries are tropical glycine lines which were bred for short day length and high temperatures that characterised production centres on the continent (Ibrahim, 2011). Thus, introducing resistant gene from poorly adapted sources may delay varietal release as loci associated with undesirable characteristics such as photoperiod sensitivity, pod shattering, and undesirable seed coat colour may take a while to eliminate. Therefore, it is important to find sources of resistance to bacterial leaf pustule in the Guinea savannah ecology where most of the country's soyabean is produced.

The objectives of this study were to

1. evaluate diverse lines for resistance to bacterial leaf pustule under field conditions
2. select bacterial leaf pustule resistant lines for cultivar development

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5.2 Materials and Methods

5.2.1 Materials

Plant materials used in this study involved a 250 soyabean lines comprising of 131 plant introductions (PI) obtained from the USDA; 66 advanced breeding lines collected from the International Institute for Tropical Agriculture (IITA); 31 soyabean lines obtained from Zimbabwe in SARI's holding; 14 Brazilian accessions, 3 Chinese accession lines, and 5 Ghanaian released varieties.

5.2.2 The study area

The study was conducted at the experimental fields of the Savannah Agriculture Research Institute, Akukeyili in the Tolon district. The area is characterised by Ferric Luvisols of the Tingoli series which is well drained, devoid of concretions, and brown in colour (Atakora and Kwakye, 2016).

5.2.3 Experimental design and field establishment

The experiment was laid at the same site (N09°24.388; W000°59.281) for two cropping seasons, 2017 and 2018. The field trials were established on the 24th of June and 18th of June for 2017 and 2018, respectively. In both years, the trial was established on the same plot.

The trial was laid in an alpha lattice design in two replicates with each replication comprising 25 blocks of 10 lines per block. Each line was planted in single a single row of 5m long with a plant spacing of 5cm. Rows were planted at 50cm apart. A spreader row using the susceptible genotype, PI81042 a highly susceptible genotype which susceptibility was confirmed in 2016 during seed multiplication on the field was planted between lines under evaluation. Phosphorus in the form of Triple Superphosphate (TSP) was applied at a rate of 60kg of P_2O_5 at emergence. Weed control was manually done with hoes.

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5.2.4 Data collection and analysis

The number of days to 50% flowering was recorded as the time each line took from planting to when 50% of its stands had at least one flower opened.

At flowering, fifty plants of each line were tagged and used to estimate the incidence of bacterial leaf pustule, twenty of which were scored for severity. Severity was scored once in every 14 days for four consecutive times. The scoring for severity occurred over a period of 56 days upon flowering. Disease severity score recording was based on 1-9 scale (Subrahmanyam, 1990) where a score of 1 denotes no spots and 9 denotes complete defoliation or death of the infected plant. Concurrently, four chlorophyll content readings were taken from 20 record plants per lines for disease severity using SPAD meter. Chlorophyll content reading also started at flowering.

The four severity score values were used to estimate the area under disease progressive curve (AUDPC) according to the formula prescribed by Emge and Shrum (1979).

$$\text{AUDPC} = \sum_{i=1}^{n} \frac{y_i + Y_{i+1}}{2} \times (t_{i+1} + t_i) \quad \ldots . eqn\ 1$$

Where n= number of observations, yi= severity score at the ith observation, and ti= time at the ith observation. The area under disease progress curve obtained for each line was used to represent the overall reaction of the line to bacterial leaf pustule infection.

Using equation 1 above, the area under the chlorophyll retention curve (AUCRC) was estimated for each line. The AUCRC value obtained for each was used to represent the depletion of chlorophyll content due to bacterial leaf pustule infection and development on each line. Values of AUDPC and AUCRC obtained were analysed using the R statistical software (version 3.5.1) (R Core Team, 2018).

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Analyses of AUDPC was first carried out on yearly bases and ranking of genotypes compared. When genotypes ranked the same in both years, combined analyses of variance was carried out to assess the effect of year, and interaction effect of year and genotypes.

Both year, and interaction between year and genotypes were not significant. The AUDPC data for both years were then standardised as described by Forbes, *et al*. (2014) and combined for analyses where years were considered as replications and thereby increasing the total number of replications to four to increase statistical power.

Genotypic effects were estimated using a linear mixed-effect model (LMEM) with restricted maximum likelihood (REML) based on the equation below:

$$y_{ijk} = g_i + ry_j + by_k + \varepsilon_{ijk} \qquad eqn\ 2$$

Where y_{ijk}, g_i, r_j, b_k and $\mathcal{E}_{ijk}$ denote observation on genotype i in block k nested within replication j, an effect of genotype i, effect of replication j, effect of block k nested within replication k and the effect of random error [$\sim N\ (\mu = 0,\ \sigma^2 = 1)$]. The model was fitted using the lme4 package (Bates *et al.*, 2015) of R.

Soyabean line, PI606440A described as resistant with few lesions was used as a check (intercept) for pairwise comparison and identification of resistant lines. The difference between the best linear unbiased estimate of AUDPC for each line and the intercept were subjected to pairwise t-test using the Satterthwaite's method in R.

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5.3 Results

Best linear unbiased estimates (BLUES) of the area under disease progress curve for the 250 lines as compared to that of the intercept (PI606440A) is presented in the (Appendix 2). Table 5.1 presents the result of a sub-sample of 35 of the 250 lines evaluated for bacterial leaf pustules disease under field condition.

The reaction of the line, PI606440A used as intercept, to bacterial leaf pustule under field condition recorded over time and presented in the form of AUDPC (61.50) was statistically higher than zero (Table 5.1). The check, PI606440A recorded a maximum score of 2.5 on a 1-9 severity score scale and considered as resistant, but not highly resistant (Appendix 1). The lines with estimated AUDPC values less or equal to that of that of the intercept were considered resistant. The lines with lower AUDPC values than the intercept recorded negative fixed effect estimates and were therefore considered to be more resistant than the check (Table 5.1) whilst lines that had positive values that were statistically similar to the intercept were considered to have the same of level of resistance as the intercept PI606440A). 28 of 250 soyabean lines evaluated recorded negative fixed effect estimates ranging from -28.8 to - 24.8 relative to the intercept (PI606440A) (Table Appendix 2) constituting 10.7% of lines screened. All the 28 lines recorded severity score of "1" (Appendix 1), a reaction described as resistant without lesions by Grant *et al.*, (2010). As indicated in Table 5.1., SARISOY 5(-28.8) and SARISOY 20B (-28.57) from SARI's soyabean germplasm assembly recorded the least estimates of AUDPC relative to the intercept and even though, their fixed effect estimates were not statistically different from the intercept, they were highly resistant than intercept as they recorded no lesions (Appendix 2) dues to *Xanthomona axonopodis pv. glycine* infection. These were followed by the plant introductions, BRS 8660A, BRS-7980B and PI274954B (Table 5.1). The tropical glycine lines TGX-1990-21F (-26.94) and TGX-1989-48FN (-26.90) from IITA were among the most resistant lines (Table 5.1)

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Two released soyabean varieties in Ghana, Afayak (-24.8) and Sal-1(-24.8) were among the highly resistant lines. Jenguma, a widely cultivated variety was moderately resistant having recorded a fixed effect estimate of -3.7 relatives to the intercept and a severity score of 3. Two varieties, Quarshie (83.84) and Songdaa (97.82) also released in Ghana were highly susceptible with AUDPC fixed effect estimates significantly ($p<0.01$) higher than the intercept (Table 5.1).

The lines PI468919, SARISOY 10, TGX 1805-8F, 1990-80F, TGX 19988-3F and BRS 8660 with fixed estimates of 1.11, 1.60, 3.27, 4.70, 5.06, 29.48, respectively were statistically similar to the intercept in their reaction to bacterial leaf pustule disease. However, PI605791A (158.97), PI417120A (162.6), PI81042 (166.19), PI417120 ((176.5), PI605854B (177.7), PI606405 (183.3), PI398825 (190.5), PI506938 (190.5), and PI567516C (209.8) were among the most susceptible of the 250 lines evaluated (Table 5.1). The area under disease progress curves of these lines was higher than that of the intercept and the differences between them and the intercept was statistically significant ($p<0.001$) (Table 5.1)

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Table 5.1: Fixed effect estimates of AUDPC for soyabean lines in relation to the intercept

Line	Estimates	Std. Error	Df	t-value	Pr (>|t|)
SARISOY 5	-28.8	31.66	211.18	-0.91	0.364
SARISOY 20B	-28.57	31.66	211.18	-0.9	0.368
BRS 8660A	-28.23	31.66	211.18	-0.89	0.374
BRS-7980B	-28.2	31.66	211.18	-0.89	0.374
PI274954B	-28.2	31.66	211.18	-0.89	0.374
TGX-1990-21F	-26.94	25.84	210.99	-1.04	0.298
TGX-1989-48FN	-26.9	25.84	211	-1.04	0.299
PI506677	-26.77	25.84	210.99	-1.04	0.301
ISS-8203	-26.49	25.84	211	-1.03	0.306
Afayak	-24.8	25.84	211	-0.96	0.338
Sal-1	-24.875	31.66	211.18	-0.78	0.435
TGX 1990-57F	-15.987	25.84	211	-0.61	0.54
JENGUMA	-3.7	25.84	211	-0.14	0.886
TGX 1989-20FA	-2.02	25.84	210.99	-0.08	0.938
PI468919	1.11	25.84	211	0.04	0.966
SARISOY 10	1.6	25.84	211	0.06	0.951
TGX 1805-8F	3.27	31.66	211.18	0.1	0.918
TGX 1990-80F	4.7	25.84	211	0.18	0.856
TGX 1988-3F	5.06	25.79	199.7	0.2	0.845
BRS 8660	29.48	25.84	211	1.14	0.255
(Intercept)	61.5	18.38	205.69	3.35	0.001**
Quarshie	78.47	25.84	211	3.04	0.003**
PI159925	83.84	25.84	211	3.25	0.001**
Songdaa	97.82	25.84	211	3.79	0.000***
TGX-1989-68FN	102.82	25.84	211	3.98	1E-04***
PI605791A	158.97	25.84	210.99	6.15	3.8E-09***
PI417120A	162.6	25.84	210.99	6.29	1.8E-09***
PI81042	166.19	25.84	211	6.43	8.3E-10***
PI417120	176.5	25.79	199.8	6.84	9.3E-11***
PI605854B	177.7	31.66	211.18	5.61	6.2E-08***
PI606405	183.3	25.84	210.99	7.09	1.9E-11***
PI398825	190.49	25.84	211	7.37	3.8E-12***
PI506938	197.5	25.84	211	7.64	7.3E-13***
PI567516C	209.58	25.84	211	8.11	4.1E-14***

Note: *, **, and *** represent significant at 5, 1 and 0.1 percent, respectively.

Grouping of the lines based on AUDPC estimates placed the lines into two major groups, I and II (Fig 5.1). Group I consist of a subset of the susceptible lines whilst group II is comprised of resistant lines. Resistance to bacterial leaf pustule could be placed into three categories including highly resistant, resistant, and moderately as indicated in Fig 5.1. The highly resistant

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category was made up of lines that recorded no lesions, and therefore had an immune response to the pathogen, *Xanthomona axonopodis pv. glycine* under natural infestation conditions on the field. Of 250 lines evaluated, 28 lines comprising of 10 plant introductions (PI587886, PI274954B, PI398512, PI398874, PI416778A, PI416810A, PI416939, PI417126A, PI506677, ISS-8203, PI506939); three Brazilian lines (BRS 8660A, BRS-7980B, PARANGOINA); five SARI's germplasm holding (SARISOY13A, SARISOY15, SARISOY20B, SARISOY2A and SARISOY5); eight advanced breeding lines from IITA (TGX 1989-20FC, TGX 1990-80FA, TGX-1835-10E, TGX-1989-40FB, TGX-1989-48FN, TGX-1989-48FNA, TGX-1990-21F and TGX1989-68FN); and one variety released in Ghana, Sal-1 had severity score of 1 and were classified as highly significant (Fig 5.1 & Appendix 2).The resistant category was composed of lines that had a negative fixed effect estimate relative to the intercept with a mean severity score in the range of 1.5 to 2.

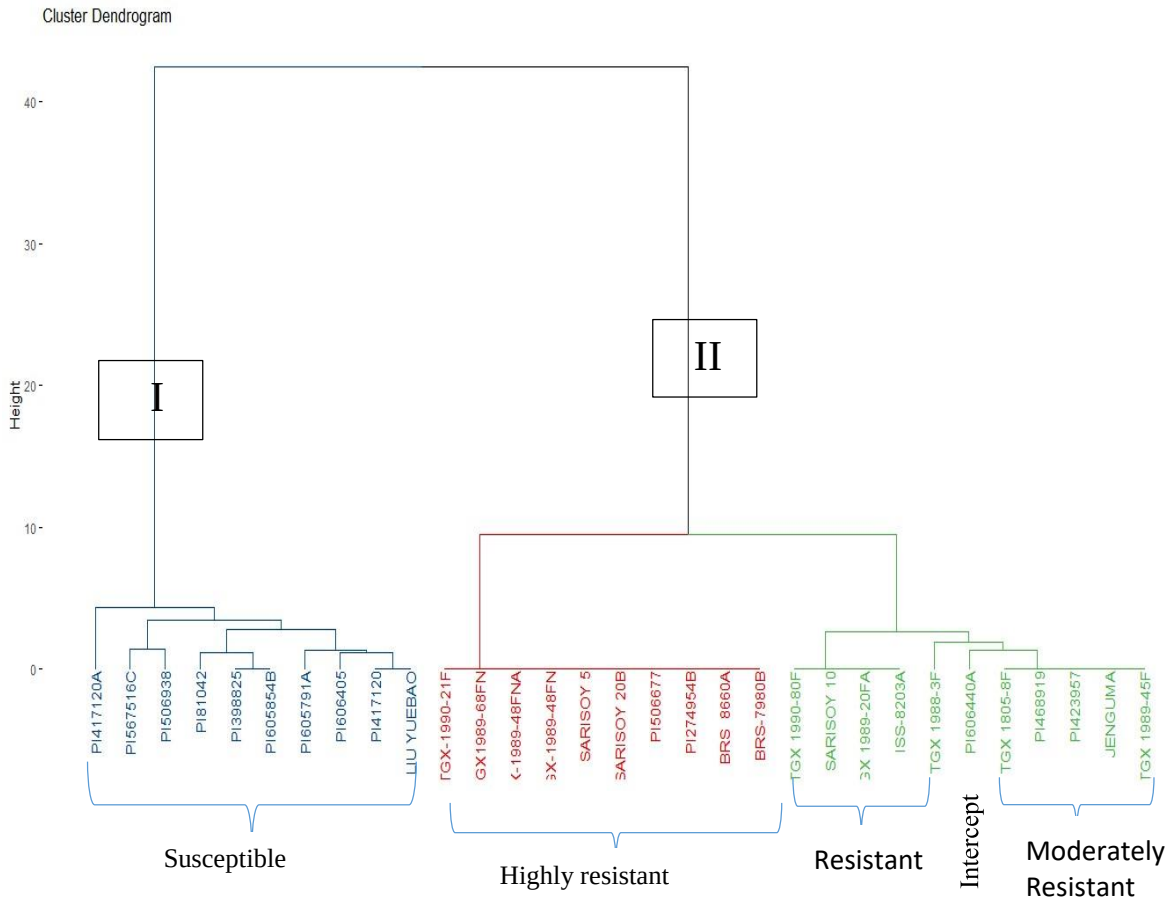

Fig. 5: 1: Grouping of soyabean lines based on fixed effects estimates for AUDPC

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The number of days it took soyabean lines from emergence to flowering (DTFR) had a significant (P<0.001) negative correlation (r=-0.45) with the area under disease progress curve (AUDPC) (Fig 5.2) recorded for the lines. On other hand, DTFR had a strong (P value<0.001) positive association (r= 0.64) with area under chlorophyll reduction curve (AUCRC). Also, the area under chlorophyll retention (AUCRC) had a significant (P<0.001) but negative association (r=-0.52) with AUDPC (Fig 5.2).

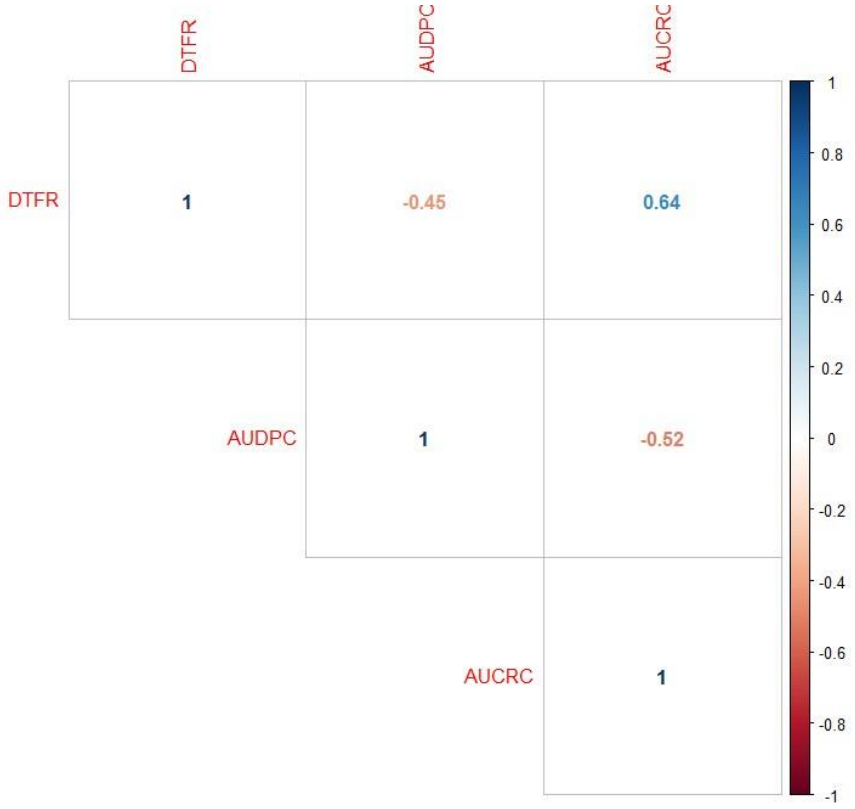

Fig 5.2: Associations between DTF, AUDPC, and AUCRC

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5.4 Discussion

The result of the field evaluation revealed reactions of the lines to bacterial leaf pustule disease. This was through natural infestation for two consecutive years. Disease pressure was high in both years which enabled successful characterisation of the lines with regards to the pathogen, *Xanthomonas axonopodis pv. glycine.*

Broadly, lines were grouped into two classes, resistant and susceptible. The resistant class was further separated into highly resistant or immune, resistant, and moderately resistant or tolerant. 28 lines had an immune response to the pathogen *Xanthomonas axonopodis pv. glycine* infection, and were therefore classified as highly resistant. Of the 28 highly resistant lines, eight including TGX 1989-20FC, TGX 1990-80FA, TGX-1835-10E, TGX-1989-40FB, TGX1989-48FN, TGX-1989-48FNA, TGX1989-68FN, TGX-1990-21F were advanced breeding lines sourced from IITA. Five Zimbabwean accessions, SARISOY 13A, SARISOY 15, SARISOY 20B, SARISOY 2A and SARISOY 5 were also part of the 28 highly resistant lines. Only one Ghanaian genotype Sal-1(Salintuya I), a variety released in 1985 (CSIR, 2015) was part of the 28 highly resistant lines identified in this study.

Most of the lines in the 28 highly resistant lines were plant introductions sourced from the USDA such as PI506677, PI506939, PI398512, and PI398874. These lines had a smaller area under the disease progress curve and therefore recorded negative fixed effect estimates relative to the intercept (check), although, their AUDPC scores did not differ significantly from that of the intercept

The second class of resistant lines simply designated as resistant, as grouped by AUDPC estimates were synonymous to lines that had a mean severity score of 1.5 to 2 on a 1-9 severity score scale. This tallies with lines classified as resistant with few lesions in the GRIN database (GRIN, 2018). Lines classified as moderately resistant based on AUDPC estimates had severity

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score ranging from 2.5 to 4. Lesions were conspicuous in the lower canopy of lines in this category but only a few lesions were observed in their upper canopy. Lines in this category were not significantly different from the intercept (PI606440A) according to a pairwise T-test based on AUDPC estimates. Jenguma, the most cultivated Ghanaian variety released in 2003 (National Seed Council, 2015) was grouped in this category based on AUDPC estimates.

The susceptible group could be further divided into susceptible and highly susceptible. Lines in the susceptible category such as PI230970, PI398387, and LG 04-600 had severity scores of 5. Both lower and upper canopy of lines in this group were covered by lesions of bacterial leaf pustule. Lesions were observed on petioles and pods but no leaf defoliation occurred as reported by Hartman and Murithi (1999). These lines differed significantly from the intercept in the pair-wise T-test. On the other hand, leaf defoliation varying from few leaves to complete defoliation were observed, and in some lines, premature maturation and death occurred in a the highly susceptible category. Lines in this category recorded severity score from 6 to 9. AUDPC estimates for lines in this class differed significantly from the intercept.

In this study, natural infestation provided the needed disease pressure which led to the successful characterisation of the 250 lines with regards to their reaction for a bacterial pustule. This result agrees with Abawi (2010) who noted that field screening for resistance provides for evaluation under a variety of interacting pressures including pathogenic and non-pathogenic organisms. Narvel *et al.,* (2001) successfully mapped bacterial leaf pustule resistant gene (Rxp) in a segregation population through natural disease infestation under field condition.

It was observed that some of the plant introductions (PIs) from the USDA recorded different reactions to bacterial leaf pustule as compared to their classification based on the disease as stated in The Germplasm Resources Information Network" (GRIN)database

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(https://npgsweb.ars-grin.gov/gringlobal/descriptors.aspx). For instance, PI417120 was classified as resistant with few lesions according to information on the GRIN database, but it was classified as susceptible and per its high AUDPC estimate, it actually belonged to the very susceptible group with a mean severity score of 8.5 in this study. However, some other lines recorded similar reaction and rating in this trial as their previous reactions stated on GRIN database. This included PI506677 classified as resistant with no lesion which also recorded immune response and was classified as highly resistant in this study. Also, PI81042 classified as completely covered was also classified as susceptible in this trial with a severity score of 8.5.

The observed changes in the reaction to a bacterial pustule recorded for some lines in this trial as compared to previous reports could be due to break down of resistance. Pathogens including *Xanthomonas axonopodis pv. glycine* has the inherent ability to evolve to overcome the resistance offered by plants. The breakdown of resistance is often induced by the emergence of a new race or physiological form of the pathogen through mutation or introduction of a different race of the pathogen from another area (Rimbaud, 2018). Resistance breakdown is frequent with resistance conferred by a single or few genes as compared to resistance due to many genes (Brun *et al.*, 2010). Single recessive genes were reported to be the source of resistance to bacterial leaf pustule in PI96188, and CNS soy (Hartwig-1951; Kim *et al.*, 2011). It is therefore not surprising that some of the lines had a complete breakdown of resistance in this study.

It was observed that most of the lines classified as resistant in this study flowered after 40 days. This resulted in a significant negative association between AUDPC and days to flowering (DTF). Higher values of AUDPC connotes susceptibility, hence the negative correlation between DTF and AUDPC implies that increases in DTF resulted in decreases in AUDPC.

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Most of the lines classified as resistant in the GRIN database belonged to maturity groups MGV, MGVI, MGVII, and MGVII. Lines in these maturity groups require a higher number of days to mature and this generally agreed with results obtained in this study. However, there were few lines that had resistant disease score but flowered earlier than 40 days. These lines could be evaluated and used to develop a bacterial pustule resistant early maturing cultivar for the Guinea and Sudan savannah ecologies of Ghana.

SPAD readings taken during disease evaluation were used to estimate the area under chlorophyll retention (AUCRC). AUCRC had a significant but negative association with AUDPC. Chlorophyll depletion increased with disease severity and therefore, resistant lines retained higher chlorophyll content in their leaves than susceptible lines. Also, AUCRC had a positive association with DTF. This observation agrees with the association between DTF and resistance to bacterial pustule. In general, late maturing lines were resistant to bacterial pustule infection and therefore, there was less depletion of chlorophyll content of their leaves. Thus, a higher number of days to flowering generally resulted in higher chlorophyll content recorded over time which resulted in the positive association between the two traits.

Foliar diseases generally degrade leaf tissues including chlorophyll as observed in yellowing or browning of leaves as disease severity increases. Ghose *et al*. (2010) reported a reduction of 53.24, 51.65, 56.55, and 58.04% for total chlorophyll, chlorophyll a, chlorophyll b and βcarotene, respectively in mulberry leaves due to blight infection. Due to the arbitrariness and errors associated with the visual assessment of disease severity, more accurate methods such as the light photography, digital imagery and hyperspectral techniques (Bock, 2010) are commonly used in advance research programmes for screening genotypes for resistance to diseases. However, the high cost and sophistication of some of this equipment is out of reach to most research programmes. Chlorophyll content, a trait easily measured with simple

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equipment such as SPAD, could be a more accurate trait to indirectly access the reaction of plants to foliar diseases like a bacterial pustule.

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5.5 Conclusion

Natural infestation under field conditions provided an effective environment for characterising the reactions of the 250 lines to bacterial leaf pustule. The 1-9 severity rating scale covered the different classes of response to the disease including defoliation and death which the 1-5 scale compounded with other reactions.

The area under disease progress curve resolved the ordinal data into a quantitative one. Grouping of lines based on AUDPC estimates was efficient and resulted in the identification of resistant lines. Twenty-eight highly resistant lines were identified. Fourteen of the twentyeight highly resistant lines were tropical glycine lines obtained from IITA and Zimbabwe. These lines could readily be deployed in breeding programmes since they are adapted to the short-day length and high temperatures that characterises major soyabean production centres in Ghana.

This study also identified a bacterial pustule resistant plant introductions (PIs) obtained from the USDA that had their resistance broken down and will therefore not be useful for developing bacterial pustule resistant cultivars for northern Ghana. The area under chlorophyll retention curve, a trait computed in this study, had a strong association with AUDPC and could, therefore, be further developed for use to indirectly screen soyabean lines for resistance to bacterial pustule.

This result presents a useful information on SARI's soyabean germplasm assembly including released varieties for scientists and farmers. It is highly recommended that the 28 highly resistant lines are further evaluated across bacterial pustule prevalent locations reported in Chapter three to identify stable sources of resistant for cultivar development.

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CHAPTER SIX

6.0 Field evaluation of soyabean genotypes for resistance to Phomopsis Seed decay (PSD)

6.1 Introduction

The current soyabean production in Ghana stands at 143, 000 t from a total cultivated area of 87,000ha (MoFA-SRID, 2016). However, efforts are underway to make soyabean a major staple as well as a cash crop for the country (https://www.feedthefuture.gov/country/ghana). One of the eight key objectives of SIL was the utilization of soyabean for human nutrition through the provision of the high protein content of soyabean supplement for the eradication of malnutrition among children (Goldsmith, 2014). The high protein, carbohydrates, and vitamin A food complement is targeted at babies of six months to augment breastfeeding. Availability of high-quality grains, free of infection will be required for the realisation of this objectives. Though many biotic stresses including pests, diseases, and weeds constitute major production constraint to soyabean world-wide, diseases are by far the most important biotic constraint (Harman, 1999). Globally, many diseases, especially seed, and seed-borne diseases have been reported to cause significant yield loss, reduced grain quality, poor seed germination and high seedling mortality (Mengistu *et al.*, 2012). Many of the pathogens of economic importance to soyabean are associated with its seed (Mengistu *et al.*, 2012). Fungi in *Diaporthe –Phomopsis* complex (DPC) causes Phomopsis seed decay (PSD) and was considered the most important pre-harvest disorder of soyabean seed worldwide (Sinclair, 1999). PSD causes significant yield loss, and reduced grain quality in many countries (Riccioni *et al.*, 2003; Medic´-Pap *et al.*, 2007; Cui *et al.*, 2009).

Many control and management strategies have been explored to limit the effects of PSD. However, the use of resistant genes remains the most cost effective and environmentally sustainable approach to controlling crop diseases including PSD of soyabean (Ali, *et al.*, 2013

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& Li *et al.*, 2015). Many sources of resistance to PSD have been reported (Sun *et al.*, 2013; Brown et al. 1987; Zimmerman and Minor, 1993; Li *et al*., 2011; Minor *et al.*, 1995; Walters *et al.*, 1973; Athow, 1987; Ross, 1986; Brown *et al.*, 1987; Li *et al.*, 2015). However, frequent breakdown of disease resistance requires continuous search for new sources of resistant for disease control.

The first step toward deploying resistant genes is the identification of sources of resistance. The two exploited methods are, artificial inoculation of plants, and natural infestation of pathogens under field conditions. In soyabean, inoculating the stem of seedlings has been a method of screening for resistance to stem canker (Cui *et al.*, 2009; Li *et al.*, 2010) caused by members in the DPC of fungi. A modification of the inoculation of the immature stem of seedlings method has been developed by Li *et al.*, (2018) for rapid evaluation of soyabean lines for resistance to PSD called the cut-seedling assay method. On the other hand, Li *et al.*, (2015) successfully screened and recorded differences among 135 soyabean lines by spraying them with conidiospores to inoculate the lines under field conditions. Abawi (2010) noted that field evaluation of crops for resistance provides for evaluation under a variety of interacting pressures including pathogenic and non-pathogenic organisms. Thus, field evaluation mimics what pertains of on field conditions.

Classifying lines as resistant or susceptible to PSD could be based on visual assessment of seeds of PSD infected plants for symptoms and signs of the pathogen on a 1-5 scale (Li *et al.*, 2015). The other approach involves plating seeds of infected plants on agar medium and culturing it. The growth of the pathogen on seed is recorded and used to estimate the infection rate (Sun *et al.*, 2013).

Li *et al.*, (2015) evaluated 135 soyabean lines at three locations in three years, and recorded a differential reaction of the lines to PSD infection between locations and years. This was

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attributed to differences in the pathogens across trial locations. The existence of different physiological races of pathogens has been linked to differential response of sources of resistance often leading to a breakdown in resistance as observed with Asian soyabean rust (Maphosa *et al*, 2012). In order to find stable sources of resistance to soyabean seed decay, it is imperative to screen assembled germplasm using prevailing strains of PSD in a target environment where the identified resistant genes will be deployed.

It is therefore important to identify sources of resistance to Phomopsis seed decays (PSD) under prevailing conditions of northern Ghana where most of the country's soyabean is produced. The goal of this study was to find sources of resistance to soyabean seed decay for development of PSD resistant cultivars.

Objectives of the study were to;

1. Screen and identify PSD resistant lines for varietal development
2. determine the relationship between growth traits and PSD infection

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6.2 Materials and Methods

6.2.1 Plant materials

Plant materials used in this study involved a 250 soyabean lines comprising 131 plant introductions (PI) obtained from the USDA; 66 advanced breeding lines collected from the International Institute for Tropical Agriculture (IITA); 31 soyabean lines obtained from Zimbabwe in SARI's holding; 14 Brazilian accessions, 3 Chinese accession lines, and 5 Ghanaian released varieties

6.2.2 Experimental design and field establishment.

The trial was laid in an alpha lattice design in three replicates with each replication comprising of 25 blocks of 10 lines per block. Each line was planted in a single row of 5m long with a plant spacing of 5 cm. Rows were planted at 50cm apart. Phosphorus in the form of Triple Superphosphate (TSP) was applied at a rate of 60kg of P_2O_5 at emergence. Weed control was manually done with hoes.

Planting was staggered based on the number of days to flowering data obtained when the lines were grown in the 2016 cropping season. This was done to ensure lines flowered at the same time to facilitate inoculation of lines with spores of *Diaporthe sp*.

6.2.3 Culturing of pathogen to obtain inoculum

Stored *Diaporthe sp.* isolate MTB-1 were cultured on Acidified Potato Dextrose Agar (APDA) prepared. Media was prepared by suspending 15g of Agar in 1litre of distilled water. The resultant was heat sterilised by autoclaving at 121°C for 15 minutes. The prepared medium was acidified by amending it with 50% lactic acid (VWR International) and pH adjusted to 4.8. The acidified PDA was poured into 9cm- diameter petri dishes, cooled to room temperature and inoculated with stored mycelia of MTB-1 strain. The inoculated media was incubated at a temperature range of 25-30℃ and subjected to 12hr light/12hr dark phase. Several cultures

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were incubated simultaneously to obtain sufficient pycnidia from which to collect conidia. Cultures normally produced pycnidia between 15 to 25 days.

6.2.4 Preparation of inoculum and inoculation

Culture plates bearing pycnidia were flooded with sterile water. Suspending pycnidia were agitated with a sterilised ceramic pestle. The resulting suspension of mycelia and conidia were filtered through a cheese cloth into a 1-liter conical flask. Hemocytometer readings and sterile water were used to adjust the concentration of conidia to 1x 10^5. Field inoculation was carried out on rainy days late in the afternoon between 5 and 6:30 pm, using flashlights. Three inoculations were carried out. The first inoculation was carried out at full flowering (R2) stage. The second inoculation was carried out during pod formation (R4) stage and the last inoculation carried at the seed development stage (R5). Five plants of each line were inoculated. Inoculations were targeted at the lower canopy. based on the planting date, flowering occurred in July, the rainiest month of the year during which relative humidity ranged from 72-93% with a mean of 83%. The mean temperature was 27.1 ºC, providing a good condition for infection and development of pathogen. The 12 hours between inoculation and sunshine also provided good conditions for infection whilst dense planting of 50 cm x5 cm ensured canopy closure, giving high humidity in the lower canopies of the plants.

6.2.5 Data collection and analyses

Data were taken on the number of days to flowering and number of days to maturity for each line. Plants were harvested at the R8 stage. Pods were manually plucked and threshed. The seeds were visually assessed and rated on a 1-5 scale as described by Li and Chen (2013) using seed quality parameters including wrinkling, mottling, molding, discoloration, and shrinking where 1= excellent (no bad/infected seed); 2= good (less than 10%/infected seed); 3 =fair

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(1130% bad/infected seed); 4= poor (31-50% bad/infected seeds); and 5= very poor (> 50% bad/infected seeds). Visual assessment was carried out with the aid of a 100 watts LED bulb (Philip).

6.2.6 Plating of seeds and estimation of infection rate

Plating was done according to the general procedure as described in Li and Chen (2013). Twenty-five seeds were randomly counted from each line**.** Seeds were surface disinfected with 70% ethanol, sterilised in 1 % sodium hypochlorite for 2 minutes and then rinsed in distilled water twice. Sterilised seeds were plated on the prepared APDA medium and incubated at a temperature range of 25-30℃ and 12hr light/ 12hr dark phase for 7 days. 25 seeds for each line were plated at a rate of 5 seeds per plate in 5 replications. Seeds with *diaporthe sp*. growth were counted and expressed as a percentage of the number of seeds plated. The scores from the 5 replications of seeds from each line were averaged to represent the score for the line.

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6.3 Result

Most of the lines had visual assessment score of 3. Forty lines had a score of 2 with 2 lines having a score of 1 (Fig 6.1). Infection rate *Diaporthe MTB-1* range from 0 to 93 % with a mean of 53% and a median of 50%.

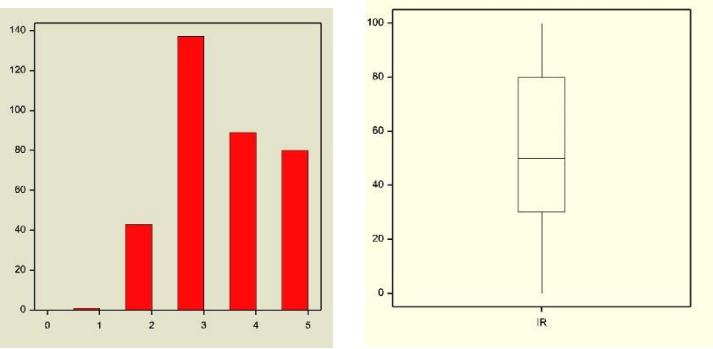

Fig 6. 1: Distribution of visual assessment score and infection rate of soyabean seeds; A (histogram of visual assessment score); B (Box plot of seed infection rate)

Seed weight had a very strong positive relation with seed quality but associated weakly with PLP. Seed quality had a positive significant association with seed infection. Seed quality also related to days to maturity, plant height at maturity and length at first pod and in all cases, the associations were significant. Seed infection rate of the pathogen, *Diaporthe sp* (MTB-1) had a significant correlation with days to 50% flowering, days to maturity and plant height. With the exception of 100 seed weight, height at first pod had a significant positive association with all the other traits.

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Table 6. 1: Pair-wise relation among growth traits, seed quality and seed infection rate

DTFF	0.07					
DTM	0.32***	0.72***				
IR	0.1	0.15*	0.11*			
PHT	0.04	0.26***	0.23**	0.17*		
PLP	0.09	0.27***	0.22***	0.07	0.32**	
VQ	0.30***	0.05	0.22***	0.13*	0.21**	0.10*
	%100SW	DTFF	DTM	IR	PHT	PLP

Note: DTF (days to 50% flowering), DTM (days to maturity), IR (seed infection rate), PHT (plant height at maturity), PLP (Plant height at first pod), and VQ (visual quality assessment), 100SW(100 seed weight)

Pair-wise t-test was performed in which the fixed effects estimate for each line was compared to that of the intercept, Jenguma, a variety that is moderately resistant to the PSD infection (Table 6.2). When analyses were performed on visual quality score, only ten lines recorded significantly lower scores than the intercept. Songdaa was the only locally released variety, and the only TGX line in SARI's germplasm holding that had a significantly lower score than the intercept. Lines that differed significantly from the intercept had lower score. Jenguma had a score of 3 which implies a fair score with infection rate between 11-30%. The ten lines presented in Table 6.2 had a mean score in the range of 1-2.5 and are the most resistant lines.

Table 6: 2 : Fixed effect estimates of visual scores (VQ) for lines relative to check

Line	Estimate	Std. Error	df	t value	Pr(>|t|)	
PI476905A	-3	8E-01	1E+02	-3E+00	1E-03	**
PI416873B	-2	9E-01	1E+02	-2E+00	2E-02	**
PI398825	-2	9E-01	1E+02	-2E+00	2E-02	**
PI605891A	-2	8E-01	1E+02	-3E+00	1E-02	**
PI594767B	-2	8E-01	1E+02	-3E+00	1E-02	**
Songdaa	-2	8E-01	1E+02	-3E+00	1E-02	*
FT christa	-2	8E-01	1E+02	-3E+00	1E-02	*
PI567516C	-2	8E-01	1E+02	-3E+00	1E-02	*
PI423958	-2	9E-01	1E+02	-2E+00	5E-02	*
PI379621	-2	7E-01	1E+02	-2E+00	3E-02	*
Jenguma(check)	4	6E-01	1E+01	7E+00	5E-10	***

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Pair-wise test of fixed estimates of infection rates of lines against the intercept showed 59 lines had significantly lower fixed estimates than the check (Table 6.3). Infection rates were generally higher. Only 3 lines had mean infection rate below 10%. These were the lines with fixed effect estimates of -8. Fixed estimates for lines that performed better than the intercept ranged from -8 to -4, revealing five classes of lines that performed better than intercept. Songdaa had a fixed estimate of -6 and belonged to the third class of lines that performed better than the check. 9 lines obtained from Zimbabwe had the same estimates as songdaa. 28 of the 59 lines that had performed better than the intercept were 'TGX' lines from Zimbabwe and IITA. Full list of the fixed effect estimates of line based on both visual assessment and infection rate are presented in Appendix 3 and Appendix 4, respectively.

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Table 6. 3: Fixed estimates of infection rate (IR) relative to the check

Line	Estimate	Std. Error	df	t value	Pr(>|t|)	Sig
Liu Yuemang	-8	2E+01	1E+02	-4E+00	3E-04	***
PI224271	-8	2E+01	1E+02	-5E+00	1E-05	***
PI417120	-8	2E+01	1E+02	-4E+00	3E-04	***
PI423958	-7	2E+01	1E+02	-4E+00	3E-04	***
BRS 361	-7	2E+01	1E+02	-4E+00	4E-05	***
PI417013	-7	2E+01	1E+02	-4E+00	4E-05	***
PI567516C	-7	2E+01	1E+02	-4E+00	4E-05	***
Liu Yuebao	-7	2E+01	1E+02	-4E+00	1E-04	***
Parangoina	-7	2E+01	1E+02	-3E+00	2E-03	**
PI567091A	-7	2E+01	1E+02	-4E+00	1E-04	***
SARISOY 10	-7	2E+01	1E+02	-3E+00	2E-03	**
SARISOY 15	-7	2E+01	1E+02	-3E+00	2E-03	**
SARISOY 18	-7	2E+01	1E+02	-3E+00	2E-03	**
SARISOY 21	-7	2E+01	1E+02	-3E+00	2E-03	**
SARISOY 26	-7	2E+01	1E+02	-3E+00	2E-03	**
SARISOY 5	-7	2E+01	1E+02	-3E+00	2E-03	**
SARISOY 21	-7	2E+01	1E+02	-3E+00	2E-03	**
TGX 1993-4FN	-7	2E+01	1E+02	-4E+00	1E-04	***
Songdaa	-6	2E+01	1E+02	-4E+00	4E-04	***
BRS 313	-6	2E+01	1E+02	-3E+00	1E-03	**
PI200492	-6	2E+01	1E+02	-3E+00	7E-03	**
PI230970	-6	2E+01	1E+02	-3E+00	1E-03	**
PI416810	-6	2E+01	1E+02	-3E+00	7E-03	**
PI605791A	-6	2E+01	1E+02	-3E+00	1E-03	**
PI605891A	-6	2E+01	1E+02	-3E+00	1E-03	**
PI628850	-6	2E+01	1E+02	-3E+00	1E-03	**
SARISOY 27	-6	2E+01	1E+02	-3E+00	7E-03	**
SARISOY 13	-6	2E+01	1E+02	-3E+00	7E-03	**
SARISOY 18	-6	2E+01	1E+02	-3E+00	7E-03	**
TGX 1903-7F	-6	2E+01	1E+02	-3E+00	1E-03	**
PI416810A	-5	2E+01	1E+02	-3E+00	8E-04	***
PI 534545	-5	2E+01	1E+02	-3E+00	3E-03	**

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Table 6.3: Cont'd

PI417126	-5	2E+01	1E+02	-3E+00	3E-03	**
TGX 1989-75N	-5	2E+01	1E+02	-3E+00	3E-03	**
TGX 2010-11F	-5	2E+01	1E+02	-3E+00	3E-03	**
TGX1989-68FN	-5	2E+01	1E+02	-3E+00	3E-03	**
Zamboani	-5	2E+01	1E+02	-3E+00	3E-03	**
PI200526	-5	2E+01	1E+02	-2E+00	3E-02	*
PI398825	-5	2E+01	1E+02	-2E+00	3E-02	*
PI399096	-5	2E+01	1E+02	-3E+00	7E-03	**
PI416806A	-5	2E+01	1E+02	-2E+00	3E-02	*
PI416806B	-5	2E+01	1E+02	-2E+00	3E-02	*
PI416939	-5	2E+01	1E+02	-3E+00	7E-03	**
PI518295	-5	2E+01	1E+02	-3E+00	7E-03	**
PI584796	-5	2E+01	1E+02	-3E+00	7E-03	**
PI594538A	-5	2E+01	1E+02	-3E+00	7E-03	**
PI603176A	-5	2E+01	1E+02	-2E+00	3E-02	*
SARISOY 23	-5	2E+01	1E+02	-2E+00	3E-02	*
SARISOY 10	-5	2E+01	1E+02	-2E+00	3E-02	*
SARISOY 4	-5	2E+01	1E+02	-2E+00	3E-02	*
TGX 1990-95F	-5	2E+01	1E+02	-2E+00	3E-02	*
PI159925	-4	2E+01	1E+02	-2E+00	2E-02	*
PI416778	-4	2E+01	1E+02	-2E+00	2E-02	*
PI416886	-4	2E+01	1E+02	-2E+00	2E-02	*
PI567058D	-4	2E+01	1E+02	-2E+00	2E-02	*
PI567188	-4	2E+01	1E+02	-2E+00	2E-02	*
PI606405	-4	2E+01	1E+02	-2E+00	2E-02	*
PI635999	-4	2E+01	1E+02	-2E+00	2E-02	*
TGX 1989-42F	-4	2E+01	1E+02	-2E+00	2E-02	*
Jenguma(check)	8	1E+01	1E+02	6E+00	2E-09	***

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6.4 Discussions

Most of the lines had very low seed quality which resulted in fewer lines recording a score between 1-2.5, the category that could be described as resistant to moderately resistant. Infection rate which scores for the presence of a specific pathogen was high but had more lines performing better than the intercept. Seed quality significantly correlated with day to maturity and so was infection rate. The association between seed infection rate and seed quality was positive but not significant. This implies that the scored pathogen was not solely responsible for the signs and symptoms that determined seed quality.

The positive significant association between seed quality and infection rate, and days to maturity recorded in this trial is contrary to what has been reported. It is generally held that early maturing lines were more susceptible to PSD infection than late maturing ones (Sun *et al.,* 2013). Thus, a reduction in infection rate with increasing days to maturity would have yielded a negative relationship. The positive association could be due to the prevailing conditions during the trial.

Divilov (2014) recorded a positive non-significant correlation between PSD incidence and days maturity and concluded that plant with different maturity periods could be inoculated at the same time without any negative effect on the outcome. The incidence of PSD in this trial was very high. This may be due to high disease pressure. High disease pressure observed in this trial was due to early planting that saw the maturity period coinciding with the heavy September rainfall that created high humidity amidst rising temperature when crops were between the R6 to R8 stage. Li *et al.,* (2015) noted that frequent rainfall during late season caused high infection by different pathogens.

The early planting approach adopted in this study affected late maturing lines as the maturity occurred in late September to early October. A period where the late September rains creates

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considerable humidity amidst rising temperatures. This could be the reason for the positive significant correlation between PSD incidence and days to maturity as the late rains provided humid and hot conditions for PSD development. This observation agrees to report by Rupe, (1990) that warm weather during seed development increase seed infection rate. Seeds developing under warm weather record higher PSD incidence and severity than seeds developed under cooler conditions. This is because whilst latter rains provide high pod moisture for infection (Rupe and Ferriss, 1986), warm temperature enhances development of the pathogen (Tekrony, 1984). Therefore, high disease pressure due to suitable condition at seed formation stages could have led to the breakdown of the resistance in some of the lines. Li *et al.*, (2011) noted that consistent maintenance of suitable conditions for PSD development could result in breakdown of resistance. Jenguma, the check was completely susceptible as revealed by infection rate score and so was the susceptible check. Nonetheless, some lines recorded zero PSD infection and could be considered as highly resistant.

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6.5 Conclusions

Prevailing weather conditions during field evaluation created high disease pressure. In late maturing lines seed development met a condition of high moisture and warm temperature. This led to high PSD incidence among late maturing line resulting in a positive significant correlation between seed infection and days to maturity.

The high presence of saprophytic fungi caused a difference between visual assessment and disease infection. This makes visual assessment less reliable for identification of resistance to specific pathogen. The lines PI416806B, PI224271, PI417120 BRS 361, PI417013, TGX 1903-7F, PI594767B, PI423958, TGX 1485-1D, TGX 2006-3F and Liu Yuemang were highly resistant to PSD infection under field conditions. These lines could be used in breeding programmes to develop early maturing PSD resistant cultivars.

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CHAPTER SEVEN

7.0 Genotyping by sequencing, and genome-wide association studies of PSD and BP resistance, and high PLP.

7.1 Introduction

Genotyping-by-sequencing (GBS) offers a more flexible and an efficient approach to genomewide level identification of genetic variation in crops (Fu and Peterson, 2011; Peterson *et al.*, 2014). GBS yields high-density genotypic information (Poland, 2012) on samples which is suitable for genome-wide association studies, and genomic selection (Poland and Rife, 2012; He *et al.*, 2014; Gore *et al.*, 2009; Yu and Buckler, 2006). GBS method provides the numerous generations of ancestral recombination events required for direct mapping of variation in natural populations without generating populations through crosses (Yu and Buckler, 2006). Chang *et al.*, (2016), successfully conducted genome-wide association studies to characterise USDA's soyabean germplasm for resistance to diseases including bacterial leaf pustule.

SARI has accumulated considerable amount of germplasm in which exist wide variation for various growth and reproductive traits, and reaction to disease as revealed in chapters 3, 4, 5 and 6 of this study. A combination of GBS and GWAS presents an opportunity to simultaneously finger print lines in the germplasm assembly for conservation and identify loci associated with traits of interest including disease resistance to support marker assisted and genomic selection. This will lead to efficient utilisation of assembled germplasm for cultivar development.

Bacterial leaf pustule is wide spread in most *soyabean* production centres worldwide including neighbouring Benin (Kime *et al.*, 2011; Zinsou *et al.*, 2015). In Ghana, recent disease incidence survey identified bacterial leaf pustule to be wide-spread, confirming earlier report by Offei *et al*. (2008). Yield losses in the range of 15-50% due to bacterial leaf pustule have been reported

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(Rahayu, 2007). Field screening of SARI's germplasm for resistance to bacterial pustule presented in Chapter five showed differential response of lines to the disease. Field screening activity identified resistant lines including lines that scored 1 which describes lines with no observable lesions on 1-9 scale. Mapping of loci in identified resistant lines and their linked markers will be required to facilitate resistant cultivar development through marker assisted selection.

Phomopsis seed decay (PSD) is the most important cause of poor seed quality in *soyabean* (Li 2011, Koenning, 2009; Wrather & Koenning, 2009). The incidence of PSD in Ghana was first reported by Asante *et al. (*1998) and confirmed in Chapter three of this thesis. Favourable conditions for PSD infection and development (Balducchi and McGee, 1987; Suli *et al.,* 2013) are common to *soyabean* production centers in Ghana. Secondly, early maturity cultivars which are suited for the production systems of the Guinea and Sudan savannah ecologies where most of the country's *soyabean* is produced are reported to be more susceptible to PSD infection (Mayhew and Caviness, 1994 and Suli *et al.,* 2013). It is therefore imperative to incorporate PSD resistance into ongoing varietal development efforts in Ghana. To efficiently incorporate PSD resistant genes into cultivar development programmes, knowledge of resistant loci and linked markers will be very useful.

High pod clearance is key to limiting yield loss when combine harvesters are deployed to harvesting *soyabean* (http://www.seedcogroup.com/sites, 2018). It is observed that most of the locally released cultivars have low pod clearance. Whilst low pod clearance has no consequence on yield losses in production systems where manual harvesting is practiced, it is very important in commercial production systems that use combine harvesters. As the country gradually makes effort to transit its agriculture from subsistence system into a commercial one, plant architecture that lend itself for mechanization has to be considered and incorporated into breeding

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programmes. To this end, Soyabean genetic resources available at SARI were evaluated for pod clearance (PLP) as reported in chapter four of this study. Wide range of height at first pod (PLP) was observed including lines that combined high pod clearance with earliness. With the aid of molecular markers, the architecture of future cultivars could be modified to have high pod clearance to facilitate the use of combine harvesters. Genome-wide association study which facilitates the identification of loci of desired traits in assembled germplasm could be used to scan the genomes of individuals within SARI's germplasm collection for loci and markers linked to high PLP.

The goal of this research work was to map loci associated with PB and PSD resistance, and high PLP through genome-wide association studies.

The specific objectives of this study were to:

1. genotype a representative panel of SARI's *soyabean* germplasm holding
2. identify loci associated with pod clearance
3. identify loci associated with resistance/tolerance to bacterial leaf pustule
4. identify loci associated with resistance/tolerance to Phomopsis seed decay

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7.2 Materials and Methods

7.2.1 Genotyping-by-sequencing 96 sub-sample of 250 lines

7.2.2 Plant materials

A 96 representative panel comprised of 49 soyabean genotypes introduced from USDA, 22 IITA lines, 15 Zimbabwean line in SARI's germplasm holding, 5 Brazillian lines, 5 locally released varieties and 1 Chinese line

7.2.3 Sampling of plant tissue for genotyping-by-sequencing

Sample collection kit comprising a collection box, a cutting mat, tubes with perforated strip caps, a cutting tool and a bag of silica gel (desiccant). For each of the 96 selected lines, leaf tissues were obtained by cutting 8 leaf discs with the cutter. These were placed in a labelled tube, covered with perforated strip cap and a desiccant containing bag fastened to it. The collected tissues were sent to LGC for genotyping-by-sequencing.

7.2.4 Genotyping-by-sequencing

DNA of individuals lines was digested using the two-enzyme modification of the original genotyping-by-sequencing (GBS) protocol (Elshire *et al.*, 2011; Poland, Brown, Sorrells, & Jannink, 2012). *Pst*I-HF and *Hind*III-HF were used as rare cutters in separate digests, and MsIII was used as the common cutter in each case. The digestion yielded fragment sizes of approximately 200bp. Sequencing of fragment was performed on the Illumina NextSeq500 equipment with paired –ends of 200bp in length. A total of 144 million reads at an average of 1.5 million reads per sample were realized.

SNP calling was performed using the TASSEL GBS pipeline (Glaubitz *et al.*, 2014), using bwa (H. Li & Durbin, 2009) to align them to the standard *soyabean* reference genome, Glycine_max_v2.0. at a mapping rate of 98.74%.

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A total of 158,592 SNPs were realized across all the 96 samples. TASSEL software was used to filter out genotypes with a depth of less than eight, sites with a minor allele count of less than three, and SNP position and taxa with more than 90% missing data.

7.2.5 Phenotypic data

Phenotypic data for PLP, BP and PSD were generated as described in Chapter 4 (PLP), Chapter 5 (BLP) and Chapter 6 (PSD), respectively.

7.2.6 Data analysis

Data were analyzed using the R statistical software (version 3.5.1) (R Core Team, 2018). A total of 32,638 single nucleotide polymorphisms (SNPs) spread across the 20 chromosomes of soyabean and scaffolds from the genotyping-by-sequencing (GBS) analysis were used. An initial genome-wide association study (GWAS) analysis was ran to identify and select the appropriate model using seven (7) models and two (2) phenotypic traits (namely: area under disease progress curve and flower colour). The selected model was deployed to analyse resistance to bacterial pustule based on AUDPC, seed decay due to *Diaporthe sp*. and PLP at a threshold value of $-\log_{10}\left(\frac{0.01}{32638}\right)$ to determine the existence of significant association. After which, detailed GWAS analysis was carried out on the traits with significant associations. The detailed association analysis was carried out in GAPIT (version 3.0, released: 2018.08.18) using the compressed mixed linear model (CMLM) (Z. Zhang *et al.,* 2010) which is based on the following equation:

$$Y = Xa + Qb + Ku + e$$

Where Q is a matrix of principal component scores representing population structure, K is the kinship matrix calculated based on the VanRaden method (VanRaden, 2008; Lipka *et al.,* 2012), X represents the genotype and Y the phenotype allowing associated values of each SNP to be calculated (X. Zhang *et al.,* 2017).

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Different distance measures (mean, maximum, minimum and median) and agglomeration methods (average, complete and ward minimum variance) were used to arrive at the optimum cluster algorithm to group individuals based on their kinship. Identity of significant SNPs were graphically determined using interactive Manhattan plot. Residual variance and heritability estimates were determined at optimum cluster algorithm.

7.3 Results

7.3.1 General properties of SNP markers and individuals in the representative panel

A plot of the first three principal components revealed six distinct groupings and few outliers (Fig 7.1). A heat map of the kinship matrix showed extent of correlation among subpopulations and among individuals within sub-population. Hierarchical clustering based on the kinship matrix revealed six clusters which separated into nine clusters, similar to what was inferred by cluster analysis in chapter four. Correlation within groups was high whilst correlation among sub-population was low.

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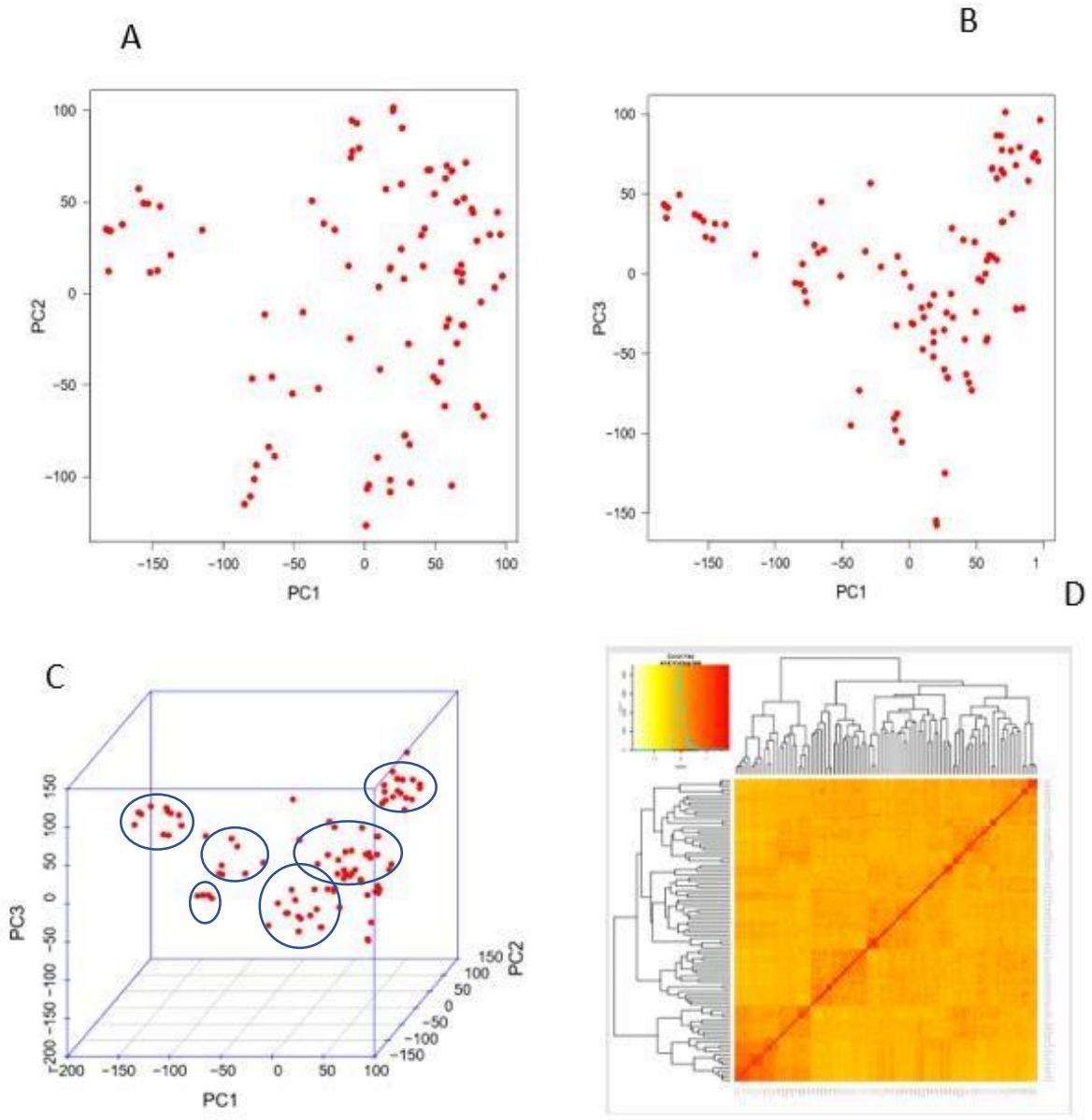

Fig.7.1: Population structure and kinship; A (PC1 VP C2), B(PC1VPC3); C (3D-plot of PC1, PC2 and PC3); D (Pair-wise correlation among 96 lines).

Heterozygosity was generally low for both individuals and as well as SNP markers used (Fig 7.2).55 lines had heterozygosity values between 0.2 and 0.25 whilst 35 lines had heterozygosity between 0.15 and 0.2. Very few individual lines had heterozygosity above 0.25 with maximum heterozygosity value of 0.45 recorded. Though SNP markers were generally homozygous, few markers recorded heterozygosity value as high as 0.95.

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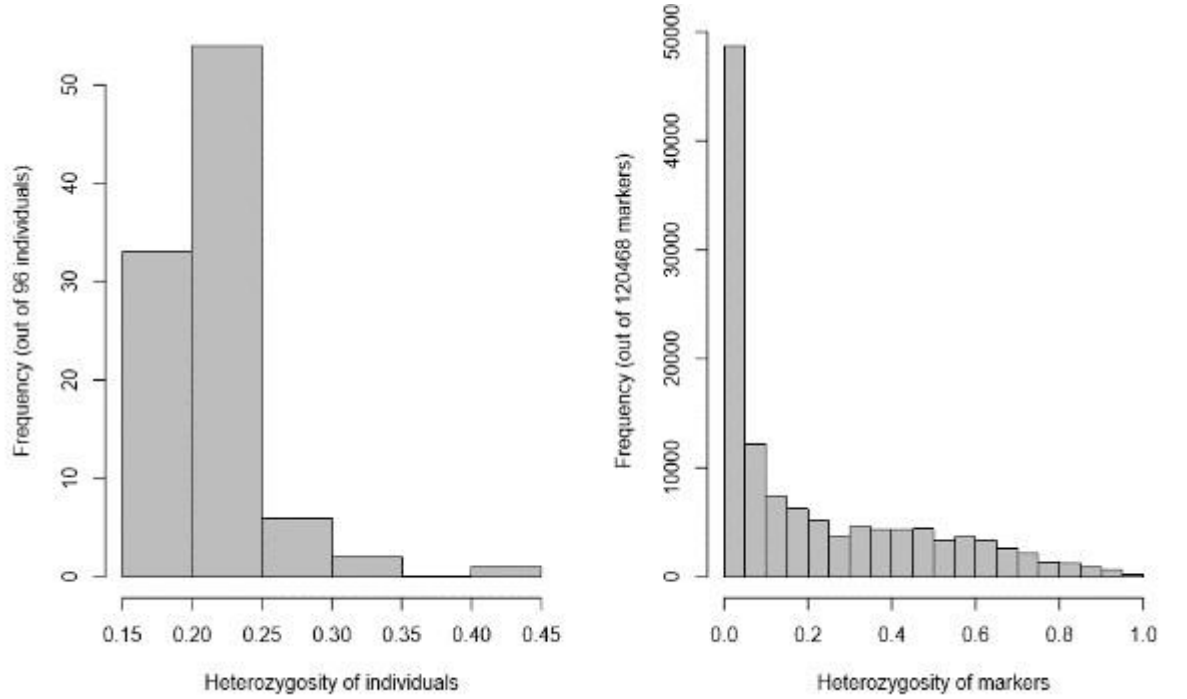

Fig.7.2: Extent of heterozygosity of A (individual lines) and B (SNP markers)

The chance of encountering at least one SNP marker at a map distance of 1million centimogan was certain with 140000 SNP markers. This likelihood reduces to 0.4 with 50000 SNP markers and to 0.2 with 2000 SNP markers

The non-random association between adjacent SNP markers, linkage disequilibrium was high at lower map distance with high accumulation of markers and generally decreased as map distance increased. Coefficient of determination (R^2) was 0.6 at maximum linkage disequilibrium (LD). LD decreased to half its maximum at 280kb with variations among individual lines.

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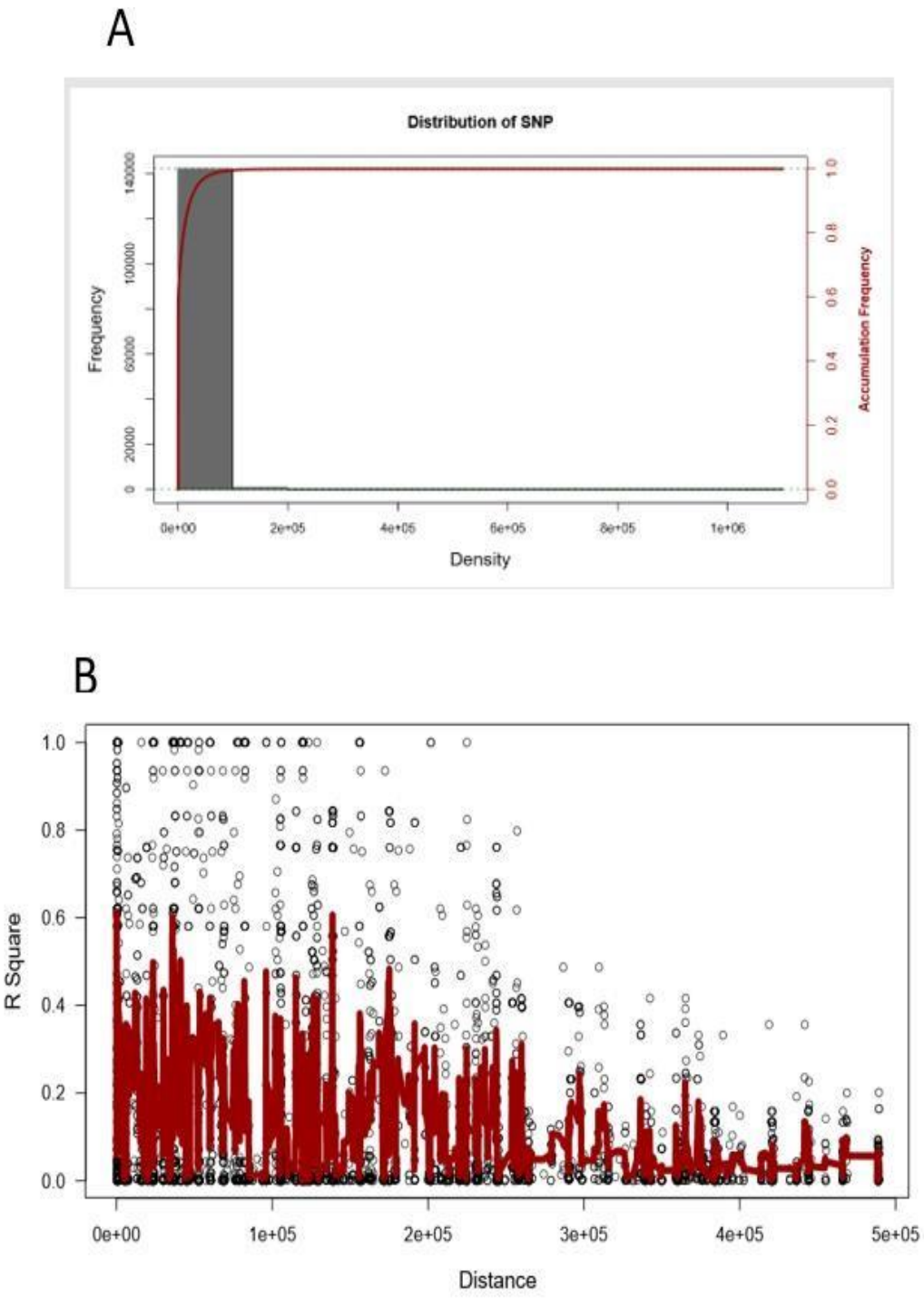

Fig.7.3: A (SNP marker density), and B (decay of linkage disequilibrium with map distance)

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7.3.2 Discovery of SNP markers associated with PLP

Estimated genetic variance based on the compressed mixed linear model was 66.8% giving a moderately high broad sense heritability of 0.668 for PLP(Fig 7.4).

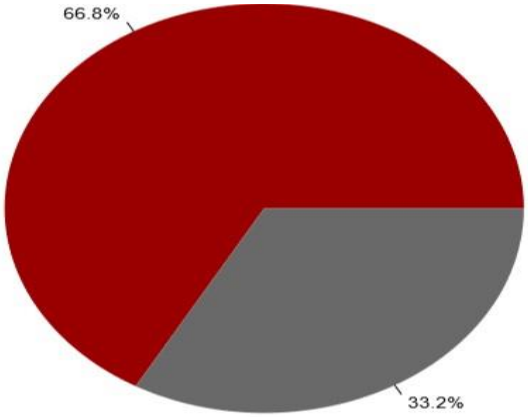

Fig.7 4: Estimated variance components of PLP; genetic variance (66.8%) and Residual (33.2%)

The Quantile-Quantile plot showed that most of the observed values were not in linkage disequilibrium with causal polymorphism. However, there were a few observations that were in linkage disequilibrium with causal polymorphism which produced significant P-values which deviated from that of the uniform distribution.

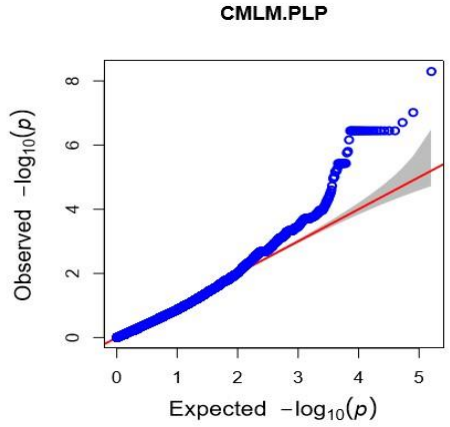

Fig.7 5: Cross validation of significantly associated SNPs with PLP through quantile-quantile plot

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Further cross validation of the significance of the association between markers and PLP was carried out through a plot of the observed P-values against a range of minor allele frequencies of markers used (Fig 7.5). The most significant association occurred at a minor allele frequency of 0.15. All significant markers recorded in the QQ plot maintained their status.

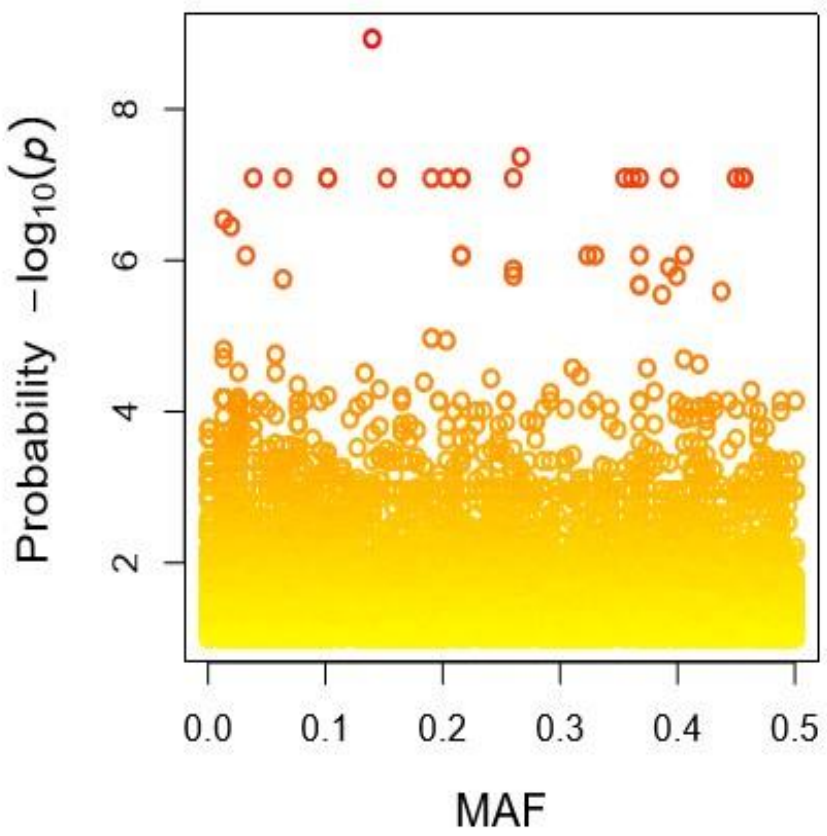

Fig.7 6: The effect of Minor allele frequency on the significance of association between markers and PLP

Chromosome 3 had to two markers that were significantly associated with PLP as they occurred above the threshold (Fig 7.7). There were seven more associated SNP markers but they were exactly on the threshold. The most significant marker, S3_1485048, occurred at the physical map position of 1485048 in the standard *soyabean* reference genome (Glycine_max_v2.0). Coefficient of determination (R^2) was high for S3_1485048 in the model. The second marker, S3_1991311 which occurred at the physical map position 1991311 had a lower observed Pvalue than S3_1485048 but its P-value was above a threshold computed at allele frequency

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of 0.01. Both markers had lower P-values than the false discovery rate. S3_1485048 and S3_1991311 both had negative allelic effect on plant length at first pod (Table 7.1).

The physical map positions of the markers were probed in *Phytozome*12 (https://phytozome.jgi.doe.gov/phytomine/begin.do) and in *Glycine max* genome assembly version Glyma.Wm82 a2 (https://soybase.org/search/index.php) for the presence of candidate genes. Furthermore, the SNP sequences of the markers were aligned to deposited sequences through BLAST. The candidate gene, Glyma.03g014800, a member of the SAURlike auxin-responsive protein was identified (Fig 7.7). The candidate gene Glyma.03g014800 was found in the region between 1493449-1494342 which is about 8401 base pairs upstream of the physical position 1485048 linked to the marker S3_1485048. SAUR family of genes positively regulate cell expansion to promote hypocotyl growth (Spartz *et al.*, 2012). The putative candidate gene was flanked by the markers, Sat_379 and BARC-042969-08479 on chromosome 3 in the soyabean reference genome.

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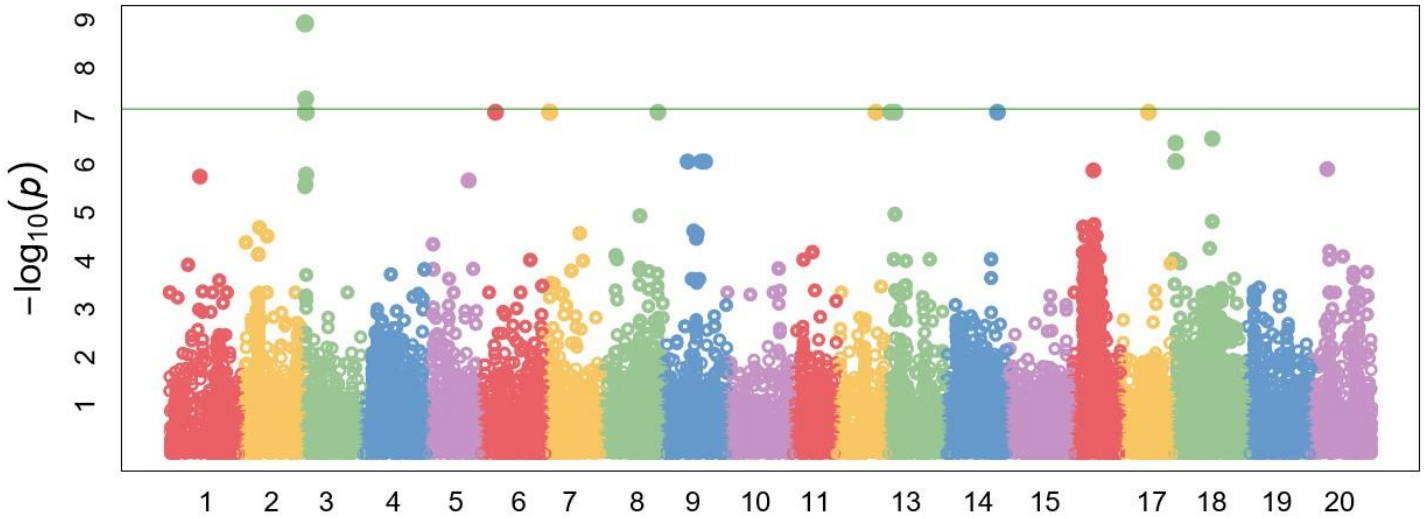

Fig.7 7**:** Manhattan plot displaying significant associations between markers and PLP

Table 7 1: Properties of significant SNP markers and their allelic effects

SNP	Chromosome	Position	P.value	Rsquare.of. Model.without.SNP	Rsquare.of .Model.with. SNP	FDR_Adjusted _P-values	Allelic Effect Estimate
S3_1485048	3	1485048	1.17E-09	0.0154	0.6511	0.00017	-26.28
S3_1991311	3	1991311	4.32E-08	0.0154	0.5049	0.00058	-23.28

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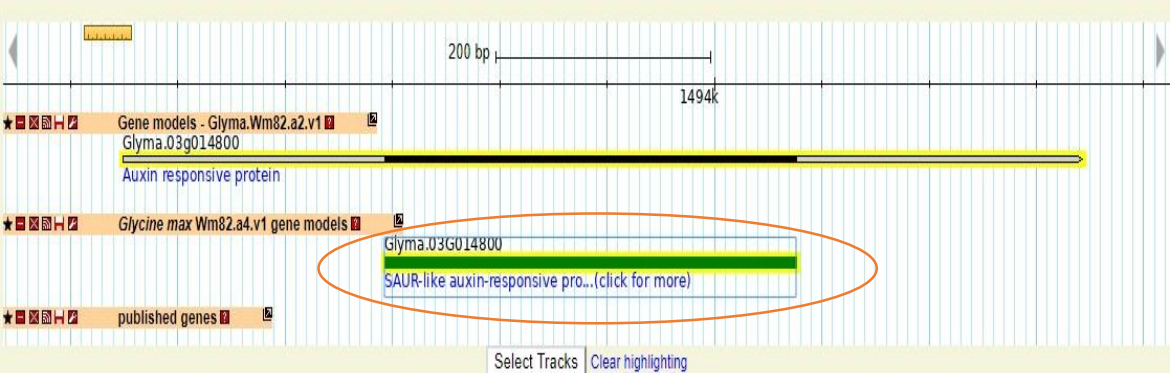

Fig.7 8: Candidate gene, Glyma.0.3G.14800 associated with PLP

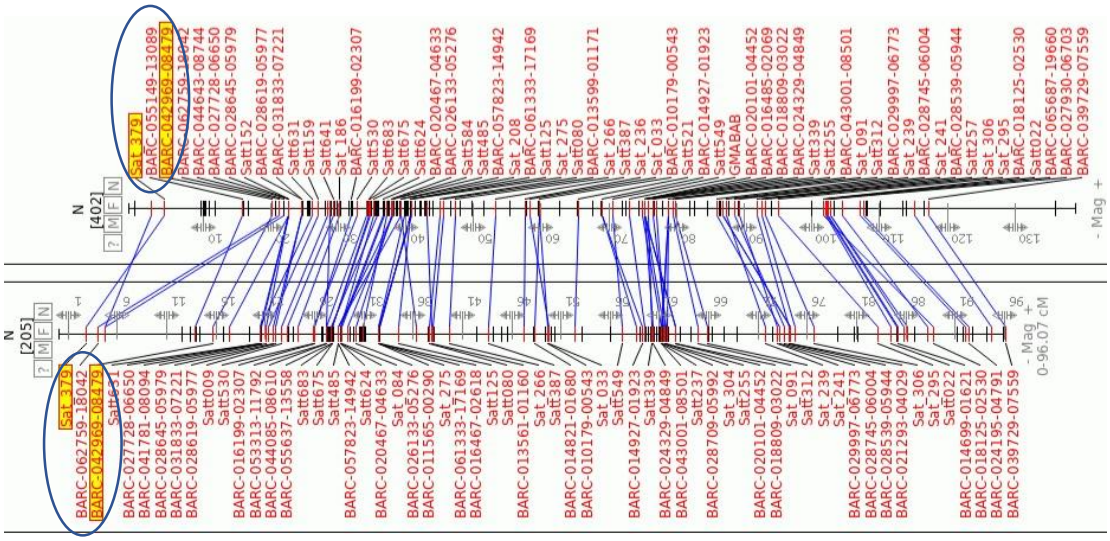

Fig.7 9**:** SSR markers flanking the identified candidate gene

Best linear unbiased predictor and linear best unbiased estimate were generated for the lines to support selection. Prediction of individual PLP was carried out using a combination of kinship data and phenotypic data (Table 7.1). SARISOYS27 had the highest random estimate and predicted mean PLP value. With the exception of SARISOYS27, random effects estimates were generally lower than fixed estimates. The difference between SARISOYS27 and the next, PI416873B in terms of random effect of PLP was very wide. An indication that SARISOY27 was exceptionally good.

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Table 7. 2:Genomic prediction of PLP for soyabean genotypes

Line	RefInf	BLUP	PEV	BLUE	Prediction	Pred Heritable
SARISOYS27	1	12.80	16.00	11.35	24.14	24.14
PI416873B	1	5.69	12.90	12.26	17.95	17.95
TGX-2011-3F	1	4.66	15.26	11.31	15.97	15.97
TGX2008-4F	1	4.32	14.68	12.72	17.04	17.04
PI567104B	1	4.30	16.13	9.49	13.79	13.79
TGX-2004-10F	1	3.26	16.26	13.11	16.37	16.37
Songdaa	1	3.20	16.98	10.53	13.74	13.74
SARISOYS30	1	1.93	17.39	10.05	11.98	11.98
PI423960A	1	1.61	15.46	11.45	13.05	13.05
TGX1988-3F	1	1.58	13.14	10.17	11.75	11.75
PI567099A	1	1.57	15.44	9.86	11.43	11.43
PI567069A	2	1.47	-39.82	12.88	14.35	14.35
PI588053B	1	1.47	16.10	11.69	13.15	13.15
PI567123A	1	1.39	17.17	10.19	11.58	11.58
PI567053	1	1.37	15.55	10.45	11.82	11.82
PI3984-77A	2	1.32	-55.29	12.76	14.09	14.09
PI567068A	1	0.91	16.37	9.96	10.87	10.87
PI398477	1	0.85	17.00	10.33	11.18	11.18
PI605824A	1	0.80	14.97	13.08	13.87	13.87
PI605891B	1	0.79	19.60	7.36	8.15	8.15
SARISOYS-14	1	0.77	16.20	10.47	11.24	11.24
PI657753	1	0.65	16.28	13.29	13.94	13.94
PI594760B	1	0.61	17.43	8.69	9.30	9.30
Sal-1	2	0.60	-89.58	12.16	12.76	12.76
BRS-326	1	0.59	15.67	9.13	9.72	9.72
TGX-1990-57F	1	0.53	14.19	13.04	13.56	13.56
TGX-1844-22E	2	0.50	-20.15	10.50	11.00	11.00
PI567076	1	0.46	14.18	11.81	12.27	12.27
PI567046A	2	0.45	-23.47	10.47	10.92	10.92
BRS-7980	1	0.35	17.32	11.13	11.49	11.49
PI606397B-2	2	0.33	-99.38	6.69	7.02	7.02
TGX-1988-3F	1	0.32	16.89	8.80	9.12	9.12
TGX1989-40F	2	0.31	-109.13	9.18	9.49	9.49
PI567129	1	0.31	19.42	7.17	7.48	7.48
SARISOY18	1	0.26	14.94	11.32	11.58	11.58
Liu yeumang	1	0.14	17.04	8.37	8.51	8.51
PI606397B	2	0.13	-96.09	6.62	6.75	6.75
PI417013	1	0.11	13.88	11.56	11.66	11.66
SARISOYS29	1	0.10	15.91	12.09	12.19	12.19
SARISOYS-1	1	0.10	14.97	11.93	12.03	12.03
PI462312-2	2	0.05	-87.86	10.41	10.46	10.46
SARISOYS-5	1	0.05	16.49	9.01	9.06	9.06
PI561381	1	0.05	16.88	10.74	10.79	10.79

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7.3.3 Genome-wide scanning for loci associated with bacterial leaf pustule

Estimate of heritability of lines reaction to bacterial pustule was moderate (Fig 7.8). Mahanttan plot based on the supper model revealed peaks on chromosomes 16, 8, and 10 (Fig 7.9). Two markers S16_34242886 and S16_35202484 with minor allele frequencies of 0.36 and 0.38, respectively had associations with area under bacterial pustule progress curve but the associations were not significant. The peaks found on chromosome 8 were due to the markers, S8_11831127 and S8_11842172 with minor allele frequencies of 0.30 and 0.33, respectively. On chromosome 10, the SNP marker, S10_12149388 produced the observed peak. In all cases, the associations between the markers and reaction to bacterial pustule infection were not significant.

Association analyses was also repeated using the compressed mixed linear model (CMLM). The resulting manhanttan plot revealed oustanding peaks on chromosomes 1, 3, 6, 13, and 16. The markers that yielded the observed peaks were S1_38672307, S3_33047652, S6_6701252, S13_4394677, and S16_20916972 on chromosomes 1, 3, 6, 13, and 16, respectively.

The peaks on chromosomes 1, 13 and 16 had higher P-values. They were however not significant as they did not significantly deviate from the expected distribution. It is noteworthy that in both models, the peak on chromosome 13 was observed. Also, the marker S8_11831127 that yielded the peak 8 on chromosome in the supper model also appeared in the compressed mixed linear model but at lower p-value.

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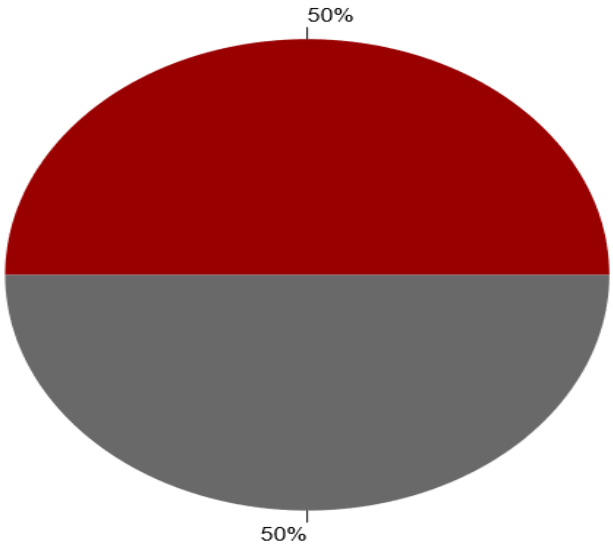

Fig.7.10**:** Estimate of heritability of reaction of soyabean lines to bacterial pustule infection; genetic variance (50%) and residual variance (50%)

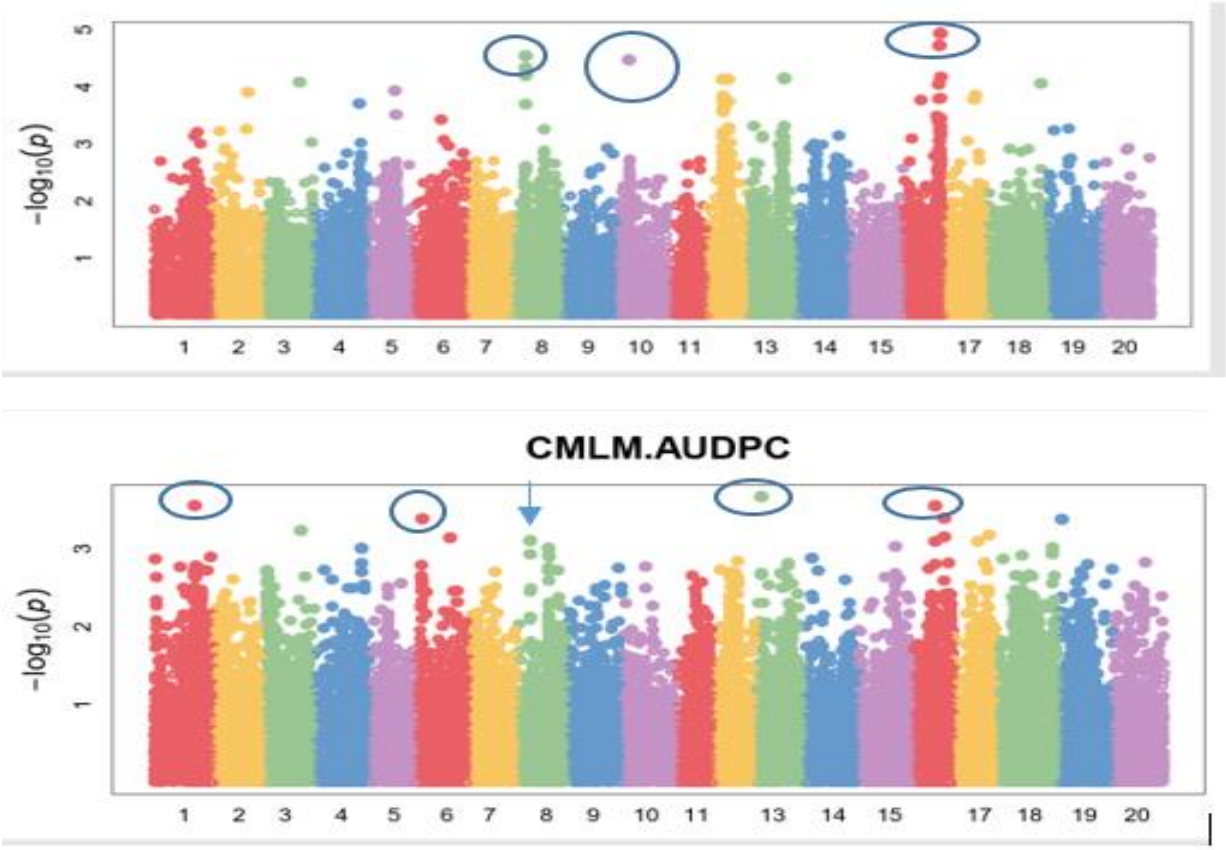

Fig.7.11: A (Manhattan plot based on the super model); B (Manhattan plot based on the compressed mixed linear model**)**

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P-values obtained from test of association based on the super model did not deviate from that of the uniform test (Fig 7.9). P-values of test of association based on the compressed mixed linear model showed deviation from that of the uniform test. The deviations were however not significant at the genome-wide association level. Peaks indicating association were observed at all frequencies of allele (Fig 7.9). Noticeable peaks were recorded at minor allele frequencies in the ranges of 0.1 to 0.5 with the highest peak occurring at 0.22. All peaks of associations did not pass the false discovery rate, irrespective of allele frequencies of markers involved.

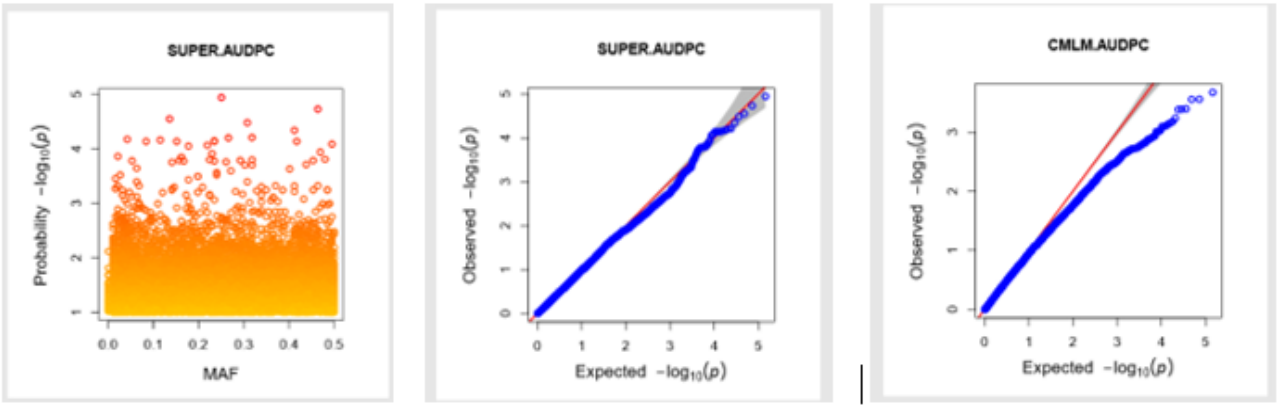

Fig 7.12: Effect of allele frequencey on false discovery rate, and test of statisitical significance of association between genomic regions and resistance to bacterial pustule

Although recorded peaks were not significant associations between genomic regions and bacterial pustules resistance, the physical positions of SNPs which yielded the highest peaks were probed on the reference genome (Fig 7.13). One leucine-rich repeat receptor-like protein kinase resistant gene candidate (Glyma.01go93200) was found within the LD region of S1_386723 on chromosome 1. Two leucine-rich repeat receptor-like protein kinase resistant gene candidates (Glyma.16g187500 and Glyma16.g187400) were found within the LD regions of S16_34242886 and S16_35202484 on chromosome 16.

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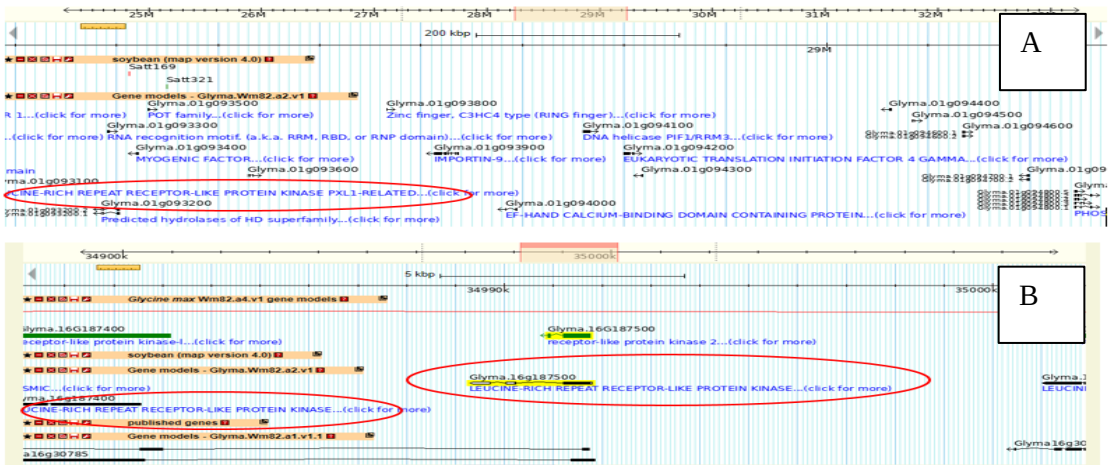

Fig.7.13: Candidate genes associated with BP resistance A(candidate gene on chromosome 1), B(candidate gene on chromosome 16)

7.3.4 Genome-wide scanning for loci associated with PSD resistance

Seed decay due to *Diaporthe sp.* was accessed using visual assessment, and assessment of pathogen infection rate through seed plating. Genomic estimate of heritability was higher for visual assessment than for pathogen infection rate based on seed plating (Fig 7.10). Inheritance of seed decay was moderate to moderately high based on method of assessment.

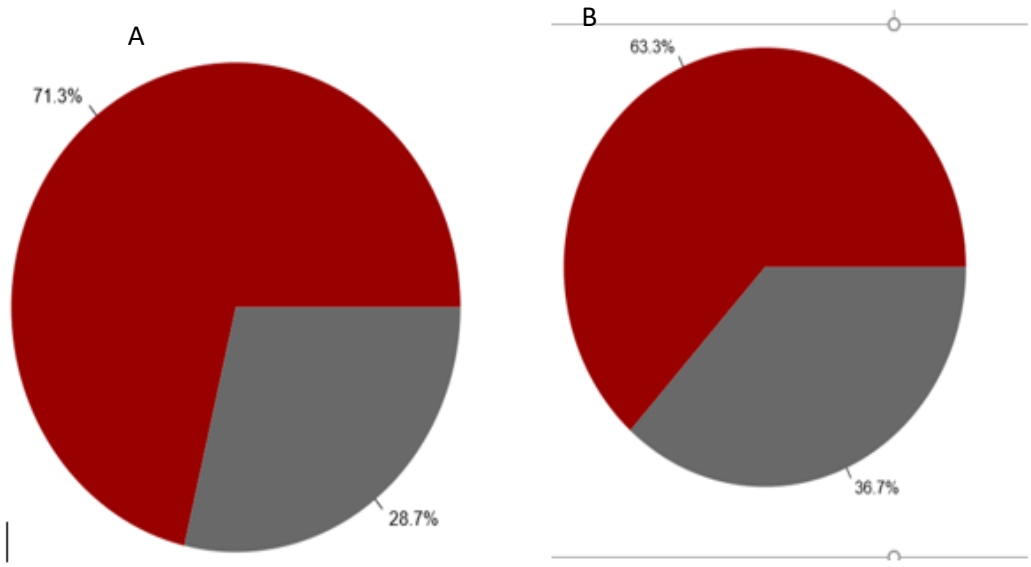

Fig.7.14**:** Genetic and residual variance of components of soyabean seed decay due to Diaporthe sp**.** (variance componentof seed decay based on visual assessment), B (variance components of seed decay based on infection rate of pathogen)

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Three peaks were recorded on the Manhattan plot based on data from infection rate (Fig 7.16). The peaks occurred on chromosome 2 (S2_19811300), chromosome 7(S7_1562611), and on chromosome 18(S18_7792728). All peaks observed did not amount to significant associations between genomic regions and seed infection rate. Data obtained from visual assessment did not produce conspicuous peaks.

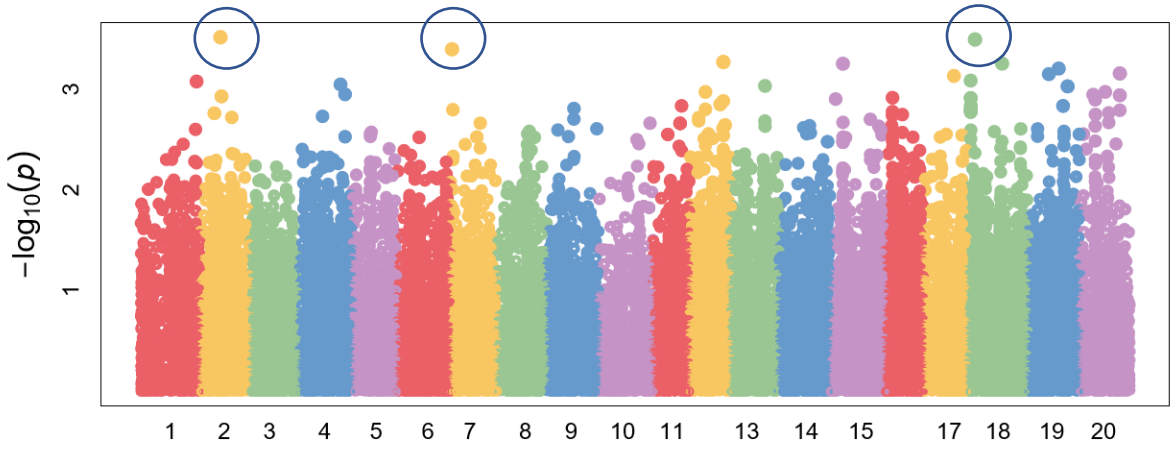

Fig.7.15: Manhattan plot depicting associated with Diaporthe sp seed decay resistance on soyabean chromosomes

Probing lack of significant association was performed through quantile-quantile plots (Fig 7.17). In both studies, the distribution of observed p-values clearly deviated from that of the ideal distribution. The deviations however did not point to any significant association between genomic regions and resistance to seed decay in soyabean.

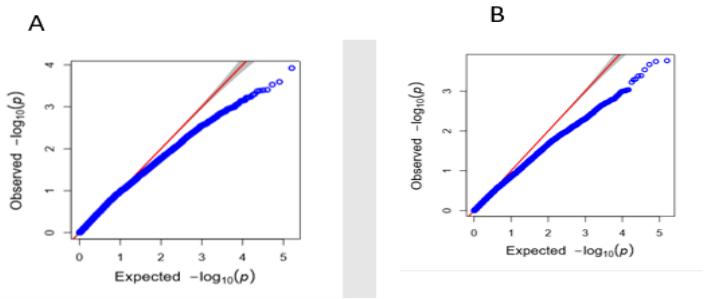

Fig 7. 16. Test of significance of observed p-values of association studies. A (QQ plot of p-values from) infection rate association studies), B (QQ plot of p-values from visual assessment association studies)

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Though not significant, physical positions of SNPs associated with conspicuous peaks were probed for identification of genes on *SoyBase* (https://www.soybase.org) and *Phytozome2* (https://phytozome.jgi.doe.gov/pz/portal.htmland). The candidate gene Glyma.18g082100 was identified within the vicinity of the physical position of the SNP, S18_7792728 located on chromosome 18. Glyma. 18G082100 which is located at about 0.2M upstream of S18_7792728, codes for Leucine rich repeat-containing proteins and disease resistance protein.

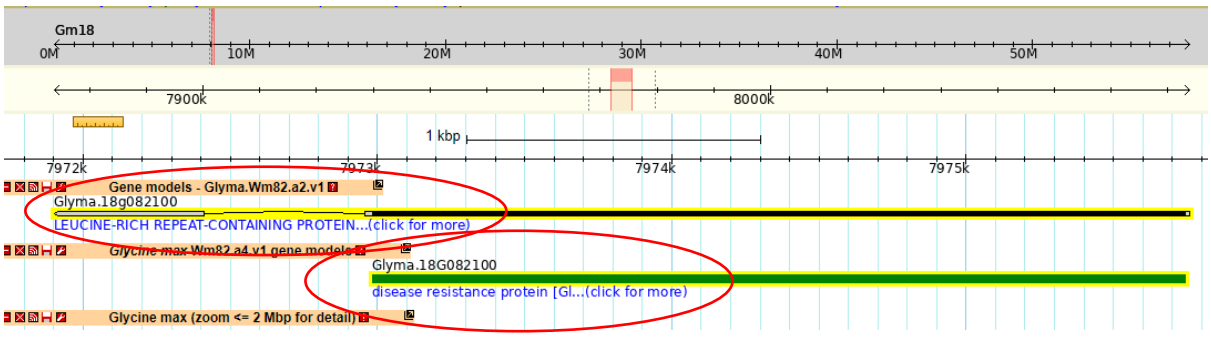

Fig 7. 17. Candidate genes identified in the chromosomal region linked to S18_7792728 on chromosome 18

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7.4 Discussion

7.4.1 Characteristics of samples and GBS generated SNP markers

Breeding for crop improvement thrives on genetic diversity. Detailed evaluation of available germplasm facilitates crop improvement through identification of the underlying allelic variation affecting crop phenotype (Zhou *et al.*, 2015). In this study a representative sample of 96 lines from SARI's *soyabean* germplasm holding were genotyped through GBS.

A biplot of the first two principal components did not show clear clustering patterns among the lines but a 3D plot involving the first three principal components revealed 6 clusters with few outliers. A pair-wise correlation based on a heat map of the kinship matrix indicates low correlation among sub-groups within the representative sample. This result points to high diversity among the lines in the sample. The high genetic diversity increases the chance for capturing the allelic variation driving phenotypic variation in traits like days to maturity, seed size, and reactions to diseases like bacterial pustule.

Individuals lines within the sample had low observed heterozygosity with more than 90% of them recording heterozygosity values in the range of 0.0 to 0.25. This implies that majority of lines in the sample were almost fixed across all loci and therefore little or no further segregation is expected. Thus, they are in a good state for conservation, and for studies that involve evaluating lines for multiple years as it is normally done for quantitative mapping like GWAS. 48000 (30.2%) of SNPs generated through GBS on the representative sample had heterozygosity in the range of 0.0 to 0.05. Markers with zero heterozygosity were not informative and were therefore filtered out before performing association studies.

Non-random association of alleles at different loci among the lines was maximum at R^2=0.6. Decay of disequilibrium was rapid reaching half its maximum value at a map distance of 2.8+e5(R^2=0.3). The R^2 at maximum LD was higher among lines used in this study than was

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reported by Liu *et al*. (2016) who obtained R2 of 0.48 at for 146 *soyabean* accessions. Strong LD exists within the 96 lines. LD is said to be stronger among cultivars than in landraces, and so the presence of advance breeding lines in the sample evaluated might have accounted for the high LD. Whilst some of the lines, especially, plant introductions from USDA were land landraces, IITA lines were advanced breeding lines. They were normally received in batches of lines from a single or few populations. Such lines with common ancestry harbour common linkage blocks of non-random associating alleles.

7.4.2. Associations studies involving PLP

66.8% of variation in PLP among the lines was due to genetic effect. This suggests that considerable gain could be made through hybridization and selection. QQ plot revealed the presence of significant deviations from uniform distribution, pointing to the existence of excess relatedness among some lines in the sample evaluated.

Manhattan plot showed two markers were strongly associated with plant length at first pod on chromosome 3. The extend of the strength of the association between the two markers was pronounced in both observed p values and in R^2 when each was included in the model. The most significant marker, S3_1485048 had observed p-value of 1.1e-9 and its inclusion in the model increased R^2 from 0.0154 to 0.651. The second marker, S3_119311 also had p-value of 4.32E-08 and increased R^2 from to 0.0154 to 0.5049. Both markers had large allelic effect on PLP. The allelic effects of both markers were negative with S3_1485048 having a higher effect. They will therefore be useful for selecting against low pod clearance but not for high clearance. Further probe at the physical map locations where these markers occurred revealed a few candidate genes including Glyma.03G014800. The candidate gene Glyma.03G014800 is

SAUR like-auxin responsive protein. SAUR family of genes regulate a wide range of growth and developmental processes in plants (Ren and Gay, 2015). McClure and Guilfoyle (1987)

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observed that SUAR genes were associated with hypocotyl elongation in soyabean. Subsequent findings confirmed SAUR family genes positively regulate cell expansion to promote hypocotyl growth (Spartz *et al.*, 2012; Stamm and Kumar, 2013). Cell expansion, cell division, elongation growth, tropic growth among others have been identified as the functions of genes in the SAUR family (Ren and Gray, 2015).

The hypocotyl is the primary organ of extension of the young plant and develops into the stem (Das, *et al.*, 2016). In soyabean, hypocotyl pulls the cotyledon out of the soil and determines how high above the ground the cotyledon reaches before the unifoliate leaf and its auxiliary bud are formed (Shaun, 2010). Low pod clearance (PLP) has to do with formation of pods close to the ground which suggest short hypocotyl or short distance between the cotyledon and the first trifoliate. Discovery of SNP markers linked to the SAUR family of genes responsible for elongation of the hypocotyl and extension of young plant could be useful for selection for high clearance.

One source of yield loss to combined harvesting is cutting below the cut-height (Hanna, 2010). As noted in Seedco Farmer's Guide (2018), the height of the pod off the ground determines the extent of yield loss with combined harvester. Height of soyabean crop correlates positively with days to maturity (Malik *et al.*, 2007). This limits the choice of breeding for taller varieties to overcome low pod clearance because of the short cropping season in which most of the country's *soyabean* is produced. The putative candidate gene and its linked markers could facilitate breeding for increased pod clearance without necessarily increasing plant height and its consequent effect on maturity period. A number of lines with predicted high pod clearance have been identified. Most of these lines had pod clearance of more than 10 cm and could be considered in breeding programmes to breed for increased pod clearance.

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7.4.3 Loci associated with bacterial leaf pustule

The compressed mixed linear model (Zhang *et al.,* 2010) was used for evaluating the association between bacterial leaf pustule infection and genomic regions of lines. Peaks were observed on chromosomes 1, 3, 6, 13, and 16. None of the observed peaks amounted to a significant association. Quantile-quantile plot revealed a clear deviation of the observed pvalues from that of the uniform distribution but there was no sufficient statistical power to give statistical significance.

A second plot was carried out based on the super model (Wang, 2014) which boast of statistical power when even small number of genetic markers are used. Manhattan plot based on the super model identified peaks on chromosomes 16, 8 and 10 with minor allele frequency in the range of 0.30 to 0.36. All peaks identified were not significant. Different peaks were recorded for the two models except the peak on the chromosome 16, which was present in both models.

Although association as revealed by Manhattan plot were not significant, a cursory probe of the physical position of the peaks on chromosome 1 and 16 indicated the LD within these regions harbours resistant genes. Three putative candidate genes, Glyma.01go93200, Glyma.g187500 and Glyma.g187400 were identified on chromosomes 1 and 16, respectively. All three-candidate genes codes for leucine-rich repeat receptor-like protein kinases. Leucine rich repeat receptor like protein kinase are leucine-rich repeat receptors localised in plant cell membrane. They play a key role in plant response to pathogen infection by recognising Pathogen Associated Molecular Patterns (PAMP) and endogenous Damage Associated Molecular Patterns (DAMP) and elicit immune response (Yamaguchi *et al.,* 2006; Huffaker *et al.,* 2006).

Chang *et al.* (2016) reported of the markers ss715580342, ss715609404 and ss715628133 on chromosomes 1, 11 and 17, respectively linked to genomic regions offering resistance to

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bacterial pustule disease. These markers were identified in a genome-wide association studies and they occurred in genomic regions codding for leucine-rich repeat receptor-like protein kinases. Before then, Kim *et al* 2010 fine mapped the rxp gene identified by Narvel *et al.* (2001) to a 33kb region and identified two candidate genes, Glyma17g09780 and Glymag09790. Glyma17g09780 is membrane protein whilst Glymag09790 was identified as a zinc finger**.**

With regards to PSD, the candidate gene, Glyma.18G082100 was observed upstream of the physical position of the SNP S18_7792728. A number of molecules including IPR000767 (Disease resistance protein), IPR025875 (Leucine rich repeat 4) and GO:0006952 (defense response) were associated with Glyma.18G082100. Additionally, PTHR23155 (Leucine-Rich Repeat-Containing Protein), and AT3G07040.1 (NB-ARC-domain-containing disease resistance protein) were found within the physical map position of SNP S18_7792728.

Recognition of invading pathogen is the important step towards plant response to diseases. Plants have thus evolved mechanisms to recognize and elicit rapid response to invading pathogen leading to basal resistance (Freeman and Beattie, 2008). Recognition of invading pathogen is achieved through resistant proteins, most of which contain central nucleotide-binding domain (NBARC domain-containing disease resistance protein) (Ooijen et al., 2008). All the molecules associated with candidate gene, Glyma.18G082100 are involve in recognition and activation of basal or immune response to pathogens (Alywin and Rammik, 2011).

The presence of LD regions harbouring Leucine-rich repeat receptor like protein kinase in genomic regions confirms that deviation from the normal as depicted in QQ plot were due to presence of LD blocks. The failure to obtain significant associations for observed peaks could be as a result of lack of statistical power due to small sample size and or structure of the representative panel. Chang *et al.*, (2016) noted that large population size does not necessarily translates into significant results despite high power. In their trial, the same significant SNP was

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detected with a sample size of 79 as with 9,865 samples. Furthermore, (Hart and Griffiths 2015) identified resistance to viral disease in common bean with just 84 recombinant inbred lines, and botrytis immunity in *Arabidopsis* with 96 accessions. Representative diversity panel has been as suggested as equally important as population for GWAS (Chang *et al.*, 2016). Hence, failure to obtain significant association in this study could be due to the structure of 96 representative panel used. Similar pattern was observed for seed decay due to *Diaporthe sp*. Although, the panel was diverse, structure for traits such as maturity period existed in the panel.

The gene-for-gene theory suggests that for every gene that conditions reaction in the host, there is corresponding gene that conditions pathogenicity in the parasit). The implication of this is an arm race between host and pathogen. This often leads to breakdown of resistance and the emergence or discovery of new sources of resistance (Brun *et al.*, 2010). This warrants the continuous search of sources of resistance to control area specific races or newly emerged races of a pathogen. Twenty-eight of 250 lines screened for bacterial leaf pustule infection recorded no lesion despite high disease pressure on the field. They were classified as highly resistant. They could be very useful for bacterial pustule resistant cultivar development.

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7.5. Conclusion

GBS generated over 150000 markers, 32000 of which are fairly polymorphic with less than 10% missing data. This can be used for quantitative mapping including association mapping. Ninety-six diverse, highly fixed lines with strong linkage disequilibrium has also been constituted to form a representative panel of the SARI's *soyabean* germplasm holding.

Plant length at first pod (PLP) has been identified as a critical trait in the *soyabean* architecture that will influence future mechanization of *soyabean* harvesting. Two SNP markers, S3_14585048 and S3_1991311 with large negative effect on PLP has been identified to support marker assisted selection. The gene influencing PLP has been identified as a SAUR like-auxin responsive protein known to positively regulate cell expansion to promote hypocotyl growth.

Associations between genomic locations and resistance to bacterial leaf pustule were identified but the associations were not significant as a result of low statistical power due to small sample size. Nonetheless, three SNP markers, S16_34242886, and S16_35202484 and S1_38672307 were found linked to three candidate genes, Glyma.01go93200 Glyma.g187500 and Glyma.g187400). All three candidate genes code for Leucine-rich repeat receptor like protein kinases for eliciting immune response to pathogen infection. The linked markers could be validated and used for marker assisted selection.

The SNP, S18_7792728 associated with PSD resistance is linked to a genomic region that harbours genes known to play critical roles in plants response to pathogens. This confirms the existence of resistant genes for PSD among the lines in the representative panel. The identified SNP could be validated to aid with marker assisted selection.

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CHAPTER EIGHT

8.0 General conclusion and recommendation

8.1Conclusion

Understanding the prevalence and distribution of plant diseases across production centres is required for effective prevention and control of diseases. This is even more critical when resistant genes are to be deployed to controlling diseases because of the resources and time required to identify and transfer resistant genes into cultivars. In order to ascertain the incidence, severity and distribution of soyabean diseases in Ghana, a survey was carried out covering production centres in the Northern, Upper East, Upper West, Brong Ahafo and Ashanti**.** The survey identified seven fungal diseases, two viral diseases and one bacterial disease. Of the ten diseases identified during the survey, bacterial leaf pustule, *cercospora* leaf blight, frog eye leaf spot and two viral diseases were present at varying levels of incidence and severity at almost all 15 locations surveyed. Bacterial leaf pustule and soyabean mosaic virus occurred at all 15 locations surveyed whilst frog eye leaf spot and *cercospora* leaf blight occurred at 13. Malzire, Karaga and Gushegu all in the northern region had higher severity of fungal and bacterial diseases encountered than other locations. Viral diseases were more important at Ejura and Wenchi than other locations surveyed. Binduri, Navrongo and Manga in the Upper east region generally recorded lower incidence and severity of identified disease than locations in the other regions. The study also identified soyabean seed decay disease, and successfully confirmed the causal agent to be *Diaporthe sp.* and tentatively designated the isolate as MTB-1. This is the first report of soyabean seed decay due to Diaporthe/Phomopsis complex of fungi in Ghana.

SARI's germplasm was screened for resistance to bacterial pustule which was the widest spread disease identified during the survey. Sources of resistance to bacterial leaf pustule exist within the assembled germplasm including twenty-eight lines that elicited immune response to

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bacterial leaf pustule. This study also identified bacterial pustule resistant plant introductions (PIs) obtained from the USDA that had their resistance broken down and will therefore not be useful for developing bacterial pustule resistant cultivars for northern Ghana. The area under chlorophyll retention curve, a trait computed in this study, had a strong association with area under disease progress curve and could therefore be further developed to indirectly screen soyabean lines for resistance to bacterial pustule.

Identification of resistant loci and linked markers are required for marker assisted selection. GWAS was carried out on a 96 representative panel to identify genomic regions associated with resistance to bacterial leaf pustule. Three SNP markers, S16_34242886, and S16_35202484 and S1_38672307 linked to bacterial leaf pustule resistance were identified. Although, the association between these SNP markers and bacterial pustule resistance was not statistically significant, three candidate genes, Glyma.01go93200 Glyma.g187500 and Glyma.g187400 found at the physical positions of these markers code for Leucine-rich repeat receptor like protein kinases known for eliciting immune response to pathogen invasion.

In order to support pre-emptive breeding efforts against the newly identified disease of soyabean in Ghana, Phomopsis seed decay (PSD), assembled germplasm was screened for sources of resistance. Eleven resistant lines were identified to aid with PSD resistant cultivar development. GWAS identified the SNP S18_7792728 linked to the candidate gene, Glyma.18G082100 which codes for central nucleotide-binding domain (NB-ARC domaincontaining disease resistance protein) also known for stress response including invading pathogens. In both cases of bacterial leaf pustule and PSD resistance, the association studies did not identify significant SNP due to lack of statistical power as a result of small size and structure of the representative panel.

Morphological evaluation showed wide diversity among accessions in SARI's germplasm holding and observed variation were largely under genetic control. Number of pods per plant,

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number of seed per plant, and days to flowering were the main drivers of variation among the assembled germplasm. Unbiased structure analysis of a 96-line representative panel revealed that accessions in the germplasm belonged to nine sub-populations with high diversity among lines in the panel which indicate that wide variations observed in morphological traits were due to genetic effect. Ninety-six diverse, nearly fixed lines with strong linkage disequilibrium has also been constituted to form a representative panel of the SARI's *soyabean* germplasm holding. GBS generated over 150000 markers, 32000 of which are fairly polymorphic with less than 10% missing data. This can be used for quantitative mapping including association studies.

Shaping the architecture of plant is an important objective in plant breeding. Pod clearance influences losses when combined harvesters are used for harvesting. SARI's soyabean germplasm was evaluated for pod clearance and the result showed that clearance was highly heritable and some accessions combined high pod clearance with higher number of pods per plant. Two SNP markers, S3_14585048 and S3_1991311 with large negative allelic effects on PLP has been identified to support marker assisted selection. The gene influencing PLP has been identified as a SAUR like-auxin responsive protein known to positively regulate cell expansion to promote hypocotyl. The sequence of the candidate gene could be used to design more primers for marker assisted selection. Lines with high pod clearance have been identified through prediction based on random effects estimate. These lines could be exploited to develop productive cultivars that will be suitable for mechanisation including the use of combine harvesters.

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8.2 Recommendation

The resistance lines identified for bacterial leaf pustule and Phomopsis seed decay should be further evaluated in multi-location trials to identify stable sources of resistance for cultivar development. The information on diseases, their severity and distribution should inform extension strategies for soyabean disease management for specific locations.

Also, the 96 lines genotyped and the inferred groupings should form the bases of conservation efforts at SARI. Furthermore, SNP markers generated through genotyping by sequencing could be used to genotype additional lines to increase the number of lines in the representative panel to attain the required statistically power for GWAS. SNP markers and candidate genes identified could be further probed and validated to aid with marker assisted selection.

Information on pod clearance generated in this study including linked markers and predicted genotypes could be used to alter the architecture of future varieties to make them suitable for mechanisation, especially, combine harvesting.

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APPENDICES

Appendix.1: severity scores of 250 lines in reaction to bacterial leaf pustule infection

Line	DS	Line	DS	Line	DS	Line	DS
BRS 8660A	1	BRS 313	1.5	PI567069A	2	TGX 19110-6F	3
BRS-7980B	1	BRS TIANA	1.5	PI605824A-2	2	TGX 1989-20FA	3
ISS-8203	1	PI200492	1.5	SAL-II	2	TGX 1990-80F	3
P587886	1	PI379621	1.5	SARISOY 12	2	PI398477	3.5
Paragoina	1	PI416873B	1.5	SARISOY 2	2	PI605823	3.5
PI274954B	1	PI59025A	1.5	TGX 1799-8FA	2	TGX 1844-22E	3.5
PI398512	1	SARISOY 14	1.5	TGX 1990-52F	2	TGX 1988-3F	3.5
PI398874	1	SARISOY 19	1.5	TGX 1990-95F	2	TGX 1989-19FA	3.5
PI416778A	1	SARISOY 27	1.5	TGX 1993-4FN	2	TGX 1989-42F	3.5
PI416810A	1	SARISOY 4	1.5	TGX 2008-4F	2	BRS 361	4
PI416939	1	TGX 1799-8F	1.5	PI588053B	2.3	HUANGZADO	4
PI417126A	1	TGX 1903-7F	1.5	PI200488	2.5	PI416810	4
PI506677	1	TGX 1989-20F	1.5	PI416810-1	2.5	PI459025A	4
PI506939	1	TGX 1990-57F	1.5	PI417503	2.5	PI468919A	4
Sal-1	1	TGX 1990-78F	1.5	PI423957	2.5	PI507004	4
SARISOY 13A	1	TGX 2006-37F	1.5	PI606440A	2.5	PI507009	4
SARISOY 15	1	TGX 2011-3F	1.5	TGX 1989-11F	2.5	PI561356	4
SARISOY 20B	1	TGX1845-10E	1.5	TGX 1989-45F	2.5	PI603170	4
SARISOY 2A	1		1.5	WAMINI	2.5	SARISOY 23	4
TGX-198948FNB							
SARISOY 5	1	ZAMBOANI	1.5	ISS-8203A	3	SARISOY 24	4
TGX 1989-20FC	1	BRS 90-8360	2	JENGUMA	3	TGX 1834-5E	4
TGX 1990-80FA	1	BRS PEROLA	2	PI468919	3	TGX 1844-19F	4
TGX-1835-10E	1	BRS-360	2	PI594538A	3	TGX 2007-11F	4
TGX-1989-40FB	1	BRS-7980	2	SARISOY 1	3	TGX-1989-40F	4
TGX-1989-48FN	1	FT christaline	2	SARISOY 10	3	TGX-198968FNA	4

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TGX-1989-48FNA	1	PI224270	2	SARISOY 26	3	TGX-1990-46F	4
TGX1989-68FN	1	PI379621A	2	TGX 1445-3E	3	TGX-1990-55F	4
TGX-1990-21F	1	PI3984 77A	2	TGX 1485-1D	3	BRS- 7525	4.5
Afayak	1.5	PI567020A	2	TGX 1740-2F	3	BRS 8660	4.5
PI506491	4.5	SARISOY 7	5.5	PI567099A	6.5	PI594767A	7.5
SARISOY 17	4.5	SARISOY 9	5.5	PI567104B	6.5	PI594767B-1	7.5
TGX 1805-8F	4.5	TGX 1988-5E	5.5	PI567190	6.5	PI605865B	7.5
TGX 1989-19F	4.5	TGX 1989-41F	5.5	SARISOY 28	6.5	PI605891B-1	7.5
TGX 1989-20FB	4.5	TGX 2010-11F	5.5	Songdaa	6.5	PI60591B	7.5
TGX 1989-75N	4.5	SARISOY 8	5.75	TGX 1990110FN	6.5	PI81042A	7.5
TGX 1990-93F	4.5	CROTON	6	TGX-1989-68FN	6.5	SARISOY 20	7.5

Appendix 1 Cont'd: Severity scores of 250 lines in reaction to bacterial leaf pustule

Line	DS	Line	DS	Line	DS	Line	DS
TGX 2004-10F	4.5	PI 534545	6	PI594760B	7	PI594767B	8
TGX1987-10F	4.5	PI203398	6	PI628850	7	PI605824A	8
BRS-326	5	PI224271	6	PI628932	7	PI605829	8
LG 04 -6000	5	PI398594	6	PI635999	7	PI605854B	8
PI230970	5	PI399096	6	SARISOY 16	7	PI606397B	8
PI274954	5	PI462312	6	SARISOY 18	7	TGX 184419FB	8
PI398387	5	PI506491A	6	SARISOY 30	7	PI398825	8.5
PI398836C	5	PI5670466	6	TGX 1990 -114 FN	7	PI417120A	8.5
PI41219B	5	PI567076	6	TGX 2004-13FB	7	PI506938	8.5
PI423963	5	PI567104	6	OZARK	7.5	PI567056A	8.5
PI506491C	5	PI567123A	6	PI398836	7.5	PI567188	8.5
PI603176A	5	PI567191A	6	PI417085	7.5	PI567516C	8.5
PI605824A-1	5	PI584796	6	PI417126	7.5	PI587886	8.5
PI605865B-2	5	PI594538A-1	6	PI423960A	7.5	PI605865B-1	8.5
PI657753	5	Quarshie	6	PI476905A	7.5	PI605891A	8.5
SARISOY 21	5	SARISOY 11	6	PI567053	7.5	PI605891B	8.5

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TGX 1987-62F	5	SARISOY 13	6	PI567129	7.5	PI81042	8.5
TGX 2004-13FA	5	SARISOY 22	6	PI567191	7.5	PI427120	8.5
TGX-1989-40FA	5	TGX 1988-5EB	6	TGX 2006-3F	7.5		
TGX1989-48FN	5	TGX 1990-93FB	6	PI274454	8		
TGX 2004-13F	5.3	TGX-1990-40F	6	PI398836A	8		
PI398836B	5.5	159925	6.5	PI416806	8		
PI417119	5.5	TGX-1990-49F	6.75	PI416810B-1	8		
PI518295	5.5	BRS-7980A	7	PI417013	8		
SARISOY 25	5.5	PI416778	7	PI417134	8		
SARISOY 29	5.5	PI423972	7	PI423958	8		
PI200526	6.5	PI567058D	7	PI561381	8		
PI340898A	6.5	PI567090	7	PI567034	8		
PI567054C	6.5	PI578457A	7	PI567046A	8		
PI567068A	6.5	PI588053A	6.5	PI587905	8		

Appendix. 2: Fixed effects estimates of AUDPC for soyabean lines relative to the check

Line	Estimate	Std. Error	df	t value	Pr(>|t|)
SARISOY 5	-28.8	31.7	211	-0.91	0.364
SARISOY 20B	-28.6	31.7	211	-0.90	0.368
BRS 8660A	-28.2	31.7	211	-0.89	0.374
BRS-7980B	-28.2	31.7	211	-0.89	0.374
PI274954B	-28.2	31.7	211	-0.89	0.374
TGX-198948FNA	-28.2	31.7	211	-0.89	0.374
TGX1989-68FN	-27.0	25.8	211	-1.05	0.296
TGX-1990-21F	-26.9	25.8	211	-1.04	0.298
TGX-1989-48FN	-26.9	25.8	211	-1.04	0.299
PI506677	-26.8	25.8	211	-1.04	0.301
PARANGOINA	-26.7	25.8	211	-1.03	0.303
PI416810A	-26.5	25.8	211	-1.03	0.306
PI416939	-26.5	25.8	211	-1.03	0.306
ISS-8203	-26.5	25.8	211	-1.03	0.306
TGX-1835-10E	-26.5	25.8	211	-1.02	0.307
P587886	-26.4	25.8	211	-1.02	0.308

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PI398512	-26.4	25.8	211	-1.02	0.309
PI506939	-26.1	25.8	211	-1.01	0.313
PI398874	-25.9	25.8	211	-1.00	0.318
SARISOY 2A	-25.4	31.7	211	-0.80	0.423
BRS 313	-25.1	25.8	211	-0.97	0.333
SARISOY 13A	-25.0	31.7	211	-0.79	0.430
SARISOY 15	-24.9	31.7	211	-0.79	0.432
TGX 1903-7F	-24.9	25.8	211	-0.96	0.337
PI59025A	-24.8	25.8	211	-0.96	0.337
TGX 1989-20FC	-24.8	31.7	211	-0.78	0.434
Afayak	-24.8	25.8	211	-0.96	0.338
PI379621	-24.8	25.8	211	-0.96	0.338
PI417126A	-24.7	31.7	211	-0.78	0.435
Sal-1	-24.7	31.7	211	-0.78	0.435
SARISOY 14	-24.6	25.8	211	-0.96	0.341
ZAMBOANI	-24.6	25.8	211	-0.95	0.341
TGX1845-10E	-24.6	25.8	200	-0.95	0.342
BRS TIANA	-24.6	25.8	200	-0.95	0.342

Appendix 2 Cont'd.

Line	Estimate	Std. Error	df	t value	Pr(>|t|)
PI416778A	-24.6	31.7	211	-0.78	0.439
TGX-1989-40FB	-24.6	31.7	211	-0.78	0.439
TGX 1990-80FA	-24.6	31.7	211	-0.78	0.439
TGX 1799-8F	-24.5	25.8	211	-0.95	0.343
PI200492	-24.5	25.8	211	-0.95	0.345
BRS-360	-22.8	25.8	211	-0.88	0.378
PI416873B	-21.8	25.8	211	-0.85	0.399
TGX-198948FNB	-21.5	25.8	211	-0.83	0.407
TGX 2011-3F	-21.4	25.8	211	-0.83	0.408
PI379621A	-21.2	31.7	211	-0.67	0.503
SARISOY 4	-21.2	25.8	211	-0.82	0.413
TGX 2006-37F	-21.1	25.8	211	-0.82	0.415
SARISOY 19	-21.0	25.8	211	-0.81	0.418
PI567020A	-20.9	31.7	211	-0.66	0.510
BRS-7980	-19.7	25.8	211	-0.76	0.447

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TGX 2008-4F	-19.4	25.8	211	-0.75	0.454
PI567069A	-19.2	25.8	211	-0.75	0.457
SAL-I	-17.8	31.7	211	-0.56	0.574
TGX 1990-57F	-15.9	25.8	211	-0.61	0.540
TGX 1990-78F	-15.9	25.8	211	-0.61	0.540
PI605824A-2	-14.3	31.7	211	-0.45	0.651
SARISOY 2	-14.3	25.8	211	-0.55	0.580
PI3984 77A	-14.3	25.8	211	-0.55	0.581
TGX 1990-52F	-14.2	25.8	211	-0.55	0.584
SARISOY 12	-14.0	25.8	211	-0.54	0.587
TGX 1993-4FN	-13.9	25.8	211	-0.54	0.590
TGX 1799-8FA	-13.8	31.7	211	-0.44	0.664
PI416810-1	-12.6	25.8	211	-0.49	0.626
TGX 1989-11F	-12.4	25.8	211	-0.48	0.632
FT christa	-11.1	25.8	211	-0.43	0.669
PI588053B	-9.9	23.6	208	-0.42	0.674
TGX 1989-20F	-9.2	25.8	211	-0.36	0.723
PI417503	-8.9	25.8	211	-0.35	0.730
PI200488	-8.8	25.8	211	-0.34	0.734
TGX 1990-95F	-7.2	31.7	211	-0.23	0.820

Appendix 2 Cont'd.

Line	Estimate	Std. Error	df	t value	Pr(>|t|)
SARISOY 27	-7.1	25.8	211	-0.28	0.784
BRS 90-8360	-6.0	25.8	211	-0.23	0.816
PI224270	-5.4	25.8	211	-0.21	0.836
BRS PEROLA	-5.3	25.8	211	-0.20	0.839
TGX 19110-6F	-3.9	31.7	211	-0.12	0.903
JENGUMA	-3.7	25.8	211	-0.14	0.886
TGX 1989-20FA	-2.0	25.8	211	-0.08	0.938
ISS-8203A	-1.9	25.8	211	-0.07	0.942
TGX 1989-45F	-0.3	25.8	200	-0.01	0.992
PI423957	0.0	25.8	211	0.00	1.000
PI468919	1.1	25.8	211	0.04	0.966
SARISOY 10	1.6	25.8	211	0.06	0.951
TGX 1805-8F	3.3	31.7	211	0.10	0.918
TGX 1990-80F	4.7	25.8	211	0.18	0.856
TGX 1988-3F	5.1	25.8	200	0.20	0.845
TGX 1485-1D	5.4	25.8	211	0.21	0.834

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PI468919A	5.5	25.8	211	0.21	0.833
TGX 1740-2F	6.8	25.8	211	0.26	0.792
TGX 1445-3E	6.8	25.8	211	0.26	0.792
TGX 1844-22E	8.6	25.8	211	0.33	0.738
PI398477	8.9	25.8	211	0.34	0.732
TGX 1989-42F	12.1	25.8	211	0.47	0.641
WAMINI	12.2	25.8	211	0.47	0.639
PI561356	13.3	31.7	211	0.42	0.675
SARISOY 24	13.4	31.7	211	0.42	0.672
TGX-1989-40F	13.5	25.8	211	0.52	0.601
SARISOY 1	13.6	25.8	211	0.53	0.599
TGX-1990-55F	13.8	25.8	211	0.53	0.594
TGX 1989-19FA	15.4	25.8	211	0.60	0.552
BRS 361	15.5	25.8	211	0.60	0.549
SARISOY 26	16.9	25.8	211	0.65	0.514
GIN HUANGZADOU	16.9	25.8	211	0.66	0.513
TGX 1834-5E	20.7	25.8	211	0.80	0.425
TGX-198968FNA	21.1	31.6	191	0.67	0.505
TGX-1990-46F	21.2	25.8	211	0.82	0.413
PI605823	24.1	25.8	211	0.93	0.351

Appendix .2: Cont'd.

Line	Estimate	Std. Error	df	t value	Pr(>|t|)	Sig.
PI507004	24.8	25.8	200	0.96	0.337	
TGX-1989-40FA	27.4	31.7	211	0.87	0.387	
PI459025A	27.8	25.8	211	1.07	0.284	
TGX 1844-19F	27.9	25.8	211	1.08	0.281	
BRS 8660	29.5	25.8	211	1.14	0.255	
TGX1987-10F	30.9	25.8	211	1.20	0.233	
SARISOY 23	31.0	25.8	211	1.20	0.231	
PI398836C	31.2	31.7	211	0.99	0.326	
TGX 1989-19F	31.7	25.8	211	1.23	0.222	
BRS- 7525	31.7	25.8	211	1.23	0.222	
TGX 2007-11F	32.7	25.8	211	1.27	0.207	
PI416810	32.9	25.8	211	1.27	0.204	
TGX 1990-93F	36.4	25.8	211	1.41	0.160	
PI603170	36.5	22.4	210	1.63	0.104	

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TGX 1987-62F	40.4	25.8	200	1.57	0.119	
SARISOY 22	41.7	25.8	211	1.61	0.108	
TGX 1990-93FB	41.7	31.7	211	1.32	0.189	
PI567104B	41.7	25.8	211	1.62	0.108	
PI594538A	43.1	25.8	211	1.67	0.097	
BRS-326	43.8	25.8	200	1.70	0.091	
PI567191A	47.0	25.8	211	1.82	0.071	
SARISOY 9	47.2	25.8	211	1.83	0.069	
PI584796	48.7	31.7	211	1.54	0.126	
SARISOY 17	48.7	25.8	200	1.89	0.060	
PI567123A	48.8	25.8	211	1.89	0.060	
TGX 1989-41F	49.0	25.8	200	1.90	0.059	
PI657753	50.6	25.8	211	1.96	0.052	
PI398836B	50.7	25.8	211	1.96	0.051	
PI603176A	52.0	25.8	211	2.01	0.045	*
PI507009	52.2	31.7	211	1.65	0.101	
PI567090	52.2	25.8	211	2.02	0.045	*
TGX 1989-20FB	52.2	25.8	211	2.02	0.044	*
PI605865B-2	52.8	31.7	211	1.67	0.097	.
TGX 2004-13F	55.1	25.8	211	2.13	0.034	*
SARISOY 21	55.6	25.8	211	2.15	0.032	*
TGX 1988-5EB	55.8	25.8	211	2.16	0.032	*
PI567104	57.6	25.8	211	2.23	0.027	*

Note: *, **, *** represents significance at 5, 1 and 0.1 %, respectively.

Appendix.2: Cont'd

Line	Estimate	Std. Error	df	t value	Pr(>|t|)	Sig.
TGX 2004-10F	57.8	25.8	211	2.24	0.026	*
PI423963	58.9	25.8	211	2.28	0.024	*
PI398387	59.1	25.8	211	2.29	0.023	*
TGX1989-48FN	59.1	25.8	211	2.29	0.023	*
SARISOY 7	59.3	25.8	211	2.30	0.023	*
PI203398	59.4	25.8	211	2.30	0.022	*
SARISOY 29	59.5	25.8	211	2.30	0.022	*
PI506491C	59.6	31.7	211	1.88	0.061	.
PI417119	60.4	25.8	211	2.34	0.020	*
PI399096	61.1	25.8	211	2.37	0.019	*
(Intercept)	61.5	18.4	206	3.35	0.001	***
PI594538A-1	62.8	25.8	211	2.43	0.016	*
PI41219B	63.1	31.7	211	1.99	0.048	*

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PI506491	64.5	25.8	211	2.50	0.013	*
PI230970	66.4	31.7	211	2.10	0.037	*
SARISOY 25	67.8	25.8	211	2.62	0.009	**
PI274954	68.1	25.8	211	2.63	0.009	**
PI506491A	68.2	25.8	211	2.64	0.009	**
PI628850	69.4	25.8	211	2.69	0.008	**
TGX-1990-40F	69.6	25.8	211	2.69	0.008	**
PI567076	71.5	25.8	200	2.77	0.006	**
PI567190	73.2	25.8	211	2.84	0.005	**
PI224271	73.2	25.8	211	2.84	0.005	**
PI605824A-1	73.3	31.7	211	2.32	0.022	*
CROTON	74.3	25.8	211	2.88	0.004	**
LG 04 -6000	74.8	25.8	211	2.90	0.004	**
TGX 1989-75N	76.4	25.8	211	2.96	0.003	**
TGX 2004-13FA	76.5	31.7	211	2.42	0.016	*
SARISOY 20	76.7	25.8	211	2.97	0.003	**
PI567058D	77.1	31.7	211	2.43	0.016	*
Quarshie	78.5	25.8	211	3.04	0.003	**
PI567099A	80.2	25.8	211	3.11	0.002	**
PI567054C	80.2	25.8	211	3.11	0.002	**
PI340898A	80.3	25.8	200	3.11	0.002	**
PI578457A	80.4	31.7	211	2.54	0.012	*

Note: *, **, *** represents significance at 5, 1 and 0.1 %, respectively

Appendix. 2 :Cont'd.

Line	Estimate	Std. Error	df	t value	Pr(>|t|)	Sig
TGX 1988-5E	80.5	25.8	211	3.12	0.002	**
PI518295	80.5	25.8	211	3.12	0.002	**
SARISOY 8	81.3	25.8	211	3.15	0.002	**
PI416778	81.8	25.8	211	3.17	0.002	**
PI628932	82.0	25.8	211	3.17	0.002	**
TGX 1990 -114 FN	82.5	25.8	211	3.19	0.002	**
PI398836A	83.8	31.7	211	2.65	0.009	**
PI398594	83.8	31.7	211	2.65	0.009	**
159925	83.8	25.8	211	3.25	0.001	**
SARISOY 11	84.2	31.7	211	2.66	0.008	**
PI462312	85.6	25.8	211	3.31	0.001	**
SARISOY 16	88.7	25.8	211	3.43	0.001	***
PI423972	88.9	25.8	211	3.44	0.001	***
BRS-7980A	90.3	31.7	211	2.85	0.005	**

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PI587905	90.6	31.7	211	2.86	0.005	**
PI588053A	90.7	25.8	211	3.51	0.001	***
SARISOY 18	90.9	25.8	211	3.52	0.001	***
PI200526	92.2	25.8	211	3.57	0.000	***
TGX 2010-11F	92.4	25.8	211	3.58	0.000	***
TGX 2004-13FB	93.9	31.7	211	2.97	0.003	**
SARISOY 30	93.9	25.8	211	3.64	0.000	***
PI 534545	94.3	25.8	211	3.65	0.000	***
PI567053	96.0	25.8	211	3.71	0.000	***
SARISOY 28 TGX 1990-110FN	96.0	25.8	211	3.72	0.000	***
	96.2	25.8	211	3.72	0.000	***
SARISOY 13	97.8	25.8	211	3.78	0.000	***
Songdaa	97.8	25.8	211	3.79	0.000	***
PI567068A	99.5	25.8	211	3.85	0.000	***
PI417085	99.6	25.8	211	3.85	0.000	***
PI605824A	99.9	25.8	211	3.87	0.000	***
TGX-1990-49F	100.3	25.8	211	3.88	0.000	***
PI594767B	100.9	25.8	200	3.91	0.000	***
PI635999	101.0	25.8	211	3.91	0.000	***
TGX 2006-3F	101.3	25.8	211	3.92	0.000	***
PI605829	101.5	25.8	211	3.93	0.000	***

Note: *, **, *** represents significance at 5, 1 and 0.1 %, respectively.

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Appendix 2 Cont'd.

Line	Estimate	Std. Error	df	t value	Pr(>|t|)	Sig
PI567191	102.6	25.8	211	3.97	0.000	***
TGX-1989-68FN	102.8	25.8	211	3.98	0.000	***
OZARK	106.1	25.8	211	4.11	0.000	***
PI594767A	106.5	25.8	211	4.12	0.000	***
PI605865B	106.7	25.8	211	4.13	0.000	***
PI5670466	111.5	31.7	211	3.52	0.001	***
PI81042A	111.5	25.8	211	4.32	0.000	***
PI567129	111.9	25.8	211	4.33	0.000	***
PI606397B	113.3	25.8	211	4.38	0.000	***
TGX 1844-19FB	114.9	31.7	211	3.63	0.000	***
PI398836	115.3	25.8	211	4.46	0.000	***
PI476905A	115.6	25.8	211	4.47	0.000	***
PI416810B-1	115.6	31.7	211	3.65	0.000	***
PI417126	118.8	25.8	211	4.60	0.000	***
PI594760B	120.7	25.8	200	4.68	0.000	***
PI594767B-1	121.9	25.8	211	4.72	0.000	***
PI60591B	122.1	25.8	211	4.72	0.000	***
PI605865B-1	122.3	25.8	211	4.73	0.000	***
PI567034	122.5	31.7	211	3.87	0.000	***
PI423958	124.1	25.8	211	4.80	0.000	***
PI567046A	129.0	25.8	211	4.99	0.000	***
PI423960A	131.1	25.8	211	5.07	0.000	***
PI417013	132.8	25.8	211	5.14	0.000	***
PI567188	134.5	25.8	211	5.21	0.000	***
PI417134	138.2	25.8	211	5.35	0.000	***
PI416810B	139.4	25.8	211	5.40	0.000	***
PI274454	141.3	25.8	211	5.47	0.000	***
PI567091A	143.2	31.7	211	4.52	0.000	***
PI605891A	143.3	25.8	211	5.55	0.000	***
PI567180	144.6	25.8	211	5.60	0.000	***
LIU YUEMANG	146.6	25.8	211	5.67	0.000	***
PI416806	148.3	25.8	211	5.74	0.000	***
PI605891B	150.3	25.8	211	5.82	0.000	***
PI605891B-1	150.5	25.8	211	5.83	0.000	***
PI567025A	151.9	25.8	200	5.89	0.000	***
PI567056A	152.1	25.8	211	5.89	0.000	***
PI561381	153.7	25.8	211	5.95	0.000	***
PI587886	154.1	25.8	211	5.97	0.000	***
PI605791A	159.0	25.8	211	6.15	0.000	***
PI417120A	162.6	25.8	211	6.29	0.000	***
PI81042	166.2	25.8	211	6.43	0.000	***

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LIU YUEBAO	176.4	25.8	211	6.83	0.000	***
PI417120	176.5	25.8	200	6.84	0.000	***
PI605854B	177.7	31.7	211	5.61	0.000	***
PI606405	183.3	25.8	211	7.09	0.000	***
PI398825	190.5	25.8	211	7.37	0.000	***
PI506938	197.5	25.8	211	7.64	0.000	***
PI567516C	209.6	25.8	211	8.11	0.000	***

Note: *, **, *** represents significance at 5, 1 and 0.1 %, respectively.

Appendix. 3: Fixed effects estimates of lines' reaction to PSD based visual assessment

Line	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	4.01E+00	5.65E-01	8.02E+01	7.101	4.50E-10
Croton	-1.58E-02	7.71E-01	1.31E+02	-0.02	0.98368
Wamini -	8.71E-01	7.72E-01	1.31E+02	-1.128	0.26146
Afayak	8.44E-01	9.45E-01	1.31E+02	0.893	0.37352
BRS- 7525	1.13E+00	9.45E-01	1.31E+02	1.194	0.23458
BRS- 7525B	-1.15E+00	9.45E-01	1.31E+02	-1.22	0.22464
BRS-326	4.90E-01	7.71E-01	1.31E+02	0.636	0.52582
BRS-7980	9.80E-01	7.71E-01	1.31E+02	1.271	0.20595
BRS 8660	9.86E-01	7.71E-01	1.31E+02	1.28	0.20298
BRS 8660A	8.39E-01	9.45E-01	1.31E+02	0.888	0.3764
BRS Perola	-1.15E+00	9.45E-01	1.31E+02	-1.219	0.22497
BRS TIANA	-1.01E+00	7.71E-01	1.31E+02	-1.309	0.19292
BRS 313	-2.10E-02	7.71E-01	1.31E+02	-0.027	0.97836
BRS 361	-1.01E+00	7.71E-01	1.31E+02	-1.303	0.19472
BRS 90-8360	-1.32E-02	7.71E-01	1.31E+02	-0.017	0.98633
FT christa	-2.01E+00	7.71E-01	1.31E+02	-2.608	0.01017

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ISS-8203	-1.15E+00	9.45E-01	1.31E+02	-1.22	0.22464
Jenguma	9.78E-01	7.71E-01	1.31E+02	1.269	0.2066
LG 04 -6000	-2.54E-02	7.71E-01	1.31E+02	-0.033	0.97375
Liu Yuebao	-1.02E+00	7.71E-01	1.31E+02	-1.319	0.18953
Liu Yuemang	-1.16E+00	9.45E-01	1.31E+02	-1.227	0.22202
NE 3400	-1.01E+00	7.71E-01	1.31E+02	-1.31	0.19233
Ozark	1.12E+00	9.45E-01	1.31E+02	1.185	0.23823
OZARK	8.59E-01	9.45E-01	1.31E+02	0.908	0.36528
Parangoina	-8.68E-01	9.45E-01	1.31E+02	-0.918	0.36009
PARANGOINA	8.33E-01	9.45E-01	1.31E+02	0.881	0.38001
PI 534545	4.72E-01	7.71E-01	1.31E+02	0.612	0.54158
PI159925	-5.45E-03	7.71E-01	1.31E+02	-0.007	0.99437
PI200492	1.15E+00	9.45E-01	1.31E+02	1.218	0.2256
PI200526	-1.15E+00	9.45E-01	1.31E+02	-1.219	0.225
PI224271	-5.15E-01	7.71E-01	1.31E+02	-0.668	0.50557
PI229358	-9.96E-01	7.71E-01	1.31E+02	-1.292	0.19849
PI230970	-9.98E-01	7.69E-01	1.21E+02	-1.297	0.197
PI274454	-2.45E-03	7.71E-01	1.31E+02	-0.003	0.99747
PI274954A	8.54E-01	9.42E-01	1.14E+02	0.907	0.36634
PI340898A	-5.21E-01	7.71E-01	1.31E+02	-0.675	0.50064
PI379621	-1.51E+00	6.68E-01	1.31E+02	-2.258	0.0256
PI398387	9.80E-01	7.71E-01	1.31E+02	1.271	0.20587
PI398477	-8.68E-01	9.45E-01	1.31E+02	-0.918	0.36027
PI398512	-1.01E+00	7.71E-01	1.31E+02	-1.313	0.19154
PI398543	-1.15E+00	9.45E-01	1.31E+02	-1.219	0.2251
PI398594	-1.01E+00	7.71E-01	1.31E+02	-1.31	0.1925
PI398825	-2.15E+00	9.45E-01	1.31E+02	-2.277	0.0244
PI398836	-1.01E+00	7.71E-01	1.31E+02	-1.304	0.19455
PI398874	-1.02E+00	7.71E-01	1.31E+02	-1.327	0.18681
PI399096	-5.12E-01	7.71E-01	1.31E+02	-0.664	0.50784

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PI416778	-9.97E-01	7.71E-01	1.31E+02	-1.294	0.19805
PI416806A	-1.15E+00	9.45E-01	1.31E+02	-1.219	0.225
PI416806B	1.01E+00	7.71E-01	1.31E+02	1.303	0.19475
PI416810	-1.15E+00	9.45E-01	1.31E+02	-1.219	0.22516
PI416810A	-9.75E-01	7.04E-01	1.31E+02	-1.386	0.16821
PI416810B	-8.64E-01	9.45E-01	1.31E+02	-0.915	0.36211
PI416810B-1	-1.15E+00	9.45E-01	1.31E+02	-1.218	0.22526
PI416810C	-1.53E-01	9.45E-01	1.31E+02	-0.162	0.87155
PI416810E	8.63E-01	9.45E-01	1.31E+02	0.913	0.36279
PI416873B	-2.17E+00	9.45E-01	1.31E+02	-2.292	0.02351
PI416886	4.97E-01	7.71E-01	1.31E+02	0.645	0.52015
PI416939	-5.07E-01	7.69E-01	1.21E+02	-0.659	0.51118
PI417013	-1.52E+00	7.69E-01	1.22E+02	-1.977	0.05035
PI417119	-1.00E+00	7.71E-01	1.31E+02	-1.299	0.19624
PI417120	-8.54E-01	9.42E-01	1.14E+02	-0.907	0.36634
PI417120A	-8.70E-01	9.45E-01	1.31E+02	-0.92	0.35931
PI417126	-1.02E+00	7.71E-01	1.31E+02	-1.326	0.18721
PI417134	-1.50E+00	7.71E-01	1.31E+02	-1.947	0.05373
PI423958	-1.88E+00	9.45E-01	1.31E+02	-1.984	0.04938
PI423960A	4.98E-01	7.71E-01	1.31E+02	0.646	0.51956
PI423972	-2.43E-02	7.71E-01	1.31E+02	-0.031	0.97493
PI459025A	-5.21E-01	7.71E-01	1.31E+02	-0.675	0.50068
PI462312	8.31E-01	9.45E-01	1.31E+02	0.879	0.38082
PI468919A	-1.56E-01	9.45E-01	1.31E+02	-0.165	0.86918
PI476905A	-2.51E+00	7.71E-01	1.31E+02	-3.255	0.00144
PI506677	-1.37E-01	9.45E-01	1.31E+02	-0.145	0.88519
PI506938	-1.00E-02	7.71E-01	1.31E+02	-0.013	0.98963
PI506939	-1.37E-01	9.45E-01	1.31E+02	-0.145	0.88519
PI507004	4.83E-01	7.71E-01	1.31E+02	0.627	0.53199
PI507004A	-1.03E+00	7.71E-01	1.31E+02	-1.333	0.18498

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PI518295	-1.01E+00	7.69E-01	1.21E+02	-1.319	0.18975
PI561381	-1.01E+00	7.71E-01	1.31E+02	-1.315	0.19069
PI567025A	4.76E-01	7.71E-01	1.31E+02	0.617	0.53818
PI567053	-1.35E-02	7.71E-01	1.31E+02	-0.018	0.98606
PI567054C	-1.02E+00	7.71E-01	1.31E+02	-1.32	0.1893
PI567056A	-1.13E-02	7.71E-01	1.31E+02	-0.015	0.98829
PI567058D	-5.09E-01	7.71E-01	1.31E+02	-0.66	0.51016
PI567068A	-1.51E+00	7.71E-01	1.31E+02	-1.961	0.05204
PI567076	-1.50E+00	7.71E-01	1.31E+02	-1.947	0.05371
PI567090	-1.02E+00	7.71E-01	1.31E+02	-1.324	0.18781
PI567091A	-1.51E+00	7.71E-01	1.31E+02	-1.952	0.05307
PI567099A	-1.01E+00	7.71E-01	1.31E+02	-1.316	0.19052
PI567104B	-1.89E-02	7.71E-01	1.31E+02	-0.025	0.98044
PI567123A	-2.19E-02	7.71E-01	1.31E+02	-0.028	0.97738
PI567129	-1.02E+00	7.71E-01	1.31E+02	-1.319	0.18939
PI567188	-5.27E-01	7.71E-01	1.31E+02	-0.683	0.49555
PI567190	-1.52E-01	9.45E-01	1.31E+02	-0.161	0.8722
PI567191	-1.87E+00	9.45E-01	1.31E+02	-1.977	0.05018
PI567516C	-2.01E+00	7.71E-01	1.31E+02	-2.604	0.01028
PI578457A	1.00E+00	7.71E-01	1.31E+02	1.297	0.19681
PI584796	-9.48E-03	7.71E-01	1.31E+02	-0.012	0.9902
PI587886	-1.16E+00	9.45E-01	1.31E+02	-1.223	0.22352
PI587905	-1.01E+00	7.71E-01	1.31E+02	-1.313	0.19152
PI588053A	-1.87E+00	9.45E-01	1.31E+02	-1.976	0.05024
PI588053B	-1.01E+00	7.71E-01	1.31E+02	-1.315	0.19071
PI594538A	-1.00E+00	7.71E-01	1.31E+02	-1.3	0.19577
PI594760B	-1.50E+00	7.71E-01	1.31E+02	-1.949	0.05347
PI594767A	-1.51E+00	7.71E-01	1.31E+02	-1.964	0.05164
PI594767B	-2.02E+00	7.71E-01	1.31E+02	-2.625	0.00969
PI603170	-7.67E-03	7.71E-01	1.31E+02	-0.01	0.99207

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PI603176A	1.51E-01	9.45E-01	1.31E+02	0.159	0.87353
PI605791A	-1.02E+00	7.71E-01	1.31E+02	-1.321	0.18874
PI605823	1.00E+00	7.71E-01	1.31E+02	1.297	0.19706
PI605824A	-1.69E-01	9.45E-01	1.31E+02	-0.179	0.85846
PI605829	-1.01E+00	7.71E-01	1.31E+02	-1.315	0.19081
PI605854B	-1.50E+00	7.71E-01	1.31E+02	-1.944	0.05402
PI605891A	-2.03E+00	7.71E-01	1.31E+02	-2.626	0.00966
PI605891B	-1.02E+00	7.71E-01	1.31E+02	-1.32	0.18925
PI605891B-1	-1.52E-01	9.45E-01	1.31E+02	-0.161	0.87213
PI606405	-1.50E+00	7.71E-01	1.31E+02	-1.945	0.05386
PI628850	-4.47E-03	7.71E-01	1.31E+02	-0.006	0.99538
PI628932	-5.18E-01	7.69E-01	1.22E+02	-0.674	0.50179
PI635999	-9.99E-01	7.71E-01	1.31E+02	-1.296	0.19717
PI657753	4.85E-01	7.71E-01	1.31E+02	0.628	0.5308
PI81042	-1.13E+00	9.45E-01	1.31E+02	-1.196	0.23397
Quarshie	1.32E-01	9.45E-01	1.31E+02	0.14	0.88884
SARISOY 27	1.33E-01	9.45E-01	1.31E+02	0.141	0.88823
SARISOY 1	1.33E-01	9.45E-01	1.31E+02	0.141	0.88828
SARISOY 10	1.51E-01	9.45E-01	1.31E+02	0.159	0.87353
SARISOY 12	-8.94E-01	9.45E-01	1.31E+02	-0.946	0.34602
SARISOY 13	-8.68E-01	9.45E-01	1.31E+02	-0.918	0.36008
SARISOY 14	1.13E+00	9.45E-01	1.31E+02	1.197	0.23329
SARISOY 15	1.13E+00	9.45E-01	1.31E+02	1.197	0.2333
SARISOY 16	-3.57E-01	7.72E-01	1.31E+02	-0.462	0.64482
SARISOY 17	1.10E-01	7.72E-01	1.31E+02	0.143	0.88669
SARISOY 18	1.32E-01	9.45E-01	1.31E+02	0.139	0.88945
SARISOY 19	1.12E+00	9.45E-01	1.31E+02	1.185	0.23823
SARISOY 21	-8.66E-01	9.45E-01	1.31E+02	-0.916	0.36131
SARISOY 23	1.33E-01	9.45E-01	1.31E+02	0.141	0.88823
SARISOY 25	-1.87E+00	9.45E-01	1.31E+02	-1.975	0.05036

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SARISOY 26	-8.67E-01	9.45E-01	1.31E+02	-0.917	0.36074
SARISOY 28	1.06E-01	9.45E-01	1.31E+02	0.112	0.91082
SARISOY 30	1.13E+00	9.45E-01	1.31E+02	1.194	0.23458
SARISOY 4	1.52E-01	9.45E-01	1.31E+02	0.161	0.87239
SARISOY 5	1.13E+00	9.45E-01	1.31E+02	1.196	0.2339
SARISOY 7	-8.64E-01	9.45E-01	1.31E+02	-0.914	0.36219
SARISOY 9	1.13E+00	9.45E-01	1.31E+02	1.197	0.2333
SEEDCO 27	-1.66E-01	9.45E-01	1.31E+02	-0.176	0.86065
SEEDCO 8	-1.57E-01	9.45E-01	1.31E+02	-0.166	0.86841
SEEDCO 1	8.51E-01	9.45E-01	1.31E+02	0.9	0.36969
SEEDCO 10	-1.15E+00	9.45E-01	1.31E+02	-1.216	0.22633
SEEDCO 11	8.48E-01	9.45E-01	1.31E+02	0.897	0.37126
SEEDCO 12	8.33E-01	9.45E-01	1.31E+02	0.881	0.38001
SEEDCO 13	-1.17E+00	9.45E-01	1.31E+02	-1.242	0.21657
SEEDCO 14	8.34E-01	9.45E-01	1.31E+02	0.882	0.37936
SEEDCO 15	8.51E-01	9.45E-01	1.31E+02	0.9	0.36969
SEEDCO 16	3.29E-01	7.72E-01	1.31E+02	0.426	0.67047
SEEDCO 17	-1.15E+00	9.42E-01	1.14E+02	-1.216	0.2264
SEEDCO 18	8.51E-01	9.45E-01	1.31E+02	0.9	0.36969
SEEDCO 19	-1.13E+00	9.45E-01	1.31E+02	-1.199	0.23275
SEEDCO 21	-1.15E+00	9.42E-01	1.14E+02	-1.216	0.2264
SEEDCO 25	-1.15E+00	9.45E-01	1.31E+02	-1.218	0.22526
SEEDCO 26	-1.57E-01	9.45E-01	1.31E+02	-0.166	0.86841
SEEDCO 28	8.40E-01	9.45E-01	1.31E+02	0.889	0.37561
SEEDCO 29	-1.37E-01	9.45E-01	1.31E+02	-0.145	0.88519
SEEDCO 30	8.49E-01	7.72E-01	1.31E+02	1.099	0.27368
SEEDCO 4	-1.16E+00	9.45E-01	1.31E+02	-1.227	0.22202
SEEDCO 5	8.48E-01	9.45E-01	1.31E+02	0.897	0.37155
SEEDCO 7	-1.46E-01	9.45E-01	1.31E+02	-0.155	0.87719
SEEDCO 9	8.54E-01	9.45E-01	1.31E+02	0.903	0.36811

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Songdaa	-2.01E+00	7.71E-01	1.31E+02	-2.609	0.01013
TGX-1835-10E	-5.10E-01	7.71E-01	1.31E+02	-0.662	0.50906
TGX-1989-40FA	-1.87E+00	9.45E-01	1.31E+02	-1.978	0.05005
TGX-1989-48FN	-1.61E-01	9.45E-01	1.31E+02	-0.17	0.86497
TGX-1990-21F	-1.02E+00	7.71E-01	1.31E+02	-1.32	0.18899
TGX-1990-40F	1.01E+00	7.69E-01	1.21E+02	1.311	0.19243
TGX-1990-46F	-5.25E-01	7.71E-01	1.31E+02	-0.68	0.49746
TGX-1990-49F	-1.50E+00	7.71E-01	1.31E+02	-1.946	0.05375
TGX-1990-55F	-1.53E-02	7.71E-01	1.31E+02	-0.02	0.9842
TGX 1445-3E	-1.01E+00	7.71E-01	1.31E+02	-1.311	0.19203
TGX 1485-1D	1.52E-01	9.45E-01	1.31E+02	0.161	0.87239
TGX 1740-2F	4.86E-01	7.71E-01	1.31E+02	0.63	0.52961
TGX 1799-8F	-7.08E-03	7.71E-01	1.31E+02	-0.009	0.99269
TGX 1805-8F	-1.01E+00	7.71E-01	1.31E+02	-1.313	0.19162
TGX 1805-8F-1	-1.56E-01	9.45E-01	1.31E+02	-0.165	0.86918
TGX 1834-5E	4.86E-01	7.71E-01	1.31E+02	0.631	0.52922
TGX 1844-19F	-5.10E-01	7.71E-01	1.31E+02	-0.661	0.50967
TGX 1903-7F	-9.98E-01	7.69E-01	1.21E+02	-1.298	0.19682
TGX 19110-6F	-1.00E+00	7.71E-01	1.31E+02	-1.298	0.19666
TGX 1987-62F	9.89E-01	7.71E-01	1.31E+02	1.283	0.20161
TGX 1988-3F	-1.06E+00	7.04E-01	1.31E+02	-1.499	0.13622
TGX 1988-3FA	-1.37E-01	9.45E-01	1.31E+02	-0.145	0.88519
TGX 1989-11F	-5.08E-01	7.71E-01	1.31E+02	-0.659	0.51096
TGX 1989-19F	-5.18E-01	7.71E-01	1.31E+02	-0.671	0.50316
TGX 1989-20FA	-1.14E+00	9.45E-01	1.31E+02	-1.203	0.2313
TGX 1989-41F	-1.15E+00	9.45E-01	1.31E+02	-1.219	0.22516
TGX 1989-42F	-5.08E-01	7.71E-01	1.31E+02	-0.66	0.51072
TGX 1989-75N	-5.31E-01	7.71E-01	1.31E+02	-0.688	0.49244
TGX 1990-110FN	-1.61E-01	9.45E-01	1.31E+02	-0.17	0.86497
TGX 1990-52F	4.75E-01	7.71E-01	1.31E+02	0.617	0.53853

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TGX 1990-57F	2.68E-01	7.04E-01	1.31E+02	0.381	0.7037
TGX 1990-78F	4.98E-01	7.71E-01	1.31E+02	0.646	0.51958
TGX 1990-80F	-3.78E-02	7.71E-01	1.31E+02	-0.049	0.96097
TGX 1990-93F	-1.13E+00	9.45E-01	1.31E+02	-1.199	0.23275
TGX 1990-95F	1.11E+00	9.45E-01	1.31E+02	1.172	0.24351
TGX 1990 -114 FN	-5.05E-01	7.71E-01	1.31E+02	-0.655	0.51347
TGX 1993-4FN	-2.91E-03	7.69E-01	1.21E+02	-0.004	0.99699
TGX 2004-10F	9.78E-01	7.71E-01	1.31E+02	1.268	0.20706
TGX 2004-13F	-5.20E-01	7.71E-01	1.31E+02	-0.674	0.5016
TGX 2006-3F	-7.63E-01	6.68E-01	1.31E+02	-1.143	0.25497
TGX 2007-11F	-1.50E+00	7.71E-01	1.31E+02	-1.949	0.05348
TGX 2008-4F	-5.10E-01	7.71E-01	1.31E+02	-0.662	0.50935
TGX 2010-11F	-1.01E+00	7.69E-01	1.22E+02	-1.31	0.19278
TGX 2011-3F	-1.61E-02	7.71E-01	1.31E+02	-0.021	0.98342
TGX1845-10E	-1.02E+00	7.71E-01	1.31E+02	-1.322	0.1886
TGX1987-10F	-1.27E-04	7.71E-01	1.31E+02	0	0.99987
TGX1989-48FN	-4.14E-03	7.71E-01	1.31E+02	-0.005	0.99572
TGX1989-68FN	-1.03E-02	7.71E-01	1.31E+02	-0.013	0.98938
Zamboani	-2.48E-02	7.71E-01	1.31E+02	-0.032	0.97441

Appendix. 4: Fixed effect estimate of lines based PSD infection rate

Line	Estimate	Std. Error	df	t value	Pr(>|t|)	Sign.
(Intercept)	7.50E+01	1.17E+01	1.31E+02	6.419	2.31E-09	***
Croton	2.50E+00	1.65E+01	1.31E+02	0.151	0.879966	
Wamini	-2.00E+01	1.65E+01	1.31E+02	-1.21	0.228283	
Afayak	-3.00E+01	2.02E+01	1.31E+02	-1.483	0.140608	
BRS- 7525	-1.50E+01	2.02E+01	1.31E+02	-0.741	0.459868	
BRS-326	-3.00E+01	1.65E+01	1.31E+02	-1.816	0.071705	
BRS-7980	-2.50E+01	1.65E+01	1.31E+02	-1.513	0.13267	

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BRS 8660	-3.00E+01	1.65E+01	1.31E+02	-1.816	0.071705	
BRS 8660A	-5.00E+00	2.02E+01	1.31E+02	-0.247	0.80523	
BRS Perola	2.50E+01	2.02E+01	1.31E+02	1.235	0.218886	
BRS TIANA	2.00E+01	1.65E+01	1.31E+02	1.21	0.228283	
BRS 313	-5.50E+01	1.65E+01	1.31E+02	-3.329	0.001133	**
BRS 361	-7.00E+01	1.65E+01	1.31E+02	-4.237	4.25E-05	***
BRS 90-8360	1.50E+01	1.65E+01	1.31E+02	0.908	0.365628	
FT christa	-1.00E+01	1.65E+01	1.31E+02	-0.605	0.546074	
Jenguma	2.50E+01	1.65E+01	1.31E+02	1.513	0.13267	
LG 04 -6000	-3.50E+01	1.65E+01	1.31E+02	-2.118	0.036038	*
Liu Yuebao	-6.50E+01	1.65E+01	1.31E+02	-3.934	0.000135	***
Liu Yuemang	-7.50E+01	2.02E+01	1.31E+02	-3.706	0.000309	***
NE 3400	-2.50E+01	1.65E+01	1.31E+02	-1.513	0.13267	
Ozark	1.50E+01	2.02E+01	1.31E+02	0.741	0.459868	
OZARK	5.00E+00	2.02E+01	1.31E+02	0.247	0.80523	
Parangoina	-6.50E+01	2.02E+01	1.31E+02	-3.212	0.001658	**
PARANGOINA	-5.00E+00	2.02E+01	1.31E+02	-0.247	0.80523	
PI 534545	-5.00E+01	1.65E+01	1.31E+02	-3.026	0.002983	**
PI159925	-4.00E+01	1.65E+01	1.31E+02	-2.421	0.016852	*
PI200492	-5.50E+01	2.02E+01	1.31E+02	-2.718	0.007459	**
PI200526	-4.50E+01	2.02E+01	1.31E+02	-2.224	0.027878	*
PI224271	-7.50E+01	1.65E+01	1.31E+02	-4.539	1.26E-05	***
PI229358	2.50E+01	1.65E+01	1.31E+02	1.513	0.13267	
PI230970	-5.50E+01	1.65E+01	1.31E+02	-3.329	0.001133	**
PI274454	-1.50E+01	1.65E+01	1.31E+02	-0.908	0.365628	
PI274954A	-3.50E+01	2.02E+01	1.31E+02	-1.73	0.08606	.
PI340898A	-3.00E+01	1.65E+01	1.31E+02	-1.816	0.071705	.
PI379621	-3.75E+01	1.43E+01	1.31E+02	-2.621	0.009812	**
PI398387	-2.50E+01	1.65E+01	1.31E+02	-1.513	0.13267	
PI398477	1.50E+01	2.02E+01	1.31E+02	0.741	0.459868	

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PI398512	-1.00E+01	1.65E+01	1.31E+02	-0.605	0.546074	
PI398543	-2.50E+01	2.02E+01	1.31E+02	-1.235	0.218886	
PI398594	-6.90E-13	1.65E+01	1.31E+02	0	1	
PI398825	-4.50E+01	2.02E+01	1.31E+02	-2.224	0.027878	*
PI398836	-2.50E+01	1.65E+01	1.31E+02	-1.513	0.13267	
PI398874	1.50E+01	1.65E+01	1.31E+02	0.908	0.365628	
PI399096	-4.50E+01	1.65E+01	1.31E+02	-2.724	0.00734	**
PI416778	-4.00E+01	1.65E+01	1.31E+02	-2.421	0.016852	*
PI416806A	-4.50E+01	2.02E+01	1.31E+02	-2.224	0.027878	*
PI416806B	-4.50E+01	2.02E+01	1.31E+02	-2.224	0.027878	*
PI416810	-5.50E+01	2.02E+01	1.31E+02	-2.718	0.007459	**
PI416810A	-5.17E+01	1.51E+01	1.31E+02	-3.425	0.00082	***
PI416810B	5.00E+00	2.02E+01	1.31E+02	0.247	0.80523	
PI416810B-1	5.00E+00	2.02E+01	1.31E+02	0.247	0.80523	
PI416873B	-2.50E+01	2.02E+01	1.31E+02	-1.235	0.218886	
PI416886	-4.00E+01	1.65E+01	1.31E+02	-2.421	0.016852	*
PI416939	-4.50E+01	1.65E+01	1.31E+02	-2.724	0.00734	**
PI417013	-7.00E+01	1.65E+01	1.31E+02	-4.237	4.25E-05	***
PI417119	2.00E+01	1.65E+01	1.31E+02	1.21	0.228283	
PI417120	-7.50E+01	2.02E+01	1.31E+02	-3.706	0.000309	***
PI417120A	-3.50E+01	2.02E+01	1.31E+02	-1.73	0.08606	.
PI417126	-5.00E+01	1.65E+01	1.31E+02	-3.026	0.002983	**
PI417134	-1.50E+01	1.65E+01	1.31E+02	-0.908	0.365628	
PI423958	-7.47E+01	2.02E+01	1.31E+02	-3.69	0.000328	***
PI423960A	-2.50E+01	1.65E+01	1.31E+02	-1.513	0.13267	
PI423972	2.50E+01	1.65E+01	1.31E+02	1.513	0.13267	
PI459025A	-2.00E+01	1.65E+01	1.31E+02	-1.21	0.228283	
PI462312	5.00E+00	2.02E+01	1.31E+02	0.247	0.80523	
PI468919A	-5.00E+00	2.02E+01	1.31E+02	-0.247	0.80523	
PI476905A	5.00E+00	1.65E+01	1.31E+02	0.303	0.762664	

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PI506938	-7.01E-13	1.65E+01	1.31E+02	0	1	
PI507004	-5.00E+00	1.65E+01	1.31E+02	-0.303	0.762664	
PI507004A	-3.00E+01	1.65E+01	1.31E+02	-1.816	0.071705	.
PI518295	-4.50E+01	1.65E+01	1.31E+02	-2.724	0.00734	**
PI561381	-3.00E+01	1.65E+01	1.31E+02	-1.816	0.071705	.
PI567025A	-3.50E+01	1.65E+01	1.31E+02	-2.118	0.036038	*
PI567053	-2.50E+01	1.65E+01	1.31E+02	-1.513	0.13267	
PI567054C	-3.50E+01	1.65E+01	1.31E+02	-2.118	0.036038	*
PI567056A	-1.50E+01	1.65E+01	1.31E+02	-0.908	0.365628	
PI567058D	-4.00E+01	1.65E+01	1.31E+02	-2.421	0.016852	*
PI567068A	5.00E+00	1.65E+01	1.31E+02	0.303	0.762664	
PI567076	2.00E+01	1.65E+01	1.31E+02	1.21	0.228283	
PI567090	-1.00E+01	1.65E+01	1.31E+02	-0.605	0.546074	
PI567091A	-6.50E+01	1.65E+01	1.31E+02	-3.934	0.000135	***
PI567099A	-7.13E-13	1.65E+01	1.31E+02	0	1	
PI567104B	-2.00E+01	1.65E+01	1.31E+02	-1.21	0.228283	
PI567123A	-1.50E+01	1.65E+01	1.31E+02	-0.908	0.365628	
PI567129	-3.00E+01	1.65E+01	1.31E+02	-1.816	0.071705	.
PI567188	-4.00E+01	1.65E+01	1.31E+02	-2.421	0.016852	*
PI567190	5.00E+00	2.02E+01	1.31E+02	0.247	0.80523	
PI567191	-3.50E+01	2.02E+01	1.31E+02	-1.73	0.08606	.
PI567516C	-7.00E+01	1.65E+01	1.31E+02	-4.237	4.25E-05	***
PI578457A	-5.00E+00	1.65E+01	1.31E+02	-0.303	0.762664	
PI584796	-4.50E+01	1.65E+01	1.31E+02	-2.724	0.00734	**
PI587886	-5.00E+00	2.02E+01	1.31E+02	-0.247	0.80523	
PI587905	2.50E+01	1.65E+01	1.31E+02	1.513	0.13267	
PI588053A	-1.50E+01	2.02E+01	1.31E+02	-0.741	0.459868	
PI588053B	-6.92E-13	1.65E+01	1.31E+02	0	1	
PI594538A	-4.50E+01	1.65E+01	1.31E+02	-2.724	0.00734	**
PI594760B	-2.50E+01	1.65E+01	1.31E+02	-1.513	0.13267	

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PI594767A	-3.00E+01	1.65E+01	1.31E+02	-1.816	0.071705	.
PI594767B	-6.00E+01	1.65E+01	1.31E+02	-3.631	0.000403	***
PI603170	-3.00E+01	1.65E+01	1.31E+02	-1.816	0.071705	.
PI603176A	-4.50E+01	2.02E+01	1.31E+02	-2.224	0.027878	*
PI605791A	-5.50E+01	1.65E+01	1.31E+02	-3.329	0.001133	**
PI605823	-1.00E+01	1.65E+01	1.31E+02	-0.605	0.546074	
PI605824A	-3.00E+01	2.02E+01	1.31E+02	-1.483	0.140608	
PI605829	5.00E+00	1.65E+01	1.31E+02	0.303	0.762664	
PI605854B	-2.50E+01	1.65E+01	1.31E+02	-1.513	0.13267	
PI605891A	-5.50E+01	1.65E+01	1.31E+02	-3.329	0.001133	**
PI605891B	-1.50E+01	1.65E+01	1.31E+02	-0.908	0.365628	
PI605891B-1	-5.00E+00	2.02E+01	1.31E+02	-0.247	0.80523	
PI606405	-4.00E+01	1.65E+01	1.31E+02	-2.421	0.016852	*
PI628850	-5.50E+01	1.65E+01	1.31E+02	-3.329	0.001133	**
PI628932	-1.50E+01	1.65E+01	1.31E+02	-0.908	0.365628	
PI635999	-4.00E+01	1.65E+01	1.31E+02	-2.421	0.016852	*
PI657753	1.00E+01	1.65E+01	1.31E+02	0.605	0.546074	
PI81042	-3.50E+01	2.02E+01	1.31E+02	-1.73	0.08606	.
Quarshie	5.00E+00	2.02E+01	1.31E+02	0.247	0.80523	
SARISOY 27	-3.50E+01	2.02E+01	1.31E+02	-1.73	0.08606	.
SARISOY 1	-1.50E+01	2.02E+01	1.31E+02	-0.741	0.459868	
SARISOY 10	-6.50E+01	2.02E+01	1.31E+02	-3.212	0.001658	**
SARISOY 12	2.50E+01	2.02E+01	1.31E+02	1.235	0.218886	
SARISOY 13	-3.50E+01	2.02E+01	1.31E+02	-1.73	0.08606	.
SARISOY 14	-5.00E+00	2.02E+01	1.31E+02	-0.247	0.80523	
SARISOY 15	-6.50E+01	2.02E+01	1.31E+02	-3.212	0.001658	**
SARISOY 16	-2.00E+01	1.65E+01	1.31E+02	-1.21	0.228283	
SARISOY 17	-3.50E+01	1.65E+01	1.31E+02	-2.118	0.036038	*
SARISOY 18	-6.50E+01	2.02E+01	1.31E+02	-3.212	0.001658	**
SARISOY 19	-2.50E+01	2.02E+01	1.31E+02	-1.235	0.218886	

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SARISOY 21	-6.50E+01	2.02E+01	1.31E+02	-3.212	0.001658	**
SARISOY 23	-4.50E+01	2.02E+01	1.31E+02	-2.224	0.027878	*
SARISOY 25	-1.50E+01	2.02E+01	1.31E+02	-0.741	0.459868	
SARISOY 26	-6.50E+01	2.02E+01	1.31E+02	-3.212	0.001658	**
SARISOY 28	2.50E+01	2.02E+01	1.31E+02	1.235	0.218886	
SARISOY 30	-5.00E+00	2.02E+01	1.31E+02	-0.247	0.80523	
SARISOY 4	-3.50E+01	2.02E+01	1.31E+02	-1.73	0.08606 .	
SARISOY 5	-6.50E+01	2.02E+01	1.31E+02	-3.212	0.001658	**
SARISOY 7	-2.50E+01	2.02E+01	1.31E+02	-1.235	0.218886	
SARISOY 9	1.50E+01	2.02E+01	1.31E+02	0.741	0.459868	
SEEDCO 27	-5.50E+01	2.02E+01	1.31E+02	-2.718	0.007459	**
SEEDCO 8	-2.50E+01	2.02E+01	1.31E+02	-1.235	0.218886	
SEEDCO 1	-1.50E+01	2.02E+01	1.31E+02	-0.741	0.459868	
SEEDCO 10	-4.50E+01	2.02E+01	1.31E+02	-2.224	0.027878	*
SEEDCO 11	-5.00E+00	2.02E+01	1.31E+02	-0.247	0.80523	
SEEDCO 12	1.50E+01	2.02E+01	1.31E+02	0.741	0.459868	
SEEDCO 13	-5.50E+01	2.02E+01	1.31E+02	-2.718	0.007459	**
SEEDCO 14	-5.00E+00	2.02E+01	1.31E+02	-0.247	0.80523	
SEEDCO 15	-5.00E+00	2.02E+01	1.31E+02	-0.247	0.80523	
SEEDCO 16	-6.89E-13	1.65E+01	1.31E+02	0	1	
SEEDCO 17	-3.50E+01	2.02E+01	1.31E+02	-1.73	0.08606 .	
SEEDCO 18	-5.50E+01	2.02E+01	1.31E+02	-2.718	0.007459	**
SEEDCO 19	-3.50E+01	2.02E+01	1.31E+02	-1.73	0.08606 .	
SEEDCO 21	-6.50E+01	2.02E+01	1.31E+02	-3.212	0.001658	**
SEEDCO 25	-5.00E+00	2.02E+01	1.31E+02	-0.247	0.80523	
SEEDCO 26	2.50E+01	2.02E+01	1.31E+02	1.235	0.218886	
SEEDCO 28	2.50E+01	2.02E+01	1.31E+02	1.235	0.218886	
SEEDCO 29	5.00E+00	2.02E+01	1.31E+02	0.247	0.80523	
SEEDCO 30	-2.50E+01	1.65E+01	1.31E+02	-1.513	0.13267	
SEEDCO 4	-4.50E+01	2.02E+01	1.31E+02	-2.224	0.027878	*

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SEEDCO 5	-3.50E+01	2.02E+01	1.31E+02	-1.73	0.08606	.
SEEDCO 7	5.00E+00	2.02E+01	1.31E+02	0.247	0.80523	
SEEDCO 9	1.50E+01	2.02E+01	1.31E+02	0.741	0.459868	
Songdaa	1.50E+01	1.65E+01	1.31E+02	0.908	0.365628	
TGX-1835	-10E1.50E+01	1.65E+01	1.31E+02	0.908	0.365628	
TGX-1989-40FA	5.00E+00	2.02E+01	1.31E+02	0.247	0.80523	
TGX-1989-48FN	-2.50E+01	2.02E+01	1.31E+02	-1.235	0.218886	
TGX-1990-21F	-1.00E+01	1.65E+01	1.31E+02	-0.605	0.546074	
TGX-1990-40F	2.00E+01	1.65E+01	1.31E+02	1.21	0.228283	
TGX-1990-46F	-6.92E-13	1.65E+01	1.31E+02	0	1	
TGX-1990-49F	-1.50E+01	1.65E+01	1.31E+02	-0.908	0.365628	
TGX-1990-55F	-1.00E+01	1.65E+01	1.31E+02	-0.605	0.546074	
TGX 1445-3E	-2.50E+01	1.65E+01	1.31E+02	-1.513	0.13267	
TGX 1740-2F	2.50E+01	1.65E+01	1.31E+02	1.513	0.13267	
TGX 1799-8F	5.00E+00	1.65E+01	1.31E+02	0.303	0.762664	
TGX 1805-8F	-2.50E+01	1.65E+01	1.31E+02	-1.513	0.13267	
TGX 1805-8F-1	-1.50E+01	2.02E+01	1.31E+02	-0.741	0.459868	
TGX 1834-5E	-6.99E-13	1.65E+01	1.31E+02	0	1	
TGX 1844-19F	-1.00E+01	1.65E+01	1.31E+02	-0.605	0.546074	
TGX 1903-7F	-5.50E+01	1.65E+01	1.31E+02	-3.329	0.001133	**
TGX 19110-6F	-1.00E+01	1.65E+01	1.31E+02	-0.605	0.546074	
TGX 1987-62F	2.50E+01	1.65E+01	1.31E+02	1.513	0.13267	
TGX 1988-3F	-5.00E+00	1.51E+01	1.31E+02	-0.331	0.740798	
TGX 1989-11F	-1.50E+01	1.65E+01	1.31E+02	-0.908	0.365628	
TGX 1989-19F	-1.50E+01	1.65E+01	1.31E+02	-0.908	0.365628	
TGX 1989-20FA	-2.50E+01	2.02E+01	1.31E+02	-1.235	0.218886	
TGX 1989-41F	1.50E+01	2.02E+01	1.31E+02	0.741	0.459868	
TGX 1989-42F	-4.00E+01	1.65E+01	1.31E+02	-2.421	0.016852	*
TGX 1989-75N	-5.00E+01	1.65E+01	1.31E+02	-3.026	0.002983	**
TGX 1990-110FN	-5.00E+00	2.02E+01	1.31E+02	-0.247	0.80523	

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TGX 1990-52F	-3.00E+01	1.65E+01	1.31E+02	-1.816	0.071705	
TGX 1990-57F	-2.17E+01	1.51E+01	1.31E+02	-1.436	0.153247	
TGX 1990-78F	-6.88E-13	1.65E+01	1.31E+02	0	1	
TGX 1990-80F	-3.50E+01	1.65E+01	1.31E+02	-2.118	0.036038	*
TGX 1990-93F	-1.50E+01	2.02E+01	1.31E+02	-0.741	0.459868	
TGX 1990-95F	-4.50E+01	2.02E+01	1.31E+02	-2.224	0.027878	*
TGX 1990 -114 FN	-3.00E+01	1.65E+01	1.31E+02	-1.816	0.071705	
TGX 1993-4FN	-6.50E+01	1.65E+01	1.31E+02	-3.934	0.000135	****
TGX 2004-10F	-1.00E+01	1.65E+01	1.31E+02	-0.605	0.546074	
TGX 2004-13F	-2.00E+01	1.65E+01	1.31E+02	-1.21	0.228283	
TGX 2006-3F	-9.00E+00	1.43E+01	1.31E+02	-0.629	0.530462	
TGX 2007-11F	-2.00E+01	1.65E+01	1.31E+02	-1.21	0.228283	
TGX 2008-4F	2.50E+01	1.65E+01	1.31E+02	1.513	0.13267	
TGX 2010-11F	-5.00E+01	1.65E+01	1.31E+02	-3.026	0.002983	**
TGX 2011-3F	-1.00E+01	1.65E+01	1.31E+02	-0.605	0.546074	
TGX1845-10E	-5.00E+00	1.65E+01	1.31E+02	-0.303	0.762664	
TGX1987-10F	-3.00E+01	1.65E+01	1.31E+02	-1.816	0.071705	.
TGX1989-48FN	5.00E+00	1.65E+01	1.31E+02	0.303	0.762664	
TGX1989-68FN	-5.00E+01	1.65E+01	1.31E+02	-3.026	0.002983	**
Zamboani	-5.00E+01	1.65E+01	1.31E+02	-3.026	0.002983	**