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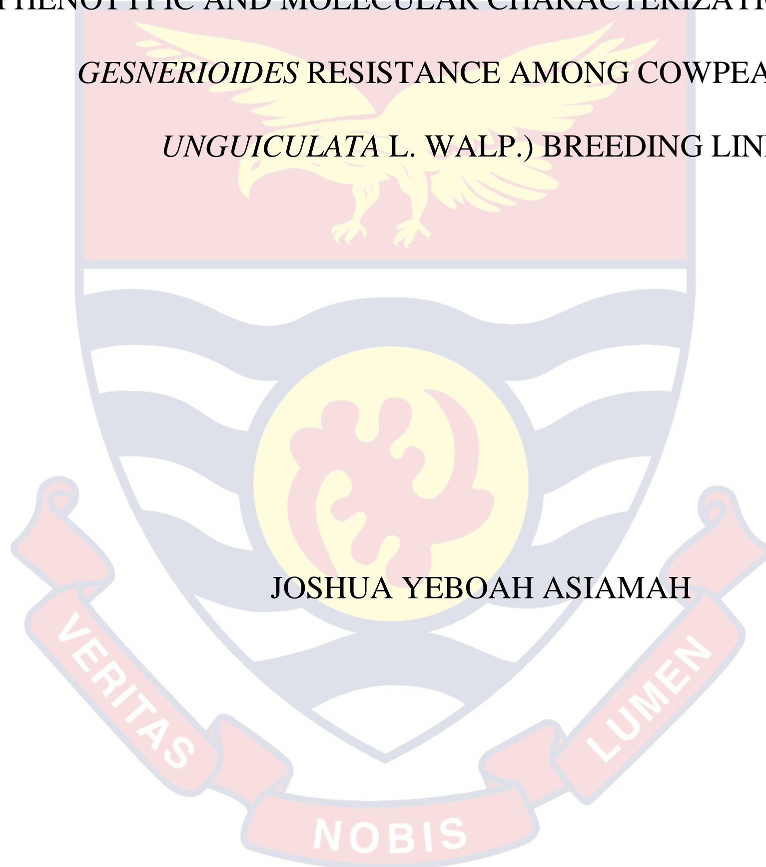
PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF *STRIGA GESNERIOIDES* RESISTANCE AMONG COWPEA (*VIGNA UNGUICULATA* L. WALP.) BREEDING LINES



JOSHUA YEBOAH ASIAMAH

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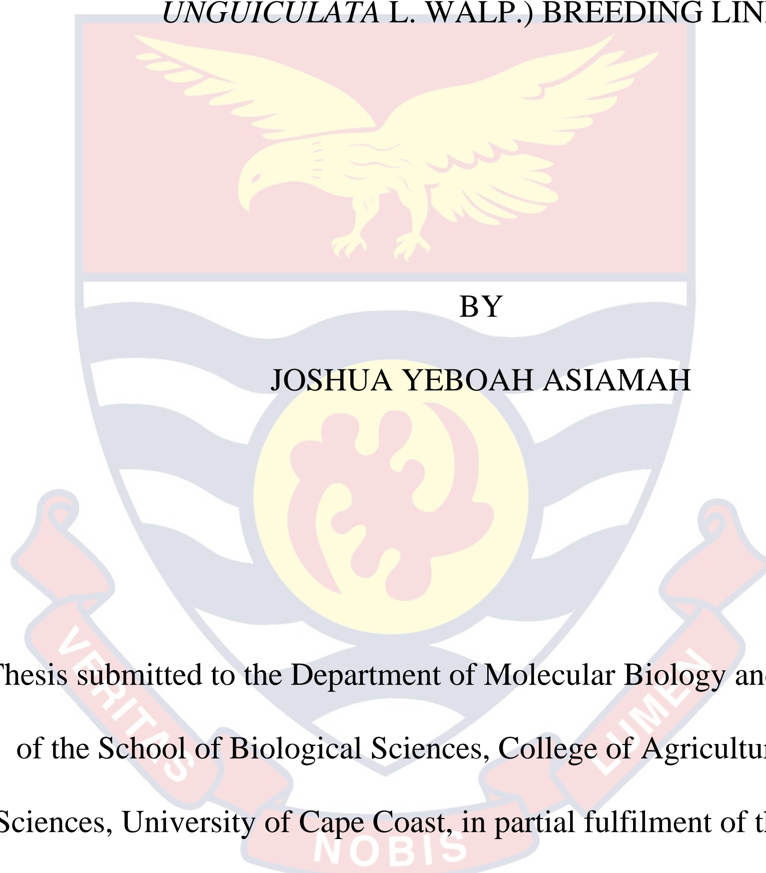

BY

JOSHUA YEBOAH ASIAMAH

Thesis submitted to the Department of Molecular Biology and Biotechnology of the School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Molecular Biology and Biotechnology

SEPTEMBER, 2020

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: .... Date: ....

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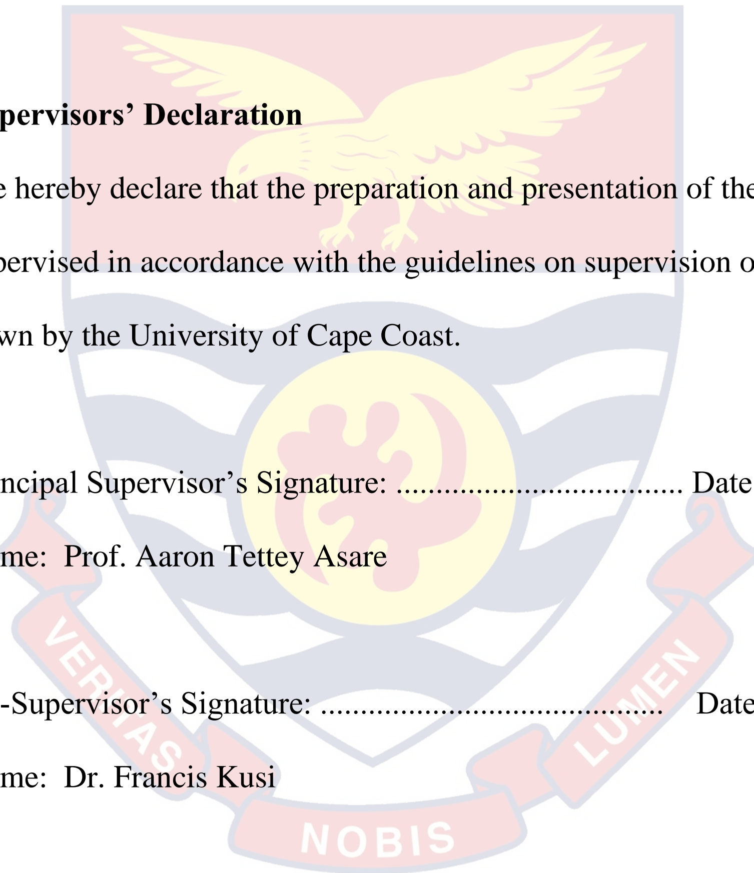

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: .... Date: ....

Name: Prof. Aaron Tettey Asare

Co-Supervisor's Signature: .... Date: ....

Name: Dr. Francis Kusi

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ABSTRACT

In Ghana, cowpea (*Vigna unguiculata*) production is constrained by *Striga gesnerioides* infestation. Though some *Striga*-resistant cowpea varieties exist, they are predominantly small to medium seed sizes, but consumer preference is tailored towards large to extra-large seeds. This study aimed to evaluate cowpea breeding lines and select for *Striga* resistance and improved agronomic traits. Data from the field were subjected to analysis of variance (ANOVA) and correlation. The variations in the quantitative and qualitative traits and molecular markers distinguished the cowpea genotypes. Genetic diversity and *Striga*-resistant cowpeas among the breeding populations were determined by SSR markers. Agro-morphogenetic variations exist among the cowpea breeding lines. The 100 seed weight differed significantly ($P < 0.001$) among the cowpea breeding lines, ranging from 11 to 26.8 g with a mean of 20.4 g. Grain yield ranged from 1.04 t ha^{-1} - 2.92 t ha^{-1}. The highest coefficient of variation (CV % > 100) was among the *Striga* response parameters. *Striga* resistance efficiency test by pot screening was consistent with the marker-assisted selection (MAS) protocol but not so with the field screening test. SSR-1, C42-2B, CLM1320 and LRR8 were considered to have the best discrimination efficiency (74%-85.5%) to *S.gesnerioides* resistance. The alleles per primer pair of 2 to 7 with an average of 3, PIC of 0.41 and gene diversity of 0.25 were evidence of genetic variations. On the whole, UC15-01, UC15-02, UC15-19, UC15-22, UC15-28, UC15-35, UC15-43, UC15-43 UC15-47 and UC15-49 associated with large seed sizes, high yield and *Striga*-resistance traits and were the best-improved cowpea progenies selected for further evaluation.

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KEYWORDS

Breeding lines

Discriminating efficiency

Progenies

Resistant

Striga gesnerioides

Susceptible

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ACKNOWLEDGEMENTS

I express my intense gratitude to my supervisors, Prof. Aaron Tettey Asare (Principal Supervisor) and Dr. Francis Kusi (Co-supervisor) of the University of Cape Coast (UCC) and Council for Scientific and Industrial Research,-Savannah Agricultural Research Institute (CSIR-SARI) respectively, for their contributions, constructive criticism, encouragement and advice towards the execution of this work.

I am also grateful to Mr. Patrick Attamah (CSIR-SARI), Mr. Frank Essem (UCC), Mr. Theophilus Abonyi Mensah, Mr. Samuel Acheampong (UCC), Mr. Samuel Adjei (NDSU), Mr. Kofi Kudiabor Ntsunyo, Mr. Emmanuel Halm and Mr. Adamu for their unflinching support and dedication towards the success of this work. Am thankful to all lecturers of the department of molecular biology and Biotechnology.

I lengthen my sincere gratitude to the Ghana National Petroleum Corporation Foundation for funding my Postgraduate education. I am also grateful to FAO- ITPGRFA (sponsored Cowpea Project) and Samuel and Emelia Butler Brew grant for funding this work through the University of Cape Coast, Ghana.

I am sincerely grateful to all my colleagues, staff and Technicians of CSIR-SARI, -Manga station - Bawku, Lab Technologist and Technicians of UCC, Teaching and Senior Research assistants for their support

Finally, I would also want to thank my family and friends for their support especially, my mother, Mrs. Elizabeth Yeboah Asiamah and Mrs. Rejoice Dzirasa Asare, for their support, prayers and encouragement.

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DEDICATION

To my mother, Elizabeth Yeboah Asiamah and my late father, Mr. Charles Yeboah Asiamah

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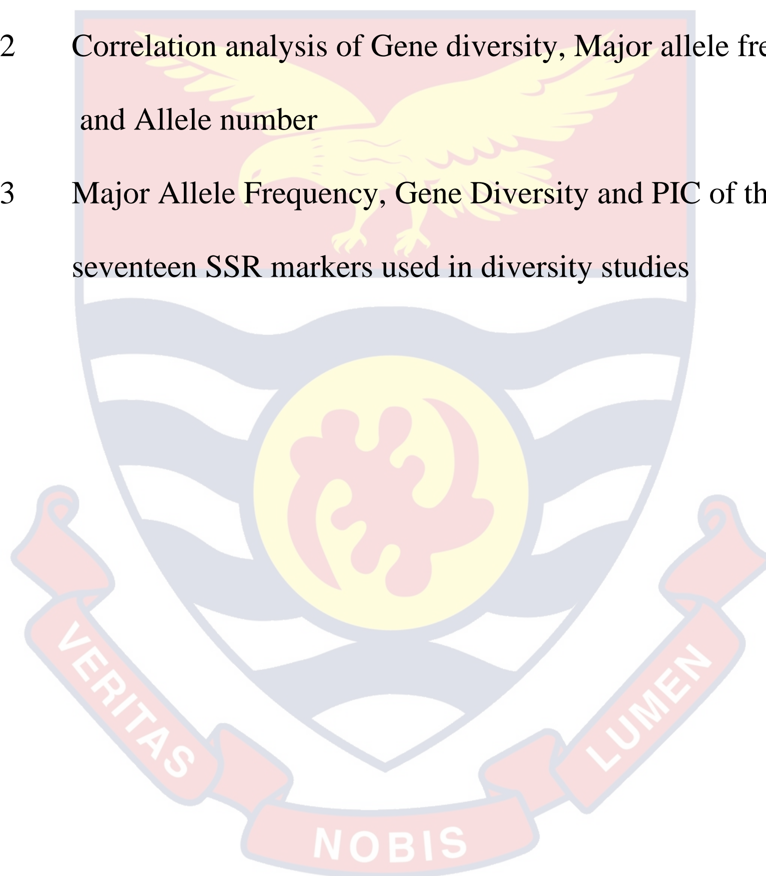

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LIST OF ACRONYMS

%	Percentage
Cm	Centimeters
CSIR-SARI	Council for Scientific and Industrial Research, Savannah Agriculture Research Institute
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
GH-BINDURI	*Striga gesnerioides* found and collected in the Binduri district
IBPGR	International Board for Plant Genetic Resources
IITA	International Institute of Tropical Agriculture
LSD	Least Significant Difference
MoFA	Ministry of Food and Agriculture
MT	Metric Tonnes
PCR	Polymerase Chain Reaction
PIC	Polymorphism Information Content
SCAR	Sequence Characterized Amplified Region
SSR	Simple-sequence repeats
UPMGA	Unweighted Pair Group Method with Arithmetic Mean
µL	Microlitre
CTAB	Cetyl Trimethylammonium Bromide

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CHAPTER ONE

INTRODUCTION

Chapter one covers the general introduction of the current work, the study's background, problem statement, justification, objectives and the associated hypothesis, and the study's significance.

1.1 Background to the study

Cowpea (*Vigna unguiculata* L. Walp.) is a source of protein and directly increases in soil fertility through nitrogen fixation when grown in rotation with cereals (Sanginga *et al.*, 2003). Moreover, cowpea production is suitable for subsistence farming systems in which low inputs are involved due to its ability to thrive on relatively poor soil (Pasquet, 1999; PRONAF, 2003). It has a high adaptation level due to its inherent ability to withstand drought, tolerate shade, and fix atmospheric nitrogen (Singh, Chambis & Sharma, 1997).

Regardless of cowpea's huge potential to ensure food security and good soil nutrient turnover, several abiotic and biotic factors affect its production. The low productivity of cowpea is mainly due to intense biotic stress by insects and other pests. A significant biotic constraint is *Striga. gesnerioides* infestation (Asare, Galyuon, Padi, Otwe & Takrama, 2013).

S. gesnerioides is amongst the world's worst obligate parasitic weed, reducing the yield of legumes, especially in semi-arid areas of the world (Botanga & Timko, 2006). *S. gesnerioides* is the only species of the genus *Striga* that is virulent on dicots (Mohamed, Musselman & Riches, 2001). Yield losses ranging from 83 – 100 % have been reported on farmers'

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fields due to infestation by *S. gesnerioides* (Asare *et al.*, 2013). This represents an annual loss of about 7 billion dollars (Hearne, 2009). However, no strategy is completely sufficient in the control of this parasitic weed. Host plant resistance appears to have merit in effectively and economically controlling the parasite in that it is affordable to farmers (Omoigui *et al.,* 2007). Hence, breeding for resistant genotypes has become necessary. Breeding programs are enhanced by molecular markers, mostly simple sequence repeats (SSR) markers, to facilitate the introgression of the selected trait of interest. Apart from breeding cowpea for *Striga* resistance, consumers' preference for grain characteristic need to be considered. Consumer preference is one of the dictators of cowpea production and marketing. There are several visual characteristics of cowpeas that have been known to be on the checklist of consumers. One of the important desirable traits of cowpea that consumers look out for in West Africa is large seed size (Langyintuo *et al.*, 2003; Tchiagam, Bell, Nassourou, & Njintang, 2011; Egbadzor *et al.*, 2013). However, many breeding objectives have not directly focused on seed size compared with traits such as biotic and abiotic stress tolerance (Orawu, Melis, Liang, & Derera, 2013). Size, shape, colour, and textures are critical features of these market classes and should be the breeders' target for developing demand- led market-driven cultivars. Developing cowpea with *Striga* resistance alone while ignoring consumer-preferred traits could defeat the researcher's aim in the Ghanaian market by reducing its acceptance and subsequent adoption significantly. It is therefore essential to incorporate consumer-preferred traits during cowpea breeding programmes in Ghana. Timko *et al.* (2007) reported that some regional landraces of cowpea appear to

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be invulnerable to some *Striga* races, with resistance due to a single dominant gene. The first *Striga*-resistant cowpea landrace genotype, GH3684, first reported by Asare *et al.* (2013), appears to have resistance to multiple races of *S. gesnerioides* (Essem, 2017). However, GH3684 is an unimproved local landrace that produces seeds that are small and red. Hence, the need to explore *Striga*-resistance traits of GH3684 in a breeding programme in Ghana to develop improved varieties associated with consumer-preferred traits.

1.2 Problem Statement

The existing *Striga*-resistant cowpea varieties are predominantly small to medium seed sizes but consumer preference is driven towards large to extra-large seeds. The production constraints by the parasitic weed, *Striga gesnerioides* and its devastating effects on cowpea yield loss (80-100 %) in the dry Savannah regions of Northern Ghana warrant robust breeding programmes to meet the preference of both farmers and consumers preference. Currently, there is a lack of market-driven local cowpea varieties associated with *Striga gesnerioides* resistance. Hence, the Ghanaian market seems to be dominated by imported large-seeded white cowpea varieties from neighboring countries (Mishili *et al.*, 2009). However, these imported cowpea varieties are susceptible to *Striga* and are not adapted to Ghana's local environmental conditions due to genotype-environment interaction (, Lane, Bailey & Terry, 1991, MoFA, 2016). The *Striga*-resistance gene of GH3684 has been introgressed into PADI-TUYA, UCSO1 and SARC-1-57-1 (with consumer-preferred traits) as well as Songotra (IT97K-499-35). The *Striga*-resistance status of F_4 breeding lines of these cowpeas, as well as their genetic

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relatedness, agronomic and morphological characteristics, have not been assessed to pre-select farmer and consumer-preferred traits.

1.3 Justification

To combat *Striga* infestation in the dry Savanna Agro-Ecological Zones of Northern Ghana, improved cowpea varieties with desirable grain qualities and resistance to the parasitic weed, have to be developed and made available to farmers to cultivate. F_4 cowpea breeding lines have been developed and stored in the Molecular Biology and Biotechnology department, University of Cape Coast, but lack characterization and the *Striga* resistance status is unknown.

1.4 Research Objectives

1.4.1 Main Objective

This study's main objective was to characterize cowpea breeding lines and select for improved *Striga* resistance and agronomic traits.

1.4.2 Specific Objective

The specific objectives were to:

1. Evaluate phenotypic variations among cowpea breeding lines.
2. Identify *Striga*-resistant cowpea lines and validate SSR markers linked to *Striga* resistance across the genome of the cowpea breeding lines.
3. Assess genetic diversity among cowpea breeding lines.

1.5 Research questions

1. Are there phenotypic variations among the cowpea breeding lines?
2. Are there any specific SSR markers linked to *Striga* resistance?
3. Do genetic variations exist in the F_4 cowpea breeding lines?

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4. Is there observed inheritance of *Striga* resistance and large seed size trait among cowpea breeding lines?

1.6 Significance of the study

1. The identified cowpea genotypes showing resistance to the parasitic weed in this study could be subjected to multi-locational evaluation and subsequently released as varieties for cultivation in Ghana
2. The *Striga*-resistant cowpea breeding lines found in this study could be used in back-cross breeding programmes to develop more improved cowpea varieties resistant to *S. gesnerioides*.
3. The cowpea breeding lines that may be resistant to *Striga* gesnerioides in Ghana could be further tested with other *Striga* races in Sub-Saharan Africa.
4. The genetic variations within the cowpea breeding lines could be ample and better adapt the crop to the farmer and consumer preference.

1.7 Organization of the Study

The study is organized into six chapters. Chapter one presents a general overview of the study. It briefly describes the cowpea (*Vigna unguiculata* L. Walp) plant, the problem which guided the study and the importance and application of the study. The objectives to be achieved and the reseach questions are also outlined. Chapter two mainly focused on the literature review. Chapter three focused on phenotypic characterization to explore the traits of the cowpeas. Chapter Four dealt with the screening of cowpea genotypes for *Striga*-resistance using SSR markers, pot-testing as well as evaluation of cowpea breeding lines at a *Striga*-hotspot in the Savannah Agriculture Research Institute, Manga-station. Chapter five deals with the

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genetic diversity studies of the cowpeas using 100 SSR primers. Chapter six gives summary and general conclusions of the study and recommendations.

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CHAPTER TWO

LITERATURE REVIEW

This chapter provides insight into the origin, morphology and phenology, importance of cowpea, production and constraints to production of the crop in Ghana, consumer preference of cowpea, *S. gesnerioides* parasitism in cowpea and characterization.

2.1 Origin, domestication and diversity

The precise origin of cultivated cowpea has been argued for a very long time. Inadequate archaeological evidence has resulted in contradicting opinions supporting Africa, Asia, and South America as the center of origin and domestication of cowpea (Coetzee, 1995; D'Andrea, Kahlheber, Logan, & Watson, 2007; Boukar *et al.*, 2015). According to the World Cowpea Conference (2010) held in Senegal, the history of cowpea dates to ancient West African cereal farming, five to six thousand years ago, where it was closely associated with the cultivation of sorghum and pearl millet (Tignegre, 2010). It has mostly been reported that cowpea originated in Africa and is widely grown in Africa, Latin America, Southeast Asia and the southern United States (Xiong *et al.*, 2016). Padulosi and Ng (1997) and Pasquet (1999) proposed that cowpea is likely to be domesticated only once, probably in West Africa, about 2000 B.C. and that the originator or parent of cultivated cowpea was the wild cowpea *V. unguiculata* var. *spontanae*. Again, archaeological evidence reveals that cowpea may have originated and domesticated in central Ghana, Kintampo (D'Andrea, *et al.*, 2007). The carbon dating of wild cowpea remnants from the rock shelter in Kintampo shows the existence of cowpea

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gathering by African hunters and gatherers as early as 1500 BC (D'Andrea *et al.*, 2007).

Most of the world's cowpea is cultivated in West Africa (Rawal 1975; Timko, Ehlers, & Roberts, 2007; Kamara, Omoigui, Kamai, Ewansiha, & Ajeigbe, 2018). Rawal (1975) reported that many weedy forms of cowpea are found in West Africa, which exhibits similar characteristics with the truly wild forms and the very small-seeded cultivated forms and can be described as intermediates. Allen and Obura (1983) reported that cowpea was introduced to the Indian sub-continent from West Africa about 2000 to 3000 years ago. Ba, Pasquet and Gept (2004) also supported that cowpea was domesticated in West Africa. Padulosi and Ng (1997) revealed that cowpea had reached Northern Africa and Europe from Asia before 300 BC. Molecular studies based on amplified fragment length polymorphism (AFLP) have shown evidence of domestication, which occurred in northeastern Africa (Coulibaly, Pasquet, Papa & Gepts, 2002). It is believed that the crop reached the southern USA in the early 18th century as a result of the slave trade in West Africa but had already reached West India in the 17th century by the Spanish during the slave trade.

Again, the center of maximum diversity of domesticated *Vigna unguiculata* L. Walp. is found in West Africa, in an area within the Savanna regions (Ng and Marechal, 1985). The highest diversity in wild relatives of cowpea has been found in southeastern Africa. It may have been the center of speciation of *Vigna unguiculata* due to the presence of most primitive subspecies in a region surrounding Namibia from the west, across Botswana, Zambia, Zimbabwe and Mozambique to the east, and South Africa and

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Swaziland to the south (Padulosi and Ng, 1997). Presumably, the crop has been first introduced in India during the Neolithic period because India is reported to be the secondary center of genetic diversity of wild cowpea (Pant, Chandel, & Joshi, 1982). Many primitive traits such as hairiness, the small seed size, perennially, outbreeding hard seeds and pod shattering are going extinct because of domestication (Magloire, 2005).

2.2 Importance and uses

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the most important crops in the world. Cowpea is a quick-growing, warm-season and very nourishing legume in Sub-Saharan Africa (Timko *et al.*, 2007; Timko & Singh, 2008). All parts of the plant, such as the fresh or dried seeds, leaves, matured and immature pods, are consumed by humans and animals (Timko *et al.*, 2007).

Cowpea is a food and animal feed crop grown in most parts of the world, especially in Africa. Cowpea is cultivated in the semi-arid tropics of Africa, Asia, North and South America as grain, vegetable and fodder crop. The name cowpea is believed to have been derived when it was very substantial in livestock feed in the United States of America (Small, 2009). It has been an important food source and favourite crop to date because of its extensive adaptation and tolerance to numerous stress. It is one of the major sources of protein for over 200 million people in Sub-Saharan Africa and amongst China's top ten fresh vegetables (Singh Ajeigbe, Tarawali, Fernandez & Abubakar, 2003). Cowpea is adaptable to different soils and intercropping systems (Mortimore, Singh, Harris, & Blade, 1997). It can improve the soil by fixing atmospheric nitrogen through symbiotic interaction with soil rhizobia

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(Saliou-Sarr, Fujimoto & Yamakawa, 2015), prevent soil erosion and its drought-tolerant trait makes it an economically important crop in many agricultural regions. The low level of the glycaemic index, high protein and fibre of cowpea makes it highly nutritious and potentially beneficial to health (Xu and Chang, 2012; Aguilera *et al.*, 2013; Xiong, Yao, & Li, 2013). Cowpea is reported to be one of the quality of protein crops for human consumption (MOFA, 2005). The seed contains protein, carbohydrate, fat, fibre and minerals, including calcium, phosphorus, selenium, vitamin and iron, making it an excellent food even if in small quantity (NARP, 1993).

Cowpea grain is rich in protein (23 - 32%) and a significant amount of vitamins (folic acid and vitamin B) and minerals essential for preventing congenital disabilities during pregnancy (Nielson, Brandt & Singh, 1993; Hall *et al.*, 2003). Cowpea contains fibre and fat in amounts required for preventing heart disease (Phillips *et al.*, 2003). Cowpea protein is rich in essential amino acids tryptophan, leucine, arginine and lysine and can largely fulfill the essential amino acid requirements in the human diet (FAO, 2004; MOFA, 2005). Cowpea seed is valued as an expensive nutritional supplement to cereals and an extender of animal proteins (MOFA, 2005; Alayande, Mustapha, Dabak & Ubom, 2012), and due to the high protein content, the crop is essential in alleviating problems of protein deficiency and malnutrition. Cowpea leaves are reported to contain a good trace of ash, fats, proteins and fibre, for which reason, young leaves of cowpea are important food sources for most people in East Africa and are cooked as a potherb, like spinach (Gerrano *et al.*, 2019). Immature pods are used as snap-beans and are usually eaten with other foods. Cowpea is used to prepare a wide range of dishes and snacks such

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as Akara (Nigeria), Moin-Moin (Nigeria), Waakye (Ghana), Danwake, Adayi, Akidi-na Oka, cowpea bread, cowpea cake (Africa and USA) Gbegiri, Baiao de dois (Brazil) (Asif, Rooney, Ali, R., & Riaz, 2013).

Additionally, cowpea is known to be sacred among the Hausa and Yoruba tribes in Nigeria and are used for sacrifices to abate evil and pacify the spirits of sickly children (Ige, Olotuah and Akerele, 2011; Carvalho, Lino-Neto, Rosa, & Carnide, 2017). In some parts of Africa, the Edos and Hausas use cowpea medicinally; one or two seeds are ground and mixed with soil or oil to treat persistent bowel and stomach diseases (Nkouannessi, 2005).

Another importance of the cowpea crop is the creation of jobs through production, processing and sales. Nagai (2008) identified typical markets opened in Benin, Togo, Burkina Faso and Ghana for cowpea trade. He reported jobs were created for commission agents who mediate cowpea surplus selling from rural assemblers to small and large wholesalers. Retailers also buy from wholesalers or commission agents or sometimes at harvest time directly from farmers and sell to consumers.

2.3 Production in Ghana

Cowpea is the most important legume in Ghana besides groundnut, in terms of quantity produced, the area under cultivation and quantity consumed annually (Langyintuo *et al.*, 2003). The area under cowpea cultivation in Ghana as of 2010 was at 163,700 ha (MOFA, SRID, 2011). However, as of 2016, the region under cowpea cultivation in Ghana has reduced to around 147,000 ha. The annual yield has also reduced from 219,300 MT in 2010 to 206,000 MT in 2016 (MOFA, SRID, 2016). This reduction in production may be due to various factors ranging from cultivation to post-harvest constraints,

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which can be biotic and abiotic factors. MOFA (2016) predicted a decline in production by about 11.1% from 2015 to 2020 since production is concentrated in Ghana's Guinea Savanna zone. Cowpea consumption is higher than it is produced in Ghana. A report in 2010 shows that Ghana imports about 3380MT of cowpea to supplement the country's demand; 30% from Burkina Faso and the rest from Niger (Egbadzor, Yeboah, Offei, Ofori, & Danquah, 2013). The cause of low production in Ghana is multi-faceted as production is concentrated in the Upper West, Upper East, Savanna and Northern regions with many farming constraints (MoFA-SRID, 2016). Another major factor affecting the production and consumption of cowpea in Ghana could be varietal preference (Langyintuo *et al.*, 2003). Ghanaians prefer medium to large cream or white seeded cowpea (Quaye, Adofo, Madode & Abdul-Razak, 2009). The most common varieties cultivated are the local types, namely "Kirkhouse Benga", "Wangkae", "Mallam Yaya", "Pangaabu", "Alancash", "Yekoyenim", "Uganda", "Yaminu", "Burkina" and "Adamu akese" (Karim, 2016; Gulbi, 2019).

2.4 Challenges to production

Agriculture is at a crossroads due to climate change, population pressure and environmental degradation. Even though cowpea is one of the world's important legume crops, it suffers biotic and abiotic stresses. Diseases and insect pests are major cowpea production constraints (Rusoke & Rubaihayo, 1994; Omongo, Adipala, Ogenga-Latigo, & Kyamanywa, 1998; Singh *et al.*, 2003). During the past 40 years, the West African National Agricultural Research Systems (NARS) and the International Institute of Tropical Agriculture (IITA) have aligned with American and some African

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universities to study major biotic constraints and develop sustainable solutions to address them. Despite the billions of dollars spent on research, Africa's average cowpea yield is still far below the potential yield (Akibode & Maredia, 2012). The majority of farmers in Africa cultivate cowpea without insect pest protection (IPM) measures, leading to severe yield reduction due to insect pests damage (Singh, 2006; Timko *et al.*, 2007). In Ghana, farmers do not have access to high-yielding varieties for cultivation and lack appropriate technologies for commercial cultivation of the crop (Yirzagla *et al.,* 2016).

2.4.1 Abiotic Constraints

Abiotic constraints affecting cowpea production, especially in Africa, include soil complications (such as low fertility, low and high pH, soil salinity), quality of seeds, poor plant protection, seed access and distribution of improved varieties, access to inputs, excessive rainfall water stress), drought and heat stress. Even though cowpea is inherently more tolerant to drought stress than some other food crops, it still suffers considerable damage due to frequent drought in regions where rainfall periods are short and irregular (Ram *et al.*, 2005). Wittig, König, Schmidt and Szarzynski (2007) reported that, in Sudan and Sahelian semi-arid regions, the intensity and frequency of drought have increased over the past 30 years. This has resulted in morphological, physiological and metabolic changes in the crop leading to poor yield. Seed production, which is positively correlated with leaf area (Rawson & Turner, 1982), is reduced by drought-induced stress. Drought is estimated to cause up to 21-30% yield loss (FAO, 2009). In Ghana, cowpea production is hampered by recurrent drought, especially in the Northern regions that are the center of production (Callo-Concha, Gaiser, Ewert 2012

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and Batieno, 2014). However, early maturing cowpeas tend to be very sensitive to the drought that occurs during the early stages of the reproductive phase (Thiaw & Parker, 1993). Early maturity in cowpea cultivars is desirable and has proven to be useful in some dry environments because of the ability to escape terminal drought (Singh, 1994),

2.4.2 Biotic constraints

Cowpea is one of the major legume crops that have been plagued and damaged by insect pest, fungal diseases, viral diseases, bacterial diseases and parasitic weeds (Muleba, Ouedraogo & Drobo, 1996; Mortimore, Singh, Harris and Blade, 1997). Dabiré *et al.* (2012) reported that these constraints could cause up to 200 kg ha^{-1} loss of grain yield under traditional farming conditions in many West African countries.

Insect pest is one of the major constraints to cowpea production. A wide range of insects decimates the crop at all growth stages (Jackai & Raulston, 1988). These insects include legume bud thrips (*Megalurothrips sjostedti)*, bean fly, whitefly, aphids (*Aphis craccivora)*, flower bud thrips and complex of pod sucking bugs (*Clavigralla tomentosicollis)*, and they cause low yields in cowpea production, especially in Africa (Olatunde, Biobaku, Ojo, Pitan & Adegbite, 2007).

In Africa, several bees have been observed on cowpea flowers, causing flowers to fall and affecting yield (Pasquet *et al.*, 2008; Ige, Olotuah and Akerele, 2011). Earlier research showed that insect attack on cowpea is different in different agro-ecological zones (Dabiré & Suh, 1988). Ba *et al.* (2009) reported that legume borers are present in Sudan-Sahelian and Sahelian zones during the rainy season. Legume pod borers migrate from the South-

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Sudanian zones to the rest of the country. Drought spells escalate the outbreak of aphids on cowpea farms. Thrips (*M. sjostedti*) are also known to be found in areas where legume borers are found. Tamo, Baumgärtner, Delucchi & Herren, 1993) reported that most host plants for *M. vitrata* that might be competing with *M. sjostedti* in for scarce resources are noticed in cowpea production. However, some mature pods have chemicals like trypsin and cynogenics in them that inhibit the development of some insects, such as *C. tomentosicollis* at their developmental stage (Dabiré *et al.,* 2012).

During storage, one major constraint fronting cowpea farmers are insect infestation. The major insect pest causing losses to stored cowpea in West Africa is the cowpea weevil (*Callosobruchus maculatus*). Hermetic storage is now known to be the best method for preserving grain (Murdock, Margram, Baoua, Balfe, & Shade, 2012).

Cowpea is infected by about 140 viruses worldwide (Hughes & Shoyinka, 2003), of which only nine had been reported to occur in Africa (Taiwo, 2003). Losses due to viral infections are estimated to be between 10 and 100% (Singh & Rachie, 1985) and the complete loss of irrigated cowpeas in northern Nigeria had been attributed to virus infection (Rossel, 1977). In West Africa, cowpea is threatened by parasitic weeds such as *Striga gesnerioides* and *Alectra sp*., which can cause up to 100 % yield loss (Asare *et al.*, 2010).

2.5 Morphological Characterization

The diversity of cowpea can be assessed by characterizing their morphological features, thus measuring the variation in the plant's phenotypic traits. These inherited traits may be quantitative (such as plant height, yield

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potential and stress tolerance, disease resistance, number of branches, number of peduncles, protein content and seed size) and qualitative (flower colour, growth habit and seed coat colour) (Rao & Singh, 2004). Characterization is aimed to select a trait of direct interest to users. Plant specific descriptors are used for morphological characterization. In cowpea, the descriptor list by the International Board for Plant Genetic Resources (IBPGR), now Biodiversity International (IBPGR, 1983; Johnson & Hodgkin, 1999) is used in germplasm characterization and preliminary evaluation. Morphological characterization of germplasm is very important in establishing each germplasm's descriptive features and aids in identifying identical, duplicate, or closely related germplasms (phylogenetic studies), detect unique traits and the population structure for breeding and conservation purpose (Smýkal *et al.*, 2015). The environment may influence morphological characteristics. Therefore, their variations must be confirmed by either biochemical or molecular methods to provide adequate information for comparison, identification and selection of genotypes (Huamán, 1999).

2.6 Molecular Characterization

Advances in molecular biology have provided the needed tools to detect genetic variations among progenies in a population easily. This has highly facilitated the analysis of plant genome structure and their evolution, including relationships among the Legumioseae (Gepts *et al.*, 2005). This, in turn, has contributed significantly to our current understanding of the cowpea genome organization and evolution.

Molecular techniques such as Marker Assisted Selection (MAS) is dependent on the identification of DNA sequences near or within genes

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controlling a trait (s) of interest that are not easily observed phenotypically (Ibitoye & Akin-Idowu, 2010). MAS allows a more efficient way of identifying alleles of interest in an improved cultivar, thereby increasing the overall efficiency and effectiveness of crop improvement programs (Charcosset & Moreau, 2004; Moreau, Lemarie, Charcosset, & Gallais, 2000; Boopathi, 2020). A marker may be monomorphic and invariable in all organisms but when a marker shows differences in molecular weight, enzyme activity, structure or restriction site, it is polymorphic and can be used as a basis for characterization (Semagn *et al.,* 2006).

2.7 *Striga* gesnerioides

2.7.1 Taxonomy and Description

Striga gesnerioides is a major problem to cowpea production by farmers in West Africa (Ghana, Mali, Burkina Faso, Niger, Nigeria, Senegal, Togo and Benin) (Timko *et al.*, 2007). *Striga gesnerioides* is an angiospermic, hemiparasite belonging to the family Orobonchaceae (formerly Scrophulariaceae). However, Vatke used the earlier specific name, *gesnerioides*, to combine with the genus name *Striga* to form the world wide name *Striga gesnerioides*. Among all *Striga* species, *Striga gesnerioides* differ significantly in being parasitic, without expanded leaves, and with a pale-green or yellowish colour. In vigorous plants, as cowpea, the stems branch mainly below the soil and emerge as a cluster of generally unbranched, fleshy, erect shoots 10-20 cm high, with scale leaves only a few millimetres long (Riches & Parker, 1995) (Figure. 2.1). On other hosts, shoots may be single. Much of the shoot comprises the spike-like inflorescence. Flowers, generally in opposite pairs, subtended by bracts 4-6 mm long, are sessile with a tubular

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calyx, 4-6 mm long with five ribs and corolla 5-15 mm long with corolla lobes expanding to about 5 mm across. Flower colour in *S. gesnerioides* forms that attack cowpea is usually mauve (Figure 2.1) but occasionally white, whereas it may be reddish, purple or even yellow (Musselman, & Parker, 1981). Up to 5 mm long, the capsule develops several hundred-minute seeds about 0.25 mm long, not readily distinguishable from *S. asiastica* (Musselman, & Parker (1981). Seed production per plant was estimated to be over 60,000 (Hartman & Tanimonure, 1991). *S. gesnerioides* also differs from most other *Striga* species in developing a substantial haustorium at least several millimetres across, about 1 cm on tobacco and often up to 3-4 cm in diameter on cowpea. The root system is rudimentary and has a chromosome number (2n) = 40.

Figure 2. 1: Vigorous growing unbranched erect shoot of *Striga gesnerioides* plants parasitizing cowpea. (Dugje, Omoigui, Ekeleme, Kamara & Ajeigbe, 2009).

2.7.2 Races of *Striga* gesnerioides

Striga gesnerioides has been categorized on the bases of its genetic relatedness and capability to differentially parasitize varieties of cowpea as well as landraces (Parker & Polniaszek, 1990; Lane et al., 1996; Botanga &

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Timko 2007). Parker & Polniaszek (1990) revealed that three races of the parasite weed were initially recognized on the bases of their differential responses of two *Striga*-susceptible and three *Striga*-resistant cowpea lines to the parasite. A fourth race was reported shortly after that (Lane, Moore, Child & Cardwell, 1996). Lane *et al.* (1996) examined 48 *Striga* populations collected from 1984 to 1993 from seven countries in West Africa and propose the presence of at least five races (SG1 to SG5) (Table 4) of the parasite. This was based upon the differential responses of one *Striga* susceptible and three *Striga*-resistant cowpea lines to parasitism. Races SG1 and SG5 are reported to be the most widespread, while SG4 is only detected in Zakpota region in Benin (Lane *et al.,* 1996). Botanga and Timko (2006) reassessed *S. gesnerioides* race structure among 24 populations by combining the results of differential parasitism of six resistant and three susceptible cowpea lines with molecular analysis of *Striga* genetic diversity and concluded that intra- and interpopulation variability was low in *S. gesnerioides.* Researchers had proposed SG4 for race of *Striga* in the greater part of Benin and SG4z for the race from Zakpota (Table 2.1 and Figure 2.2). This designation is based on the nearly identical genotypes of populations within and outside of Zakpota region and the ability of these populations to parasitize unique hosts differentially. In their analysis, Botanga and Timko (2006) did not account for the fact that the newly defined SG4 population had parasitized the same cowpea varieties as the SG1 race previously defined by Lane *et al.* (1996). They also suggested that *Striga* populations from Senegal be designated SG6 based primarily on genotypic characteristics and geographic location. However, the differential parasitism of variably resistant cowpea lines indicated SG6 corresponded to

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the SG1 designation by Lane *et al.* (1996). This could stem from the fact that differential parasitism of cowpea lines was only tested for *Striga* populations collected from Senegal by Botanga and Timko (2006). It is unknown how the other genotyped *Striga* population host compatibilities correspond to races designated by Lane *et al.* (1996).

Essem, Ohlson, Asare and Timko, (2019), reported that the phenotypic segregation in cowpea analysis suggests that the SG3 resistance gene and the *Striga* race's resistance gene found in Ghana (SG-GH) could be different. However, the low recombination frequency (4.2 %) between these two resistant genes demonstrates tight linkage, indicating these genes can be pyramided with relative ease. On the other hand, SG-GH has been reported to be the same as the SG5, found in Burkina Faso, Cameroon, Nigeria and Togo (Table 2.1, Figure 2.2; Ohlson and Timko, 2020)

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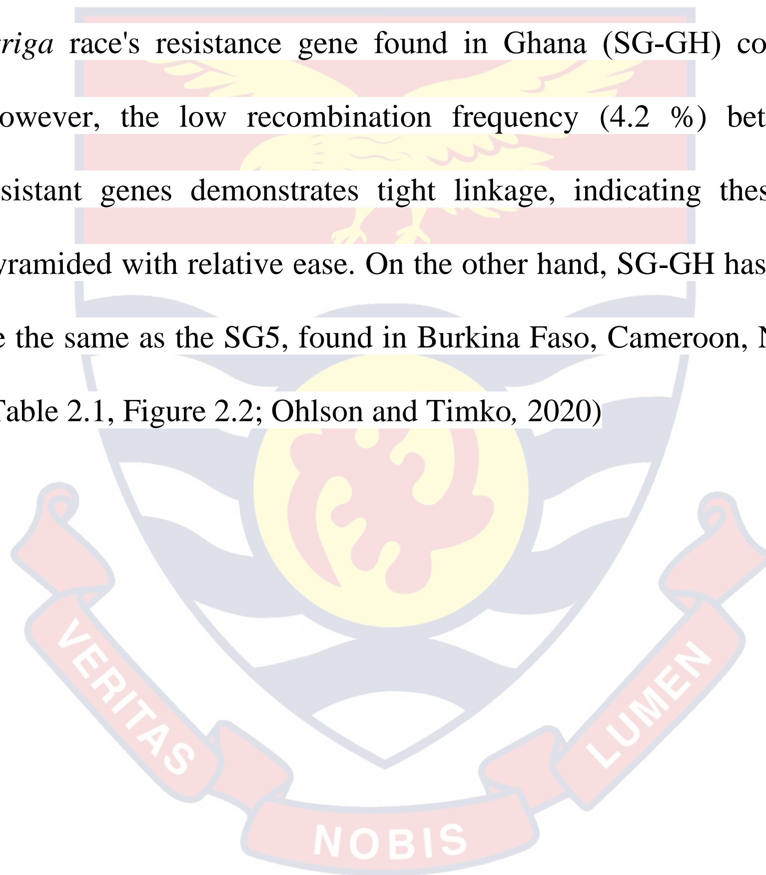
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Table 2. 1: Trend of race designation for *Striga* gesnerioides in West Africa based on differential host parasitism

Timko and Ohlson (2019)		Botanga and Timko (2006)		Lane *et al.* (1996)	
Race	Country	Race	Country	Race	Country
SG1	BJ, BF, CM, GH, NG, TG	SG1	BJ, BF, ML,NG,TG	SG1/SG4/SG6	BF, BJ, SN
SG2	ML, SN, TG	SG2	ML	SG2	ML
SG3	CM, NE, NG, TG	SG3	NE, NG	SG3	NE, NG
SG4	BJ	SG4	BJ	SG4z	BJ
SG5	BF, CM, GH, NG, TG	SG5	BJ, BF, CM, NG	SG5	CM
SG6	NG				

Abbreviations: BF- Burkina Faso; BJ- Benin; CM- Cameroon; GH-Ghana; ML- Mali; NE- Niger; NG- Nigeria; SN- Senegal; TG- Togo

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Figure 2.2: Striga gesnerioides sampling locations and race distribution across West Africa (Ohlson & Timko, 2020)

2.7.3 Effect of *S. gesnerioides*

Striga gesnerioides is an obligate root-parasitic flowering plant that attacks cowpea, tobacco and other legumes. This parasite has been devastating across many West Africa countries, causing significant loss of yield and spreading and intensifying in some areas. In a survey of cowpea farmers in the Kano district, northern Nigeria, not less than 25 % of farmers recounted serious infestation of *S. gesnerioides* (Bottenburg, 1995). However, Emechebe, Singh, Leleji, Atokple and Adu,. (1991), also revealed that many farmers' lands across the northern part of Nigeria had been completely destroyed. In a field trial to evaluate the yield loss in cowpea, a number of varieties exhibited yield that were 56 % lower in the most susceptible lines parasitized by *S. gesnerioides* (Aggarwal & Ouedraogo, 1989). This parasitic

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weed represents a critical danger to cowpea production, especially in Northern Ghana (Larweh *et al.*, 2017). Cowpea yield is reduced because of *S. gesnerioides* infestation and this could be up to 70% dependent upon the degree of harm and level of infestation (Aggarwal & Ouedraogo, 1989; Alonge Lagoke & Ajakaiye, 2005). It has been reported that susceptible cultivars could record yield losses of 100% when *S. gesnerioides* population was more than ten plants for each host plant (Kamara Chioke, Ekeleme, Omoigui & Dugie, 2008). Notably, yield reduction brought about by *S. gesnerioides* in dry Savanna of Sub-Saharan Africa has been evaluated in millions of tons every year and the spread of the parasite is relentlessly expanding. The harm caused by *S. gesnerioides* occurs at different parts of cowpea plants, influencing the physiological and developmental processes in the crop (Alonge *et al.*, 2005). *Striga* infestation of cowpea causes a decrease in leaf area, plant necrosis and chlorosis, inadequate blooming and podding, and reduce seed advancement (Alonge *et al.*, 2005). Such harm is frequently escalated by transpiration of the parasite when dry spell predominates. Once *S. gesnerioides* invades a field, the underground seed stock will build up (Singh, 2006; Dugje *et al.*, 2009), which sets up a situation of potential yield loss in the future (Cardwell & Lane, 1995).

2.7.4 Control measures

Eradication of *Striga* has been difficult because of its unique environmental adaptation, and complexity of the host-parasite relationship. Cultural, biological and chemical methods used in controlling *Striga* were explored. Successful control depends on eliminating the soil seed bank of the *Striga* species. In the 1980s, scientists began working on *Striga* control

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strategies appropriate for small-holder farmers, with efforts focused mainly on breeding for resistance (Oswald, 2005).

Cultural control

Small scale farmers have been hand-pulling *Striga* on the field (Doggett, 1965; Leandre, 2018). Uprooting the *Striga* by hand every 10 days to 2 weeks has been recommended to control its damage (Doggett, 1965; Leandre, 2018). It can be a burdensome task and its effect is short-lived. If the *Striga* is already flowering and fruiting, viable seeds may well be broadcast by the uprooted plant. Hand pulling may be valuable where *Striga* plants in the crop field are few. It is quite a futile exercise in a heavily infested field. Again the use of farmyard manure to boast the grain yield has long been known to be effective against *Striga* infected fields. In addition, crop rotation has been known to be effective in a long term practice but is rarely practicable and there has been little research on the potential for trap crops, although pigeon pea, velvet bean (Mucuna species), sorghum and soybean have been suggested (Igbinnosa & Okonkwo, 1991; Berner & Williams, 1998).

Chemical control

The potential of herbicide seed treatments for parasitic weed control was first demonstrated by Berner, Awad and Aigbokhan, (1994) using imazaquin on cowpea parasitized by cowpea (*S. gesnerioides* (Willd.) Vatke). Subsequently, seed treatments have been shown to improve control of crenate broomrape (*Orobanche crenata Forsk.*) on broad bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) using other imidazolinone herbicides (Jurado-Expósito, Castejón-Muñoz, & García-Torres, 1996). Even though some herbicides have shown moderate promise for conventional pre-emergence application, farmers

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affected by *S. gesnerioides* are not generally in a position to use these and there have been no field trials (Riches, & Parker, 1995). A suggested alternative is by introducing synthetic analogs of strigol applied to the germination stimulant isolated from cotton (*Gossypium hirsutum*) into *Striga* infested soil to stimulate the parasite's seed suicidal germination (Cook *et al.,* 1966; Zwanenburg, Mwakaboko, Reizelman, Anilkumar & Sethumadhavan, 2009). However, these analogs' instability in soil precludes their usefulness (Babiker, Hamudoun, Rudwan, Mansi & Faki, 1987).

Biological control

S. gesnerioides is often very heavily affected by *Smicronyx* gall-forming weevils (Compendium, 2020). Though unexploited, it has been noted that this natural control can be adversely affected by insecticide use. There have been reports on the use of *Sclerotium rolfsii* as biological control of *S. gesnerioides* affecting tobacco (Oswald, 2005). However, this could not be feasible because *S. roffsii* is a pest to many crops and can attack the next crops and cause substantial yield losses. The only better effort towards biological control has been the testing of the ethylene-generating bacterium *Pseudomonas syringae*, which might be useable as soil amendments as a means of inducing suicidal germination of *Striga* seed (Berner, Schaad, & Völksch, 1999).

Host plant resistance

Currently, no cultural, biological and chemical method is economically feasible to control *Striga* very effectively (Aly, 2007), although research efforts have demonstrated that real progress can reduce the devastating effects of *Striga* (Aly, 2007). The main *Striga* control measure available is host plant

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resistance. The first cowpea reported to be resistant to *S. gesnerioides* were cultivars Suvita-2 (formerly Gorom Local) and 58-57 from Burkina Faso (Aggarwal, Muleba, Drabo, Souma & Mbewe, 1984). However, both cultivars showed susceptibility to other *S. gesnerioides* in other countries, igniting the idea of the presence of different biotypes or races of the parasite (Aggarwal *et al.*, 1984). The most important known sources of resistance are the landraces B301 (Riches & Parker 1995) and GH3684 (Asare *et al.*, 2013; Essem *et al.*, 2017). The landrace B301 is originally selected for its high-level resistance to *A. vogelii* in West Africa (based on two dominant genes) as well as to *S. gesnerioides* (based on a single dominant gene) (Atokple *et al.,* 1995; Li, Lis & Timko, 2009; Boukar *et al.*, 2019; Essem, Ohlson, Asare, Timko, 2019). B301 shows resistance to all biotypes of *Striga gesnerioides* in West Africa except that occurring locally in southern Benin (SG4) (Lane *et al.* 1996; Botanga & Timko, 2006; Li & Timko, 2009). The landrace GH3684 from Ghana has been tested and found to be resistant against most races of *Striga gesnerioides* in West Africa, but the genetic bases of resistance of GH3684 are still under study (Essem, 2017). The challenge to breeding programmes is that there are limited *Striga*-resistant sources in cowpea germplasm to mitigate the six known *S. gesnerioides* biotypes, varying in their virulence on different 'resistant' varieties of cowpea (Timko & Ohlson, 2019).

2.7.5 Mechanisms involved in the resistance to *Striga gesnerioides*

The life cycle of *Striga* before it emerges above the soil comprises germination, haustorial induction, attachments to the host root and the penetration of the host vascular cells. All these stages are critical for the successful development of *Striga* (Botanga & Timko, 2006). The study of

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Striga growth '*'in-vitro''* using parasitized hosts could shed more light on the underlying mechanisms of resistance to *S. gesnerioides* at different developmental stages.

Resistance at germination and attachment level

There is a very close interaction between the host and the parasitic weed. Germination of the *S. gesnerioides* seed is initiated by Strigolactones exuded by the host roots (Muller, Hauck & Schildknecht, 1992). After germination, the rootlet extremity turns into a haustorium (Okonkwo & Nwoke, 1978), which attaches itself to the host root and penetrates its vascular tissue. Mechanism of resistance to *S. gesnerioides* has been studied and in all cases, results show that there are at least two mechanisms of resistance. Yet, neither reduces parasite germination nor fails haustorial formation at the potential host (Lane *et al.*, 1991; Botanga & Timko, 2005): Parasite seeds germinate as usual and the radicles attach to the roots, but the resistant roots do not permit haustorium development.

Rapid necrosis of the host cells around the point of infection causes the death of the parasite in 3 to 4 days. In a report by Lane et al. (1991) involving genotype 58-57, it was observed that there was first level of resistance which occured as a result of death of tissue(necrosis) before root cortex fixation by the parasite. Fixation starts with haustorium formation and the growth of the tubercle tip. Botanga and Timko (2006), confirmed that the *S. gesnerioides* tubercle growth could be stopped for weeks with no connection to the host vascular system. Hood, Condon, Timko and Riopel (1998) considered such a resistance mechanism as supported host reaction, which was generally expressed at the root cortex level. Such effects were termed as hypersensitive

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responses shown in plant-pathogen interaction, which indicates vertical resistance and therefore, single genes might be involved.

Resistance at the penetration of the host vascular

It has been shown that a cellulose-rich wall layer accumulation in the host roots following contact with the invading parasite cells can be a form of resistance to *Striga* (Maiti *et al.*, 1984). In cowpea, a similar resistance mechanism was observed with resistant cowpea genotype B301; the *Striga* seed germinated, formed haustorium, but developed no *Striga* stems (Lane *et al.*, 1991). Benin's SG4 developed haustoria and stems, but these did not develop further (Lane *et al.*, 1993). This type of mechanism is similar to antibiosis, resulting in an incompatibility between cowpea and *Striga* (Hood *et al.*, 1998). Hood *et al.* (1998) suggested that such a mechanism of resistance is durable in that the resistance involved is due to the lack of chemical signals or nutrients produced by the host, as a prerequisite to further development of *Striga*. In another form of resistance, host tissues alter their structure in response to the infestation (Olivier *et al.,* 1991). However, in susceptible genotypes, such a response is very slow to stop the penetration (Olivier *et al.*, 1991). The use of *Striga* resistant or tolerant varieties is the most feasible and sustainable approach for mining the losses caused by this parasitic weed (De Vries, 2000; Badu-Appraku Menkir & Lum 2005). According to Parker (1991), resistant varieties are probably the most appropriate way for subsistence farmers to control *S. gesnerioides*.

2.7.6 Breeding for resistance to *Striga gesnerioides*

Achieving successful crosses is a prerequisite to any genetic study. Crossing cowpea is relatively easy compared to other grain legumes, but its

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success rate is 10-20 % under natural conditions (Singh, Ehlers, Sharma, & Freire Filho, 2002). Usually, a successful cross produces a pod with 8-12 seeds. Singh *et al.*, 2002, reported that synchronizing flowering under cool temperatures (early in the morning) and high humidity may increase the success of hand crossing to 50 %. In cowpea flowers, anthesis takes place just before the opening of the corolla. Hence, flower buds destined to open the following morning are ready for emasculation (Myers, 1996). These buds have now reached their maximum unopened size and have started to pale slightly from their original deep rich green color in earlier development. Cool nights provide better conditions for fertilization than the hotter daytimes. The emasculated flower should be pollinated immediately after emasculation or pollinated the following morning (Myers, 1996).

The advantages of using genetic markers and the potential value of genetic marker linkage maps and direct selection in plant breeding were first reported around 1996 (Crouch & Ortiz, 2004). DNA marker technology has dramatically enhanced the efficiency of plant breeding and genetic engineering (Joshi *et al.*, 2011). Genetic enhancement of cowpea has taken place within national research facilities and universities in a couple of West African countries, India, Brazil, USA and International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Timko *et al.*, 2007). The imbricate dispersion of *Striga* races has essential outcomes for breeding resistant cowpea. While most cowpea plants are prone to *Striga* parasitism, some native landraces and wild accessions are resistant to the parasite, and in many reports, resistance is a dominant characteristic, acquired in a monogenic way (Touré,

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Olivier, Ntare, Lane & St-Pierre, 1997; Ouédraogo *et al.*, 2001; Ouédraogo, Tignegre, Timko & Belzile, 2002; Timko *et al.*, 2007).

Breeding for *Striga*-resistant cowpea involves characterization of germplasm for *Striga* resistance, improvement of available sources of resistance for better agronomic characteristics, the transfer of resistance genes into farmer adapted selected cultivars, and pyramiding of resistance genes into elite adapted cultivars (Haussmann, Hess, Welz & Geiger, 2000).The development of molecular markers could ease marker-assisted selection (Boukar, Kong, Singh, Murdock & Ohm, 2004; Haussmann *et al.*, 2000; Ouédraogo *et al.*, 2001; Ouédraogo *et al.*, 2002b). In addition, multi-location experiments could test the identification of stable resistance across different environments (Muleba *et al.*, 1996; Haussmann *et al.*, 2000; Braun, Altin & Payne 2010; Rubiales *et al.*, 2012).

2.7.7 Techniques for *Striga* resistance screening

Various methodshave been employed for identifying resistance sources of *S. gesnerioides* in cowpea (Lane & Bailey, 1992; Muleba *et al.*, 1996; Ouedraogo *et al.*, 2002; Boukar *et al.*, 2004). These techniques comprised; (a) field and pot screenings, which involve exposing the crops to *Striga* infested fields and in pot culture, respectively, (b) the molecular screening technique, involving the use of DNA markers associated with the resistance to *S. gesnerioides* in cowpea. (Tignegre, 2010; Gulbi, 2019). (c) "*in-vitro*" screening techniques, which facilitated the study of *Striga* resistance mechanisms (Lane *et al.*, 1991)

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Field and pot-screening techniques

An effective pot-screening method for *Striga* resistance is available. The use of pot screening techniques is to simulate field conditions. Pot screening is intended to ensure that *Striga* seeds are evenly introduced to the soil, which is difficult to achieve under field conditions. There is a screening method for *Striga* that is relatively effective. It is reported that 1000 *Striga* seeds per pot (8 – 10 litre) is effective for *S. gesnerioides* screening (Musselman and Ayensu, 1984). However, before employing pot screening, pots and soils are sterilized at 150°C using an autoclave (Tignegre, 2010)

Molecular screening techniques

Marker Assisted Selection (MAS) has become an important tool in plant breeding, and it has increased transfer efficiency of genomic regions and the recovery of the recurrent parent genome (Ibitoye & Akin-Idowu 2010). Therefore, MAS's application for efficient selection systems would fast-track the breeding efforts to introgress *Striga*-resistance gene (s) into locally adapted cowpea cultivars targeted for *Striga* prone areas. A number of crop varieties or breeding lines, such as soybean (Khanh, Anh, Buu & Xuan, 2013), sorghum (Gamar and Mohammend, 2013) and cowpea (Ouedraogo *et al.*, 2002) have been developed using the MAS approach, which shortened the breeding cycle considerably.

Molecular markers that are linked to *Striga* resistance exist across the cowpea genome. Such markers are applicable and efficient for marker-assisted selection (MAS) to fast-track cowpea development with resistance to *S. gesnerioides.* Molecular markers associated with *Striga* resistance genes in cowpea have previously been reported for SG1, SG3 and SG5 (Boukar *et al.*,

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2004; Timko *et al.*, 2007). Asare *et al.* (2013) previously reported that the molecular markers 'SSR-1' and 'C42-2B' developed for *S. gesnerioides* races SG3 and SG5, respectively, were linked with resistance to *Striga* in Ghanaian germplasm (SG-GH). Essem, 2017, reported that the SSR markers: LRR9, LRR11 and CLM1320, could help identify *Striga*-resistant cowpeas.

The SSR-1 and C42-2B markers were previously found to co-segregate with *S. gesnerioides* race SG3 resistance (Omoigui *et al.*, 2007; Li & Timko, 2009). As reported, SSR-1 co-localized with the SG3 resistance gene since the marker is embedded within the resistance gene itself (Li & Timko, 2009).

2.8 Genetic Diversity

Genetic diversity provides the basis of genetic variation and relationships among cowpea genotypes. This provides information for crop resource utilization, preservation and improvement (Kameswara, 2004; Tan *et al.*, 2012). Various studies have proved that some morphological traits such as pod per plants, seed per pod and seed size are mainly used as markers, which have a significant effect on the potential yield of cowpea (Mishra, Singh, Chand, & Meene, 2002; Carnide, Pocas, Martins, & Pinto-Carnide, 2007; Siise & Massawe, 2013). Morphological markers are highly dependent on the environment for expression. Several limitations reduce their ability to estimate genetic diversity in plants. However, molecular markers are considered as an effective tool for the efficient selection of desired agronomic traits. They are based on the plant genotype sufficient in numbers, not vulnerable to environmental influences (Franco *et al.*, 2001), and not influenced by developmental stages. Evaluation of genetic diversity, variation, and genetic

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distance in cowpea genotypes has been conducted in several studies according to morphological and physiological markers (Ntundu, Shillah, Marandu, & Christiansen, 2006; Ouedraogo, Thiombiano, Hahn-Hadjali, & Guinko, 2008; Siise and Massawe, 2013; Stoilova & Pereira, 2013), and molecular markers such as Amplified Fragment Length Polymorphism (AFLP) (Coulibaly, Pasquet, Papa, & Gepts, 2002; Tosti & Negri, 2002), Random Amplified Polymorphism DNA (RAPD; Nkongolo, 2003; Fall *et al.*, 2003), DNA amplification fingerprinting (Simon, Benko-Iseppon, Resende, Winter & Kahl, 2007) and simple sequence repeats (SSRs; Wang *et al.*, 2008; Ogunkanmi *et al.*, 2008; Xue *et al.*, 2010) or sequence-tagged microsatellite sites (Choumane, Winter, Weigand & Kahl, 2000; Li, Weinberg, Darden & Pedersen, 2001; Abe, Xu, Suziki, Kanazawa & Shimamoto, 2003). Among molecular markers providing useful tools for studying genetic variation and examining the relationship between and within genotypes, SSR markers have proven to be particularly useful since they are highly polymorphic, inherited co-dominantly and reproducible, as well as abundantly distributed throughout eukaryotic genomes (Kalia, Rai, Kalia, Singh, & Dhawan, 2011). SSRs have also been extensively used in genotype identification, seed purity evaluation and variety protection (Brown *et al.*, 1996; Senior, Murphy, Goodman & Stuber, 1998), pedigree analysis (Ayres *et al.*, 1997; Bowers *et al.*, 1999), and genetic mapping of simple and quantitative traits and MAS (Blair & McCouch, 1997; Chen, Temnykh, Xu, Cho & McCouch, 1997). Moreover, in some studies, a combination of different markers, including morphological and microsatellites (Kuruma, Kiplagat, Ateka, & Owuoche, 2008; Shehzad, Okuizumi, Kawase & Okunu, 2009; Siise and Massawe, 2013), SSR along

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with RAPD marker (Diouf & Hilu, 2005) EST-derived SSR markers (Chabane, Abdalla, Sayed, & Valkoun, 2007) and assessment of genetic diversity at DNA level have been studied (Reif *et al.*, 2003). The SSR markers are reproducible (Heckenberger, Van Der Voort, Peleman & Bohn, 2003) and reveal a high polymorphism level (Smith *et al.*, 1997). This allows the application of automated analysis systems (Mitchell, Kresovich, Jester, Hernandez, & Szewc-McFadden, 1997).

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CHAPTER THREE

ASSESSMENT OF PHENOTYPIC VARIATIONS AMONG COWPEA BREEDING LINES

3.1 Introduction

Cowpea (*Vigna unguiculata* L. Walp) is largely cultivated in about sixteen countries in Africa (Abate *et al.*, 2012). It provides income, food for people and their livestock and nourishment for the next crop. However, cowpea demand and consumption is higher than it is produced in Ghana. A report in 2010 showed that Ghana imported about 3380MT of cowpea to supplement the country's demand; 30% from Burkina Faso and the rest from Niger (Egbadzor *et al.*, 2013). The major factor affecting the production and consumption of cowpea in Ghana include varietal preference (Langyintuo *et al.*, 2003) biotic and abiotic factors, hence, the need to breed market-driven cowpeas and select for improved desirable traits through characterization. (Asseng *et al.*, 2020).

Phenotypic characterization involves recording heritable characters that can be seen easily by the eye and are expressed in all environments (Flamarique Cheng, Bergstrom & Reimchen, 2013). Many selection methods are available, but no one method is completely the best for general use with all crops. The efficacy of the selection procedure during successive generations is the most essential role of any breeding program. A standard descriptor format for cowpea characterization exists (IBPGR, 1983). Using this descriptor and following it closely will help identify and select the preferred traits of interest in a breeding programme. Many selection methods are available, but no method is completely the best for general use with all crops. The efficacy of

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the selection procedure during successive generations is the most essential role of any breeding program.

Over the years, cowpea programmes in West Africa have been developing disease and pest resistant cowpeas (Horn & Shimelis, 2020). However, most of these varieties of cowpea are medium seed types, but the market preference for cowpea in Ghana and for that matter West Africa is driven towards large, white, brown and cream cowpea (Langyintuo, 2003). A novel source of *Striga*-resistance local cowpea, GH3684, is said to have broad resistance to *Striga* races in West Africa (Asare *et al.,* 2013; Essem, 2017) and was incorporated into a breeding programme to develop four populations in the University of Cape Coast, Ghana. Therefore, it has become necessary to characterize and pre-select to advance breeding lines with potential preferable phenotypic traits. Selection of progenies during suitable generations by inbreeding increases homozygosity since the crop is self-pollinated. The Single Seed Descent (SSD) model approach used in generating the cowpea breeding populations involved a single seed from each plant, bulking the individual seeds, and planting out the next generation (Funada, Helms, Hammond, Hossain & Doetkott, 2013). This is a cost-saving and efficient method in breeding and advancing progenies (Haddad & Muehlbauer, 1981). Analysis of variance is a useful tool in separating observed variance data into different components for useful additional test such as Least Significant Difference and correlation. Multivariate analysis is significant in phenotypic studies which seeks to capture not only changes of individuals between different populations, but also utilize dependence structures between the individual groups within the population (Wold, Esbensen, & Geladi, 1987;

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Christensen, 1996; Cox & Solomon, 2002; Sharma *et al.*, 2019; Nadeem *et al.,* 2020).

Moreover, Cluster analysis decreases the number of individual variable units by arranging them into groups, which are translated into a dendrogram based on the coefficient of similarity (Tatineni, Cantrell, & Davis, 1996). It determines the relationships between genotypes and hierarchical mutually exclusive grouping such that similar descriptions are mathematically gathered into the same cluster (Ariyo, 2007). The objective of this study was to assess the phenotypic variations among F_4 progenies from a half-diallel crosses scheme of five cowpea parents to pre-select breeding lines with improved agronomic and yield traits.

3.1 Materials and Methods

3.1.1 Experimental Site

The experiment was conducted at the Teaching and Research Farm of the School of Agriculture, University of Cape Coast (5 0 10’N, 1.2 0 50’W), under the Coastal Savanna Agro-Ecological Zone of the Central Region, Ghana. The soil was an acrisol with sandy-loam texture Nitrogen 0.07 %, Phosphorous 56.64 ug/g, Organic carbon of 1.04%, pH of 6.51, Potassium(K) of 0.28 cmol/kg, Sodium (Na) of 0.44 cmol/kg and Calcium of 1.89 cmol/kg.

The site recorded an average rainfall of 111.85 mm, during the experimental period from June to October 2019 and mean monthly temperatures ranging between 24 °C and 24.1°C (Ghana metrological agency, June 2020).

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3.1.2 Experimental Material

Seeds of fifty (50) F_4 breeding lines of four cowpea populations developed by diallel cross-breeding and advanced by single seed descent methods were obtained from the Department of Molecular Biology and Biotechnology, University of Cape Coast, as indicated in Table 3.1.

Table 3.1: Sources and pedigree of F4 cowpea breeding lines

Genotype Name	Source	Seed Coat colour	Parents
UC15-01	UCC	White	
UC15-02	UCC	White	
UC15-03	UCC	White	
UC15-04	UCC	White	**GH3684**
UC15-05	UCC	White	**X**
UC15-06	UCC	Cream	**IT97K-499-35**
UC15-07	UCC	White	**(Population 1)**
UC15-09	UCC	White	
UC15-10	UCC	White	
UC15-11	UCC	Deep brown	
UC15-12	UCC	Speckle Purple	
UC15-13	UCC	White	
UC15-14	UCC	White	**GH3684**
UC15-15	UCC	White	**X**
UC15-16	UCC	White	**PADI-TUYA**
UC15-17	UCC	White	**(Population 2)**
UC15-18	UCC	Cream	
UC15-19	UCC	White	
UC15-20	UCC	White	
UC15-21	UCC	White	
UC15-22	UCC	Cream	
UC15-23	UCC	White	
UC15-24	UCC	White	**GH3684**
UC15-25	UCC	Brown	**X**
UC15-26	UCC	White	**SARC-1-57-1**
UC15-27	UCC	Red	**(Population 3)**
UC15-28	UCC	Cream	
UC15-29	UCC	White	
UC15-30	UCC	Brown	
UC15-31	UCC	White	

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Table 3.1 cont'd

UC15-32	UCC	Deep brown	**GH3684 X UCSO1 (Population 4)**
UC15-33	UCC	Cream	
UC15-34	UCC	Cream	
UC15-35	UCC	White	
UC15-36	UCC	Purple	
UC15-37	UCC	Cream	
UC15-38	UCC	Brown	
UC15-39	UCC	White	
UC15-40	UCC	White	
UC15-41	UCC	White	
UC15-42	UCC	Brown	
UC15-43	UCC	White	
UC15-44	UCC	Brown	
UC15-45	UCC	Purple	
UC15-46	UCC	Deep brown	
UC15-47	UCC	White	
UC15-48	UCC	Deep brown	
UC15-49	UCC	Brown	
UC15-50	UCC	Speckled brown	
UC15-51	UCC	Brown	
GH3684	UCC	Purple	**Local varieties and landraces (parents)**
SARC-1-57-1	UCC	White	
IT97K-499-35	IITA	White	
PADI-TUYA	UCC	White	
UCSO1	UCC	Cream	

3.1.3 Field Establishment and Data collection

The field experiment was carried out under a rain-fed condition from June – Ocober (2019). A total land area of 1108.8 m^2 was ploughed and harrowed. It was then divided into three blocks, spaced 1.5 m apart, and each block was further divided into 55 single-row plots at 1 m apart and intra-row plant spacing of 70 cm. The cowpea seedlings were thinned out to maintain one seedling per stand two weeks after sowing seeds. The field layout was based on the randomized complete block design in three replications comprising 50 cowpea breeding lines and 5 parental lines. All agronomic practices were carried out to maintain the crops. The cowpea field was

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weeded manually at weeks 3 and 6 using hoes and spayed with K- Optimal pesticide (Lambda-Cyhalothrin 15g/l + Acetamiprid 20g/l; EC) to prevent insect and pests attack at the manufacturer's recommended rate of 40 ml/15 L knapsack. Dry pods were harvested manually and threshed. Eleven qualitative and 15 quantitative data were scored on six randomly selected plants per plot at vegetative and reproductive stages and after harvesting based on the cowpea descriptors by IBPGR now Biodiversity International (1983). The various variables assessed are indicated in Table 3.2.

Table 3.2: Qualitative and quantitative parameters and methods of measurement

Parameter Qualitative	Method of measurement
Flower pigmentation	Visualization and scoring; 1- Non pigmented (white), 2 – only wing pigmented, 3 – pigmented at the margin, 4- Completely pigmented.
Growth pattern	Visualization and scoring; 1- determinate 2- indeterminate
Terminal leaflet shape	Visualization and scoring; 1- Globose, 2 – Sub-Globose, 3- Sub- Hastate, 4- Hastate
Growth habit	Visualization and scoring; 1- erect, 2- semi- erect, 3- semi-prostrate, 4- prostrate
Matured pod pigmentation	Visualization and scoring; 1= Pale tan 2= Dark tan 3- dark purple 4= Others
Twinning tendency	Visual estimation and scoring; 0 – None, 3 – Slight, 5 – Intermediate, 7 – Pronounced
Immature pod pigmentation	Visualization and scoring; 0 – None, 1- Pigmented tip, 2 - Pigmented sutures, 3 -Pigmented valves, green sutures, 4 - Splashes of pigment, 5 - Uniformly pigmented, 6 – Others.
Flower colour	Visualization and scoring 1= white 2= violet
Pod attachment to the peduncle	Visual estimation by scoring 1 = pendent, 2= 30-90° down from erect, 3= erect
Leaf colour	Visualization and scoring 1= Pale green 2= intermediate green 3= dark green
Seed coat colour	1 - White, 2 - Cream, 3 - Brown, 4 - Red, 5 - Purple, 6 – Black, 7- multi-coloured

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Table 3.2 cont'd

Quantitative	**Method of measurement**
Plant Height	Measured the height of the main stem from the base to the shoot tip with a meter rule, and the mean determined at 6 weeks after sowing seeds
Canopy diameter	Measured the broadest canopy diameter with a meter rule for each plant at 6 weeks after sowing seeds
Number of branches at Maturity	Counted the number of branches on the main stem.
Days to 1st flower	Number of days from planting to when flowering was observed in each experimental unit.
Days to 50 % flowering	Number of days from sowing of seeds to the date when 50 % of the plants flowered.
Days to 1st Maturity	Number of days from planting to when the first matured pod was observed in an experimental unit
Terminal leaflet area	Mean of widths (W) and lengths (L) of a fully expanded terminal leaflet (cm) of six randomly selected plants. (Formula; Area = LXW)
Days to **50**% Maturity	Number of days from planting to when 50 % of the plants had matured pods.
Number of peduncles	Counted the number of peduncles on each plant at maturity
100 Seed weight	Weight of 100 seeds measured with a weighing balance (Mettler Telodo, PG203)
Pod length	Mean of length of 10 randomly selected dried pods from six selected plant.
Number of pod per peduncle	Counted the number of pods on each peduncle and the number of peduncles. The mean was recorded for each plant
Number of locules	Counted of the number of locules of 10 randomly selected dried pods from six selected plants from each experimental unit.
Number of seeds per pod	Mean of number of seeds of 10 randomly selected dried pods from six selected plants.
Grain yield	Weight of total dried seeds using a weighing balance (Mettler Telodo, PG203)

3.1.4 Data Analysis

Descriptive statistical tests were employed to analyze the qualitative data using Microsoft Excel 2016. The quantitative data were analyzed using GenStat Discovery 12th Edition statistical package using general linear model analysis of variance (ANOVA) with subsequent mean separation using Tukey's LSD in a single-step multiple comparison procedure at 95% level of

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significance. Correlation coefficients were calculated as explained by Udensi & Ikpeme (2012). Power maker (version 3.25) (Liu & Muse, 2005) was used for cluster analysis involving 26 quantitative and qualitative data to generate a dendrogram using Unweighted Pair-Group Average Method with Arithmetic mean (UPGMA) to classify cowpea genotypes by their similarity based on Nei's Genetic Distances (Nei, Tajima & Tateno, 1983) and observed in Molecular Evolutionary Genetics Analysis 4 (MEGA 4). The principal component analysis was employed to assess the percentage contribution of quantitative and qualitative trait to variation among genotypes using R statistical software version 3.6.0.

3.2 Results

3.2.1 Qualitative trait characterization

Twinning tendency

The cowpea progenies varied in terms of their twinning tendency (Appendix A9). None of the progenies exhibited a pronounced twinning tendency. However, 64% (32 progenies) showed a slight twinning, 4% of the progenies showed intermediate twinning and 32% had no twinning.

Leaf Markings

Eight-six percent of the cowpea progenies showed no marking on leaves as well as PADI-TUYA, UCSO1 and SARC-1-57-1. Only fourteen percent of progenies exhibited marking on leaves which was also observed on the leaves of GH3684 and IT97K-499-35 parental genotypes (Appendix A6).

Growth Pattern

Among the cowpea breeding lines, 52 % showed determinate growth pattern and 48% showed indeterminate growth pattern (Appendix A4).

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Growth Habit

Results showed variation in growth habit among the cowpea progenies (Appendix A5). Fourteen percent (14%) of the progenies were erect, 50% were semi-erect, 14% were prostrate and 22% were semi- prostrate. All the parental genotypes except UCSO1 and IT97K-499-35 were semi-erect.

Flower Pigmentation

Flower pigmentation observed among the cowpea progenies were white (considered as non-pigmented) and violet (considered as pigmented) (Appendix A6). However, the distribution of pigmentation differed among cowpea progenies. UCSO1 and SARC-1-57-1 exhibited white floral pigmentation together with 34 % (17) of the progenies were completely white (Appendix A6). PADI-TUYA and 22 % (11) of the progenies showed only wing pigmentation (Figure 3.1B), IT97K-499-35, as well as 18 % (9 progenies) exhibited pigmentation at margins (Figure 3.2C) whole GH3684 with 26 % of progenies showed complete violet pigmentation (Figure 3.3D).

Figure 3.4: Variation in flower pigmentation pattern among cowpea breeding lines; A- Non pigmented (white), B- Only wing pigmented, C- Pigmented at margins and D- Completely pigmented.

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Floral Raceme Position

Floral raceme position varied among cowpea progenies. Forty percent of the progenies showed mostly above canopy raceme position as well as PADI-TUYA. Besides, 54% (27) progenies showed raceme position in the upper canopy, including the parental genotypes GH3684, SARC-1-57-1, IT97K-499-35 and UCS01. Only 6% of the progenies showed raceme position throughout the canopy (Appendix A12).

Terminal Leaf Shape

The trifoliate leaves of cowpea exhibited variation in terminal leaflet shape (Figure 3.2). Twenty- six percent of cowpeas exhibited globose terminal leaflet shape as well as two of the parental genotypes, GH3684 and SARC-1-57-1. Thirty- eight percent of progenies exhibited a Sub-globose terminal leaflet shape. Thirty-two percent showed sub-hastate terminal leaflet shape similar to that of the parental genotypes; PADI-TUYA, IT97K-499-35, and UCS01. Only 4 % of the progenies exhibited hastate terminal leaflet shape (Figure 3.2).

Seed Coat Colour

Seed coat colour differed among the cowpea progenies. Eight different colours (white, cream, purple, brown, pale brown, dark brown, red and golden brown) were observed among the progenies (Figure 3.4). Among the 50 progenies, 36% of the progenies exhibited white seed coat colour same as parental genotypes PADI-TUYA, SARC-1-57-1 and IT97K-499-35. Twenty-Six percent had cream colouration same as UCSO1, 8% had brown colouration, 4% had purple, 6% had pale brown coat colouration, 12% had red

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colouration, 6% had dark brown colouration and only 2% had the golden brown colouration.

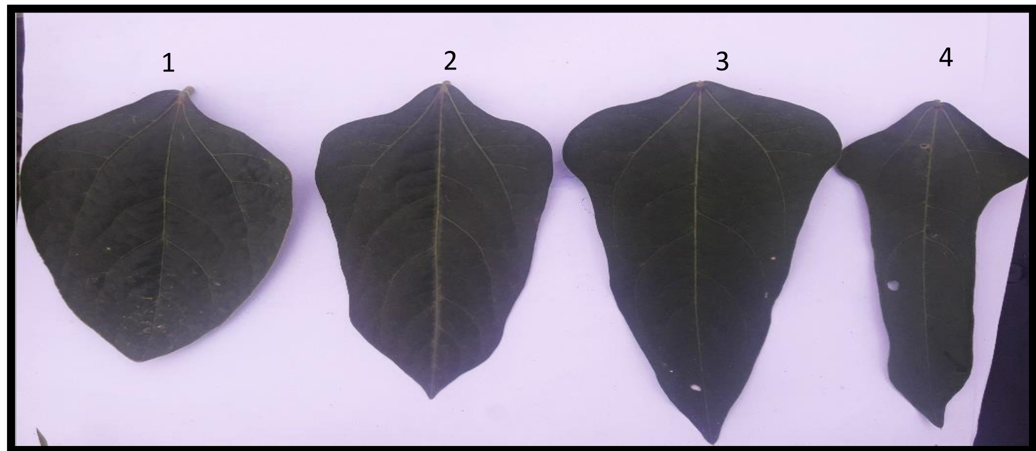

Figure 3.5: Variation in terminal leaf Shape; 1- Globose, 2- Sub-globose, 3-sub-hastate and 4-Hastate (Field survey, 2019).

Mature Pod Pigmentation

The F_5 progenies varied in terms of the pod pigmentation (Figure 3.3). 42 % (21) of the cowpea progenies as well as two parental genotypes, UCS01 and IT97K-499-35 showed pale tan pod pigmentation. 24 % (12 progenies), as well as the three parental genotypes (GH3684, PADI-TUYA and SARC-1-57-1), had dark tan pod pigmentation. Only 26 % (13 progenies) and 8 % (4 progenies) showed dark purple and other colours respectively.

Figure 3.6: Variation in pod curvature- thickness and pigmentation A-Dark tan; B and D-Tan ; C- pale tan;; E-Dark brown; F-black or dark purple.

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Figure 3.7: Variation in seed coat colour among cowpea genotypes. A-White cowpea B- Cream cowpea C- Dark brown cowpea D-White cowpea E- Pale brown cowpea F- Red cowpea G- Creamy brown cowpea, H- Golden brown.

3.2.2 Quantitative trait characterization

The cowpea breeding lines varied in terms of their agro-morphological traits. There were highly significant difference ($P < 0.001$) in all the quantitative parameters evaluated among the fifty (50) breeding lines and five (5) parental lines.

Days to first flowering and days to 50 % flowering varied significantly ($P < 0.05$) among the 50 breeding lines (Table 3.4). Days to first flowering ranged from 30 to 48 days, with a mean of 38 days. Also, days to 50 % flowering ranged from 35 to 52 days with a mean of 42 days. UC15-45 flowered very early with mean days to 50 % flowering of 37, whereas UC15-16 exhibited a late flowering trait with mean days to 50 % flowering of 52 (Table 3.4). The parental lines, PADI-TUYA, SARC-1-57-1, UCSO1, IT97K-499-35 and GH3684 recorded mean days to 50 % flowering of approximately 39.7, 41, 41, 45 and 47.3 days respectively. Again, days to 50 % flowering had a significant ($P < 0.01$) positive correlation with days to 50 % maturity (r

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= 0.211) (Table 3.6). Similarly, there was a significant ($P < 0.05$) positive correlation between days to 50 % flowering and number of peduncles (r =0.169), number of branches (r = 0.222), number of seed per pod (r = 0.245), plant height (r = 0.211) and number of locules (r = 0.207) (Table 3.6).

Terminal Leaflet Area

Terminal leaflet area ranged from 53.79 cm^2 to 132.84 cm^2 with a mean of 97.25 cm^2 (Table 3.3). The highest terminal leaflet area of 132.84 cm^2 was observed for UC15-35. The lowest was 53.79 cm^2 and this was observed in UC15-06. There was a significant positive correlation between Terminal Leaflet Area and pod length (r = 0.234) (Table 3.6).

Canopy diameter

The progenies differed significantly ($P < 0.05$) in their canopy diameter. The canopy diameter of the populations ranged from 54.8 cm to 297 cm, with a mean canopy diameter of 87.542 cm (Table 3.3). UC15-02 recorded the highest canopy diameter with a mean of 297 cm, while UC15-29 had the lowest mean canopy diameter of 54.8 cm (Table 3.4). A significant positive correlation between canopy diameter and number of branches (r = 0.221) and days to 50 % flowering (r = 0.155) were observed (Table 3.6)

Plant height

Plant height among the cowpea progenies varied significantly ($P < 0.05$). Plant heights ranged from 19.9 cm to 52.6 cm, with a mean of 40.6 cm (Table 3.3). UC15-47 had the highest height with a mean of 52.6 cm, whereas UC15-07 had the lowest height with a mean of 19.9 cm (Table 3.4). A significant positive correlation was observed between plant height and days to

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50 % flowering (r = 0.158), number of peduncles (r = 0.168) and number of seed per pod (r = 0.209) (Table 3.6).

Number of branches

Among the cowpea breeding lines evaluated, number of branches ranged from 2.3 to 6 with a mean of 5 (Tables 3.3 and 3.4). UC15-42, UC15-12, UC15-43, UC15-28, GH3684, UC15-34, UC15-25 and UC15-03 had the same highest number of branches of 6. UCS01 recorded the least mean number of branches of 2.3. Sixty- six percent of the progenies had a higher number than the parental lines except for GH3684. Again, the correlation analysis exhibited a significant positive correlation between number of branches and days to 50% flowering (r = 0.222), canopy diameter (r = 0.221), number of peduncles (r = 0. 354), terminal leaf area (r = 0.177), number of seed per pod (r = 0.117), number of locules (r = 0.142) and grain yield (r = 0.257) (Table 3.6).

Days to 50% maturity

The breeding lines varied significantly ($P < 0.001$) in terms of days to 50 % maturity. The variation ranged from 57 to 75 days with a mean of 64 days (Table 3.3). GH3684 had the shortest days to 50% maturity of 62.3 days, followed by UC15-21, UC15-31 and UC15-50, recording a mean of approximately 63 days. On the other hand, UC15-35 recorded the longest mean days to 50 % maturity of 70.3 days (Table 3.4).

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Table 3. 3: Descriptive statistics of quantitative traits of fifty-five (55) cowpea (*Vigna unguiculata*) breeding lines.

CHARACTER	RANGE	MEAN	CV%	LSD
Days to First Flowering	30.0-48.0	38.0***	3.20	2.00
Days to First Maturity	57.0-70.0	61.0***	3.40	3.30
Days to 50% Flowering	37.0-52.0	42.0***	2.70	1.90
Days to 50% Maturity	60.0-75.0	64.0***	2.70	2.80
Terminal Leaf Area(cm^2)	53.8-132.8	97.3***	19.60	30.87
Canopy Diameter (cm)	54.8 -149.5	87.5***	31.80	45.01
Plant Height (cm)	19.9- 52.6	31.0***	40.6	21.35
Number of Branches	2.0-6.0	4.7***	16.60	1.26
Number of Locules	9.3-17.5	13.6***	8.50	1.87
Number of Peduncles	25.5-50.1	27.0***	15.50	6.75
Number of Pod Per Peduncles	1.3 – 4.0	2.0****	30.00	0.955
Number of Seed per Pod	9.3 -17.0	13.4***	9.4	2.03
Pod Length (cm)	12.1-23.4	18.1**	6.90	2.02
100 Seed weight (g)	13.0- 25.8	20.4***	9.40	3.26
Grain yield (t ha^{-1})	1.1-2.7	1.17***	9.00	0.17

Significant codes: ‘***’0.00; ‘**’ < 0.001; ‘*’ <0.05; Df-164(Field data, 2019)

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Table 3.4: Variations in Morphological and Phenological traits of cowpea breeding lines.

LINES	DFFL	D50%FL	DFM	D50% MAT	PH (cm)	NB	CD (cm)	TLA (cm^2)
GH3684	44.3a-d	47.3b-c	58.7ab	62.3d	25.9ab	5.8a	98.2ab	96.9abc
IT97K-499-35	40.3d-l	45.0d-l	62.0ab	64.2bcd	30.9ab	4.4ab	108.4ab	78.4abc
PADI-TUYA	36.7k-r	39.7o-s	61.0ab	63.7bcd	29.2ab	3.9ab	87.8ab	94.1abc
SARC-1	36.7k-r	41l-s	62.7ab	64.7a-d	28.9ab	4.3ab	84.2ab	90.3abc
UC15-01	38.3g-p	42i-r	60.5ab	64.0bcd	30.4ab	5.4a	87.6ab	97.0abc
UC15-02	42.0c-h	47b-f	61.0ab	64 .5a-d	28.6ab	4.3ab	149.5a	88.8abc
UC15-03	37.0k-r	41l-s	63.0ab	68.0a-d	23.9ab	5.5a	69.5ab	88.7abc
UC15-04	38.0h-q	41.3k-r	61.0ab	64.0bcd	25.3ab	4.6ab	108.8ab	80.5abc
UC15-05	39.0f-o	41.7j-r	63.0ab	66.5a-d	23.9ab	5.2a	87.3ab	54.3cd
UC15-06	38.3g-p	41l-s	63.0ab	66.0a-d	24.2ab	4.6ab	136.4a	53.8cd
UC15-07	36.7k-r	40n-s	59.5ab	65.0a-d	19.9ab	4.2ab	91.8ab	77.0abc
UC15-09	37.3j-q	40.7m-s	60.0ab	66.0a-d	25.4ab	4.9ab	98.7ab	74.9abc
UC15-10	43.7b-e	46.7b-g	60.5ab	64.5a-d	22.2ab	4.7ab	86.7ab	96.6abc
UC15-11	40.3d-l	46b-i	62.5ab	68.0a-d	32.6ab	5.1ab	77.4ab	107.6abc
UC15-12	40.3d-l	46b-i	62.0ab	64.5a-d	30.7ab	5.9a	127.9a	116.9abc
UC15-13	38.0h-q	41l-s	60.0ab	64.0bcd	32.1ab	5.1ab	84.9ab	86.6abc
UC15-14	44.0a-d	49a-d	61.0ab	67.0a-d	35.2ab	5.0ab	76.8ab	96.2abc
UC15-15	35.0o-r	38.3o-s	57.0b	66.0a-d	29.6ab	4.2ab	100.7ab	102.1abc
UC15-16	48.0a	51.7a	63.0ab	66.0a-d	31.7ab	4.3ab	77.2ab	118.3abc
UC15-17	36.3l-r	39p-s	59.0ab	65.0a-d	28.1ab	5.3a	80.4ab	99.3abc
UC15-18	36.0m-r	38.7p-s	57.5b	63.0bcd	26.1ab	3.5ab	86.7ab	99.0abc
UC15-19	33.0r	38rs	61.5ab	66.0a-d	25.3ab	4.1ab	69.1ab	93.8abc

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Table 3.4 Cont'D

UC15-20	38.0h-q	46.3b-h	63.5ab	67.5a-d	30.0ab	4.5ab	81.8ab	108.6abc
UC15-21	37.7i-q	40.3n-s	61.5ab	65.0a-d	25.0ab	5.3a	101.6ab	125.8ab
UC15-22	38.3g-p	41.7j-r	59.5ab	64.0bcd	32.8ab	4.4ab	100.6ab	77.6abc
UC15-23	38.0h-q	40.3n-s	60.5ab	65.0a-d	29.6ab	5.4a	102.3ab	87.1abc
UC15-24	37.0k-r	40n-s	60.5ab	62.5cd	32.6a	4.7ab	88.0ab	97.4abc
UC15-25	43.7b-e	47.3b-f	61.5ab	68.0a-d	32.2a	5.5a	111.7ab	101.3abc
UC15-26	42.7c-f	46.3b-h	63.0ab	68.5ab	30.9ab	4.9ab	68.9ab	103.3abc
UC15-27	41.3c-j	46.3b-h	62.2ab	65.3a-d	30.6ab	5.1ab	73.8ab	116.3abc
UC15-28	37.7i-q	41.7j-r	63.8ab	66.5a-d	30.8ab	5.9a	77.6ab	81.8abc
UC15-29	37.0k-r	41l-s	63.3ab	67.2a-d	27.3ab	3.6ab	54.8ab	99.2abc
UC15-30	37.0k-r	40n-s	61.2ab	65.3a-d	34.5ab	5.3a	86.9ab	119.6ab
UC15-31	35.3n-r	38.7p-s	60.2ab	62.5cd	24.5ab	3.6ab	64.2ab	91.4abc
UC15-32	38.3g-p	41.3k-r	63.3ab	65.5a-d	25.0ab	4.8ab	89.3ab	130.8ab
UC15-33	36.0m-r	39.30-s	63.2ab	66.3a-d	25.6ab	4.5ab	70.2ab	132.8a
UC15-34	42.7c-f	44.7e-m	65.2a	68.3abc	33.6ab	5.7a	87.8ab	114.9abc
UC15-35	47.0ab	50a-b	65.5a	70.3a	33.6ab	4.8ab	135.2a	89.4abc
UC15-36	42.0c-h	44.7e-m	62.5ab	65.2a-d	28.3ab	4.7ab	75.4ab	122.8ab
UC15-37	38.0h-q	41.3k-r	63.5ab	66.0a-d	26.8ab	4.6ab	69.1ab	99.3abc
UC15-38	39.0f-o	44e-n	61.3ab	63.7bcd	32.9ab	5.4a	105.5ab	104.6abc
UC15-39	39.7e-m	42i-r	62.0ab	66.8a-d	46.9a	5.0ab	88.1ab	116.2abc
UC15-40	40.7c-k	43.3f-o	60.5ab	65.8a-d	30.1ab	4.6ab	76.8ab	81.9abc
UC15-41	34.3pqr	38.7p-s	60.7ab	64.0bcd	25.0ab	4.1ab	68.4ab	118.7abc
UC15-42	39.3f-n	42i-r	61.7ab	64.5abcd	34.0ab	6.1a	76.4ab	102.6abc
UC15-43	42.3c-g	45.7c-j	60.7ab	63.0bcd	35.0ab	5.9a	75.0ab	69.6abc
UC15-44	38.3g-p	41.7j-r	61.7ab	64.2bcd	30.9ab	4.4ab	61.3ab	108.4abc
UC15-45	34.3pqr	37s	62.5ab	65.5a-d	24.5ab	3.4ab	86.4ab	83.4abc

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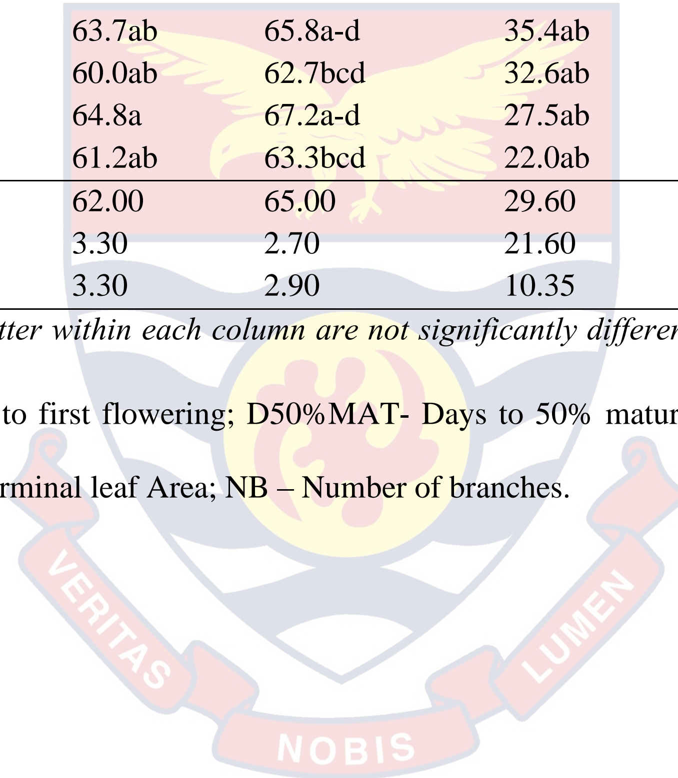
Table 3.4 Cont'D

UC15-46	38.0h-q	42.3h-q	65.0a	68.2a-d	27.4ab	4.5ab	75.3ab	121.8ab
UC15-47	39.0f-o	42.7g-p	59.3ab	64.2bcd	52.6a	5.4a	94.6ab	104.8abc
UC15-48	34.0qr	42.3h-q	61.8ab	66.7a-d	46.5ab	5.0ab	82.5ab	66.2bc
UC15-49	44.7abc	49.3a-c	63.7ab	65.8a-d	35.4ab	4.4ab	69.9ab	93.3abc
UC15-50	41.7c-i	47.7a-e	60.0ab	62.7bcd	32.6ab	5.3a	138.7a	116.9abc
UC15-51	41.3c-j	45.3c-k	64.8a	67.2a-d	27.5ab	4.5ab	96.0ab	88.8abc
UCS01	38.0h-q	41l-t	61.2ab	63.3bcd	22.0ab	2.3bc	91.0ab	97.3bc
Mean	39.00	43.00	62.00	65.00	29.60	4.76	89.10	97.60
CV%	3.10	2.70	3.30	2.70	21.60	16.40	31.40	19.60
$LSD_{(0.05)}$	2.00	1.90	3.30	2.90	10.35	1.27	30.89	30.89

NOTE: Note: Means followed by the same letter within each column are not significantly different (P = 0.05) as indicated by Tukey's method. Df-164; rep-3; confidence level- 95%.

DFFL- Days to first flowering; DFM- Days to first flowering; D50%MAT- Days to 50% maturity; D50%FL – Days to 50% flowering; PH-Plant height; CD – Canopy diameter; TLA- terminal leaf Area; NB – Number of branches.



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Number of peduncles

The number of peduncles varied significantly (P< 0.001) among the cowpeas, ranging from 25.3- 50.1 at a mean of 30 (Table 3.3). The highest number of peduncles of 50.1 and 49 were observed for the breeding lines UC15-44 and UC15-43, respectively, compared with the parental genotypes (Table 3.5). UC15-50 recorded the lowest number of peduncles 25.3. In all, 52% (26) of the breeding lines had number of peduncles than all parental lines (Table 3.5).

Number of pods per peduncle

The variation among the cowpea breeding lines in terms of number of pods per peduncle was highly significant (P < 0.001), ranging from 1.3 to 4, with a mean of approximately 2 pods per peduncle (Table 3.3). The highest number (4) of pods per peduncle was recorded for UC15-44, while UC15-33 recorded the lowest (1.3) (Table 3.5). Among progenies, 24 % had more number of pods than all parental lines. A significant (P < 0.05) positive correlation was recorded between number of peduncles and days to 50 % flowering (r = 0.169), plant height (r = 0.169), number of branches (r = 0.354) and grain yield (r = 0.167) (Table 3.6)

Number of seeds per pod

Highly significant (P < 0.001) variation was observed among the cowpea genotypes in terms of their number of seeds per pod. The number ranged from 9.3 to 17.4 with a mean of 13.4 (Table 3.3). GH3684 and IT97K-499-35 recorded the highest number of seeds per pod of 17.4 (Table 3.5). Among the breeding lines, UC15-14, UC15-01, UC15-26 and UC15-21 recorded the highest number of seeds per pods, with approximately a mean of

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16 (Table 3.5). There was a highly significant (P < 0.00) positive correlation between number of seed per pod and number of locules (r= 0.922), plant height (r = 0.209), pod length (r = 0.209) and grain yield (r = 0.161). However, the number of seeds per pod exhibited a significant negative correlation with hundred seed weight (r=-0.404) (Table 3.6).

Pod Length

Pod length varied significantly (P < 0.05) among the cowpea breeding lines ranging from 14.1 cm to 21.7 cm with a mean of 18.09 cm. UC15-38 recorded the lowest with a mean of 14.12 cm. In all, sixty percent of the cowpea progenies had pod length higher than those of parental lines (Table 3.5).

Number of Locules

Number of locules differed significantly (P < 0.001) among the cowpea genotypes, ranging from 9.3 to 17.5 with a mean of 13.4 (Table 3.3). GH3684, scored the highest number of locules of 17.5. UC15- 09 had the lowest number of locules of 9.6. (Table 3.5). Number of locules showed a significant positive correlation to grain yield (0.231), number of seed per pod (0.922), pod length (0.531) and number of branches (0.142) but a significant negative correlation with 100 seed weight (Table 3.6)

100-seed weight

Variation in 100-seed weight differed significantly (P < 0.001) among the cowpea breeding lines, ranging from 13.3 to 25.8 g with a mean of 20.4 g (Table 3.3). UC15-36, had the highest 100 seed weight at a mean of 25.8 g. GH3684 recorded the lowest with a mean of 13.3 g. 37 (74%) of breeding lines exhibited weights of more than 20 g. 22 (44%) of the progenies recorded

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values more than all the parental lines, out of which 17 of them were from a cross between GH3684 and UCSO1 (Table 3.5). There was a significant negative correlation between 100-seed weight, number of locules (r= -0.395), number of seed per pod (r= -0.404) and grain yield (r= -0.253) (Table 3.6).

Grain yield

Grain yield differed significantly (P < 0.001) among cowpea progenies ranging from 1.1 t ha^{-1} to 2.7 t ha^{-1} with a mean of 1.17 t ha^{-1} (Table 3.3). UC15-12, recorded the highest grain yield at a mean of 2.7 t ha^{-1} (Table 3.5). The first three highest lines were progenies from a cross between GH3684 and PADI-TUYA. Forty percent had grain yield higher than all parental genotypes. Out of these five progenies 8 were from GH3684 and PADI- TUYA, 6 from GH3684 and SARC-1-57-1, 4 from GH3684 and UCS01 and 2 from GH3684 and IT97K-499-35. Among the population, UC15- 33 had the lowest grain yield with a mean of 1.1 t ha^{-1}.

There was a positive correlation between grain yield and number of branches (r = 0.257), number of peduncles (r = 0.167), number of seed per pod (r = 0.161) and number of locules (r = 0.231).

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Table 3.5: Average yield and field related parameters of cowpea breeding lines

LINES	NoP	NoPP	PL(cm)	NSPP	NoL	100 SW(g)	GY (t ha^{-1})
GH3684	38.5a-k	1.4ab	18.3a-f	17.4a	17.5a	14.0mn	1.9h-n
IT97K-499-35	36.5a-k	1.6ab	18.9a-e	17.4a	15.7a-f	16.0lmn	1.3t-w
PADI-TUYA	33.1d-k	2.3ab	16.6c-f	9.6hi	9.6jk	21.7a-i	1.2vw
SARC-1	33.9c-k	2.2ab	17.8a-f	16.8ab	16.9ab	13.3n	1.7k-s
UC15-01	35.0b-k	2.0ab	19.5a-d	15.9a-d	16.1a-d	19.0g-l	1.6l-t
UC15-02	37.7 a-k	1.8ab	18.2a-f	14.8a-e	15.5a-f	19.7e-l	1.5n-v
UC15-03	35.7b-k	2.4a	18.9a-e	10.4f-i	11.8f-k	22.7a-g	1.6l-t
UC15-04	38.2 a-k	1.6ab	15.2ef	13.4a-i	13.7a-i	19.3f-l	1.7j-q
UC15-05	31.5e-k	2.0ab	20.2a-d	13.4a-i	14.4a-h	21.7a-i	1.9h-n
UC15-06	29.4h-k	2.0ab	18.6a-e	9.4i	12.5c-k	24.7ab	1.3t-w
UC15-07	34.1c-k	2.1a	16.8c-f	10.1ghi	10.9h-k	20.7b-k	1.4r-w
UC15-09	45.1a-f	2.2a	14.1f	9.3i	9.3k	19.0g-l	2.0f-l
UC15-10	39.7a-j	1.8ab	16.8c-f	14.2a-g	14.3a-h	19.3f-l	1.7j-r
UC15-11	37.9a-k	1.5ab	17.9a-f	12.4c-i	12.5c-k	19.7e-l	2.3b-f
UC15-12	44.8a-f	2.1a	18.4a-e	11.6e-i	13.5a-j	20.3c-k	2.7a
UC15-13	45.0a-f	2.1a	19.9a-d	14.8a-e	16.0a-e	20.0d-l	2.4a-d
UC15-14	38.3a-k	1.8ab	20.4abc	16.4abc	16.5abc	19.7e-l	2.4abc
UC15-15	42.0a-i	2.2a	18.0a-f	12.4c-i	13.2b-k	19.7e-l	2.5ab
UC15-16	38.7a-k	2.0ab	19.4a-d	12.1c-i	13.4b-j	23.3a-f	2.4a-d
UC15-17	40.6a-j	1.9ab	17.1b-f	12.4c-i	12.9b-k	20.7b-k	2.2b-h
UC15-18	32.9d-k	1.5ab	17.5a-f	10.1ghi	11.4g-k	21.0b-j	1.7k-s

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Table 3.5 cont'd

UC15-19	29.9h-k	2.1a	18.4a-e	11.7d-i	12.4d-k	20.3c-k	2.1c-i
UC15-20	37.2a-k	2.4a	18.2a-f	15.0a-e	15.1a-g	18.0i-m	1.4p-v
UC15-21	35.7b-k	1.9ab	19.5a-d	15.6a-e	15.8a-f	19.7e-l	2.2b-g
UC15-22	38.4a-k	1.8ab	18.3a-f	14.4a-g	14.6a-h	20.3c-k	2.0e-k
UC15-23	39.7a-j	2.1a	19.1a-e	14.9a-e	15.3a-g	17.3j-n	2.1c-i
UC15-24	36.9a-k	2.0ab	19.3a-e	14.7a-f	15.1a-g	16.6k-n	1.7i-p
UC15-25	45.2a-f	1.7ab	19.6a-d	14.7a-f	15.0a-h	22.0a-i	1.9g-m
UC15-26	29.0h-k	2.1a	18.8a-e	15.8a-e	16.1a-d	16.7k-n	2.0d-j
UC15-27	33.9c-k	2.3a	18.6a-e	15.2a-e	15.7a-f	17.3j-n	1.8i-o
UC15-28	40.4a-j	2.1a	17.1b-f	13.0b-i	13.4b-j	18.0i-m	1.8i-o
UC15-29	27.9j-k	1.8ab	18.0a-f	12.9b-i	13.3b-k	21.7a-i	1.8i-n
UC15-30	43.2a-f	2.6a	18.6a-e	15.0a-e	15.5a-f	18.3h-l	2.3b-e
UC15-31	32.2d-k	2.5a	18.3a-f	13.4a-i	13.6a-j	22.3a-h	1.4q-w
UC15-32	40.2a-j	1.6ab	18.2a-f	11.4e-i	11.9f-k	24.0a-d	1.9f-l
UC15-33	45.5a-e	1.3ab	20.6abc	15.2a-e	15.4a-g	23.9a-e	1.1w
UC15-34	40.9a-j	1.9ab	19.5a-d	13.5a-i	13.6a-i	21.0b-j	1.4t-w
UC15-35	48.5ab	1.7ab	18.9a-e	14.5a-f	14.7a-h	20.0d-l	1.6m-u
UC15-36	36.7a-k	2.5a	18.0a-f	12.7b-i	13.4a-k	25.8a	1.4q-w
UC15-37	40.4a-j	1.6ab	18.8a-e	11.7d-i	12.0e-k	23.8a-e	1.5n-v
UC15-38	45.0a-f	2.7a	21.7a	14.6a-f	14.8a-h	23.5a-f	2.1b-h
UC15-39	46.5a-d	1.9ab	21.1ab	14.3a-g	14.6a-h	23.5a-f	1.5o-v

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Table 3.5 cont'd

UC15-40	43.8a-g	2.5ab	18.2a-f	14.0a-g	14.2a-h	23.3a-f	2.0e-k
UC15-41	38.2a-k	1.7ab	18.2a-f	12.1c-i	12.3d-k	22.2a-i	1.3t-w
UC15-42	47.9abc	2.7a	18.8a-e	14.4a-g	14.5a-h	22.7a-g	1.9h-n
UC15-43	49.0ab	2.3a	17.0b-f	13.5a-i	13.4a-j	21.3b-j	1.6l-t
UC15-44	50.1a	3.0a	19.3a-e	13.8a-h	14.1a-i	23.7a-e	2.4m-u
UC15-45	31.2f-k	1.8ab	17.0b-f	12.9b-i	13.1b-k	23.3a-f	1.6n-v
UC15-46	38.9a-k	2.0ab	18.6a-e	14.0a-g	14.1a-i	24.8ab	1.3t-w
UC15-47	37.6a-k	1.6ab	20.6abc	14.2a-g	14.7a-h	24.3abc	1.3u-w
UC15-48	42.1a-j	1.7ab	16.1def	12.8b-i	13.1b-k	22.7a-g	1.6m-v
UC15-49	44.0a-f	2.0ab	17.1b-f	11.4e-i	11.9e-k	23.1a-g	1.2vw
UC15-50	25.3k	2.5a	18.7a-e	12.6b-i	12.7c-k	24.3abc	1.4s-w
UC15-51	39.0a-k	2.3a	16.8c-f	10.1ghi	10.1ijk	24.5abc	1.1vw
UCS01	26.7jk	1.3ab	20.1a-d	13.2a-i	14.9a-h	21.0b-j	1.3tw
Mean	26.98	1.97	18.10	13.40	13.60	20.98	1.17
CV%	15.50	30.00	6.90	9.30	8.50	5.90	9.00
$LSD_{(0.05)}$	6.75	0.95	2.02	2.02	1.87	1.99	0.17

NOTE: Note: Means followed by the same letter in each column are not significantly different (P = 0.05) as indicated by Tukey's method. Df-164; rep-3; confidence level- 95%; NoP– Number of Peduncles; NoPP – Number of pod per peduncle; NoL- Number of locules; PL – pod length; NSPP – Number of seeds per pod; 100SW – 100 seed weight; GY – Grain yield; BY- Bulk yield

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Table 3.6: Correlation coefficients for pairwise comparison of the relationship between morphological and yield among cowpea genotypes.

	D50% FL	D50% MAT	PH	NB	CD	NoP	NoPP	TLA	PL	NSPP	NL	100sw
D50% MAT	0.211**											
PH	0.158**	0.04										
NB	0.222**	0.064	0.142									
CD	0.155*	-0.098	0.093	0.221**								
NoP	0.169**	0.065	0.168**	0.354***	0.059							
NoPP	-0.001	-0.148	0.012	0.094	-0.15	0.015						
TLA	0.086	0.062	-0.053	0.177**	0.074	0.129	-0.084					
PL	0.115	0.034	0.058	0.112	0.072	0.053	0.019	0.234**				
NSPP	0.245**	-0.04	0.209**	0.177**	0.017	0.091	-0.011	0.134	0.471***			
NL	0.207**	-0.028	0.143	0.142**	0.013	0.015	-0.002	0.115	0.531***	0.922***		
100sw	-0.04	0.033	-0.022	-0.09	0.002	0.116	0.008	0.145	0.107	-0.404***	-0.395***	
GY ($t\ ha^{-1}$)	0.065	0.031	-0.02	0.257**	0.03	0.167**	0.08	0.089	0.079	0.161**	0.231**	-0.253***

- *Significant codes: '***'0.001; '**' < 0.01; '*' <0.05*; D50%MAT- Days to 50% maturity, D50%FL – Days to 50% flowering, PH- Pl ant height, CD – Canopy diameter, TLA- terminal leaf Area, NB – Number of branches, NoP– Number of Peduncles, NoPP – Number of pod per peduncles, PL – pod length, NSPP – Number of seeds per pod, 100SW – 100 seed weight, GY – Grain yield (Field data, 2019).

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Principal component analysis

The principal component analysis for a total of thirteen (13) agronomic traits of the breeding lines was estimated as shown in Table 3.7. Eight principal components (PC1 to PC8) explained 82.9 % of the total phenotypic variation proportion of 21.7, 12.7, 10.2, 9.1, 8.6, 8.4, 6.6, and 5.5 %, respectively. In the first component (PC1), the number of seeds per pod, number of locules and pod length had the most important contribution to variations observed with positive loadings Eigenvectors of 0.527, 0.524 and 0.350, respectively (Table 3.7; Figure 3.5). However, 100 seed weight was the only trait with a negative loading impact of -0.244. The second component was dominated by traits such as the number of peduncle and number of branches and 100 seed weight, all with positive loading impact (Figure 3.5). The number of seeds per pod and grain yield contributed negatively with the loading of -0.628 and -0.599, respectively (Table 3.7). The fourth component (PC4) had one important contributor, the number of pods per peduncle with a positively charged loading of eigenvectors 0.515. Canopy diameter and days to 50 % maturity were the highest contributors to PC5, having a loading of 0.676 and 0.646, respectively. Plant height, days to 50 % flowering and terminal leaflet area exhibited the highest contribution to PC6, PC7 and PC8, respectively (Table 3.7).

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Table 3.7: Principal Component Analysis (PCA) of agronomic traits among the cowpea breeding lines.

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
DFF	0.244	0.287	-0.008	-0.326	0.126	-0.145	-0.581	-0.218
DFM	0.030	0.245	0.151	-0.397	0.646	0.075	-0.104	0.213
PH	0.174	0.139	-0.159	-0.348	-0.176	-0.538	0.369	-0.096
NB	0.259	0.415	-0.307	0.140	-0.059	0.086	-0.051	-0.061
CD	0.096	0.272	-0.024	-0.186	-0.676	0.260	-0.301	0.237
NoPP	0.007	-0.067	-0.325	0.515	0.115	-0.476	-0.450	-0.069
NoP	0.151	0.479	-0.186	0.137	0.072	-0.147	0.445	0.095
TLA	0.152	0.290	0.388	0.312	0.032	0.282	0.086	-0.656
PL	**0.350**	-0.012	0.465	0.239	-0.039	-0.143	-0.065	0.443
NSPP	**0.527**	-0.237	0.089	-0.044	-0.017	-0.078	0.073	-0.083
NOL	**0.524**	-0.279	0.116	0.000	0.009	-0.022	0.023	0.034
100sw	**-0.244**	0.369	0.430	0.235	-0.039	-0.283	-0.015	0.267
GY	0.230	0.063	-0.379	0.254	0.227	0.418	0.059	0.344
Eigenvalue	2.821	1.6562	1.3284	1.1893	1.1182	1.0910	0.8603	0.7086
Percentage of total variation	21.7	12.7	10.2	9.1	8.6	8.4	6.6	5.5
Cumulative percentage of variation	21.7	34.4	44.7	53.8	62.4	70.8	77.4	82.9

DFF- Days to 50% flowering; DFM- Days to 50% maturity; NB- Number of branches; PH – Plant height; CD- Canopy diameter; TLA – Terminal leaf Area; NoP– Number of Peduncles; NoPP – Number of pod per peduncles; NoL- Number of locules; PL – pod length; NSPP – Number of seeds per pod; 100SW – 100 seed weight; GY – Grain yield (Field data, 2019).

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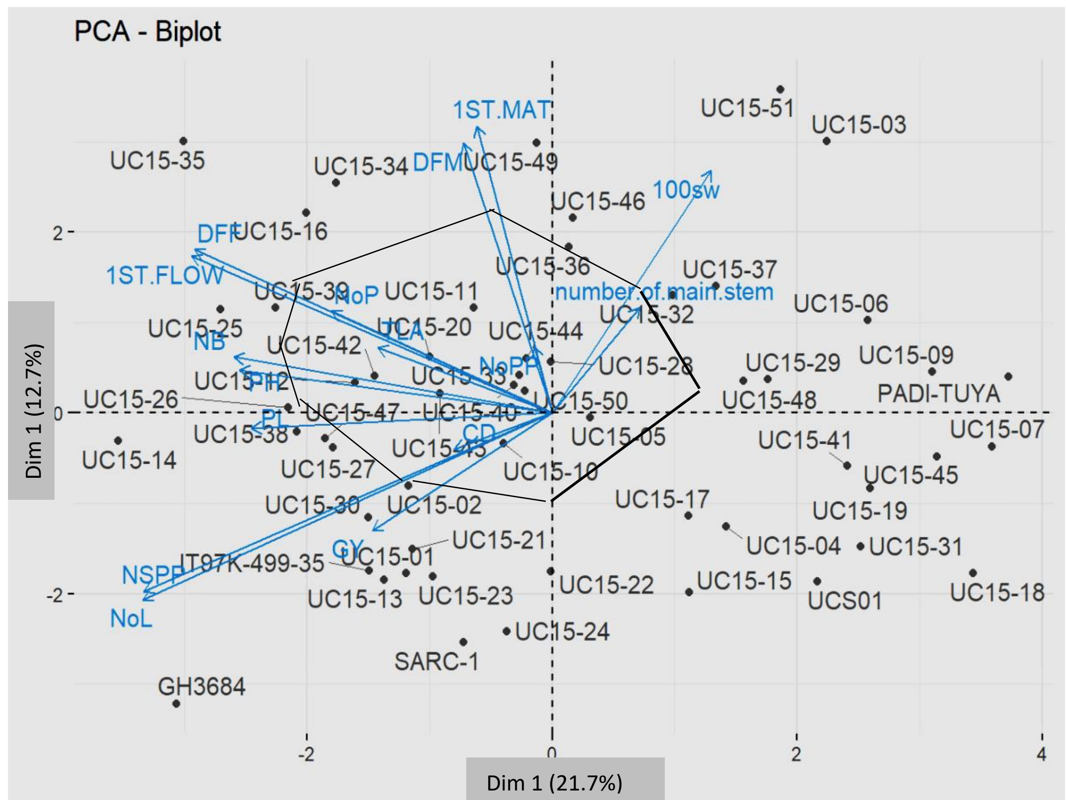

Figure 3.8: Biplot showing distribution of cowpea breeding lines, according to the first and second components from Table 3.7

3.2.3 Cluster analysis

The dendrogram (Figure 3.6) involving 12 agro-morphological traits grouped the 50 cowpea progenies and 5 parental genotypes into two main clusters based on Nei genetic distance (Nei, Tajima & Tateno, 1983) using unweighted pair grouped method with arithmetic average (UPGMA) cluster analysis at a dissimilarity of 38 % (Figure 3.6). The 55 cowpeas appeared to have emerged from common ancestry and were distinguished into two major clusters (A and B). Cluster A comprised 75 % of the cowpea accessions, which were further distinguished into 10 sub-clusters. However, cluster B consisted of about 25 % of the cowpeas that were distributed into four sub-clusters. Cluster B was predominated by cowpea breeding lines from population four (P4), the progenies from the cross between GH3684 and

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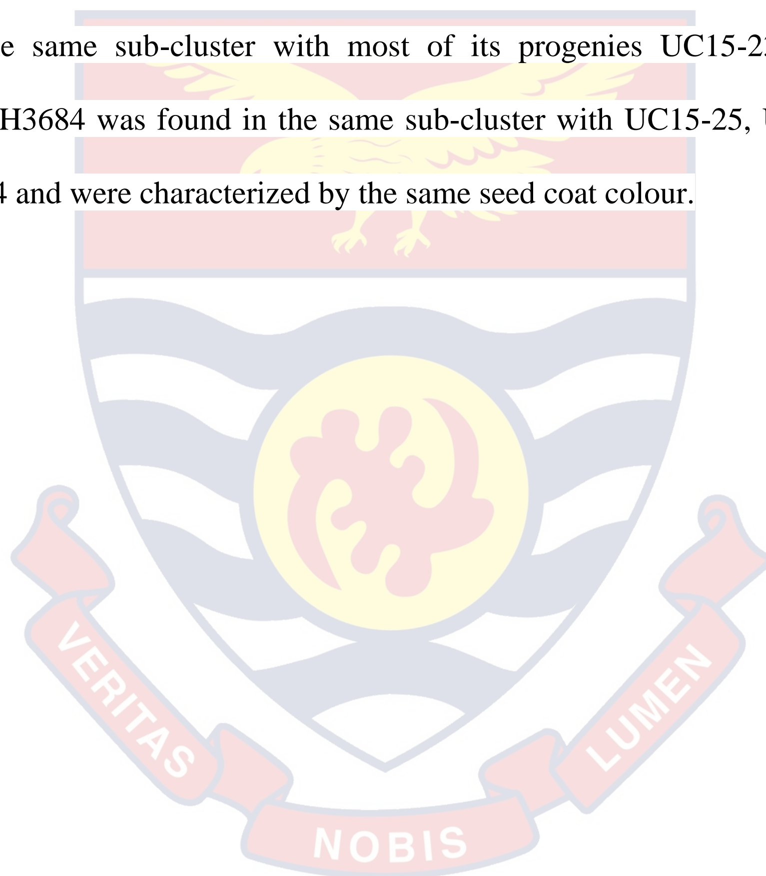
UCSO1. In cluster B, IT97K-499-35 resulted as an outlier. It had a relatively smaller seed size than all the genotypes in the cluster. All progenies found in cluster B, except for IT97K-499-35, exhibited traits of large seed size (>17g/100seeds) (Figure 3.5; Figure 3.6), similar days to 50 % flowering, approximately two pods per peduncle and comparatively lower yield as compared to cluster A. PADI-TUYA shared similar traits with its progenies (UC15-19 and UC15-18), hence were clustered together. SARC-1-57-1 shared the same sub-cluster with most of its progenies UC15-23 and UC15-20. GH3684 was found in the same sub-cluster with UC15-25, UC15-35, UC15-24 and were characterized by the same seed coat colour.



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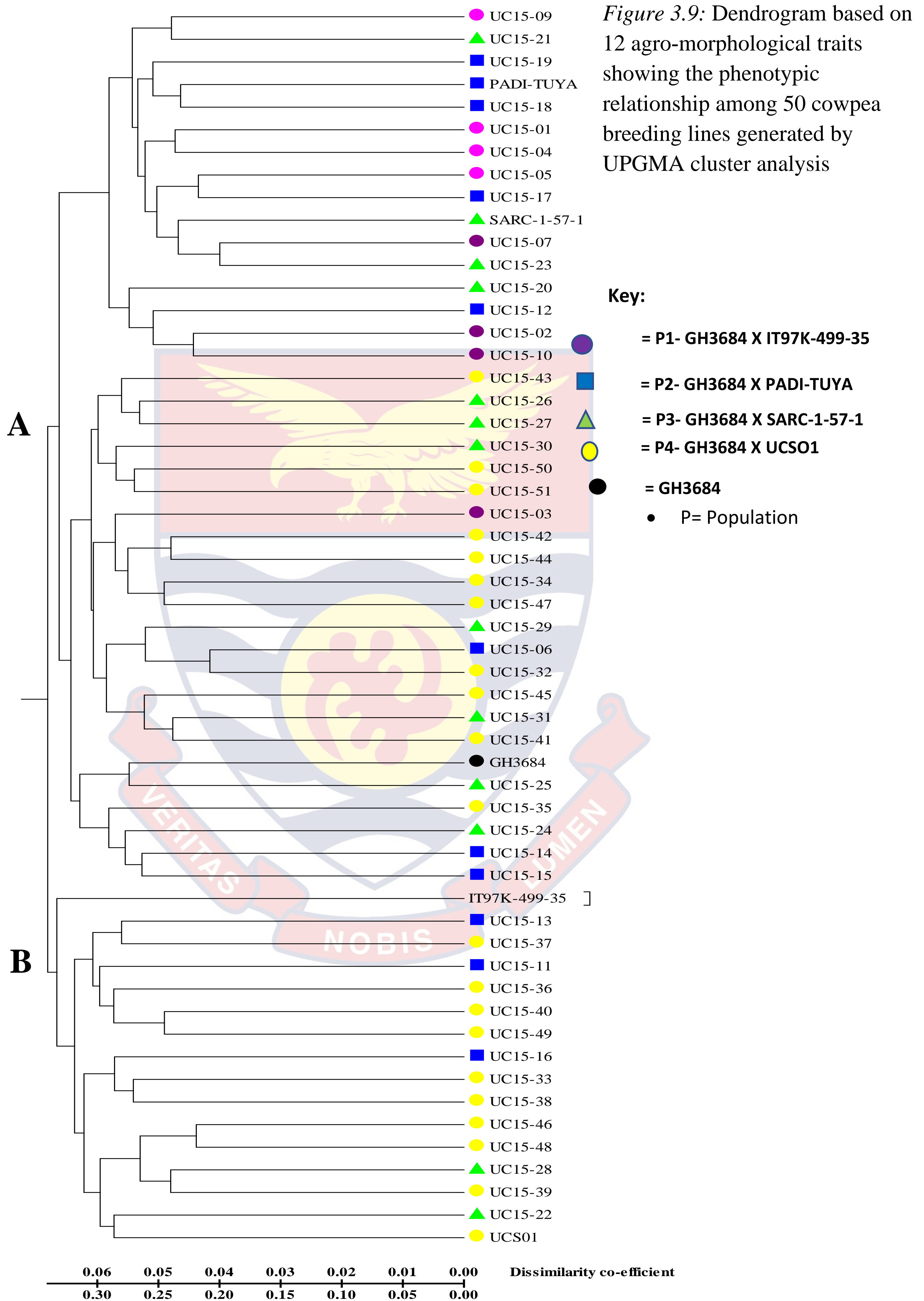

Figure 3.9: Dendrogram based on 12 agro-morphological traits showing the phenotypic relationship among 50 cowpea breeding lines generated by UPGMA cluster analysis

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3.3 Discussion

Conventionally, the first step in studying the genetic variability in any breeding programme is based on the differences in phenotypic traits (Schut, Qi & Stam, 1997; Govindaraj, Vetriventhan & Srinivasan, 2015). Variations in the qualitative and quantitative characteristics exist among the cowpea breeding lines compared to the parental genotypes. The identities of the 50 cowpea breeding lines were established based on their agro-morphological characteristics following the IBPGR standard descriptors (Makanur, Deshpande & Vyakaranahal, 2013).

Variation in the twinning tendency was evident among the cowpea breeding lines. Most of the breeding lines (64 %) produced slight twinning tendency whereas 32 % exhibited no twinning tendency. The indication is that, most of the cowpea breeding lines may not need staking and could be used for intercropping and reduce the cost of production since there will be no need for staking. A similar result was obtained by Cobbinah, Addo-Quaye and Asante (2011) on characterization, evaluation and selection of cowpea accessions with desirable traits from eight regions of Ghana. Variation in growth habit and growth pattern were evident among the cowpea breeding lines. Fifty-two percent of the progenies exhibited determinate growth pattern, which was a characteristic feature of all the parents except UCSO1. This shows a high inheritance of these traits by the cowpea progenies. Among the breeding lines, semi-erect trait was predominant (50 %). However, 22 % of the breeding lines exhibited semi- prostrate growth habit, 14 % were erect and 14 % were prostrate. Growth habit is a very important trait of cowpea because of its influence in harvesting. The semi-erect nature of most of the cowpea will

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make it easy to harvest. Prostrate and semi-prostrate cowpea are very difficult to harvest as compared with erect and semi-erect since one needs to bend down very low to harvest matured pod (Aryeetey, 1971; Cobbinah *et al.*, 2011). This observation was similar to the report by Cobbinah *et al.* (2011). In addition, Asare *et al.*, (2013) observed that segregation lines derived from $GHUCOL_{BL}$ were prostrate, semi-prostrate and semi-erect.

Raceme position is one of the traits that need to be studied among elite lines of cowpea, since those held above the canopy, aid easy visibility and harvesting of pods as compared with those held within (throughout) the canopy (Cobinnah, et al., 2011; Ibrahima, 2012). This current study indicates that the raceme positions of 54 % of the cowpea progenies were in the up canopy, 40 % were mostly above the canopy and 6 % were throughout the canopy. This conforms to the studies by Essandoh (2017), and Tettey (2017) but contrary to the study by Cobbinnah *et al.* (2011) among 134 genotypes studied. In all, 57.7 % had raceme position mostly above the canopy. Among the parental genotypes, GH3684, IT97K-499-35, UCSO1 and SARC-1-57-1 exhibited raceme position in the upper canopy, whereas PADI-TUYA recorded raceme position above the canopy. 60 % of the progenies of GH3684 x PADITUYA exhibited raceme position mostly above the canopy, showing that the inheritance of the trait may be stable.

Flower colour varied among the cowpea breeding lines. The white flower was predominant in this study, representing 52 % of the progenies, whereas 48 % produced violet flower colour (Appendix A7). These findings confirm earlier research by Essandoh (2017) and Tettey (2017) where white and violet flower colour were 57 % and 51% respectively on the cowpea

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genotypes studied. However, the findings of this present study were contrary to previous studies by Bennett-Lartey and Ofori (1999) as well as Ezueh and Nowffiah (1984) whose work reported the violet flower as predominant among the cowpea genotypes evaluated. Flower colours such as pale blue, yellow and pink have been reported by earlier research on cowpea (Gibbon & Pain, 1985; Ige *et al.*, 2011) but were not observed in the current research. Moreover, flower pigmentation pattern was diverse among the cowpea progenies in this study. It was observed that UCSO1, SARC-1-57-1 along with 34 % of the progenies had complete white flowers (Appendix A6). PADI-TUYA and 22 % of the cowpea progenies showed only wing pigmentation (Appendix A6), IT97K-499-35, as well as 18 % of the cowpea progenies, exhibited pigmentation at margins (Appendix A6) and GH3684 and 26 % of the cowpea progenies, had complete violet pigmentation (Appendix A6). The variations in flower pigmentation pattern may suggest the multi-allelic nature of the flower gene flow.

M|ean plant height, canopy diameter, terminal leaflet area, number of branches, number of peduncles, number of locules, peduncle length, number of pod per peduncle, number of seed per pod, 100 seed weight and grain yield revealed significant differences ($P<0.05$) among the cowpea breeding lines and the parental genotypes. This indicates that the cowpea progenies may be genetically diverse, which probably reflects the diversity of the different parents coupled with segregation of the traits. Plant height recorded in this study ranged from 18.9 cm to 86.9 cm with a mean of 31cm was consistent with earlier studies by Abayomi, Ajibade, Sammuel, and Saadudeen (2008) who reported height between 20.21 cm and 59.12 cm but was lower than the

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report by Basaran, Ayan, Acar, Mut, and Asci (2011), Khan, Qureshi, Gilani and Ullah (2011) and Peksin and Artik (2004) who recorded values between 101 cm and 122.4 cm. UC15-47 recorded the highest mean height of 52.7 cm. Moreover, progenies from the cross between GH3684 X UCS01 recorded the highest plant height with a mean range of 24.6 cm to 52.6 cm (Table 3.4), followed by progenies from GH3684 X PADI-TUYA with a mean range of 25.0 cm to 35.2 cm (Table 3.4). This observed variation may be due to genetic differences among the cowpeas and the influence of environmental factors. However, Timko, Ehlers, and Roberts (2007) reported that photoperiod can also affect plant height.

The number of branches (NB) ultimately determines the plant's pod bearing ability, which in turn contributes to yield (Musvosvi, 2009; Makanur, 2013). Therefore, it is important to select cowpea plants with higher number of branches. In this study, the cowpea progenies varied significantly ($P < 0.05$) in terms of their branch formation ranging from a mean of 2.3 (UCS01) to 6.1 (UC15-42). There was a low positive correlation between number of branches and canopy diameter ($r = 0.221$), number of branches and number of peduncles ($r = 0.354$), as well as number of branches and grain yield ($r = 0.257$). It was observed that most progenies such as UC15-01, UC15- 03, UC15- 12, UC15-28 and UC15- 37 which recorded higher number of branches, were among the cowpeas with yield, ranging from 2.0 t ha^{-1} -2.7 t ha^{-1}. On the other hand, among 134 cowpeas studied by Cobinnah *et al.* (2011), mean number of branches reported was 4.4 and this study recorded 4.7. Suggesting that breeding lines used in this study have a higher number of branches. However, the variation observed in number of branches could partly be due to genetic

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factors and environmental conditions (Magani & Kuchinda, 2009; Tettey, 2017).

The majority of cowpeas are sensitive to photoperiod, whereas others are day-neutral when it comes to the formation of floral buds and flower development (Timko *et al.*, 2007; Timko & Singh, 2008). The length of the reproductive period differed significantly ($P < 0.05$) among the cowpea breeding lines. UC15- 45 recorded the lowest number of days to 50 % flowering (37days) and UC15-16 recorded the highest number days to 50% flowering (51.7days). In all, 90 % of the cowpea studied exhibited days to 50 % flowering between 37-45 days. The cowpea lines studied may be considered as either early to medium maturing since none of the progenies recorded late days to flowering (90 to 100 days) (Madamba, Grubben, Asante & Akromah 2006). Parental lines recorded days to 50 % flowering between 36.7 days and 44.3 days, which suggests that most of the progenies inherited traits of early to medium days to 50 % from the parents. According to Ojomo (1974), much of the genetic variation for days to flowering is due to dominance or epitasis. Singh and Rachie (1985) reported that broad-sense heritability estimates an average of 48.3% for days to flowering and 47.8% for days to pod maturity. It was obvious in this study that all cowpea genotypes with lower days to 50% flowering recorded lower days to maturity. It has been reported that additive gene action is responsible for much of the genetic variation for earliness (Lal, Miksic, Drawbaugh, Numan, & Smith, 1976; Mak &Yap, 1980). In the study of cowpea physiology, Gonné, Venasius and Laminou, (2013), reported that a good seed yield will require varieties with short flowering periods to enable them to divert energy into the pod and seed development. Wallace *et al.*

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(1995) as cited in Shiringani (2007) observed that temperature is the dominant factor that affected flowering and maturity. Purseglove (1972) reported variations in the days to flowering among cowpea genotypes to be due to character dependent minor gene complex.

There were significant ($P < 0.05$) variations regarding both pod length (PL) and the number of seed per pod (NSPP) among cowpea breeding lines. UC15-13 recorded the highest pod length (20.4 cm) as well as number seed per pod (16.4). Most of the breeding lines with long pods recorded higher number seed per pod in this study, thus, the positive correlation ($r = 0.22$) between pod length and number seed per pod. Moreover, a positive correlation was observed between number seed per pod and grain yield as well as number of locules. Other research has reported different pod length. Khan, Bari, Khan, Hussain, and Zada (2010) recorded pod length values ranging from 10 cm to 38 cm and 7 to 21 for number seed per pod among 24 genotypes. Again, among 400 cowpea genotypes studied, Pasquet (1999), pod length varied between 9.2 cm to 43.7 cm. Despite this, Basaran *et al*. (2011) reported 14.4 cm and 14.2 cm as the highest mean pod length among nine cowpea lines used in their study, which was lower than the highest mean pod length in this study. Basaran *et al*. (2011) also recorded the highest number of seeds per pod of 9.9, which was rather lower than the highest number of seeds per pod in this study. In this study, it was observed that cowpea breeding lines with longer pods were easily visible (especially with the erect types), firmly held and makes easy harvesting. A similar observation was made by Cobbinah *et al.* (2011). According to Cobbinah *et al.* (2011), in situations where all the locules are filled up during pod development, pod length could also play a significant (P <

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0.05) role in the number of seeds per pod. Moreover, it was observed among the progenies, those with longer pods had a higher number of seeds per pod (NSPP) but comparatively small seed size to progenies with shorter pod length (PL). However, our results were in line with the report by Saviers es-Hass, (1973), that seed yield is highly and positively correlated with pod length and the number of seeds per pod

. Fery, (1985) showed that PL was highly heritable with an average heritability estimate of 75.2%. Variation in pod length and number of seed per pod may be a result of genetic and abiotic factors. Aliyu and Makinde, (2016) reported that the availability of moisture at the time of pod formation and maturity might have also influenced large and longer pods. However, since all breeding lines were exposed to similar growing conditions any variations in pod length and number of seed per pod in the population may be genetic in nature.

Seed size contributes to yield and it is also a farmer preference trait of cowpea in Ghana and other parts of the world. Ghanaian consumers tend to prefer large-seed cowpea to small medium seeds (Langyintuo *et al.*, 2003). 100-seed weight is a reflection of the seed size, which is one of the yield components of cowpea and is generally positively correlated to yield (Burris, Edje & Wahab, 1973). Seed size is a key factor in crop improvement and a component of seed quality which has an impact on the performance of the crop. 100 seed weight (100sw) among the cowpea genotypes (including parents) ranged from 11.00 g to 26.8 g with a mean of 20.4 g (Table 3.3;df =164). Burris et al. (1973), Asare (2013) and Essandoh (2017) classified the variation among 100 seed weight (seed size) as follows: 10 g to 15 g small-

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sized seeds, 15.1 g to 20 g medium-sized seeds, 20.1 g to 25g - large-sized seeds and 25.1 g and above extra-large seeds. Indeed, none of the cowpea breeding lines in the present study had small seed sizes, 36% were medium-sized seeds, 62% large-sized seeds and 2% were of extra-large seed category (Table 3.5). In all, 44% of the cowpea breeding lines had seed size greater than all the parental genotypes, among which 17 of these progenies were from a cross between GH3684 and UCSO1. Most probably, the cowpea breeding lines might have inherited large seed size from UCS01 (large-seeded parent) compared with GH3684 (small-seeded parent). Even though, the inheritance of seed size in cowpea has been proven by some researchers to be complex (Lingyintuo, 2003; Egbadzor *et al*, 2013), and governed by many genes acting mainly additively with small size partially dominant over large seed size, (present study suggests otherwise). In this study, majority of the breeding lines in each population inherited the characteristics of their better parents in terms of seed size, who conform to the finding by Drabo, Redden, Smithson and Aggarwal (1984), which observed large seed size to be dominant over small seed**.**

Grain yield differed at a highly significant ($P< 0.01$) range of 1.00 to 2.92 t ha^{-1}. UC15-12 recorded the highest yield of 2.7 t ha^{-1}. These results conform to a study by Afutu, Mohammed, Odong, Biruma, and Rubaihayo (2016), who indicated 2.7 t/ha as the highest seed yield of 100 cowpea genotypes in two different locations of Uganda. However, a previous study by Khan *et al.,* 2011 recorded grain yield ranging from 0.32 to 3.6 t ha^{-1} among the 24 cowpea genotypes studied in Pakistan. Evaluation of locally known varieties including Asumdwe, Tona, Nhyira, Asetenapa, Videza, and Hawale

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exhibited yield ranging from 1.4 t ha^{-1} to 2.2 t ha^{-1} in the major cropping season (Agyeman, Berchie, Osei-Bonsu & Fordjour, 2015). This yield range is closely similar to the results obtained in this study. However, the breeding lines under study could have performed better if similar planting distance used by Agyemang *et al*. (2014) was used. A study conducted by Essandoh, 2016 and Tettey, 2017, recorded grain yield ranging from 2.1-9.9 t ha^{-1} and 1.3-8.0 t ha^{-1}, respectively, in the same location of F_9 breeding lines. The yield recorded in this study may be influenced by genetic and seasonal environmental factors as well as plant density (planting distance per plot). Moreover, Peksen and Artuk (2004) recorded a range of yield (0.68-1.2t/ha), which was lower than the present study. In the current study there was positive correlation between grain yield and number of branches (r= 0.257), number of peduncles (r = 0.167), number of seed per pod (0.161) and number of locules (r= 0.231). Total seed yield per plant varied significantly ($P < 0.05$) among the cowpea breeding lines and correlated positively to number of branches, number of peduncles, number of seeds per pod and number of locules. Manggoel and Uguru (2011), reported a similar correlation, as grain yield showed a significant ($P < 0.05$) positive relationship with the number of peduncles, number of pods per plant and pod length. This suggests a positive association between grain yield and yield attributes in cowpea.

A large number of variables are often measured by plant breeders, some of which may not be of sufficient discriminatory power for germplasm evaluation, characterization and management (Maji & Shaibu, 2012). In such a case, principal component analysis (PCA) may be used to reveal patterns and eliminate redundancy in data sets as morphological and physiological

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variations routinely occur in crop species (Maji & Shaibu, 2012). Hotelling (1933) indicated that PCA is an exploratory tool to identify unknown trends in a multidimensional data set. For an eigenvalue greater than 1, comprising 70.8% of the total variation, the yield component was found to be an important contributor to describing the cowpea breeding lines. The first two PCA explained 34.4% of the variation and this was mainly due to the high positive loading coefficient of the number of seeds per pod, number of locules as well as pod length. These factors seemed to contribute to improved pod formation ascribing to the cowpea breeding lines' high yielding ability. The fourth PCA explained 57 % of the total variation and this was mainly due to the high positive loading of the number of pods per peduncle. However, the findings of the present conforms to an evaluation by the International Center for Tropical Agriculture (CIAT, Centro Internacional de Agricultura Tropical) Germplasm Bank on 306 common beans which found that about 43% of the variation was made up of the first three components (Singh, Gepts, and Debouck, 1991).

At PC5, days to 50% maturity was observed to be the major contributor at 62.4% of the total variation, whereas grain yield was the major contributor at 70.8% of the total variation at PC6. The terminal leaf area was observed to be the major contributor at PC8. Similarly, an observation by Doumbia, Akromah, and Asibuo (2013) suggested that the most effective characters for distinguishing among cowpea accessions include days to 50% flowering, days to 50% maturity, seed weight, plant height, pod length. However, Chiorato, Carbonell, Colombo, Dias, and Ito (2005) suggested that the greatest loading coefficient in the last component indicated a redundancy of the descriptor (trait) associated with the component and therefore, the

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terminal leaf area may be described as redundant in the characterization of the lines evaluated.

Cluster analysis provides more information about relatedness among the cowpea breeding lines. It substantiated the existence of diversity among the breeding lines for 12 agronomic traits studied. The most divergent among the cowpeas were the parents IT97K-499-35, UCS01, GH3684, SARC-1-57-1 and PADI-TUYA. Asare *et al*., (2013) reported IT97K-499-35 and GH3684 as most diverged and highly discriminated from other genotypes considered. The clustering observed in this study showed that most individual progenies were grouped according to the population to which they belong, indicating even though the population has the same parental donor (GH3684), the breeding lines may differ genetically from each other. However, the common parental donor GH3684 may cause some common characteristics of the progenies to influence the clustering observed in this study.

3.4 Conclusion

Variations in quantitative and qualitative characteristics exist among the cowpea breeding lines. The current study unveiled that pod length, number of peduncles, number of pod per peduncle, days to 50% flowering and maturity, raceme position, pod length, 100 seed weight, seed coat colour and immature pod pigmentation may be essential heritable traits for selection of breeding lines. The diversity and improvement among the cowpea progenies showed that using a single donor GH3684 to cross different recipient parents introduces wide variations. This study has brought to light the rich source of genetic diversity harbored in the parental donor GH3684, thus leading to significant phenotypic differences (such as seed size, seed colour, early

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maturity) among the populations. Breeding lines showed improved seed size traits better than released varieties on the market.

This study revealed that F_5 breeding lines comprising UC15- 02, UC15-03, UC15-06, UC15-07, UC15-15, UC15-17, UC15-18, UC15-24, UC15-25, UC15-29, UC15-30, UC15-31, UC15-35, UC15-36, UC15-37, UC15-38, UC15-41, UC15-43, UC15-45, UC15-46 and UC15-47 could be selected for their distinctive improved agronomic traits for further evaluation.

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CHAPTER FOUR

PHENOTYPIC SCREENING AND MARKER-ASSISTED SELECTION OF *STRIGA GESNERIOIDES* RESISTANCE AMONG NOVEL COWPEA BREEDING LINES

4.1 Introduction

Cowpea plays an essential role in Africa, especially among smallholder farmers, for nutrition and as a source of income (Rusike *et al.*, 2013). Cowpea production in Ghana's major production regions of the North has been challenged by biotic and abiotic factors. Among the biotic factors, *Striga gesnerioides* is the most devastating, causing approximately 100% yield loss depending on the severity (Asare *et al.*, 2013). No control method seems to be sufficient to curb the effect of this parasitic weed, except host plant resistance (Rodenburg *et al.*, 2016). Some *Striga* resistant varieties of cowpea have been developed in Ghana, but there is the need to improve diversity, resilience and seed size to meet farmer and consumer satisfaction to sustain the cowpea industry. Breeding for resistance to various races of *Striga gesnerioides* with improved seed size and yield will be the most reliable solution to control the parasitic weed. Plant breeders, however, often need to screen a large population of cowpea to identify resistant lines. This process is vital to have a successful breeding program to generate resistant varieties. *Striga* resistance screening can be performed in the field to facilitate pre-selection of adaptable breeding lines or in the greenhouse, which takes advantage of relatively controlled environmental conditions (Ejeta, 2007). However, the integration of Marker-assisted selection (MAS) in breeding for *Striga* resistance can shorten the breeding cycle and enhance precision in selecting desirable

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progenies as it directly targets the genotype without the influence of the environment to speed up the conventional selection procedures (Collard *et al.,* 2005). This allows breeders to focus on fewer high-priority lines in subsequent generations (Sreewongchai *et al.*, 2010; Matthayatthaworn *et al.*, 2011). Several molecular markers linked to target traits have been developed for cowpea to shorten the breeding cycle and enhance the selection efficiency of breeding programmes' to improve cowpea varieties in West Africa (Sreewongchai *et al.*, 2010; Matthayatthaworn *et al.*, 2011). The use of simple sequence repeats (SSRs) has significantly contributed to the development of genetic linkage maps for many important crop species, including cowpea (Fatokun, Perrino, & Ng, 1997; Menendez, Hall & Gepts 1997). Li, Lis, and Timko (2009) identified a Simple Sequence Repeat (SSR-1) marker that co-segregates with *S. gesnerioides* race 3 (SG3) resistance. The SSR-1, C42-2B, CLM1320, 61RM2 and LRR11 are known to have a varied range of discriminating ability in identifying *Striga*-resistant and susceptible cowpea genotypes in Ghana (Asare *et al.*, 2010; Essem *et al.*, 2019). SSR-1 is present in all cowpea cultivars resistant to SG3 but absent in SG3-susceptible genotypes. Whereas the SCAR (61RM2) marker has been reported to be linked to a cluster of resistance loci for *S. gesnerioides* races SG1, SG3 and SG5 (Li *et al.*, 2009) and C42-2B linked to SG5. These markers can be described as highly polymorphic, robust, automated, require a small quantity of DNA, co-dominant, and excellent for MAS use (Nadeem *et al.*, 2018).

The *Striga*-resistant landrace, GH3684, was used to introgress the resistant gene into local cultivars with large seeds to develop F_4 breeding lines, which require phenotypic and genomic analysis to track and identify *Striga*-

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resistance breeding lines. Therefore, the objective of the current work was to phenotype and genotype novel cowpea breeding populations to select *Striga*-resistant progenies and validate the discriminatory efficiency of some SSR markers.

4.2 Materials and Methods

4.2.1 Experimental materials

Fifty (50) F_4 breeding lines of cowpea developed in the Molecular Biology and Biotechnology department, University of Cape Coast, were obtained with five parental genotypes. Twenty (20) of the cowpea breeding lines were derived from a cross between GH3684 (resistant parent) and UCSO1 (susceptible parent), nine (9) were from a cross between GH3684 (resistant donor parent) and IT97K-499-35 (resistant recipient parent), nine (9) from a cross between GH3684 (resistant parent) and PADI-TUYA (susceptible parent) and twelve (12) from a cross between GH3684 (resistant parent) and SARC-1-57-1 (susceptible parent).

4.2.2 Field screening experiment

The field experiment was carried out under a rain-fed condition during the (July - September 2019) farming season. Fifty (50) progenies and five (5) parents were evaluated on a heavily infested *Striga* field at the Council for Scientific and Industrial Research - Savanna Agricultural Research Institute (CSIR-SARI) in Bawku, Manga Research Station (11^0 - 0'53" N, 0^0-15'55 W, Altitude 220 M) (Figure 4.1). The cowpeas were evaluated in an augmented block design as used by Santos, Bearzoti, Ferreira and Silva (2002); Federrer (2005). There were 5 blocks, each consisting of 12 cowpea progenies (test entries) and five parental lines (check entries). Each cowpea breeding line was

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represented by a plot size of 2 x 0.75 m. Three seeds were sown per hill at a 20 cm x 75 cm spacing and thinned to two plants per hill at two weeks after sowing. In each block, the checks (parental genotypes) were allotted randomly. Refilling was done within 7 – 10 days after planting. All other agronomic practices such as weed control, insecticide application, and reshaping of ridges were applied equally to all plots. Data were collected from ten randomly selected plants on morphological, yield and yield contributing parameters. Cowpeas were harvested when matured pods were fully dried. The pods were further dried and threshed.

Quantitative and qualitative data were taken at six weeks after sowing and after harvesting (Table 4.1). the following variables were scored; Plant height and canopy diameter were measured using a meter rule and number of branches at maturity, number of pods per peduncle, number of peduncles, number of plants with *Striga, Striga* count per plot, *Striga* count per plant, *Striga* count at maturity, days to *Striga* emergence, days to 50% flowering and days to 50 % maturity. Grain yield and 100 seed weight were determined by weighing grains. Leaf colour, seed coat colour and flower colour were recorded by visual estimation.

Figure 4.1: Cowpea field at *Striga* hotspot in CSIR-SARI, Manga station

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4.2.3 Pot screening experiment

Collection and Processing of S. gesnerioides seeds

Cowpea field severely infested with *Striga* were identified in and around communities in the Binduri district of the Upper East region of Ghana (Figure 4.2). Mature and dried floral parts of the *Striga* plants with intact healthy capsules were harvested (Figure 4.3B) into sacks and transported to the Manga research station (SARI-CSIR) for drying and threshing.

The harvested floral parts were spread and exposed to dry in the sun (Figure 4.3C) and threshed and further screened by passing it through laboratory sieves of 2.0 mm, 0.5 mm, 0.18 mm and 0.15 mm aperture to ensure that subsequent infestations with the seed were more accurate. Most of the *Striga* seeds were collected on the 0.15 mm sieved (grade one), labelled and stored in paper bags at room temperature for 3 months to break seed dormancy.

Soil Sterilization

The drum method of soil sterilization by Leandre (2018) was employed in this experiment. Sandy loam soil of ratio 2:1 (sand and loam, respectively) was used. The 90 x 60 cm metal drum was partitioned into two parts with a metal mesh; a lower one-third and an upper two-thirds. The lower part of the drum was filled with water. Two jute sacks were placed on the metal mesh to separate the water (lower part) from the upper part. The upper of the drum was filled with sandy-loam soil to the brim. The soil was then covered with more jute sacks. Heat was applied to the drum from the bottom until the water boiled. The heating was continued until the steam from the boiling water rose through the soil to the brim. The steam sterilization continued for I hour 40

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minutes at 95 °C and allowed to cool to 0 °C. The soil was transferred into perforated plastic buckets..

Figure 4.2: Striga infested cowpea field in Binduri. White Circles shows (matured) dried Striga gesnerioides plant.

Figure 4.3 Harvesting and drying of *Striga*. The white arrow pointed to the harvested *Striga* in a sac. A- Havesting of *Striga gesnerioides* plant; B- Packing of *Striga gesnerioides* plant; C- Drying of *Striga gesnerioides* plant

Pot Culture Screening of Cowpea against Striga gesnerioides

Two hundred and one (201) perforated plastic pots of diameter 20 cm base, 30 cm top and 35 cm height were filled with sterilized soil following the method used by Botanga and Timko (2006). The pots were filled with soil to about two-thirds. One-third of soil-filled pots were inoculated with 2.5 g of the *Striga* seeds. The pots were then arranged in a randomized complete block design in three replications and labeled. Four seeds of each cowpea breeding

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line were sown per pot. The seedlings were thinned out and two plants were maintained per pot at two weeks (14 days) after planting. The soil was kept moist by watering regularly or when necessary. Six (6) weeks after planting, plant height and canopy diameter were recorded. Days to 50 % flowering and maturity were also recorded. Number of peduncles per plant, number of pods per peduncle and number of pods per plant were recorded on the 8th week. Days to emergence of *Striga* and number of *Striga* per pot were recorded.

At maturity, destructive sampling was used to assess the attachment of *Striga*. Each plant-soil mass was removed from each pot, immersed into a bowl of water and gently agitated to loosen the soil mass. The roots were washed thoroughly free of soil and examined for attachment of *Striga gesnerioides* and tubercles. Plants with *Striga* attachment were recorded susceptible (S) and those that had no visible *Striga* attachment were categorized as candidate resistant (R) lines.

4.2.4 Molecular Screening

DNA extraction

DNA was extracted using a modified CTAB protocol as described by Doyle and Doyle (1987). Fresh young leaves were harvested from two weeks old plants and submerged immediately into 20 ml of absolute ice-cold ethanol. 200 mg of fresh young leaf samples from each cowpea breeding line were ground with a mortar and pestle to a fine powder and transferred into 2 ml microfuge tubes. Eight hundred microliters of 2 % CTAB with 0.1% of mercaptoethanol was added to the ground leaves**.** The samples were incubated in a 65 ^{o}C recirculating water bath for 30 minutes with intermittent vortexing. The sample was cooled at room temperature and 800 µl of chloroform isoamyl

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alcohol (24:1) was added and mixed slowly by several inversion of the tube. The sample was then centrifuged at 14000 rpm for 15 min and the aqueous phase of the sample was transferred into a clean 1.5 ml tube. 400 µl of ice-cold isopropanol was added to the sample and shook gently and kept at -20 ºC for 2 hours to precipitate the nucleic acid. The sample was centrifuged at 14000 rpm for 5 min to pellet the nucleic acid. The isopropanol was decanted and the pellet was washed with 500 µl of washing buffer on a rocking surface for 15 min and centrifuged at 6000 rpm for 4 min. The washing buffer was also decanted and the pellet was washed in 400 µl 80 % ice-cold ethanol and then centrifuged at 6000 rpm for 4 min. The ethanol was decanted and the pellets were dried at 37ºC for 10 minutes till the smell of ethanol was no longer detectable. DNA was stored in 100 µl molecular grade water. To determine the quality of DNA, 1.0% Agarose gel was prepared with 0.03 % ethidium bromide. Five microliter of the genomic DNA sample was pipetted and 1µl loading buffer was added. The sample was loaded in the wells on gel submerged in 1 x TBE buffer. The sample was run at 90 volts, 120 AMP and 50 W for forty-five minutes and photographed under UV light. A working solution of 50 ng/µl for each sample was prepared for downstream application.

Primer dilution

Four (4) Simple Sequence Repeat (SSR) primer; SSR-1 (Li & Timko, 2009), LRR8, LRR11 (Essem *et al.*, 2019) and CLM1320 (Badiane *et al.*, 2012; Essem *et al.*, 2019) and two SCAR markers 61RM2 (Omoigui, Ishiyaku, Gowda, Kamara, & Timko, 2015) and C42-2B that are known to be associated with *Striga* resistance were ordered from Metabion International AG, Germany. The primers were spun with a short spin using a centrifuge

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before the tubes were opened. This was to ensure that the dislodged pellet caused by the shipping would settle to the bottom of the tube. A master stock (100 µM) of the primers were prepared using the formula; 100 µM= X nmoles lyophilized primer + (X × 10 µl molecular grade H_2O)

The master stock primers newly suspended in the molecular grade water were kept at room temperature for 10 minutes and well mixed before they were used for working stock dilution. The primer master stocks were diluted with molecular grade water in the ratio 1:10 to form the working solution (10 µM) and stored at 4 ^{o}C. The sequences of primers used are listed in Table 4.1.

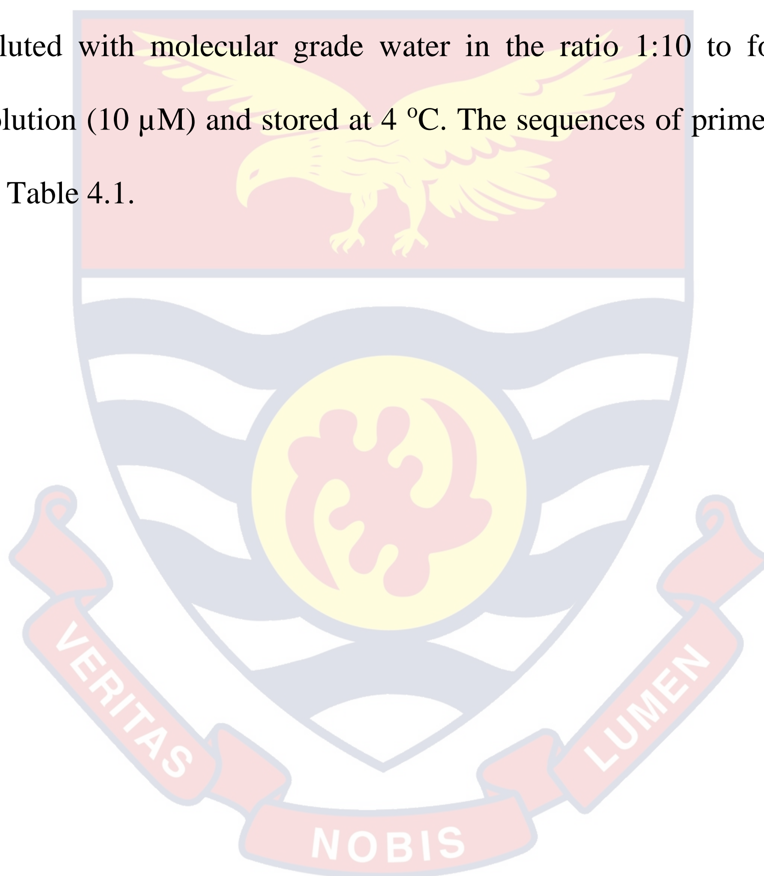

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Table 4.1: Simple sequence repeat (SSR) and sequence characterized amplified region (SCAR) markers

Marker name	Primer sequences		Annealing Temperature	*Striga* race specificity
	Forward (5'–3')	**Reverse (5–3')**		
SSR-1	CCTAAGCTTTTCTCCAACTCCA	CAAGAAGGAGGCGAAGACTG	57.7°C	Linked to SG3
LRR8	CATTCATCCACTCTCTTCCC	CCTTTGGTCATTGAATACATG	55°C	Unknown
C42-2B	CAGTTCCCTAATGGACAACC	CAAGCTCATCATCATCTCGATG	60°C	Linked to SG5
CLM1320	CACAACTTGCAACAACATGC	TACGTGGATCTGGTCTTTCC	55°C	Linked to SG-GH
LRR11	GGTAGCTCCTCTGTTGATTCAG	CATATGTCCAACCATTGCCAC AG	60°C	Unknown
61RM2	GAT TTG TTT GGT TTC CTT AAG	GGT TGA TCT TGG AGG CAT TTT	55°C	Linked to SG1, SG3, SG5

NB: [a]- Li and Timko (2009); Asare *et al.* (2013); and Larweh (2017); Gowda and Timko (unpublished), Essem *et al.*, (2019).

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PCR analysis

Each PCR reaction mixture (One Taq Quick-Load 2 x Master Mix with Standard Buffer from, "New England BioLabs®" contained 2 µl of 1x *Taq* buffer, 0.5 µl of 200 µM dNTPs, 0.5 µl of 1 unit *Taq* polymerase, 1 µl each of 1 µM forward and reverse primers, 1 µl of 50 ng genomic DNA and 14 µl Molecular Grade distilled water (MGDw) to make up a 20 µl total volume. Each of the six SSR and SCAR primers was used to amplify the DNA samples extracted from the 50 cowpea breeding lines and five parental lines. PCR amplification was carried out in "BIO RAN T100^{TM} thermal cycler (USA)". PCR conditions consisted of initial denaturation at 95 °C for 3 minutes, denaturation at 95 °C for 30 seconds, annealing at temperatures (Table 4.1) for each primer for 30 seconds and extension at 72°C for 30 seconds. This cycle was repeated 35 times and a final extension at 72°C for 5 minutes. The PCR products were further run on horizontal 2% Agarose gel electrophoresis to separate and resolve the bands.

Gel Electrophoresis and scoring

The 2 % Agarose gel was cast in a tray (27.5 cm X 24.5 cm) with barriers to retain gel and 15 well-forming combs were inserted to create wells. Forty millilitres of the agarose gel was prepared by dissolving 0.8 g of the agarose in 40 ml of ×1 TBE buffer. The mixture was stained with 3.0 µl of ethidium bromide. The mixture was then poured into the tank and distributed uniformly across the whole surface without trapping bubbles and the mixture was allowed to solidify. The whole assembly was transferred into the electrophoresis tank submerged in ×1 TBE buffer and the comb was removed. The PCR products were loaded into the wells. During loading, care was taken

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to avoid puncturing the gel' skirt.' of agarose. A 100-bp DNA ladder (N0551S) from Bioneer Biotechnology company® (USA) was used as a molecular weight-sized marker for each gel alongside the DNA samples from the progenies and the parental lines. The electrophoresis tank was covered with the lid. The PCR products were resolved for 45 minutes at 90 V, 50W and 120 mA and visualized on an "Accuris™ UV Transilluminators (USA)". DNA bands that corresponded to the marker's product size were scored present (+) and where no visible DNA band corresponded to marker were scored absent (-). The bands were photo-documented with a digital camera (Samsung J7 Neo – E4#10-1, China).

4.2.5 Data analysis

The quantitative data collected were subjected to Analysis of Variance (ANOVA) using R studio software version 3.6.0. Varietal means were compared using the Least Significant Difference at a 5% probability level (LSD 5%).

The molecular data matrix was subjected to analysis involving the Unweighted Pair Group Method of Arithmetic Averages (UPGMA) using Power marker version 3.5. The resulting dendrogram was generated in Molecular Evolution Genetic Analysis (MEGA) 4 software.

4.3 Results

4.3.1 Field and pot culture screening

Results for means value of qualitative variables are shown in Table 4.2 Days to maturity ranged from 51.8 to 68.1 days with a mean of 57.87 ± 3.99. Days to flowering ranged from 28.77 to 50.1 with a mean of 38.37 ± 4.51. Plant height ranged from 12.26cm – 26.99 cm with mean of 19.79 ± 4.08.

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Canopy diameter 9.45- 184.96 cm with mean of 78.78 ± 41.38. Days to *Striga* emergence ranged from 52– 70.73 with mean of 62.36 ± 9.11. *Striga* count per plot ranged from 0-34 with a mean of 3.15 ± 9.09. *Striga* count at maturity ranged from 0-34 with a mean of 3.096 ± 9.09. Number of pod per peduncle 1.13 - 2.3 with a mean of 1.79±0.288. Number of pods per plant 2.44 – 21.9 with a mean of 8.55 ± 3.27. 100 seed weight (g) ranged from 13.25 - 25 with a mean of 19.4±3.39 and grain yield (kg/ha) ranged from 0.41 -3.13 t ha^{-1} with a mean of 1.6t ± 0.7. The highest value of co-efficient of variation (CV) was found among the *Striga* response parameters. The co-efficient of variation among cowpea breeding lines was greater than 1 (CV% > 100). *Striga* count at maturity had the highest percentage co-efficient of variation of 325.4%. Moreover, among the agronomic traits, canopy diameter recorded the highest variation (64.02%), followed by seed weight of 40.2 % and pod per plant of 28.32%. Days to maturity had the lowest co-efficient of variation of 2.6%.

The analysis of variance (Table 4.3) revealed a significant (P < 0.05) mean sum of squares among the traits for different sources of variation. Block effect (ignoring treatment) was significant (P < 0.05) for days to maturity, days to 50% flowering and plant height. Treatment effects (eliminating blocks) were significant (P < 0.05) for 100 seed weight, days to maturity, days to 50% flowering, plant height, and pods per peduncle. Similarly, effects due to checks and progenies (test) were significant (P < 0.05) for 100SW, grain yield, days to maturity, days to 50 % percent flowering and plant height. The mean squares for progenies versus parents (Table 4.3) were not significant (P > 0.05) for all the traits except days to 50 % flowering and plant height. The standard errors of difference (Appendix B) indicate that the number of

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progenies that surpassed the best check was 3 (days to 50 % maturity), 5 (days to 50 % flowering), 9 (plant height), 6 (number of peduncles), GH3684 was the best check for all traits except days to 50 % flowering (SARC-1-57-1) and 100-seed weight (UCSO1) (Appendix B).

There was a high phenotypic co-efficient of variations in *Striga* emergence. About 27 % of the cowpea breeding lines were susceptible to *Striga* on the field. *Striga* emergence delayed on the field; the earliest was 52 days after sowing. PADI-TUYA recorded the highest *Striga* count at maturity, followed by UC15-05, UC15-03, UC15-40 and UC15-32. However, there was no significant ($P > 0.05$) effect of *S. gesnerioides* on the growth and yield of cowpeas in this study (Table 4.3).

Grain yield, number of plants with *Striga*, plant height, canopy diameter, days to *Striga* emergence, *Striga* count per plant and *Striga* count at maturity showed high phenotypic co-efficient of variations among the cowpea breeding lines (Table 4.4). Genotypic coefficient of variation (GCV) was high for number of pods per plant, plant height and days to *Striga* emergence (Table 4.4).

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Table 4.2: Descriptive statistics of quantitative traits of fifty-six (56) cowpea genotypes

Trait	Mean ± SD	Range	CV%
100 seed weight	19.409 ± 3.387	13.25 – 25	12.087
Grain yield	1619.69 ±678.612	410.14 – 3126.8	40.203
Days to 50% Maturity	57.871 ± 3.99	51.8 – 68.133	2.644
Days to 50% Flowering	38.37 ± 4.514	28.766 – 50.1	4.805
Plant Height	19.795 ± 4.079	12.26 – 26.99	14.487
Canopy Diameter	78.776 ± 41.384	9.45 – 184.96	64.017
Pod weight	2248 ± 945.23	690 -7917	39.777
Number of Pod per peduncle	1.79 ± 0.288	1.13 – 2.3	9.67
Number of pod per plant	8.555 ±3.279	2.44 – 21.9	28.316
Days to *Striga* Emergence	62.365 ± 9.112	52– 70.73	79.421
***Striga* Count Per Plot**	3.149 ± 9.086	0 – 34	321.675
***Striga* Count at Maturity**	3.096 ± 9.086	0 – 34	325.401

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Table 4.3: Analysis of variance of quantitative traits of fifty-six (56) cowpea genotypes.

Sources of variation	Df	100S WT	GY	DM	DFF	PH	CD	Pod/ Plt	Pod/P en	No. of Plt With *Striga*	*Striga*/ Plot	*Striga* At Mat.	Days To *Striga* Emerg.
Block (ignoring treatment)	4	14.05	1102x10^3	20.77***	15.36**	24.11*	4615	0.794	0.05	1.14	97.19	98.97	542.7
Treatment (Eliminating blocks)	55	15.69**	592x10^3	16.54***	21.10***	21.44**	1741	9.714	0.08**	0.56	81.76	79.57	549.7
Checks	5	57.72***	1832x10^3*	14.75**	31.89***	55.47***	3759	9.376	0.06	0.83	95.66	95.66	204.1
Test	49	10.72	495x10^3	16.27***	21.41***	17.30*	1676	9.455	0.09**	0.47	79.31	79.31	589.6
Test (progenies) Vrs Check (parents)	1	70.72	126x10^3	96.90***	37.45**	96.22**	93	1.896	0.22	0.04	25.71	25.71	867.4
Residuals	20	5.16	484x10^3	2.303	3.36	7.84	2826	6.036	0.03	1.23	108.26	108.26	321.9

- *Significant codes: '***'0.001; '**' < 0.01; '*' <0.05.* GY – Grain yield; PWT – Pod weight; DM- Days to Maturity; DFF – Days to first Maturity; PH – Plant height; CD – Canopy diameter; 100SWT- Hundred Seed Weight; Pod/Pen- Number of Pod per peduncle; Pod/Plt- Number of Pod per plant (Field data, 2019)

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Table 4.4: Phenotypic and genotypic coefficient of variation of fifty-six (56) cowpea genotypes

Trait	PCV	Category	GCV	Category
100SWT	16.866	Medium	12.147	Medium
GY	39.17	High	20.28	High
DFF	12.05	Medium	11.070	Medium
DM	6.97	Low	6.460	Low
No. Plt With *Striga*	241.17	High	NA	NA
POD/PEN	16.27	Medium	12.93	Medium
POD/PLT	35.94	High	21.62	High
PH	21.012	High	15.54	High
CD	51.974	High	NA	NA
Days To Str. Emergence.	222.949	High	150.22	High
Striga count per plant	282.791	High	NA	NA
Striga count at Maturity	283.464	High	NA	NA

GY – Grain yield; DM- Days to maturity; DFF – Days to first Maturity; PH – Plant height; CD – Canopy diameter; 100SWT- Hundred Seed Weight; POD/PEN- Number of Pod per peduncle; POD/PLT- Number of Pods per plant. (Field data, 2019)

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Figure 4.4: Striga gesnerioides infestation of cowpeas under field conditions, 7 weeks after sowing at CSIR-SARI- Manga. A- Resistant cowpea progeny (UC15-43) on the field. B - Susceptible cowpea progeny (UC15-05) showing *S. gesnerioides* emergence. The yellow circle indicates the emergence of *S. gesnerioides* on the field.

4.3.2 Effects of *Striga gesnerioides* on cowpea in pot screening

Results showed that number of peduncles differed significantly ($P < 0.05$) among the cowpea progenies under pot screening (Table 4.5). Among all the cowpeas, UC15-24 recorded the highest average number of peduncles per plant (16 peduncles), closely followed by GH3684 with an average of 14. UC15-10, UC15-07, UC15-44 and UC15-27 followed, all having an average of 13 peduncles per plant. On the whole, 70 % of the resistant cowpea progenies recorded the highest average number of peduncles per plant. UC15-37 recorded the lowest number of the peduncle (Figure 4. 5). UC15-48, PADI-TUYA, UC15-18 and UC15-37 had the lowest average number of peduncle per plant.

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There was a significant difference (P < 0.05) in the number of pods per plant among the cowpea progenies. UC15-16 recorded the highest number of pods per plant. This was followed by UC15-04, UC15-01, and IT97K-499-35 with an average number of pods of 15, 14 and 13, respectively. UC15-29, UC15-49 and PADI-TUYA recorded the lowest average number of pods per plant (1). However, susceptible cowpea progenies had low peduncle and pod formation.

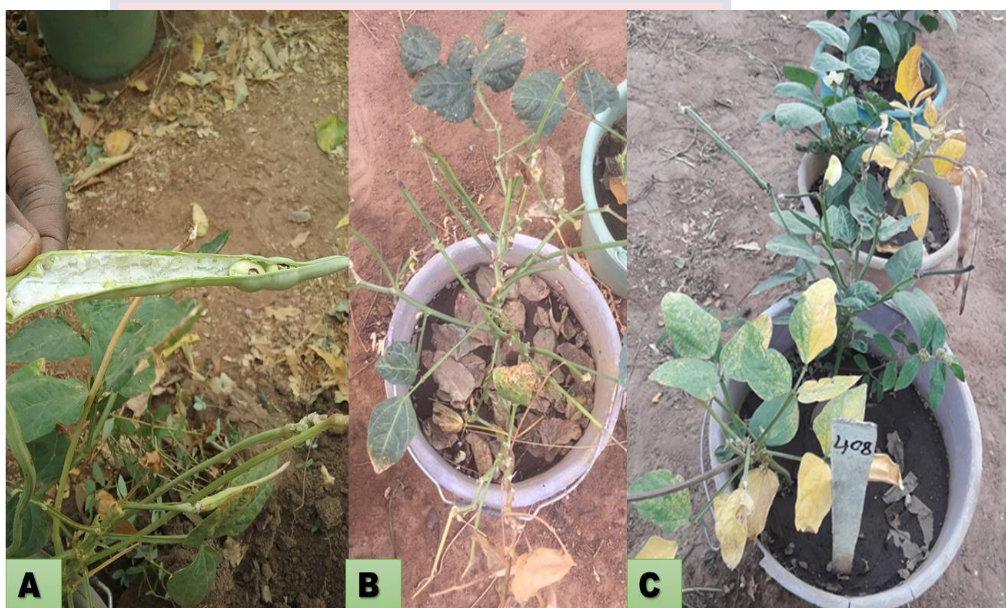

Figure 4. 6: Effect of S. gesnerioides on cowpea. A- poor pod formation; B- Low leave and peduncle formation ; C- Leaf necrosis and chlorosis

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Table 4.5: Mean values for agronomic trait and *Striga* status of cowpea progenies and their parent under pot experiment.

GENOTYPE	*Striga* Status	PH	CD	D50%F	NPED	NPOD	NPwPOD
GH3684	R	26.67	29.33	61	14	10	8
IT97k-499-35	R	30.67	39.00	64	12	13	7
PADI-TUYA	S	21.67	36.07	67	5	1	1
SARC-1	S	22.00	32.00	46	11	7	5
UC15-01	R	24.33	33.33	58	13	14	9
UC15-02	R	15.17	24.83	46	10	3	3
UC15-03	S	17.90	30.17	56	10	7	3
UC15-04	R	18.50	30.33	68	12	15	9
UC15-05	S	25.83	36.17	62	7	4	4
UC15-06	S	18.17	26.00	46	6	3	3
UC15-07	R	20.00	30.33	59	13	10	8
UC15-09	R	25.33	105.33	62	12	11	9
UC15-10	R	21.17	36.00	60	13	16	11
UC15-11	S	26.33	31.67	63	7	4	2
UC15-12	S	32.00	33.17	66	5	3	4
UC15-13	R	18.83	32.50	60	10	7	7
UC15-14	S	33.67	38.33	58	5	2	3
UC15-15	S	21.83	31.50	57	8	3	2
UC15-16	S	26.17	35.00	58	11	11	8
UC15-17	R	20.33	32.17	58	11	8	3
UC15-18	S	23.67	35.50	50	6	1	1
UC15-19	R	23.83	31.17	72	10	8	7
UC15-20	S	24.47	42.67	50	11	10	8
UC15-21	R	30.40	40.75	65	5	3	4
UC15-22	R	30.50	32.33	43	12	11	9
UC15-23	R	30.17	34.83	60	8	6	5
UC15-24	R	29.00	39.00	80	16	10	9
UC15-25	S	35.33	34.33	59	7	3	4
UC15-26	R	43.67	40.33	63	9	9	8
UC15-27	R	28.00	40.00	61	13	9	7

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Table 4.5 cont'd

UC15-28	S	22.83	32.17	62	12	11	9
UC15-29	S	23.83	30.17	56	6	1	3
UC15-30	S	27.50	38.00	60	9	4	3
UC15-31	S	22.83	32.00	46	5	4	3
UC15-32	S	28.00	36.67	66	8	5	5
UC15-33	S	28.50	41.50	59	5	3	3
UC15-34	R	34.17	40.67	57	7	4	3
UC15-35	R	27.33	34.40	67	8	7	7
UC15-36	S	33.33	42.27	66	3	2	2
UC15-37	S	24.17	34.00	59	5	2	1
UC15-38	S	35.00	39.50	64	11	4	6
UC15-39	S	21.00	33.33	64	9	4	4
UC15-40	S	23.83	33.50	65	8	3	3
UC15-41	R	26.00	26.50	60	8	6	5
UC15-42	S	35.33	31.17	62	8	10	5
UC15-43	R	26.00	29.33	47	11	9	7
UC15-44	R	19.67	30.00	70	13	11	6
UC15-45	R	19.33	30.67	47	9	7	6
UC15-46	S	34.17	42.00	41	7	3	3
UC15-47	R	27.80	42.00	57	9	3	3
UC15-48	S	41.83	36.33	45	4	1	1
UC15-49	R	39.00	31.07	64	9	11	8
UC15-50	S	30.07	34.83	53	5	3	2
UC15-51	S	30.07	40.63	61	7	4	2
UCSO1	S	26.42	36.78	69	10	3	3
Grand mean		26.71	35.9	58.95	8.90	6.27	4.95
P-value		<0.001	0.093	<0.001	<0.001	<0.001	<0.001
LSD		6.058	21.8	3.391	3.50	3.50	2.85
CV%		16.2	43.3	4.1	28.10	40.70	41.10

LSD, as indicated by Tukey's method. Df-113; rep-3; confidence level- 95%; PH – Plant Height, CD- Canopy Diameter; NPOD- number of pod per plant; NPED- Number of peduncles; NPwPOD – Number of peduncles with pod

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4.3.3 Assessment of phenotypes and genotypes associated with *S. gesnerioides* resistance

In all, 40 % of the cowpea progenies were *S. gesnerioides* resistant and 60 % were susceptible in pot screening test (Table 4.6). Cowpea breeding lines that were resistant to the parasite did not show the emergence of *S. gesnerioides* in the pots and on the field; there were no attachments of *S. gesnerioides* to the roots and no necrotic hypersensitive lesions on the roots when the roots were washed and examined (Figures 4.7C). Susceptible genotypes were characterized with germination and the emergence of *S. gesnerioides* seedlings on the surface of the in the soil pots (Figure 4.6B) and on-field or associated with tubercles attached to the roots (Figure 4.7A and 4.7B). The *Striga*-infested cowpea plants expressed varied symptoms, including stunted growth, leaf necrosis, chlorosis, senescence, reduced size of young leaves, poor flowering and poor pod and peduncle formation (Figure 4.6A and 4.6B). The resistant cowpea genotypes had normal growth and development without *Striga* attachment or emergence comparable to the resistant parental lines, GH3684 and IT97K-499-35 (Songotra) used as checks.

DNA profile indicated that band for SSR-1 and C42-2B markers were present in 29 of the cowpea progenies. Bands for the CLM1320, 61RM2, LRR8 and LRR11 were present among 30, 35, 26 and 28 progenies, respectively (Table 4.6).

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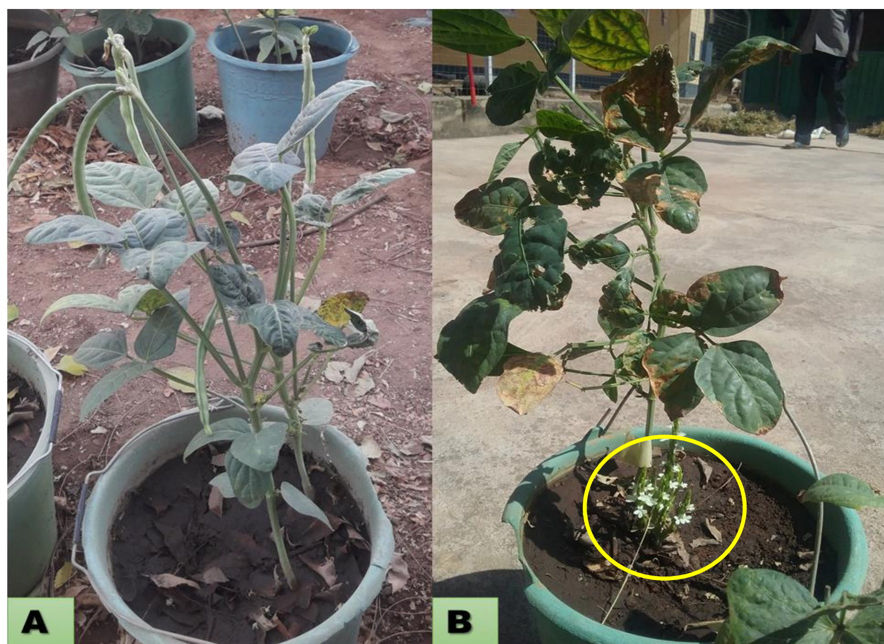

Figure 4.7: Cowpeas under pot screening test at 8 weeks after sowing. A- Resistant cowpea progeny (UC15-01) and B- Susceptible cowpea progeny (UC15-18) showing *S. gesnerioides* emergence in pot culture. The yellow circle indicates emerged *S. gesnerioides* in the pot.

Figure 4.8: Striga-infested and non-*Striga*-infested cowpea breeding lines, A and B – *Striga* seedlings and tubercules attached to roots of susceptible cowpea line. C – Resistant cowpea progeny (UC15-01).

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Table 4.6: ***S. gesnerioides*** **resistance profile of cowpea genotypes**

Cowpea genotypes	Field Screening	Pot Screening	Reaction of Molecular marker					
	Striga resistance Status	*Striga* resistance Status	SSR-1	C42-2B	CLM13 20	61R M2	LRR 8	LRR 11
GH3684	R	R	+	+	+	+	+	+
IT97K-499-35	R	R	+	+	+	+	+	+
UC15-01	R	R	+	+	+	+	+	+
UC15-02	R	R	+	+	+	+	-	-
UC15-03	S	S	-	-	-	+	+	+
UC15-04	R	R	+	+	+	+	+	+
UC15-05	S	S	-	-	+	+	-	+
UC15-06	R	S	-	-	-	+	-	-
UC15-07	R	R	+	+	+	+	+	+
UC15-09	R	R	+	+	+	+	+	+
UC15-10	R	R	+	+	+	+	+	+
PADI-TUYA	S	S	-	-	-	-	-	-
UC15-11	R	S	-	-	-	+	+	+
UC15-12	R	S	+	+	+	-	-	+
UC15-13	R	R	+	+	-	-	-	+
UC15-14	R	S	-	-	-	-	-	-
UC15-15	S	S	-	-	-	-	-	+
UC15-16	S	S	+	+	+	+	-	+
UC15-17	R	S	-	-	+	+	+	+
UC15-18	R	S	-	-	-	-	-	-
UC15-19	R	R	+	+	+	+	+	+
SARC-1-57-1	R	S	-	-	+	+	+	+
UC15-20	R	R	+	+	+	+	+	+
UC15-21	R	S	-	-	-	+	+	+
UC15-22	R	R	+	+	+	+	+	+
UC15-23	R	R	+	+	+	+	+	+
UC15-24	R	R	+	+	+	+	-	-
UC15-25	R	S	-	-	+	-	-	-
UC15-26	R	R	+	+	+	+	+	+
UC15-27	R	R	+	+	+	+	+	+

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Table 4.6 cont'd

UC15-28	R	R	+	+	+	+	+	+
UC15-29	R	S	+	+	-	+	+	-
UC15-30	R	S	-	-	+	-	-	-
UC15-31	R	S	-	-	-	-	-	-
UCSO1	S	S	-	-	-	-	+	-
UC15-32	S	S	+	+	+	+	-	-
UC15-33	R	S	-	-	-	+	+	-
UC15-34	R	R	+	+	+	+	+	-
UC15-35	R	R	+	+	+	+	+	+
UC15-36	S	S	-	-	-	-	-	-
UC15-37	R	S	-	-	-	-	-	-
UC15-38	R	S	+	+	-	+	+	+
UC15-39	S	S	-	-	-	+	+	+
UC15-40	S	R	+	+	+	-	-	-
UC15-41	R	S	-	-	-	-	-	-
UC15-42	R	S	+	+	+	+	-	-
UC15-43	R	R	+	+	+	+	+	+
UC15-44	S	S	+	+	+	-	-	-
UC15-45	R	R	+	+	+	+	-	-
UC15-46	R	S	-	-	+	-	-	-
UC15-47	R	R	+	+	-	+	+	+
UC15-48	S	S	-	-	-	-	-	-
UC15-49	R	R	+	+	+	+	+	+
UC15-50	S	S	-	-	-	+	+	+
UC15-51	S	S	-	-	-	+	-	-

R: Resistant, **S** : Susceptible, + : Marker Present, **-** : Marker Absent (Lab data, 2020)

4.3.5 Validation of Simple Sequence Repeat Markers Linked to *Striga gesnerioides* resistance among the cowpea breeding lines

The six markers, SSR-1, C42-2B, 61RM2, CLM1320, LRR8 and LRR11, co-segregated with the *Striga*-resistance allele as expressed by both cowpea progenies and parental genotypes with varied differential discrimination abilities to categorize the cowpea lines into *Striga*-susceptible and *Striga*-resistant genotypes. Cowpea progenies showing any of the markers indicated the presence of the resistance allele (s) of *Striga* (Figure

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4.8-4.19). The absence of a marker (-) denotes susceptibility to *Striga* and the presence of a marker (+) denotes resistance to *Striga* (Table 4.6) are clearly illustrated in Figure 4.8 to Figure 4.19. The product sizes of the six markers, SSR-1, C42-2B, 61RM2, CLM1320, LRR8 and LRR11, across the cowpea genome were 150 bp, 280 bp, 380bp, 380 bp, 680 bp and 550 bp, respectively. All DNA bands that corresponded to the product size of the markers were expected *Striga*-resistant cowpea genotypes.

Figure 4.9: DNA bands from PCR amplification products of LRR8 for progenies from a GH364 and IT97K-499-35 (Population 1) resolved in 2 % Agarose gel stained with ethidium bromide. GH - GH684, IT-IT97K-499-35, NT- Non template control, L- 100bp ladder (Lab data, 2020)

Figure 4.10: DNA bands from PCR amplification products of LRR8 for progenies from a GH364 and PADI-TUYA (Population 2) resolved in 2 % Agarose gel stained with ethidium bromide. P-PADI-TUYA, NT- Non template control, L- 100bp ladder (Lab data, 2020).

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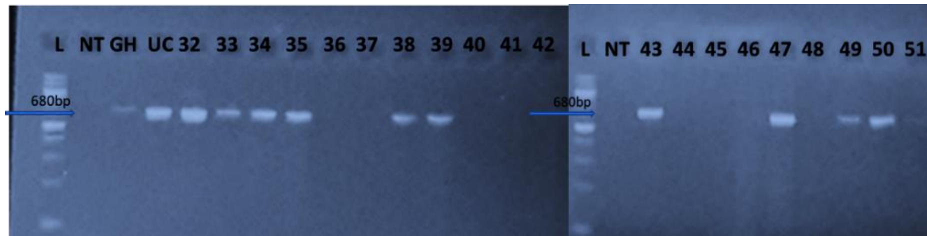

Figure 4.10: DNA bands from PCR amplification products of LRR8 for progenies from a GH364 and UCS01 (Population 4) resolved in 2 % Agarose gel stained with ethidium bromide. UC-UCS01, NT- Non template control, L-100bp ladder.

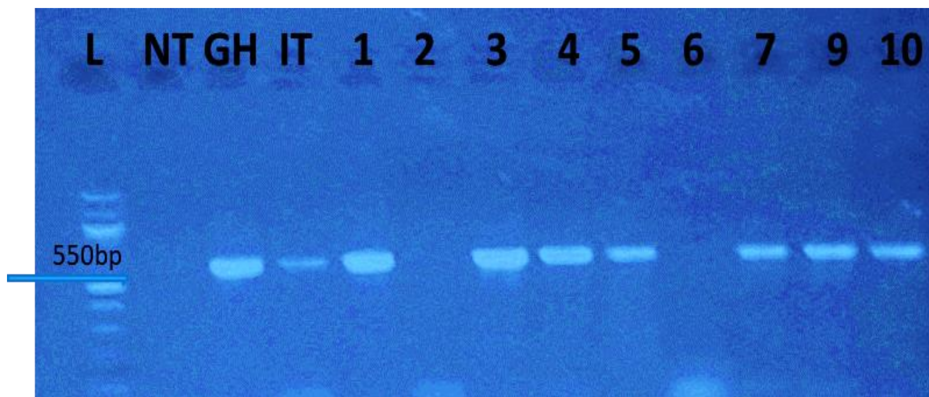

Figure 4. 11: DNA bands from PCR amplification products of LRR11 for progenies from a GH364 and IT7K-499-35 (Population 1) resolved in 2 % Agarose gel stained with ethidium bromide., IT- IT7K-499-35,NT- Non template control, L- 100bp ladder

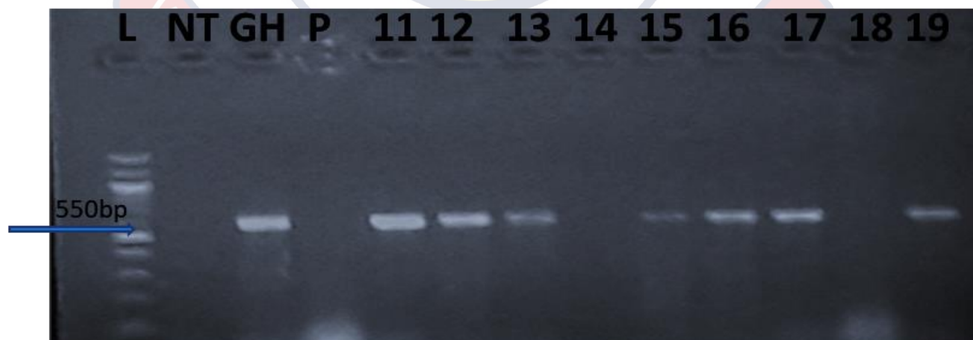

*Figure 4.12***:** DNA bands from PCR amplification products of LRR11 for progenies from a GH364 and PADI-TUYA (Population 2) resolved in 2 % Agarose gel stained with ethidium bromide. P-PADI-TUYA, NT- Non template control, L- 100bp ladder

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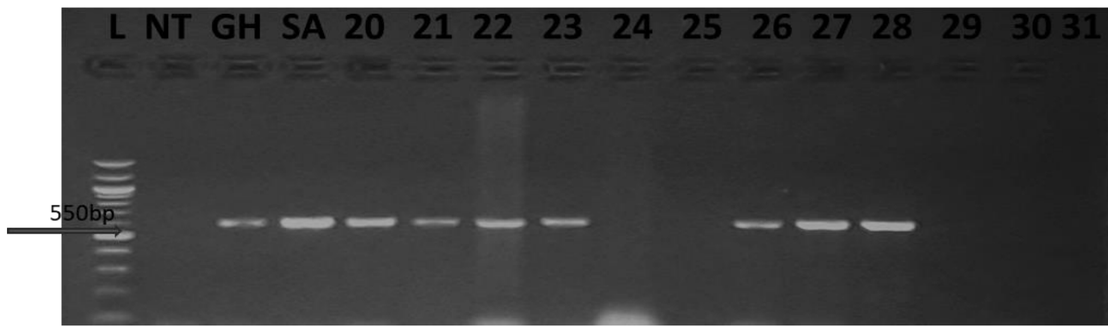

Figure 4.13: DNA bands from PCR amplification products of LRR11 for progenies from a GH364 and SARC-1-57-1 (Population 3) resolved in 2 % Agarose gel stained with ethidium bromide. SA-SARC-1-57-1, NT- Non template control, L- 100bp ladder.

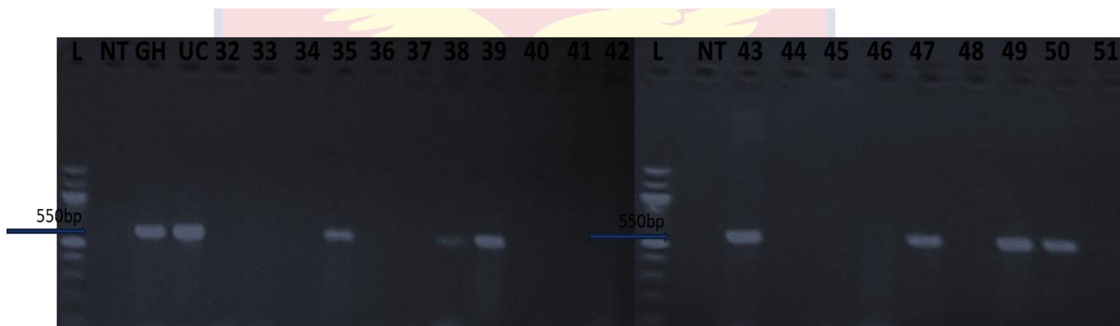

Figure 4.14: DNA bands from PCR amplification products of LRR11 for progenies from a GH364 and UCS01 (Population 4) resolved in 2 % Agarose gel stained with ethidium bromide. UC-UCS01 NT- Non template control, L- 100bp ladder.

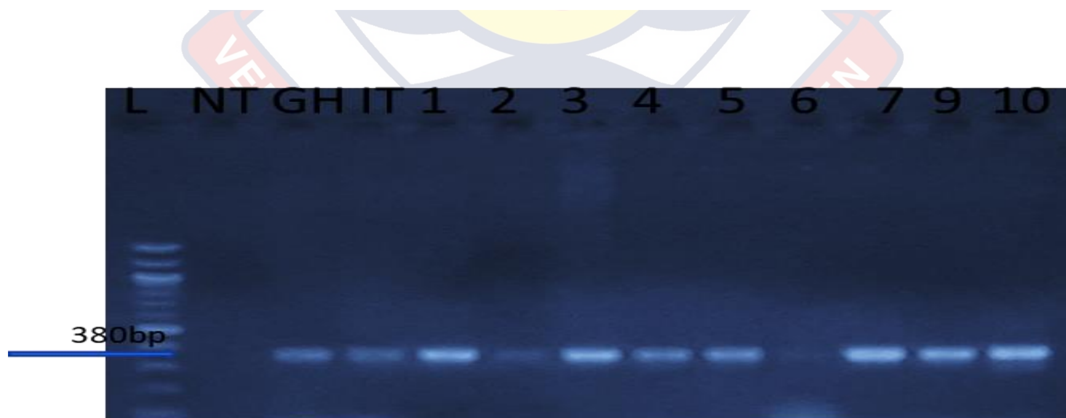

Figure 4.15: DNA bands from PCR amplification products of 61RM2 for progenies from a GH3684 and IT97K-499-35 (Population 1) resolved in 2 % Agarose gel stained with ethidium bromide., IT- IT7K-499-35,NT- Non template control, L- 100bp ladder.

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Figure 4.16: DNA bands from PCR amplification products of 61RM2 for progenies from a GH364 and PADI-TUYA (Population 2) resolved in 2 % Agarose gel stained with ethidium bromide, P-PADI-TUYA, NT- Non template control, L- 100bp ladder

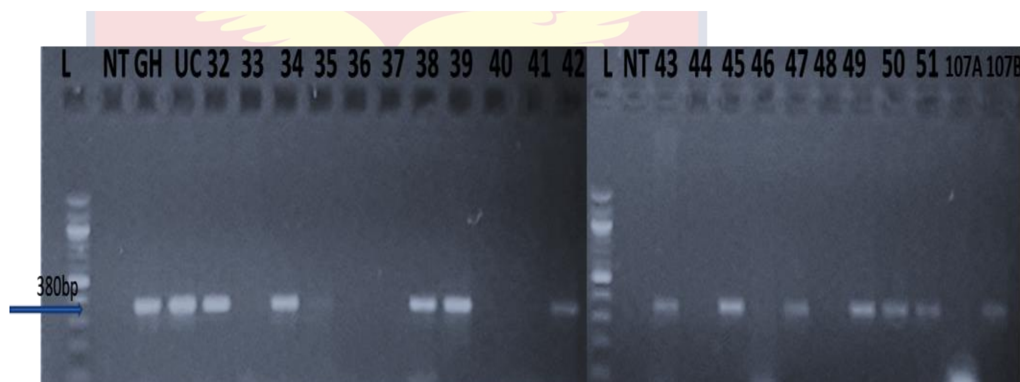

Figure 4.17: DNA bands from PCR amplification products of 61RM2 for progenies from a GH364 and UCS01 (Population 4) resolved in 2 % Agarose gel stained with ethidium bromide. UC-UCS01, NT- Non template control, L-100bp ladder.

Results for percentage discrimination efficiency of SSR and SCAR markers for the four population are presented in Table 4.8. Molecular markers studied exhibited different discrimination efficiency for *Striga* susceptibility and resistance across the genome of four cowpea populations (Table 4.7). SSR-1, C42-2B and LRR8 had 100 % discriminating efficiency for population 1 (GH3684 X IT97K-499-35). The lowest of 54.5% was scored for marker LRR11 population 2 (GH3684 X PADI-TUYA).

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Table 4.7: Percentage of discrimination efficiency of SSR and SCAR markers

Population	Discrimination Efficiency					
	SSR-1	C42-2B	CLIM1320	61RM2	LRR8	LRR11
1	100	100	90.9	81.8	100	90.9
2	81.8	81.8	63.6	65.3	72.7	54.5
3	78.5	78.5	64.3	64.3	57.1	64.3
4	81.8	81.8	77.3	68.2	68.2	72.7
Overall Efficiency	85.5	85.5	74.8	69.9	74.5	70.6

4.3.6 Phylogenetic analysis

The six *Striga*-resistant markers differentiated the 50 cowpea progenies and 5 parental genotypes into two major clusters and eighteen sub-clusters I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, XVII, XVIII (Figure 4.20). The eighteen sub-clusters had a varied proportion of recurrence of genotypes as well as marker discriminations (Table 4.9).

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Table 4.8: Cluster analysis of 55 cowpea genotypes

Cluster	Marker	Recurrence of genotype
I	SSR-1, C42-2B, 61RM2, LRR8	1
II	SSR-1, C42-2B, 61RM2, CLM1320, LRR8	1
III	SSR-1, C42-2B, 61RM2, LRR8, LRR11	2
IV	SSR-1, C42-2B, 61RM2, CLM1320, LRR11	1
V	SSR-1, C42-2B, 61RM2, CLM1320, LRR8, LRR11	17
VI	SSR-1, C42-2B, CLM1320, LRR11	1
VII	SSR-1, C42-2B, LRR11	1
VIII	SSR-1, C42-2B, CLM1320.	2
IX	SSR-1, C42-2B, 61RM2, CLM1320	5
X	61RM2, LRR8, LRR11	5
XI	61RM2, CLM1320, LRR8	1
XII	61RM2, CLM1320, LRR8, LRR11	2
XIII	61RM2, LRR8	1
XIV	LRR8	1
XV	61RM2	2
XVI	LRR11	1
XVII	CLM1320	3
XVIII	None	9

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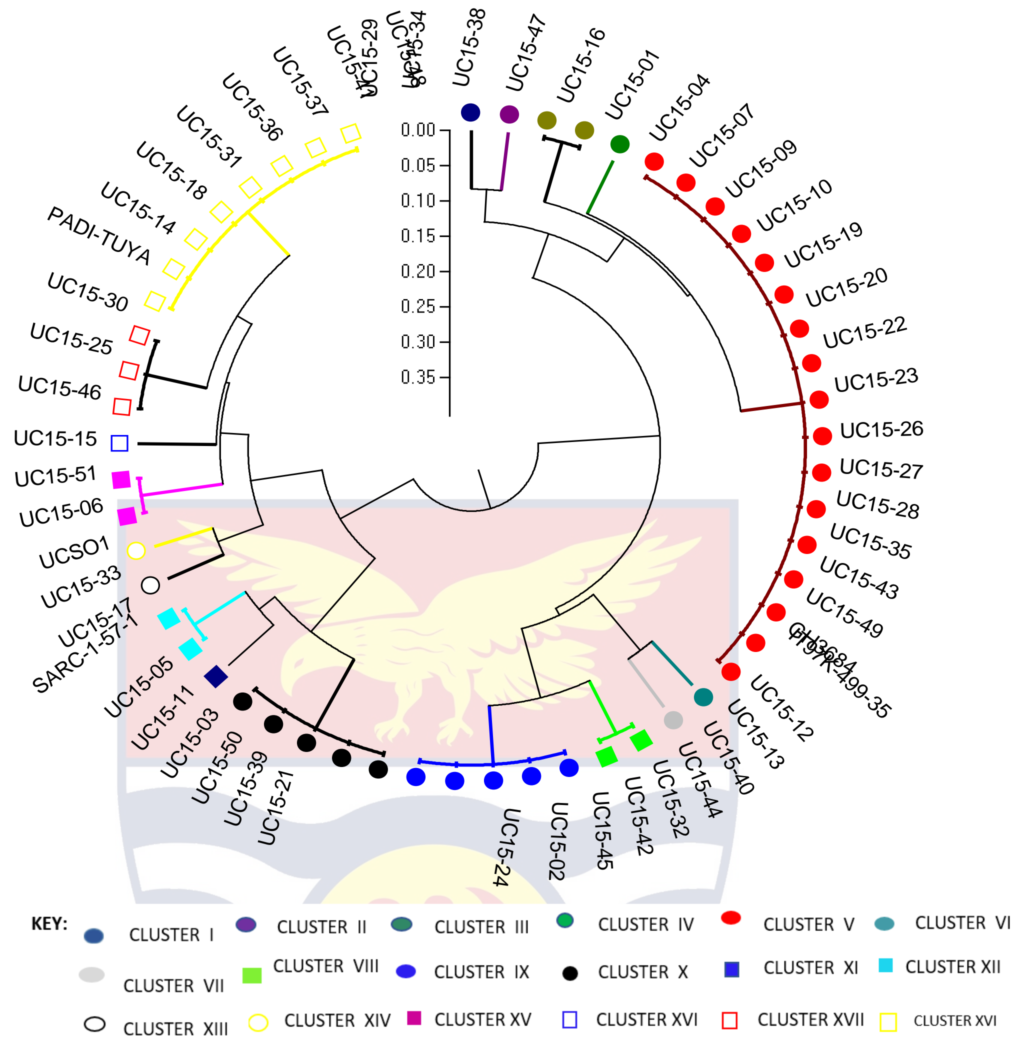

Figure 4.18: Phylogenetic relationship among 55 genotypes constructed using six informative *Striga*-resistant SSR markers with the sequential clustering algorithm (UPMGA) based on genetic similarity (Nei *et al.*, 1983) in power marker.

4.3.7 Linkage map analysis of *Striga*-resistant markers

The study revealed that all the *Striga*-resistant markers could be found on the same chromosome (9) (Figure 4.21). The six *Striga*-resistant markers and genes were linked across a total length of 63.6 cM (Figure 4.21). SSR-1 was found to be located at the same position as C42-2B on the chromosome. SSR-1 and C42-2B were 11.2 cM closest to the gene controlling the resistance of the *Striga* race collected from the Binduri district (GH-BINDURI). CLIM1320 was found to be closer to GH-BINDURI at 22.3cM. LRR11,

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LRR8 and 61RM2 were further away from GH-BINDURI resistance genes (Figure 4.21).

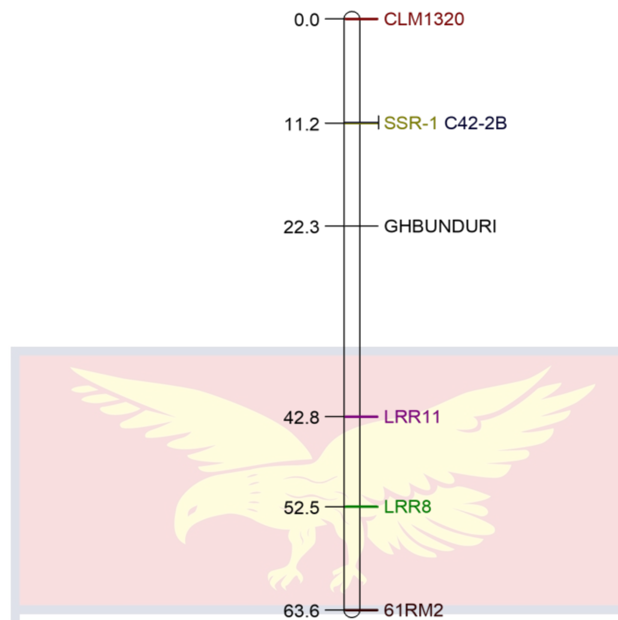

Figure 4.19: Linkage map construction output showing the position of the markers on chromosome (Lab data, 2020).

4.4 Discussions

4.4.1 Field evaluation

In the present study, 50 cowpea progenies (test entries) and five parents (checks) evaluated in an augmented block design expressed variations in growth, yield and responses to *Striga gesnerioides* infestation. The highest value of co-efficient of variation (CV) greater than 1 (CV% > 100) was found among the *Striga* response parameters. Among the agronomic traits, canopy diameter recorded the highest variation (64.02 %), followed by grain yield of 40.2%. The high co-efficient of variation observed among the *Striga* response parameters is due to the dispersion of the variables around the mean (Finlay& Wilkinson, 1963). This is due to variations or heterogeneity of the cowpeas with respect to their response to prevailing environmental conditions or perhaps the natural variations among progenies. Rana *et al.* (2015) evaluated

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4274 germplasm accessions of common beans from 58 countries and attributed observed substantial variability for the 22 traits studied to natural variation among samples. However, agronomic traits are affected by environmental factors (Baranov, Vinokurov & Fedorova, 2019); hence prevailing conditions such as climate change might have caused the progenies to naturally respond widely from mean, accounting to the observed high co-efficient of *Striga* response traits.

Consequently, analysis of variance revealed that the mean sum of square of the parents versus progenies was not significant (P > 0.05) for all the traits except days to 50 % flowering and plant height. The non-significant variation observed may be attributed to the single donor parent used to produce progenies. The mode of pre-selection of progenies for this study might be a factor since it was predominantly based on seed coat and seed size, leading to the elimination of highly contrasting progenies. This suggests that plant height and flowering responses among progenies differed from parents (checks), contrary to a report by Saba *et al.* (2017), where test entries were significantly different from checks except for days to maturity. The least significant increase (LSI) computed recommends the number of progenies that perform better than their best parental genotypes based on study traits. The present study observed that 23 progenies performed better than the best parent in terms of days to 50% maturity, days to 50 % flowering, plant height, and the number of peduncles, respectively. GH3684 was the best parent for all traits except days to 50 % flowering (SARC-1-57-1) and 100-seed weight (UCSO1).

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The emergence of *Striga* delayed on the field, with the earliest emergence at 52 days after sowing. *Striga* emergence is reported to occur at 38 to 48 days (Larweh *et al.*, 2019). PADI-TUYA recorded the highest *Striga* count at maturity, followed by UC15-05, UC15-03, UC15-40 and UC15-32. However, there was generally no significant ($P > 0.05$) effect of *S. gesnerioides* on parameters measured on cowpeas in the field study (Table 4.3). The high average rainfall (187 mm) followed by lower average temperature (21°C) observed at the time of the study (August -September) might have resulted in the observed delay of *Striga* emergence as well as the low severity of infestation on cowpea. Kust (1963) and Kuiper, Groot, Noordover, Pieterse and Verkleij (1998) reported that temperature below 25°C and above 35°C makes *Striga* seeds remain dormant and unable to germinate. Moreover, Singh, 2002 confirms that lower rainfall contributes to the severe effect of *Striga* on cowpea yields. Besides, shallow ploughing, resulting in un-uniform distribution or dissemination of *Striga* seeds and deep sowing during field setup, might have contributed to progenies escaping infestation. Ast (2006) and Gurney, Press and Scholes (1999), pointed out that the combination of shallow soil tillage, deep planting and the use of transplants in field conditions resulted in 85% reduction in *Striga*-infestation level. A study by Ast (2006) confirms that a delayed time of first *Striga* infestation contributed to the lower extent of yield reduction and other tolerant cultivar's agronomic performance. Low emergence of *Striga* and the low effects may result from too much rainfall or low *Striga* density on the field. However, it also suggests that some of the cowpeas have the potential to tolerate lower *Striga* infestation.

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Indeed, field screening under natural infestation is not always practical since parasite density and distribution on the field cannot be controlled (Hausamann *et al.* 2000). However, pot screening has become an alternate confirmation test. In this study, the number of resistant lines (including parents) observed in pot screening was reduced from 41 on the field to 22. This was due to the good control of the environment, uniformity, and high infestation of the *Striga* seeds. Tignegre *et al.* (2013) and Asare *et al.* (2013) emphasized the reliability of pot screening compared to field screening.

Ba (1983) reported that 'some cowpea genotypes stimulate the *Striga* to germinate and the haustorium penetrates the cowpea root tissues but failed to grow more. A similar observation was made by Lane (1996) in B301, parental source of *Striga* resistance in IT97K-499-35, the *Striga* seeds attached and formed haustoria but failed to grow.

4.4.2 Effect of *Striga* on plants

Comparable to GH3684 and IT97K-499-35 (positive control) (Table 4.5), pot screening showed that some susceptible progenies exhibited delayed flowering, which caused low peduncle and pod formation as a result of *Striga* infestation (Table 4.5; figure 4.5). Most of the susceptible progenies exhibited a high reduction in the number of pods per plant and the number of peduncles with pods similar to their susceptible parents, PADI-TUYA and UCSO1 (negative control), which presumably will virtually or inversely affect grain yield. This study confirms that *Striga* infestation causes stunted growth, resulting in a significant ($P < 0.05$) reduction in plant height (Figure 4.5). This study conformed to the previous study of Alonge (1999) and Press (2001), who recorded a significant ($P < 0.05$) effect of *S. gesnerioides* on plant height,

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number of pods per plant and number of peduncles. Susceptible progenies with low vegetative growth resulted from reduced photosynthetic capabilities, affecting flowering, podding and seed production (Figure 4.5). There were similar observations in the study by Alonge (1999) and Asare *et al.* (2013).

4.4.3 Marker-assisted selection and validation of simple sequence repeat (SSR) and SCAR Markers

Countless efforts have been made to detect natural sources of genetic resistance within cowpeas to enable selection and breeding for improved lines with *S. gesnerioides* resistance traits (Singh & Emechebe, 1997; Singh, Ehlers, Sharma & Freire Filho, 2002).

Identification of *Striga* resistance among novel cowpea populations based on genotypic data was necessary to confirm which progenies inherited the resistance genes from GH3684. `The SSR-1, C42-2B, CLM1320, LRR11, LRR8 and 61RM2 markers were known to co-segregate with *S. gesnerioides* resistance genes (Omoigui, 2017; Essem *et al.*, 2019). The current study showed that the six markers had different discriminating power to distinguish between the *Striga*-resistant and susceptible genotypes with the target *Striga* race in Ghana (GH-BINDURI). These markers showed a clear association with the *Striga*-resistant parental genotypes. GH3684 and IT97K-499-35 expressed the resistance allele for all the six markers employed in the current study, confirming resistance to the parasitic weed. Asare *et al.* (2013) recorded similar results when they used SSR-1 and C42-2B markers to test for association of the markers with *Striga* resistance in recombinant inbred lines of cowpea derived from IT97K-499-35 as donor. PADI-TUYA, however, lacked all the six markers indicating susceptibility to the parasitic weed.

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SARC- 1-57-1 lacked SSR-1 and C42-2B markers but expressed CLM1320, LRR11, LRR8 and 61RM2 markers though it was susceptible to the *Striga* GH- BINDURI. Besides, UCSOI conferred none of the markers except LRR8 but was susceptible to *Striga*.

It is known that race-specific resistance genes exist, with some of the genes conferring resistance to multiple races of *Striga gesnerioides* (Li *et al.*, 2009). Therefore, parents and progenies used in this study, which were associated with markers but were susceptible to the *Striga* race in this study (GH-BINDURI), may be associated or resistant to different races of *S. gesnerioides.* Consequently, the parental genotypes and progenies may also be resistant to other races of *S. gesnerioides* apart from ones found in Binduri (GH- BINDURI). It was evident that the gene controlling *Striga* resistance in GH3684 could be easily inherited through cross-breeding. It was observed in this study that marker segregations were correspondingly skewed in favour of the resistant genotype (>58%), supporting the presence of a single, race-specific *Striga* resistance gene (Barone *et al.*, 1990; Hill, Li, & Hartman, 2006; Essem *et al.*, 2019; Badu-Apraku, Adewale, Paterne, Gedil & Asiedu, 2020). Most of the progenies (58 %) in the populations inherited the *Striga*-resistance genes, suggesting that the genes controlling resistance in the parental donor GH3684 may be a single dominant gene. It is known that skewed segregation is relatively common in breeding populations (Xu, Zhu, Xiao, Huang, & McCouch, 1997; Blair, Iriarte, & Beebe, 2006). The progenies that carried and showed resistance to all the markers (34% of progenies) may carry a single dominant gene or gene complex to resist infestation by *Striga* races as their parental donor, GH3684 (Table 4.9; Essem *et al.*, 2019).

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The GH3684 (resistant) and IT97K-499-35 (resistant) derived populations had 66 % (6 out of 9) of their progenies having all markers, proving resistance to *Striga* gesnerioides (Table 4.7). The two resistant parental lines may pose a single dominant gene controlling resistance to *Striga gesnerioides*. Previous studies predicted that IT97K-499-35 has a single dominant gene controlling *Striga* resistance (Li *et al.*, 2009; Boukar *et al.*, 2019; Essem *et al.*, 2019;). The presence of susceptible progenies in this population with these two *Striga*-resistant parents (Table 4.7) can only be when the *Striga*-resistance gene in both parents (GH3684 and IT97K-499-35) is heterozygous dominant. Hence, the presence of susceptible progenies in their population (34%) suggests that the gene controlling resistance in GH3684 and IT97K-499-35 may be the same and probably a heterozygous dominant allele. In addition, the presence of susceptible lines may also result from non-allelic genes controlling resistance or even an epistatic interaction or complex genes. Neuprane *et al.* (2007) pointed the presence of susceptible progenies in subsequent filial generation apart from the $F_{1, of}$ a cross between two resistant genotypes (Chirya.3 and MS#7) shows that resistance genes in both genotypes are non-allelic. Indeed, the basis of the monogenic inheritance of *Striga* resistance cannot be overruled in the current study as in previou reports (Asare *et al.*, 2013; Essem *et al.*, 2019). This study emphasized the single dominant gene proposed by Singh and Emechebe (1990), Lane *et al.* (1993); Touré *et al.* (1997); Carsky *et al.* (2003) and Tchiagam *et al.* (2010). However, some studies identified that resistance is given by two independent dominant genes or single recessive genes (Dube, 2000).

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The six markers employed in the current study co-segregated in association with all resistance cowpea breeding lines identified by pot screening (Table 4.7). It was observed that SSR-1 and C42-2B were the most reliable predictors for *Striga* resistance. Both markers distinguished between resistant and susceptible cowpea progenies with the same discriminating power. Linkage mapping analysis predicted SSR-1 and C42-2B markers to be at the same locus on chromosome 9 (Figure 4.21). Indeed, Li and Timko (2009), Omogui *et al.* (2009) and Essem *et al.* (2017) reported that C42-2B and SSR-1 markers co-segregate. Botanga and Timko (2005) observed that C42-2B and SSR-1 markers were strongly associated with the resistance genes in the *Striga* races three (SG3) and five (SG5), respectively.

Marker discrimination efficiency differed among the population (Table4.8). SSR-1 and C42-2B recorded the highest discrimination efficiency of 85.5 %, followed by CLM1320 (74.8 %), LRR8 (74.5 %), LRR11 (70.6 %), and 61RM2 (69.9). Marker discrimination efficiency may be dependent on the population type. This study shows that SSR-1 and C42-2B markers have a high utility for introgression of *Striga* resistance through a single or a few crosses. The available marker can be used to rapidly screen for resistance without the need to plant thousands of seedlings on the field. This outcome corroborates works by Asare *et al.* (2013), Omogui *et al.* (2017) and Essem *et al.* (2019), who reported C42-2B and SSR-1 as best markers for introgression of the *Striga*-resistant genes.

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4.4.4 Linkage Analysis of the Genes Conferring *Striga* Resistance

The genetic linkage map gives breeders a clue as to how genes can be inherited together (Botanga &Timko, 2005). It further confirms the number of races the progenies might probably be resistant to. In this study, the linkage analysis using the IciMapping program showed that all the markers could be found in the same linkage group or chromosome (probably Chromosome 9) with the Ghana *Striga*-race (GH-BINDURI). The distance between the *Striga*-race in Binduri (GH-BINDURI) resistant gene and both SG3 and SG5 (represented by SSR-1 and C42-2B) was 11.30 cM. This implies that there is at least 88.3% chance that these genes could be inherited together. The *Striga* race (sampled from Binduri) resistance gene is 77.7 % likely to be inherited with CLM1320. Overall, the programe pinned the distance between the Ghana race resistance gene and the last gene (represented by 61RM2) at 41.3cM. This implies a 58.7 % chance that the genes represented by LRR11, LRR8, 61RM2, the Ghana race resistance gene in this study, will be inherited together. The result buttresses the results obtained by Botanga and Timko (2006) and Essem (2019), showing that SSR-1 and the GHrace resistance gene at the same position (12.60 cM) and may be embedded in the resistance gene (Botanga & Timko, 2005).

4.4.5 Cluster analysis

Cluster analysis substantiated based on the Six (6) markers revealed that 17 cowpea progenies that possessed all the six markers (cluster V) were *Striga*-resistant. This was consistent with Omogui *et al.* (2017) and Essem *et al.* (2019) who observed that genotypes with all the markers were resistant to pot screening. Cluster I to XIII were made up of progenies with either one or

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combinations of markers present and resistant under pot condition. Cluster XVIII indicated cowpea breeding lines that did not have any of the three markers and were susceptible under pot conditions. However, some cowpea progenies lacked consistency between the markers and the phenotypes. The markers may be present, but the cowpea lines were susceptible to *Striga* under pot condition, or cowpea genotypes were resistant to *Striga,* but no marker was expressed (Asare *et al.*, 2013, Omogui *et al.,* 2017, Essem *et al.*, 2019, Haruna, Asare & Kusi, 2020). This indicates that there might be epistatic interactions among the genes or the marker may have segregated away from the genes conferring the resistance.

4.4.6 Conclusions

There was a significant ($P < 0.05$) effect of *Striga* stress on cowpea progeny in the pot screening, causing stunted growth and low pod and peduncle formation. MAS is necessary to confirm the *Striga*-resistance gene in the cowpea breeding population. The gene responsible for *Striga* resistance and the associated molecular markers in GH3684 were transferable through conventional breeding. In addition, this study revealed that the same genic locus may be responsible for *Striga* resistance in GH3684 and IT97K-499-35 and could involve a heterozygous dominant allele, thus accounting for both *Striga*-resistant and susceptible progenies in their breeding population. SSR-1, C42-2B and LRR8 had 100 % discrimination efficiency for population 1 (GH3684 X IT97K-499-35), followed by CLM1320, LRR11 and 61RM2 exhibiting 90.9 %, 90.9% and 81.9% discrimination efficiency respectively. On the whole, the 6 SSR and SCAR markers were informative to discriminate *Striga*-resistant and susceptible cowpeas across the genome of four

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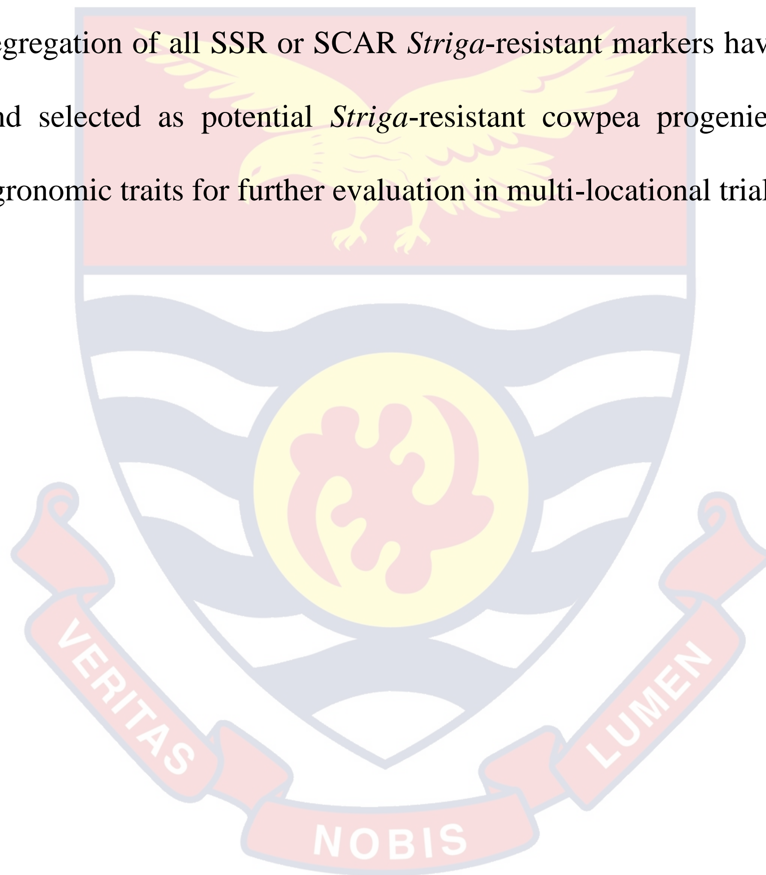
populations of cowpea. However, SSR-1 and C42-2B were the best markers with 85.5% discrimination efficiency. In addition, CLM1320 and LRR8 with discrimination efficiency of 74.8 % and 74.5 % were also informative in identifying *Striga*-resistant cowpea lines. In all, 17 advance breeding lines comprising UC15-01, UC15-02, UC15-04, UC15-07, UC15-09, UC15-10, UC15-19, UC15-20, UC15-22, UC15-23, UC15-26, UC15-27, UC15-28, UC15-35, UC15-43 UC15-47 and UC15-49, associated with consistent segregation of all SSR or SCAR *Striga*-resistant markers have been identified and selected as potential *Striga*-resistant cowpea progenies with desirable agronomic traits for further evaluation in multi-locational trials.



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CHAPTER FIVE

ASSESSMENT OF GENETIC DIVERSITY AMONG NOVEL COWPEA BREEDING LINES

5.1 Introduction

Genetic diversity studies in crops are very important for crop improvement and give vital information to enable the efficient use of available genetic resources (Mohammadi & Prasanna, 2003; Vaughan, Balazs & Heslop-Harrison, 2007). It is a platform for putting breeding population into subgroups with similar genetic characteristics (Mohammadi & Prasanna, 2003).

Cowpea is known to have a narrow genetic base (Fang, Chao, Roberts & Ehlers, 2007; Asare, 2010). This can be attributed to breeders' consistently using improved elite lines as parents in crosses to generate segregating populations in their programmes (Boukar, Fatokun, Huynh, Roberts & Close, 2016). A Cross-breeding programme to add desirable traits and utilize improved breeding lines and varieties as parents to remove weaknesses and improve cowpea varieties further narrowed the genetic base (FAO., 2010). Hence, predisposing widely distributed improved varieties to genetic vulnerability. A narrow base of genetic variation may contribute to the plateauing of some traits (such as grain yield), which compromises genetic gain (Boukar et al., 2016; Baukar *et al.*, 2020).

Along with the wide application of molecular methods, breeding programmes have remarkably expedited new cultivar releases. Nevertheless, with the high-efficiency breeding process and new variety releases, some of the traditional local varieties have been gradually eliminated, resulting in

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narrowing genetic background of crop varieties (Xiong et al., 2016). Understanding the genetic variation within and among breeding programmes involved in exchange of germplasm will provide useful information on integrating new germplasm into the programmes (Byrne, 2018). Populations from crosses between genetically diverse parents are expected to have greater genetic variation than populations developed from less diverse parents (Byrne, 2018; Xiong, 2016).

It is necessary to assess genetic variations in cowpea germplasm to eliminate similar genotypes or clones. Detection of genetic diversity in any cowpea breeding programme requires a more sensitive genomic assay involving polymorphic molecular markers. Simple sequence repeat (SSR) is a relatively new class of plant DNA marker. The highly polymorphic nature of SSR markers makes them particularly useful for genetic diversity analysis in species with a narrow genetic base (Akkaya, Shoemaker, Specht, Bhagwat, & Cregan, 1995; Basu *et al.*, 2007). SSR has been used in genetic diversity analysis of different cowpea genotypes by several researchers (Li *et al.*, 2001; Ogunkanmi, Ogundipe, Ng, & Fatokun, 2008; Asare *et al.,* 2010; Badiane *et al.,* 2012, Ali *et al.,* 2015; Doumbia, Akromah & Asibuo, 2014).

Genetic variation among cowpea breeding lines was evaluated using simple sequence repeats (SSR) markers and a high homozygosity level was detected (Li, Fatokun, Ubi, Singh & Scoles, 2001). The study revealed that some recent breeding lines derived from crosses involving several unimproved lines showed relatively higher genetic diversity levels. In the same study, Li *et al.* (2001) revealed that microsatellite marker polymorphisms from 90 IITA breeding lines indicated relatively low genetic diversity, despite the fact that

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18 of the 90 lines were developed from crosses with wild cowpea accessions. However, 51 % of the lines had one or more parents in common in their studies. Most other studies of molecular diversity in cowpea have focused on crop evolution (Panella & Gepts, 1992; Vallincourt & Weeden, 1992), cowpea taxonomy (Fatokun *et al.,* 1993; Pasquet, 1999), introgression of wild cowpea, or assessment of diversity in landrace populations (Nkongolo, 2003).

In this study, GH3684, a local landrace with a stable source of genetic variability, was used to cross four varieties of cowpea IT97K-499-35, PADI-TUYA, SARC-1-57-1 and UCSO1 to study the genetic diversity of the F_6 generation. The study aimed to detect the gene pool structure of the cowpea breeding lines and to determine the relationship among different populations according to their phylogenetic relationship to improve selection efficiency.

5.2 Materials and Methods

Young leaves at 14 days after sowing were sampled from each of 55 cowpea breeding lines and parental lines.

5.2.1 DNA extraction

A modified CTAB method was used to extract DNA from young leaves as described in 4.2.4 (Chapter 4)

5.2.2 Primer dilution

One hundred (100) Simple Sequence Repeat (SSR) primers were obtained from Metabion International AG, Germany (Appendix C). The primers were spun briefly using a centrifuge (SIGMA Laboratory centrifuge, Model: 1-14) and diluted as described in 4.2.4 (Chapter 4).

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5.2.3 Primer screening

A total of 100 SSR primers (Appendix C) were screened and optimized for polymorphism and annealing temperatures (Tm) using five parental cowpea genotypes; GH3684, IT97K-499-35, PADI-TUYA, SARC-1-57-1 and UCSO1, to ensure optimal performance. Optimal PCR amplification was within the range of 50 to 60 °C annealing temperatures. Seventeen primers (Table 5.1) that showed good and clear polymorphism in the PCR products were selected and used for the genetic diversity analysis.

Table 5.1: SSR primers used, their sequences and annealing temperatures

Name	Primer Sequence 5' ----3'	Annealing Tm
SSR-6169	F-ACCCAAGGACTTCAAGAGCA R-CGAGTGCAAGAAATGGTTCA	55.6
SSR-6172	F-GGAAGACACGCGTTATGGTT R-TTTTTCCACTAAAAGGTTTGTCA	55.6
SSR-6178	F-GAAAAAATCACACACACCAAAATTTG R-CAATCGACTGATTTCACTTAAGTC	57
SSR-6190	F-CGAGTTGCGATATCTCCCTG R-CGAAGACGACAACACAGTGG	55
SSR-6197	F-CATGGCTATCATGGGTCCTT R-TGATGTACGGAGTGAAGGAAGA	55
SSR-6201	F-TGGGCACTATTCCATGCTTT R-ATTGCAATATCAGTTTTTTC	54
SSR-6214	F-CTTCTCTCCGCACCCAATC R-GCGAAACAGGGTAGGGAATC	55.6
SSR-6229	F-TATTCCGACAACCACCCAAT R-GGGATCCATGAGGAGAGAGA	55
SSR-6240	F-TTCAATGTGGGAGGATGAGA R-GGTTCCGGATTCAATTTTCC	55
SSR-6247	F-ATATTCTGCTCCCGCTGTTG R-TCGTGCATGGGTTTATGTGT	54
SSR-6270	F-TCCTCCCACACTTGGAAATC R-TATGCGAAAAGGGATTGCTC	56
SSR-6776	F- GTAGTTAAGTTTAGAAAAATAG R- GGTGATGTTGGGAATGGTTG	55
SSR-6777	F- CGAAGCATGTGGACACGTAC R- CATTGAACAAACATCGCTGAAGC	50
SSR-6929	F-GCCCATGTAATGCTGTATAGT R-GGCGTTAGAACTACTCCAGTT	56
C49-499	F- CAATGAGCCAACAAGTCTAGAG R-GCCCTAAACTAGAATCATTGCC	57.7
SG25R	F-GGAGTTGTTGTATGAGAAGTTGC R- CGTAATAATGGATGTGTGTTTTCTC	59.3
CP01038	F- TTTTGACAGAAGAAACGTGGTGGA R-GGGGTATGTCTGAAAGTTCAACGC	59

Source: Asare *et al.,* 2013

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5.2.4 Polymerase chain reaction (PCR) and Gel Electrophoresis

The PCR assay and gel electrophoresis analysis were done as described in 4.2.4

5.2.5 Data collection and Analysis

The scoring and analysis of the data from the SSRs were done following the format used by Khosro *et al.* (2017), with slight modifications. A 100 bp DNA ladder from Invitrogen (Carlsbad, CA, USA) was used as a molecular weight-sized marker for each gel alongside the DNA samples from the progenies and the parental lines. The individual SSR fragments were scored for size and polymorphism. Amplified bands present across genotypes data matrix were subjected to further analysis. A Data matrix was created and used to calculate the genetic distance and similarity using PowerMarker software analysis (version 3.25). The related genetic parameters were computed as the number of polymorphic bands, and average alleles per locus, polymorphism information content (PIC), major allele frequency and genetic diversity.

The Unweighted Pair Group Method of Arithmetic Averages (UPGMA) on the similarity indices was performed to identify genetic variation patterns among cowpeas using PowerMarker version 3.25. Cluster analysis was carried out based on genetic distance. The resulting clusters were represented in a dendrogram and printed in Molecular Evolutionary Genetics Analysis (MEGA) version 7.0.1 software.

5.3 Results

The SSR amplified the genomic DNA sequences across the fifty (50) cowpea breeding lines and five (5) cowpea parental genotypes with high

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reliability. Some of the primer pairs identified extensive polymorphism across the cowpea genome. In all, seventeen (17) polymorphic SSR primers distinguished the 55 genotypes of the cowpea, including those with similar seed sizes and are from the same parents. The sizes of polymorphic amplicons ranged from 80 bp to 650bp. There were a total of 45 alleles amplified by the 17 SSR markers across the genome of 55 cowpea breeding lines and their parents (Table 5.3). Results for DNA analysis are presented in Table 5.2. The number of alleles detected per primer pair varied from a minimum of two (2) to a maximum of seven (7) with an average of three (3). The allele frequency yielded by the 17 SSR primers ranged from 0.38 to 0.93, with an average of 0.59 (Table 5.3). Gene diversity also ranged from 0.13 to 0.69, with an average of 0.50. The PIC varied from 0.1 to 0.69, with an average of 0.41. (Table 5.3). Only 29.4 % of the primers had PIC of 0.5 and above. There was a highly significant correlation between allele frequency and gene diversity ($r=-0.934$; $P < 0.001$) and between PIC and gene diversity ($r=0.965$; $P < 0.001$). Besides, there was a highly significant negative correlation between PIC and allele frequency ($r = -0.843$; $P < 0.001$) (Table 5.2). The five most polymorphic primer pairs, SSR-6172, SSR-6776, SSR-6247, C49-499 and CPO1038, could distinguish all lines (Figure 5.1; 5.2; 5.3 and Table 5.3).

Table 5.2: Correlation analysis of Gene diversity, Major allele frequency and Allele number

	Major Allele Frequency	Allele Number	Gene Diversity
Allele Number	-0.420		
Gene Diversity	-0.934***	0.636**	
PIC	-0.843***	0.779***	0.965***

*Significant levels; ***=pvalue < 0 .001 **= pvalue < 0 .05*

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Table 5.3: Major Allele Frequency, Gene Diversity and PIC of the Seventeen SSR Markers used in diversity studies

Marker	Major Allele Frequency	Sample Size	Allele Number	Availability	Gene Diversity	PIC
CP01038	0.38	55	4	1	0.70	0.64
SG25R	0.69	55	2	1	0.43	0.34
C49-499	0.53	55	7	1	0.67	0.64
SSR-6229	0.75	55	2	1	0.38	0.31
SSR-6179	0.53	55	2	1	0.50	0.37
SSR-6190	0.64	55	2	1	0.46	0.36
SSR-6214	0.93	55	2	1	0.15	0.13
SSR-6172	0.58	55	3	1	0.58	0.51
SSR-6169	0.64	55	2	1	0.47	0.36
SSR-6240	0.58	55	2	1	0.49	0.37
SSR-6776	0.45	55	3	1	0.63	0.55
SSR-6270	0.56	55	2	1	0.49	0.37
SSR-6929	0.55	55	2	1	0.50	0.37
SSR-6247	0.44	55	4	1	0.66	0.60
SSR-6201	0.53	55	2	1	0.50	0.37
SSR-6178	0.67	55	2	1	0.44	0.34
SSR-6777	0.53	55	2	1	0.50	0.37
Mean	**0.59**	**55**	**3**	**1**	**0.50**	**0.41**

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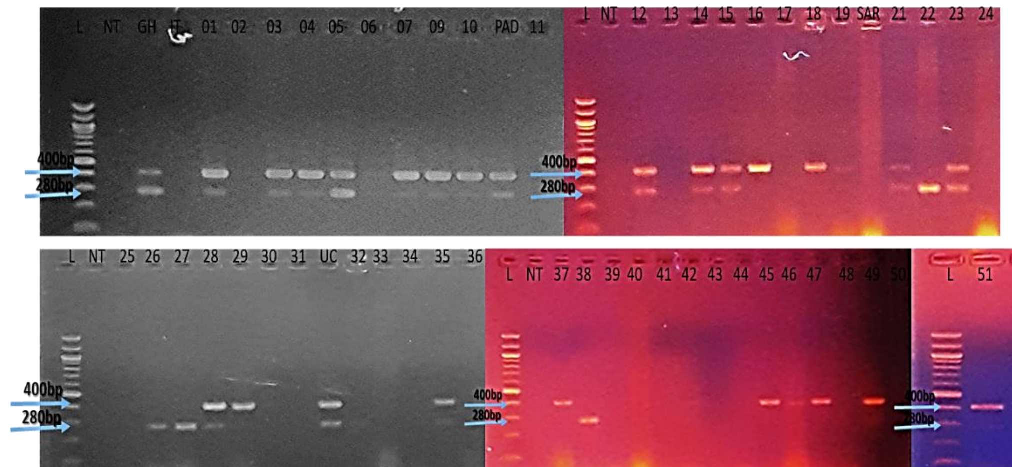

Figure 5. 1: PCR Amplified products of cowpea genomic DNA from 55 cowpea breeding lines for SSR-6247 primer resolve on agarose gel. L= 100 bp ladder, NT= Non template control, GH=GH3684, IT=IT97K-499-35, PAD= PADI_TUYA, SAR= SARC-1-57-1 and UC= UCSO1.

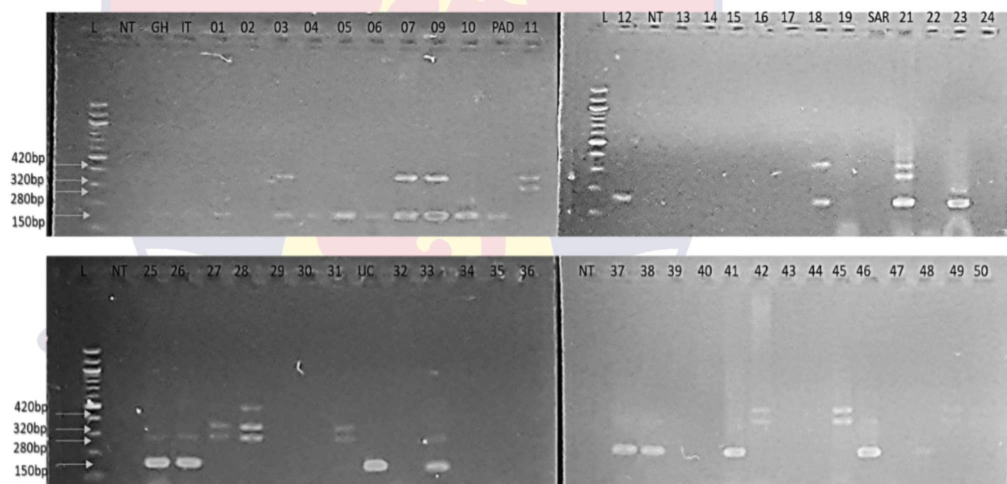

Figure 5. 2: PCR Amplified products of cowpea genomic DNA from 55 cowpea breeding lines for C49-499 primer resolved in agarose gel. L= 100 bp ladder, NT= Non template control, GH=GH3684, IT=IT97K-499-35, PAD= PADI_TUYA, SAR= SARC-1-57-1 and UC= UCSO1(Lab data, 2020).

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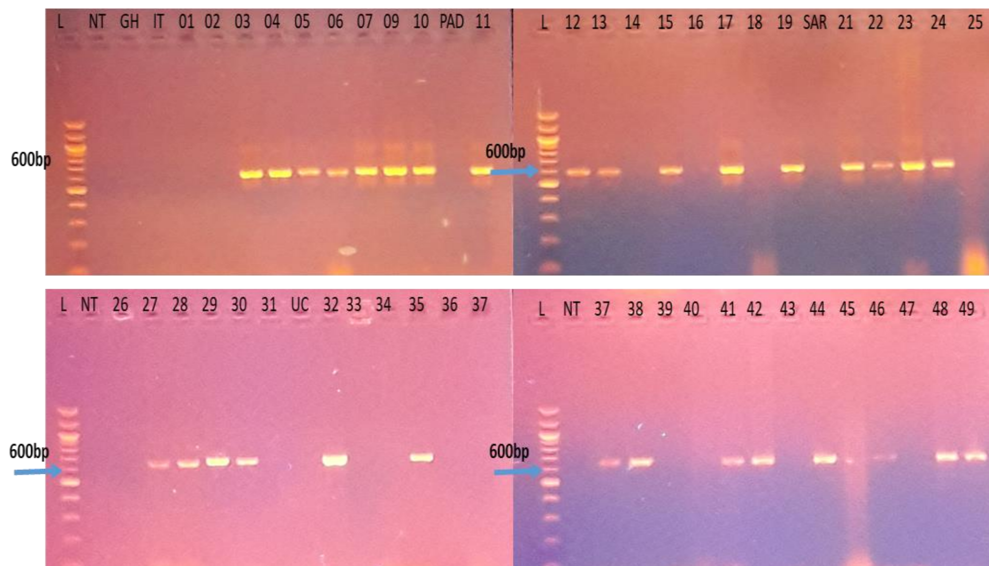

Figure 5. 3: PCR Amplified products of cowpea genomic DNA from 55 cowpea breeding lines for SSR-6169 primer resolved in agarose gel. L= 100 bp ladder, NT= Non template control, GH=GH3684, IT=IT97K-499-35, PAD= PADI_TUYA, SAR= SARC-1-57-1 and UC= UCSO1(Lab data, 2020).

5.3.1 Cluster Analysis

Seventeen (17) polymorphic primers differentiated the 55 cowpea lines genotypes into two major clusters, A and B, at 29 % dissimilarity coefficient (Figure 5.4) and five subclusters at 21% dissimilarity coefficient. Cluster B was the most extensive, comprising 69 % (38) of the 55 cowpea genotypes, out of which 15 were breeding lines from population 4; 10 breeding lines from population 3; 4 from population 2 and 8 from population 1. All five parental genotypes were found in cluster B. Generally, most of the breeding lines from population 4 clustered with their parent UCS01. About 80 % of breeding lines from population one were found to cluster together.

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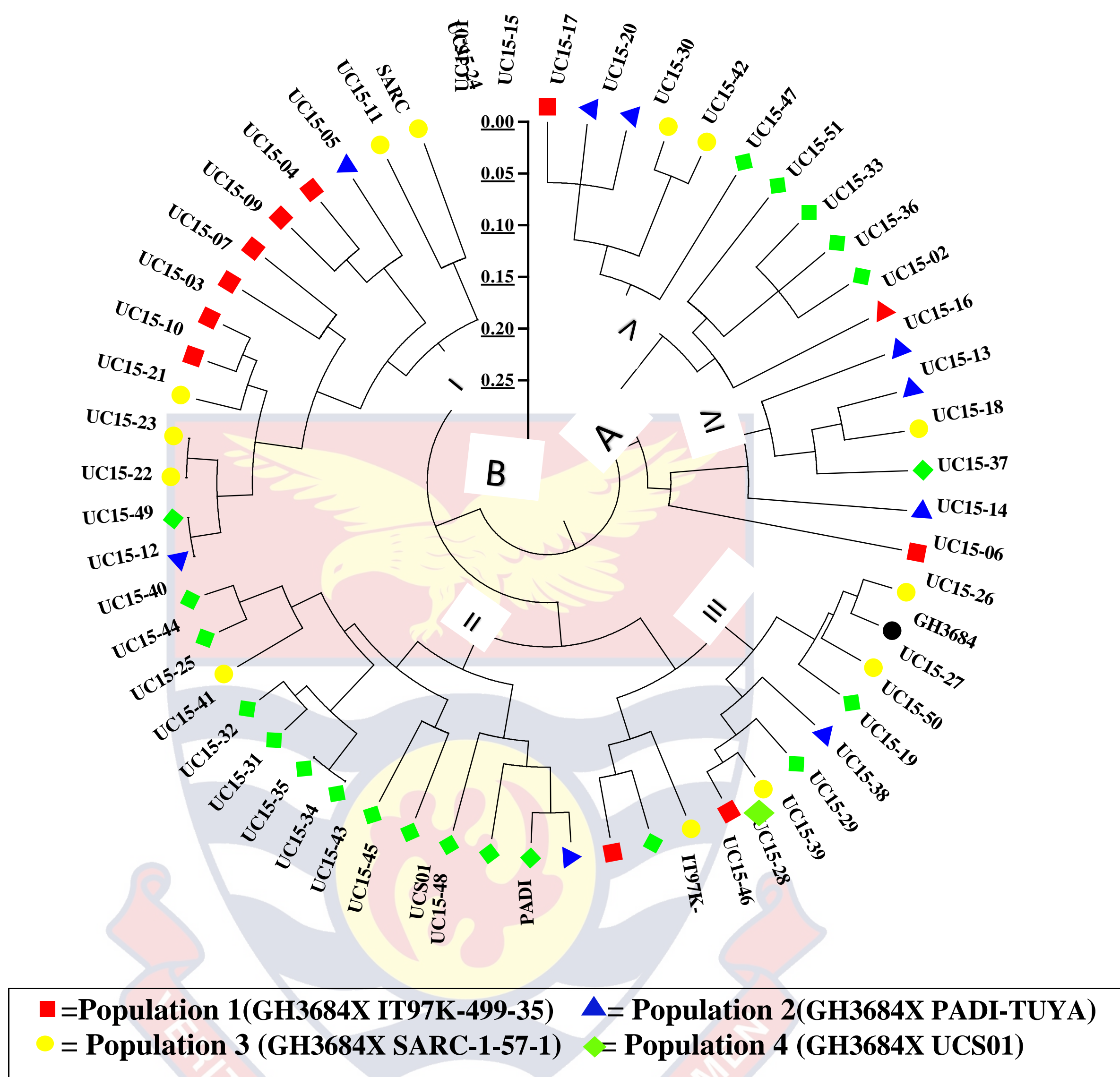

Figure 5.4 A dendrogram of 55 cowpea breeding lines constructed from PowerMarker using seventeen polymorphic markers with UPGMA tree method.

5.4 Discussions

Landraces are unexplored stores of untapped genetic resources that can be used to breed more productive and better-adapted plants (Dwivedi et al., 2016; Hour *et al.*, 2020; Pascual *et al.*, 2020). Given their evolutionary history and adaptation to local conditions, they usually harbour higher genetic diversity and environmental resilience than modern varieties (Pascual *et al.*, 2020). The study assessed the genetic diversity of four populations of cowpeas

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with the local landrace, GH3684, as the donor parent in each population. On the whole, 17 out of 100 SSR markers were polymorphic across the genome of the cowpeas. All 17 SSR markers exhibited PIC values higher than 0.3, which could be considered reasonably informative, according to Zhang, Wang and Jiang (2013), hence, their suitability for genetic studies.

The genetic diversity and phylogenetic relationships of cowpea genotypes from Ghana have been studied using Simple Sequence Repeat (SSR) markers (Asare *et al.*, 2010; Doumbia, Akromah & Asibuo, 2013; Doumbia, *et al.,* 2014). In the current study, the SSR loci can be considered multi-allelic, exhibiting alleles per primer pair of 2 to 7 with an average of 3, thus suggesting their relative potential in detecting DNA polymorphism. In comparison, this report is similar to a study by Asare *et al.* (2010), who reported 1 to 6 alleles per primer pair with a mean of 3.8 when they used 25 SSR primers to analyze 141 local cowpea lines in Ghana. Doiuf and Hilu (2005) also reported number of alleles ranging from 1 to 9 per SSR primer combination in cowpea germplasm. Sawadogo, Ouedraogo, Gowda and Timko (2010) used 16 SSR markers to examine cowpea cultivar genetic diversity from Burkina Faso and observed 5 to 12 alleles per primer combination in cowpea genotypes. The variations in numbers of alleles observed in this study can be attributed to the types of primers used and their polymorphism rate besides genetic diversity across the cowpea genome. The number of alleles per locus certainly contributed to the usefulness of these markers.

PIC identifies the discriminatory ability of the marker. It depends on the number of known (established) alleles and their distribution frequency and

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is equivalent to the gene diversity (Chesnokov & Artemyeva, 2015). There was a highly positive significant correlation (r= 0.779; P<0.05) between the number of alleles at a locus and the PIC (Table 5.2). Thus, the measure of PIC is an important component and a statistical indicator in breeding programs, ranging from 0.13 to 0.64 in this study. The average PIC of the 17 SSR markers across the genome of the cowpeas was 0.4. The SSR markers; CPO1038, C49-499, SSR- 6249, SSR-6776 and SSR-6172 exhibited the highest PIC of 0.6422, 0.637, 0.6017, 0.5511 and 0.5018 respectively. These markers can be classified as dominant markers. It is known that, even though the maximum value of PIC for dominant markers is 0.5 (Chesnokov & Artemyeva, 2015), markers with equal distribution in the population have higher PIC values and have multiple alleles, and high-frequency allele distribution (Chesnokov & Artemyeva, 2015) (Table 5.3; figure 5.1 and 5.2). Moreover, the PIC of 0.25-0.50 has been considered reasonably informative and PIC < 0.25 considered slightly informative (Botstein, White, Skonick & Davis, 1980; Zhang, Wang & Jiang, 2013). Similarly, all the SSR markers used in the current study with an average PIC of 0.41 can be classified as informative. The current results are almost similar to observation by Asare *et al.* (2010), who had PIC between 0.07 and 0.66 with a mean of 0.38 across the genome of 141 cowpea lines in Ghana. Ali *et al.* (2015) also used sixteen SSR primers to assess the genetic diversity of 252 cowpea varieties in Sudan and estimated PIC between 0.33 to 0.83 with an average value of 0.56. Khosro *et al.* (2017) also observed PIC range from 0.25 to 0.63 with an average of 0.45 involving 22 SSR markers.

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Gene diversity in this study was 0.29 on average, ranging from 0.04 to 0.49. In a study by Badiane et al., (2012), in Senegal, they observed cowpea gene diversity varied from 0.08 to 0.42 with a mean of 0.28, whereas Asare et al (2012) in Ghana, cowpea germplasm gene diversity in ranged from 0.12 to 0.68 with an average of 0.44 in cowpea germplasm from Ghana. The results of gene diversity reflect the proportion of polymorphic loci across the genome. Therefore, according to the result of the current study, the markers used were almost as polymorphic as those used by Badiane *et al.* (2012), and Asare *et al.* (2010). It was evident that there is low genetic diversity among progenies under study. This is possible because cowpeas are known to have a narrow genetic base (Fang *et al.*, 2007) due to its inherent self-pollination tendency. Furthermore, the single donor used in cross-breeding with the four elite varieties or recipients could have influenced the narrow genetic base across the genome of the cowpea populations. The results agree with previous reports in cowpea (Li, Fatokun, Ubi, Singh & Scoles, 2001; Tosti & Negri, 2002) and mung bean (Chen *et al.*, 2015). However, there were adequate observed genetic variations among cowpeas in the different populations that can be explored (Figure 5.4).

The 17 SSR polymorphic markers distinguished the 55 cowpeas and grouped them into two major clusters (A and B) at a similarity coefficient of 0.25 and five sub-clusters (I-V) with one outlier (UC15-06) at a similarity coefficient of 0.20. The sub-cluster I consisted of 80 % of the progenies of population 1(GH3684 X IT97K-499-35). Sub-cluster II was made up 95 % of progenies from population 4 (GH3684 X UCSO1). These observed clustering may be attributed to the fact that the progenies may have inherited

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similar genes from both parents. Hence, there may be a high similarity among the cowpeas within the sub-clusters I and II. The landrace GH3684 was used as a donor parent passed on similar genes that hybridized genes from the different recipient parents to produce the progenies. In this study, all of the primer combinations tested gave amplification products with 89.1% cross-progeny polymorphism; this means some of the progenies could not be distinguished by the primers (Figure 5.2). Indeed, UC15-23 and UC15- 22, UC15-49 and UC15-12 and UC15-31 and UC15-35 which could not be distinguished by the SSR markers may be genetically the same or clones.

5.5 Conclusions

The 17 SSR polymorphic markers distinguished 89.1 % of the 55 cowpea progenies, including those of the same population, seed coat colour and growth habits. The markers were observed to be highly distributed across the cowpea genome, having polymorphic DNA band sizes ranging from 80 to 650 bp. On the whole, the genetic distance among the cowpea genotypes varied from 0.00 to 0.25. UC15-23 and UC15- 22, UC15-49 and UC15-12 as well as UC15-31 and UC15-35 may be genetically similar since the SSR primers could not distinguish them. Most of the progenies in population I (90 %) clustered tighter, suggesting that GH3684 and IT97K-499-35 may have closely similar genetic traits. The alleles per primer pair of 2 to 7 with an average of 3 and mean PIC of 0.41 and gene diversity of 0.25 were evidence that genetic variations exist among the cowpeas that can be explored despite the observed narrow genetic base.

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CHAPTER SIX

SUMMARY, GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Cowpea production in Ghana is short of national demand due to the devasting effects of *Striga gesnerioides* and other constraints in the major areas of cultivation. Although *Striga*-resistant cowpea varieties exist, they are predominantly small to medium seed size (10 g – 20 g per 100 seeds), but consumer preference is driven towards large to extra-large seeds (> 20 g per 100 seeds). This study aimed to characterize cowpea breeding lines developed from diallel crosses and select *Striga* resistant and improved agronomic traits. This was achieved by assessing the phenotypic and genetic variations in the breeding populations and validating some SSR markers across the genome of the crop.

Variations in twinning tendency, growth habits and patterns, raceme positions and flower pigmentation pattern were evident among the cowpea breeding lines. The agro-morphological parameters revealed significant differences ($P < 0.05$) among the cowpea breeding lines and the parental genotypes. Variations in the quantitative and qualitative traits distinguished the cowpea progenies in a dendrogram. The 100 seed weight differed significantly ($P < 0.001$) among the cowpea breeding lines, ranging from 11 to 26.8 g with a mean of 20.4 g. UC15-36, UC15-46, UC15-06 and UC15-51 had the highest 100 seed weight with a mean of 25.8 g, 24.8 g, 24.7 g and 24.0 g respectively. UC15-12 recorded the highest grain yield of 2.7 t ha^{-1}. A significant positive correlation ($P < 0.05$) was observed between grain yield

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and number of branches (r = 0.257), number of peduncles (r = 0.167), number of seeds per pod (r = 0.161) and number of locules (r = 0.231). The study revealed that 42 % of the cowpea progenies had improved agro-morphological traits prospects for further evaluation.

The 55 cowpea lines evaluated in an augmented block design showed that the highest coefficient of variation (CV % > 100) was among the *Striga* response parameters. The emergence of *Striga* delayed on the field at 52 days after sowing probably due to late germination and environmental factors. PADI-TUYA recorded the highest *Striga* count at maturity, followed by UC15-05, UC15-03, UC15-40 and UC15-32. However, there was generally no significant (P > 0.05) effect of *S. gesnerioides* on growth and yield of cowpeas in the field study, compared to GH3684 and IT97K-499-35 (positive control). In the pot experiments, *Striga* stress caused delayed flowering, low peduncle and pod formation, seed yield and stunted growth, with a significant (P < 0.05) reduction in plant height similar to their susceptible parents, PADI-TUYA and UCSO1 (negative control).

The six SSR and SCAR markers had different discriminating power to distinguish between *Striga*-resistant and susceptible cowpea genotypes with the target *Striga* race from BINDURI in Ghana (GH-BINDURI). These markers showed a clear association with the *Striga*-resistant phenotypes. GH3684 and IT97K-499-35 as well as 17 of the progenies, expressed the resistance allele for all the six markers employed in the current study similar to their phenotypes in the field and pot tests. PADI-TUYA, however, lacked all the six markers and was highly susceptible to the parasitic weed. SARC-1-57-1 lacked SSR-1 and C42-2B markers but expressed CLM1320, LRR11,

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LRR8 and 61RM2 markers though it was susceptible to the *Striga* from Binduri (GH-BINDURI). Besides, UCSOI conferred none of the markers except LRR8 but was susceptible to the *S. gesnerioides*.

The markers, SSR-1 and C42-2B were the most reliable predictors of *Striga*- resistant traits across the cowpea genome. Both markers distinguished between resistant and susceptible cowpea progenies with the same discriminating power of 85.5 %, followed by CLM1320 (74.8 %), LRR8 (74.5 %), LRR11 (70.6 %) and 61RM2 (69.9 %). However, some cowpea progenies lacked consistency between the markers and the phenotypes. Linkage analysis using the IciMapping program predicted that all the markers could be found in the same linkage group or chromosome 9 with the *Striga*-race in Binduri (GH-BINDURI). It was evident that the gene controlling *Striga* resistance and the associated markers in GH3684 could be easily inherited through cross-breeding and that the same genic locus may be responsible for *Striga* resistance in GH3684 and IT97K-499-35 probably involving a dominant heterozygote gene.

On the whole, 17 out of 100 SSR markers were polymorphic across the genome of the cowpeas. All 17 SSR markers exhibited PIC ranging from 0.13-0.64 with an average of 0.41. Gene diversity in this study was low, 0.29 on the average, ranging from 0.04 to 0.49. The 17 SSR polymorphic markers distinguished 89.1 % of the 55 cowpeas and grouped them into two major clusters (A and B) at a similarity coefficient of 0.25 and five sub-clusters (I-V) with one outlier (UC15-06) at a similarity coefficient of 0.20. The sub-cluster I consisted of 80 % of the progenies of population 1(GH3684 X IT97K-499-35). Subcluster II is made up of 95 % of progenies from population 4 (GH3684 X

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UCSO1). In this study, all of the primer combinations tested gave amplification products with 89.1% cross-progeny polymorphism. UC15-23 and UC15- 22, UC15-49 and UC15-12 and UC15-31 and UC15-35, which could not be distinguished by the SSR markers, may be genetically the same or the primers involved failed to distinguish them.

6.2 General Conclusions

- The study revealed substantial phenotypic variations associated with different responses to *Striga gesnerioides* infestation. The immatured pod pigmentation, raceme position, flower pigmentation pattern, twinning tendency, pod length, plant height, days to 50 % flowering, number of seeds per pod, number of locules, 100 seed weight and number of peduncles were found to be important contributors to variation among the cowpea progenies based on the correlation and the principal component analysis. The use of GH3684 to cross-bred 4 different recipient parental genotypes increased variations among the cowpea breeding lines.
- Seventeen (17) of the cowpea breeding lines were confirmed to have resistance to *Striga gesnerioides* by both SSR and SCAR markers, under pot and field test screening.
- There was a significant ($P < 0.05$) effect of *Striga* stress on cowpea progenies that were susceptible to *Striga* infestation in the pot experiment, causing stunted growth, low pod and peduncle formation, and low yield.
- The pot screening method was efficient in the identification of *Striga*-resistant phenotypes of cowpea that were consistent with the *Striga*-

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resistant genotypes based on molecular analysis. Hence, the use of pot screening experiment for identifying *Striga gesnerioides* resistant phenotypes of cowpea has greater reliability than that of the field screening experiments.

- On the whole, the 6 SSR and SCAR markers were informative to discriminate *Striga*-resistant and susceptible cowpeas across the genome of four populations of cowpea. However, SSR-1, C42-2B, CLM1320 and LRR8 were considered to have the best discrimination efficiency (>74 %). Hence, they may have a high utility for introgression of *Striga*-resistant gene through single or multiple crosses.
- The gene responsible for *Striga* resistance and the associated molecular markers in GH3684 were transferable to cowpea progenies through conventional breeding. In addition, this study revealed that the same genic locus may be responsible for *Striga* resistance in GH3684 and IT97K-499-35 and could involve a heterozygous dominant gene, thus accounting for both *Striga*-resistant and susceptible progenies in their population though both parents have resistance to *Striga gesnerioides*.
- The 17 SSR polymorphic markers could be considered reasonably informative to have distinguished 89.1 % of the 55 cowpea lines, including those of the same population, similar seed coat colour and growth habits.
- The SSR loci are multiallelic associated with 2 to 7 alleles per primer pair and an average of 3 in the current study, which suggest their relative efficiency in detecting DNA polymorphism.

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- There was a significant correlation between the PIC and the number of alleles at a locus ($r = 0.78$, $P < 0.05$) and gene diversity ($r = 0.97$; $P < 0.05$). This emphasized that polymorphism probably increases with gene diversity and number of alleles. Thus, the measure of PIC is an important component and statistical indicators in breeding programs.
- Cluster analysis revealed that UC15-23 and UC15- 22, UC15-49 and UC15-12, UC15-31 and UC15-35 might be clones or closely similar genotypes since they could not be distinguished by the SSR primers used in the current study.
- Most of the progenies in population I (90 %) clustered tighter, suggesting that GH3684 and IT97K-499-35 may have closely similar genetic traits.
- The alleles per primer pair of 2 to 7 with an average of 3 and a mean PIC of 0.41 and gene diversity of 0.25 were evidence that genetic variations exist among the cowpeas and can be explored.
- On the whole, ten breeding lines comprising UC15-01, UC15-02, UC15-19, UC15-22, UC15-28, UC15-35, UC15-43, UC15-43 UC15-47 and UC15-49 with a 100-seed weight range of 19.7-24.0 g and high grain yield (> 1.7 t ha^{-1}) associated with *Striga*-resistant traits have been identified as the best-improved cowpea progenies for further evaluation.

6.3 Recommendations

1. The cowpea progenies with both large seed size and *Striga*-resistance traits should be further evaluated in multi-locational trials in *Striga* infested fields of the regions in northern Ghana.

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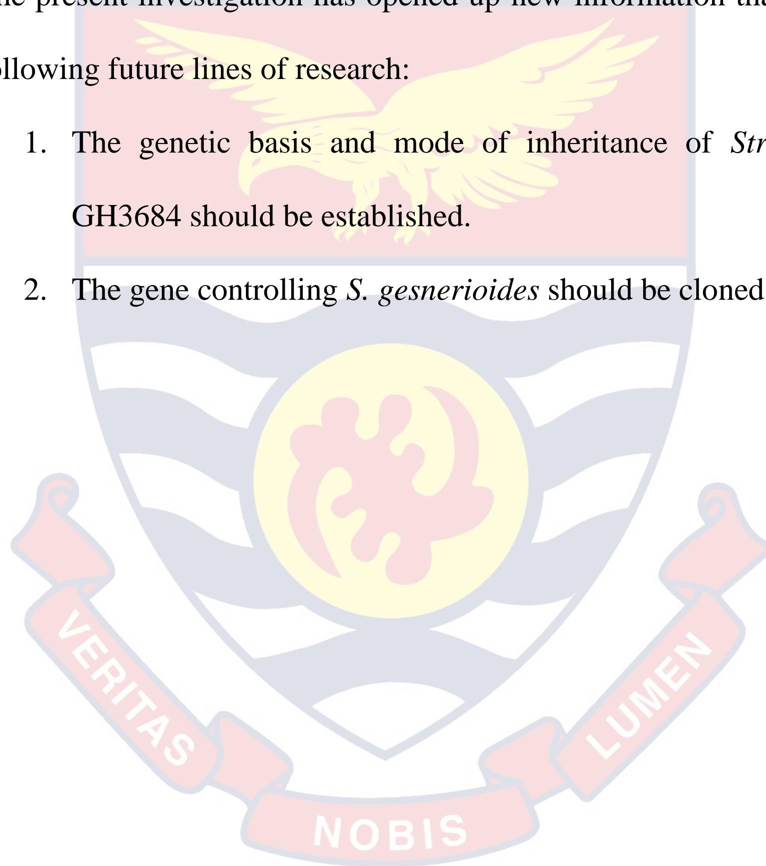
2. Drought and disease tolerance potentials of the cowpeas should be studied to ascertain adaptation to cultivation in broad agro-ecological zones.
3. The nutritional and sensory test should be carried out to establish the acceptability of the cowpea lines.

6.4 Suggestions for Further Research

The present investigation has opened up new information that can lead to the following future lines of research:

1. The genetic basis and mode of inheritance of *Striga*-resistance in GH3684 should be established.
2. The gene controlling *S. gesnerioides* should be cloned and sequenced.



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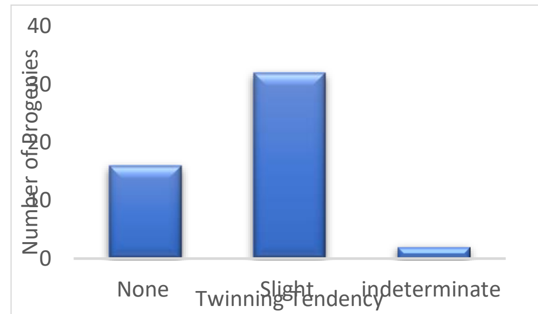
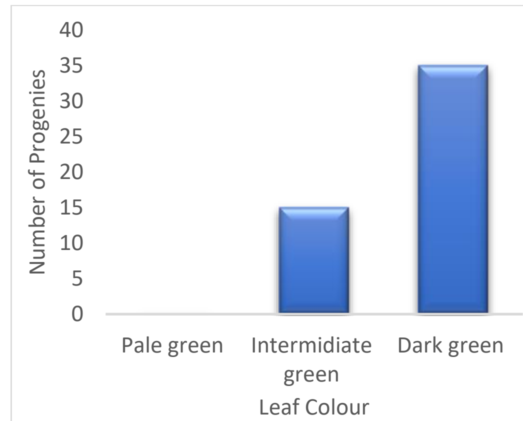
APPENDICES

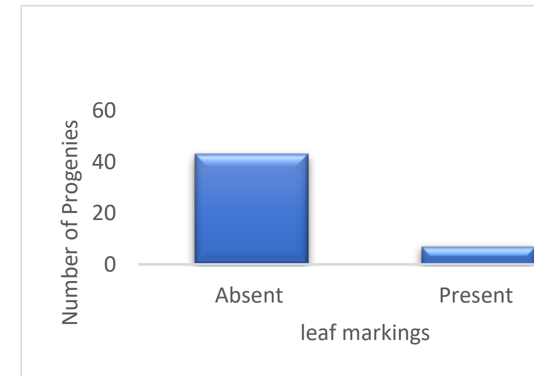
Appendix A - Qualitative trait characterization



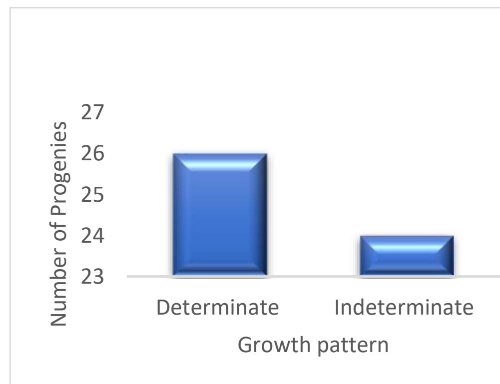
A1: Variation of twinning tendency among cowpea progenies



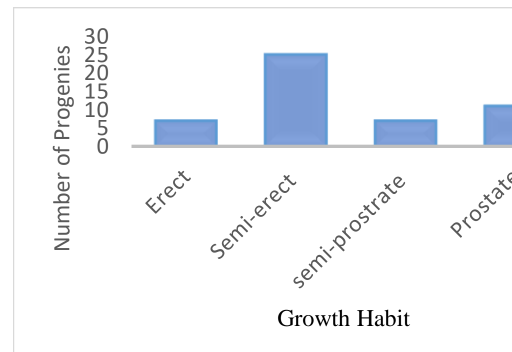
A2: Variation of leaf colour among cowpea progenies



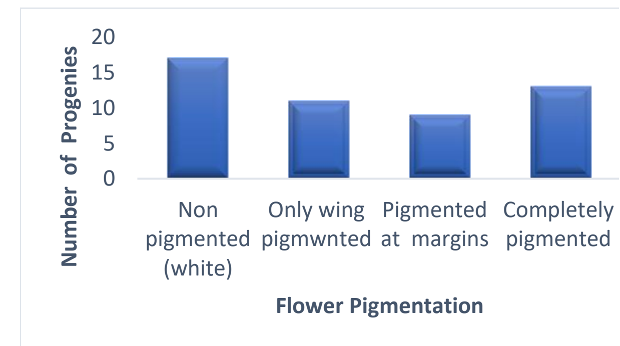
A3: Variation of leaf markings among cowpea progenies



A4: Variation of growth pattern among cowpea progenies



A5: Variation of growth habit among cowpea progenies



A6: Variation of flower pigmentation among cowpea progenies

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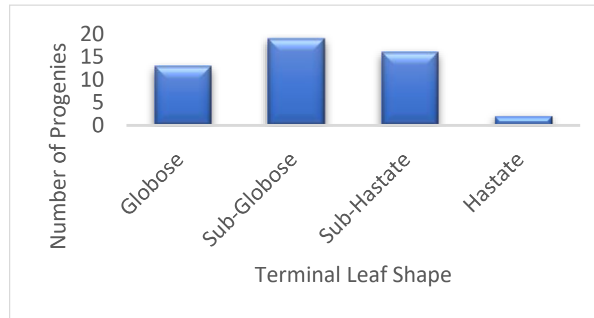
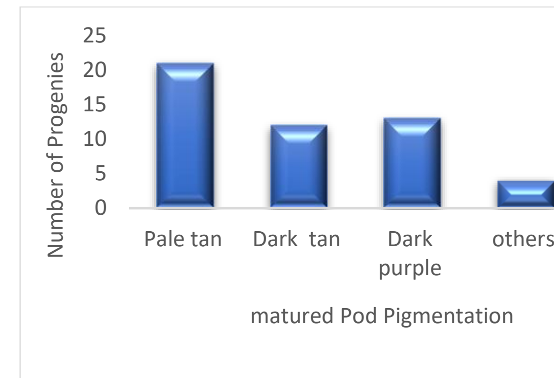
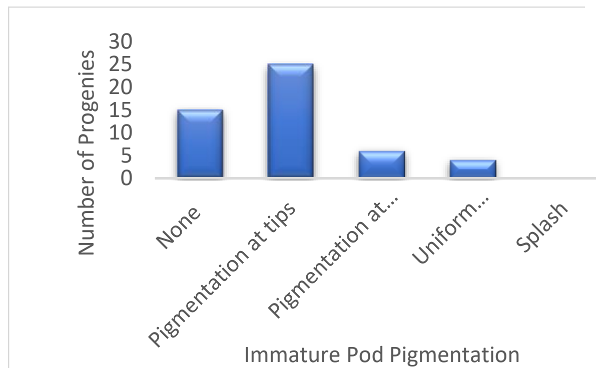
A7: Variation of flower colour among cowpea progenies



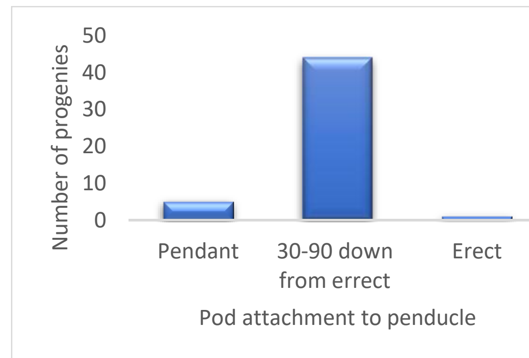
A8: Variation of terminal leaf shape among cowpea progenies



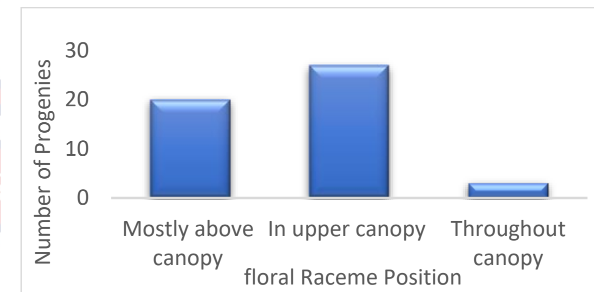
A9: Variation of matured pod pigmentation among cowpea progenies



A10: Variation of immature pod pigmentation among cowpea progenies



A11: Variation of pod attachment to peduncle



A12: Variation of floral raceme position among cowpea progenies

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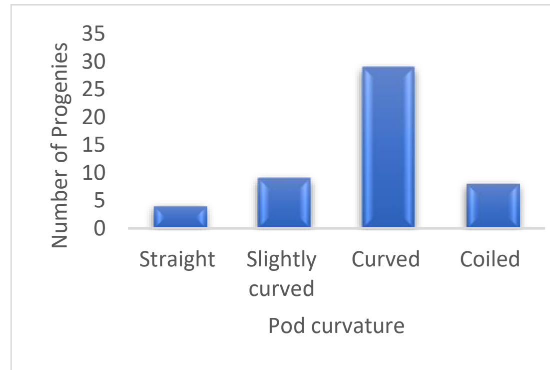
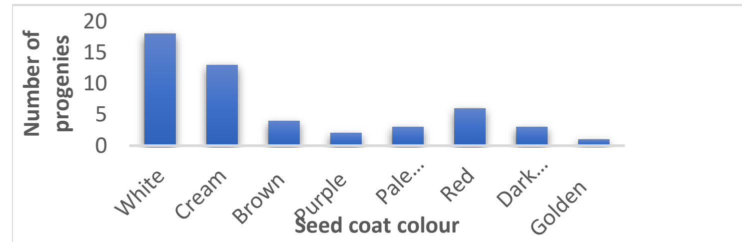
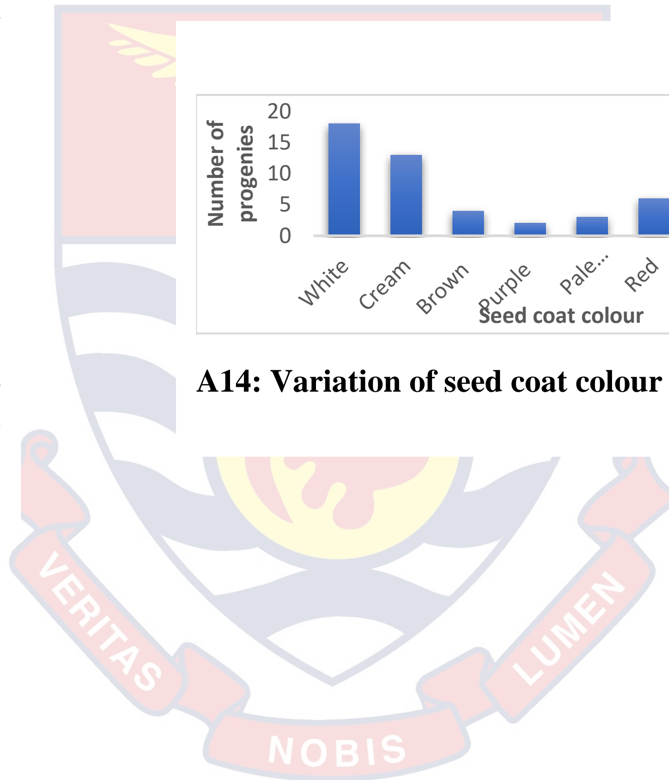


A13: Variation of pod curvature among cowpea progenies



A14: Variation of seed coat colour among cowpea progenies



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Appendix B

B1: Standard errors of the mean for comparison of adjusted means of fifty-six (56) cowpea genotypes

Standard Error	Formula	DFF	100SWT	GY	DM	PH	CD	Pod/Plt	Pod per Pen	No. Of Plt With *Striga*	*Str.* / Plot	*Str.* At Mat.	Days To *Striga* Emerg.
Difference between 2 check varieties (Sc)	√2MSE/ R	1.16	1.44	440.306	0.96	1.771	33.621	1.554	0.111	0.702	6.681	6.581	11.348
Difference between adjusted means of two Test entries in the same block (Sb)	√2MSE	2.59	3.21	984.555	2.15	3.959	75.18	3.474	0.249	1.571	14.715	14.714	25.374
Difference between adjusted means of two test entries in different blocks (Sv)	√2 (C + 1) MSE/ C	2.80	3.47	1063.44	2.32	4.276	81.203	3.753	0.269	1.696	15.894	15.893	27.407
Difference between adjusted test entry and check mean (Svc)	√{(R +1) (C + 1) MSE}/ R.C	2.12	2.62	803.88	1.75	3.233	61.384	2.836	0.204	1.282	12.014	12.014	20.717
Least Significant increase (LSI)	$t_{.05}$.Svc	3.540	4.375	1342.47	2.925	5.399	102.511	4.736	0.340	2.141	20.057	20.057	34.597

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Appendix C

APPENDIX C1- 100 SSR PRIMERS USED FOR THE DIVERSITY STUDIES

Serial No.	Name	Primer Sequence 5' ----3'
1	SSR-6169	F-ACCCAAGGACTTCAAGAGCA R-CGAGTGCAAGAAATGGTTCA
2	SSR-6170	F- ACCTGCATTGCCTCATATCC R- GCTGATTCGGCTTGTTCTTC
3	SSR-6171	F- ATTCGATCCAACCCAATGAC R- AGCGAAGGCATGTTCGTAAG
4	SSR-6172	F-GGAAGACACGCGTTATGGTT R-TTTTTCCACTAAAAGGTTTGTCA
5	SSR-6173	F-AGATCCCACGCTGATTATGG R-ACTTGACGCAGAGCCATCTT
6	SSR-6174	F-TCCTTAGAGGTCCAGCCAGA R-GGAGGAAGAGAGCACACACA
7	SSR-6175	F-GCAAGCTTTTGGAAGTTGGA R-GGCCAGAAGCATGAATCACT
8	SSR-6176	F-GCCACAAGTGCTTGAAGTGA R-CCACGTAACGAGGATCAACA
9	SSR-6177	F-GTAAGTGGGATTCTTATTGTTG R-CAAGAACCTTACTCTAGATACC
10	SSR-6178	F-GAAAAAATCACACACACCAAAATTTG R-CAATCGACTGATTTCACTTAAGTC
11	SSR-6190	F-CGAGTTGCGATATCTCCCTG R-CGAAGACGACAACACAGTGG
12	SSR-6191	F-AAACTGCTAACCAGAAACAGAAAA R-TGTCAATTTTGTTGGCCTCA
13	SSR-6192	F-AACGGGTCCTAAACGAATGA R-ATCCTTGAACTCCGTGTTGC
14	SSR-6193	F-ACCAAAGCAACACCAACACA R-GATGTGGGAAGAAGCTGAGG
15	SSR-6194	F-CACACACAAGGTGGGTCTCA R-TTTGGGACCGTGTCTTCCTA
16	SSR-6195	F-GATGCTGGTGCTTGTATGGA R-TAATTTCTACGCAAGGGAGAGAG
17	SSR-6196	F-TGAAAGAATCCTCGTCATCG R-TCAGGTCCAAAGAGCCAAAC
18	SSR-6197	F-CATGGCTATCATGGGTCCTT R-TGATGTACGGAGTGAAGGAAGA
19	SSR-6198	F-TGAAGCAAAGGGAGTTGTGA R-GAAAGCCCAAAAGGGAAAAA
20	SSR-6199	F-TGGAAAATTGGTGTTATTAAAGTAT R-ATGGGGATTTGCTTCCTTGT
21	SSR-6200	F-CCAGACAGTGCATCCCATAG R-GCGTTGATTTATGGACATTCAA
22	SSR-6201	F-TGGGCACTATTCCATGCTTT R-ATTGCAATATCAGTTTTTTC
23	SSR-6210	F-AGCGTAGTGACTTTTTCCAGATT R-TGTCTTTCTGCACTCAAAGGA
24	SSR-6212	F-GCCTATGACACATAGACCATGC R-TTGGTGGTCAAGGATGAAGA

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Serial No.	Name	Primer Sequence 5' ----3'
25	SSR-6214	F-CTTCTCTCCGCACCCAATC R-GCGAAACAGGGTAGGGAATC
26	SSR-6215	F-GCTTCCCCGCTAGAATCTTT R-GGTGCCAATGGATCAGGTAA
27	SSR-6216	F-GAGGCTAAATGTGATTGGGTCT R-TTTGCCCTAATACCTTTATCATCTC
28	SSR-6217	F-GGGAGTGCTCCGGAAAGT R-TTCCCTATGAACTGGGAGATCTAT
29	SSR-6218	F-GTGGAAGGAATGGGTCCAG R-AGGAAATTTGCATTCCCTTGT
30	SSR-6219	F-ACAATGCACAAAATGTGAATCTC R-GGGAAGCTTAGGAAAAGTTTGA
31	SSR-6220	F-ACCAGGTGCAATGCTTCTCT R-CCTTCCTGTCATCATTTCCAA
32	SSR-6224	F-GCTTTGCATGTGGATTTCCT R-GGGGAGAATGAAACTAAAGTAATGTT
33	SSR-6226	F-ATCTCAGCTTCACCCACCAC R-TGATGAAGAATTGGGGGAAG
34	SSR-6228	F-CACGTTTTCCTTTCCTCACC R-TACAATGAAATGGGCTGCAC
35	SSR-6229	F-TATTCCGACAACCACCCAAT R-GGGATCCATGAGGAGAGAGA
36	SSR-6230	F-TCCATTGACATTATAATCTTTGACG R-TCCTCCTGATTGGACCTCAC
37	SSR-6235	F-TTTTCCCTCCACCTGTTTGA R-GAAGCATTGACCAAGCAACA
38	SSR-6236	F-AGCAGCAGTGTTGCCTCATA R-TGGAATCCGTGTTTTTATCCA
39	SSR-6237	F-CGTCGCAATTCCCAATCTAA R-ATGTTCGTAAAACCGCGTTC
40	SSR-6240	F-TTCAATGTGGGAGGATGAGA R-GGTTCCGGATTCAATTTTCC
41	SSR-6242	F-TGTTGACTGGCAGAGGTTGA R-TTCCACGAATCATCGACAGA
42	SSR-6243	F-GTAGGGAGTTGGCCACGATA R-CAACCGATGTAAAAAGTGGACA
43	SSR-6245	F-CGAACATGTTTTTGGTCACG R-CTACAACCGCGTTAGCCTTC
44	SSR-6247	F-ATATTCTGCTCCCGCTGTTG R-TCGTGCATGGGTTTATGTGT
45	SSR-6248	F-GGGTGCTTTGCTCACATCTT R-TCCATGTGTTTATGACGCAAA
46	SSR-6250	F-GCTGTTATCGTTGCCTTGGT R-GGGCAAATAGGTTGAGTTGG
47	SSR-6252	F-TTTCACATTGTCCACCAGGA R-TTGGGCTTGGTTAAAAGTCG
48	SSR-6254	F-CCATCCTGTTTGCAAAGTACA R-TTACATATATTCTAGAGGGGGAATTG
49	SSR-6259	F-CCTTCATAAAGACCACGTCCA R-TGTTGCTCAAATTTCCAGCTT
50	SSR-6260	F-AAAGTTTTAATATTACCAACAACAA R-CAACCAGGCAAATGGAAATC

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Serial	Name	Primer Sequence 5' ----3'
51	SSR-6265	F-CAGAAGCGGTGAAAATTGAAC R-GCATGTTGCTTTGACAATGG
52	SSR-6266	F-AAGTTGTTCCACCCCACTGT R-TTTCCTTCCATTTTCATGGTG
53	SSR-6270	F-TCCTCCCACACTTGGAAATC R-TATGCGAAAAGGGATTGCTC
54	SSR-6273	F-CCCCCAGAACAAATAGAAACTC R-TGAATTTGAAGAAGAGATGGTTG
55	SSR-6277	F-CACCCCCGTACACACACAC R-CACTTAAATTTTCACCAGGCATT
56	SSR-6282	F-CCAAAATTAAAGTGCAAGCTCA R-TCTTTGGATGGGATGAGAGC
57	SSR-6283	F-GTGCATCGGGAAAAAGAAAA R-GAAGCGAGGGAATTATGCAG
58	SSR-6285	F-AACTATTTTCATCTTAAATATACGTCTT R-TTCATAACTCTAATTGTCACACCA
59	SSR-6287	F-GCCTTTTGGCAACTTCTGAG R-TGCAAGAGAACATTAAAAAGCCTA
60	SSR-6289	F-CCCCCAAAGTTGATGAACAC R-TTGATGGAGTTCGCATCTTCT
61	SSR-6291	F-TCATGAGTTTCCACACACCAA R-CCTTCGTATGTATATGTGGCTACTG
62	SSR-6292	F-AAGGGTGCACTGGTAGAGGA R-GCTCACTTTGTGCATGTTCC
63	SSR-6294	F-TGGTGCTTGTAAGAAAAACAGAA R-GGAGAGCAGAAGATGAAGTGAA
64	SSR-6296	F-GTGGGTGCAGTCACTCTCAA R-TCACCTTTGATCACGCTCTG
65	SSR-6299	F-GGCGCAGAAAGACAGGTTAC R-CTGCAGCACCTAACTCACCA
66	SSR-6300	F-CTGCAGCACCTAACTCACCA R-ATGCCACAACACCATCTTCA
67	SSR-6301	F-ACCTCCCAAGTCCCACTCTT R-CGGACTGGACGGAGAGAC
68	SSR-6315	F-CGCAGTGAAAAGGAAAAGGA R-ATCAGCGTCCAATCCAAAAA
69	SSR-6317	F-CTCCTTCCTCCACCTCCTCT R-AAATCGAGGGGAAAATGGAG
70	SSR-6320	F-AGGCTATGATGTACGGACACG R-TATCTCGGAGGTGCCATTTC
71	SSR-6323	F-CAAAGGGTCATCAGGATTGG R-TTTAAGCAGCCAAGCAGTTGT
72	SSR-6325	F-GGTGTCAACACCGTTGGAG R-TGCAAGCCATTAGAGAATGACA
73	SSR-6336	F-TGAAAAACAACGATATGCAGAAG R-TCAGTCTTAGAATTGAGTTTTCTTCG
74	SSR-6350	F-TGCCTATGCTTATGCCTGTG R-GATGCCTGTTACTTGCCTTCT
75	SSR-6356	F-TGCAATATGGACCAGAAGAAA R-ATGCCCCAACAACAACATTT
76	SSR-6360	F-TTTTCAATCCTCCCCTTGTC R-TGTAGTTAAAATCAGAGACTTACAGG
77	SSR-6375	F-GCTCGGATATGGTCCTGAAA R-TCAGTGTCAGCACCATACCC

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No.		
78	SSR-6518	F-GAGATGCCTCCTCAGCACTC R-TCTCACTCTCTCTAACCGACACA
79	SSR-6600	F-GAGCAGCAGATACACCTAAC R-CCTGCTTCGACCCTCTTCAG
80	SSR-6605	F-TTATCTGTTTCAACAATTTAATAAC R-GGTAAGGTTACAAAATATAAAGTC
81	SSR-6607	F-GAGAGTATCAAATGCTGTGGC R-CAATGAACTCAGACATCTCAC
82	SSR-6610	F-CGCCGATATTCATGCCAAGG R-GTTGTTGAGTGACTATGGG
83	SSR-6518	F-GAGATGCCTCCTCAGCACTC R-TCTCACTCTCTCTAACCGACACA

84	SSR-6929	F-GCCCATGTAATGCTGTATAGT R-GGCGTTAGAACTACTCCAGTT
85	SSR-6937	F-CCAGGTTCATCTAATTGGGAC R-GGTGACATCTGCGTCTAGAAG
86	SSR-6941	F-CTCTTGACCAGAAACAGGAAG R-GAGCATAAGGACATGAACACA
87	SSR-6954	F-CCAACTCTTAGGAGCATTAGT R-GTACCGGTTCTCTTCGTTTGT
88	SSR-6959	F-GTTCTTGTGGTGTCTTACATC R-CCTGCAAGGACGTAGTTTTCA
89	VuUGM02	F-AAACTAGCACCAAATCCAACA R-TCAAAAACACAGGTCCTCCA
90	VuUGM08	F- AGAACCCAGCAATACCTGCAT R- GAGCAAAAGCCTCCATCACT
91	VuUGM19	F- CATCCCGTGAAATTCAACAA R-CCTCGCCAATGATTCTGAG
92	E61R	F- AATTCACTTATGACTGAGCTATAT R- AATTCACTTATGACTGAGCTATAT
93	61RM2	F- GATTTGTTTGGTTTCCTTAAG R- GGTTGATCTTGGAGGCATTTT
94	SSR-1	F- CCTAAGCTTTTCTCCAACTCCA R- CAAGAAGGAGGCGAAGACTG
95	C42-2B	F- CAGTTCCCTAATGGACAACC R- CAAGCTCATCATCATCTCGATG
96	SSR-6965	F- GCATTCAGCTACGATGTGTTC R- GGCACTTTGTAAAAGACAGGC
97	SSR-6775	F- CAGAATATATGAGAAAGTTAAGTG R- CACATAACACTGTACGAACACG
98	SSR-6776	F- GTAGTTAAGTTTAGAAAAATAG R- GGTGATGTTGGGAATGGTTG
99	SSR-6777	F- CGAAGCATGTGGACACGTAC R- CATTGAACAAACATCGCTGAAGC
100	SSR-6778	F- GATGCTCCCAAGAAAGATAC R- CTCGATACTATTTTCCGTGG

Source; Asare et al., 2013

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Appendix D

Table 8: Polymorphism among cowpea progenies in the four populations as revealed by the six *Striga* resistance markers

Primer	Population	Population size/Total number of progenies	Number of progenies with the marker	Number of progenies without the marker	Proposed % of progenies Resistance
LRR8	1	9	**6**	**3**	66.7
	2	9	**3**	**6**	33.3
	3	12	**8**	**4**	66.7
	4	20	**9**	**11**	45.0
LRR11	1	9	**7**	**2**	77.8
	2	9	**7**	**2**	77.8
	3	12	**7**	**5**	58.3
	4	20	**7**	**13**	35.0
SSR-1	1	9	**6**	**3**	66.7
	2	9	**4**	**5**	44.4
	3	12	**8**	**4**	66.7
	4	20	**11**	**9**	55.0
C42-2B	1	9	**6**	**3**	66.7
	2	9	**4**	**5**	44.4
	3	12	**8**	**4**	66.7
	4	20	**11**	**9**	55.0
CLM1320	1	9	**7**	**2**	77.8
	2	9	**4**	**5**	44.4
	3	12	**9**	**3**	75.0
	4	20	**10**	**10**	50.0
61RM2	1	9	**9**	**0**	100.0
	2	9	**4**	**5**	44.4
	3	12	**9**	**3**	75.0
	4	20	**13**	**7**	65.0

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Appendix E -- OUTPUT for Pot Screening

GenStat Twelfth Edition
GenStat Procedure Library Release PL20.1

Analysis of variance

Variate: NPEND

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	8.222	4.111	0.66	
REP.*Units* stratum					
Genotype	55	1472.722	26.777	4.30	<.001
Residual	113	703.944	6.230		
Total	170	2184.889			

Message: the following units have large residuals.

REP 1 *units* 55	5.43 approx. s.e. 2.03	
REP 2 *units* 17		6.03 approx. s.e. 2.03
REP 2 *units* 22		-6.30 approx. s.e. 2.03

Standard errors of differences of means

Table	Genotype	
rep.	unequal	
d.f.	113	
s.e.d.	2.038	min.rep
	1.765	max-min
	1.441X	max.rep

(No comparisons in categories where s.e.d. marked with an X)

Least significant differences of means (5% level)

Table	Genotype	
rep.	unequal	
d.f.	113	
l.s.d.	4.037	min.rep
	3.497	max-min
	2.855X	max.rep

(No comparisons in categories where l.s.d. marked with an X)

Stratum standard errors and coefficients of variation

Variate: NPEND

Stratum	d.f.	s.e.	cv%
REP	2	0.269	3.0
REP.*Units*	113	2.496	28.1

Analysis of variance

Variate: NPOD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	7.520	3.760	0.58	
REP.*Units* stratum					
Genotype	55	2484.749	45.177	6.92	<.001
Residual	113	737.813	6.529		
Total	170	3230.082			

Message: the following units have large residuals.

REP 2 *units* 5	6.94 approx. s.e. 2.08	
REP 2 *units* 17		5.94 approx. s.e. 2.08
REP 2 *units* 47		-6.06 approx. s.e. 2.08
REP 2 *units* 48		6.27 approx. s.e. 2.08

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Standard errors of differences of means

Table	Genotype	
rep.	unequal	
d.f.	113	
s.e.d.	2.086	min.rep
	1.807	max-min
	1.475X	max.rep

(No comparisons in categories where s.e.d. marked with an X)

Least significant differences of means (5% level)

Table	Genotype	
rep.	unequal	
d.f.	113	
l.s.d.	4.133	min.rep
	3.580	max-min
	2.923X	max.rep

No comparisons in categories where l.s.d. marked with an X)

Stratum standard errors and coefficients of variation

Variate: NPOD

Stratum	d.f.	s.e.	cv%
REP	2	0.257	4.1
REP.*Units*	113	2.555	40.7

Analysis of variance

Variate: NPED_POD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	14.140	7.070	1.71	
REP.*Units* stratum					
Genotype	55	1120.360	20.370	4.92	<.001
Residual	113	468.026	4.142		
Total	170	1602.526			

Message: the following units have large residuals.

REP 1 *units* 17	-4.37	approx. s.e.	1.65
REP 1 *units* 48	-4.70	approx. s.e.	1.65
REP 2 *units* 17	5.04	approx. s.e.	1.65
REP 3 *units* 36	4.67	approx. s.e.	1.65

Standard errors of differences of means

Table	Genotype	
rep.	unequal	
d.f.	113	
s.e.d.	1.662	min.rep
	1.439	max-min
	1.175X	max.rep

(No comparisons in categories where s.e.d. marked with an X)

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Least significant differences of means (5% level)

Table	Genotype	
rep.	unequal	
d.f.	113	
l.s.d.	3.292	min.rep
	2.851	max-min
	2.328X	max.rep

(No comparisons in categories where l.s.d. marked with an X)

Stratum standard errors and coefficients of variation

Variate: NPED_POD

Stratum	d.f.	s.e.	cv%
REP	2	0.352	7.1
REP.*Units*	113	2.035	41.

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Appendix F- R Output

```
Augmented Design Details
========================

Number of blocks           "5"
Number of treatments       "56"
Number of check treatments "6"
Number of test treatments  "50"
Check treatments           "GH3684, IT97k-499-35, KIRKHOUSE BENGA, P
ADI-TUYA, SARC-1, UCSO1"

ANOVA, Treatment Adjusted
=========================
                                     Df  Sum Sq Mean Sq F value   Pr(
>F)
Block (ignoring Treatments)           4    56.2   14.05   2.723  0.05
853 .
Treatment (eliminating Blocks)       55   862.9   15.69   3.041  0.00
398 **
  Treatment: Check                    5   288.6   57.72  11.189 3.02e
-05 ***
  Treatment: Test and Test vs. Check 50   574.3   11.49   2.227  0.02
639 *
Residuals                            20   103.2    5.16
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

ANOVA, Block Adjusted
=====================
                               Df Sum Sq Mean Sq F value   Pr(>F)
Treatment (ignoring Blocks)    55  884.5   16.08   3.117  0.00339 **
  Treatment: Check              5  288.6   57.72  11.189 3.02e-05 **
*
  Treatment: Test              49  525.1   10.72   2.077  0.03818 *
  Treatment: Test vs. Check     1   70.8   70.76  13.717  0.00141 **
Block (eliminating Treatments)  4   34.7    8.66   1.680  0.19409
Residuals                      20  103.2    5.16
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Treatment Means
===============
         Treatment Block Means        SE r  Min  Max Adjusted Means
1           GH3684       13.58 0.3760319 5 12.8 14.8       13.58000
2     IT97k-499-35       15.56 0.3957272 5 14.8 16.9       15.56000
3  KIRKHOUSE BENGA       18.14 0.5732364 5 16.6 19.5       18.14000
4        PADI-TUYA       21.60 0.4266146 5 20.3 22.5       21.60000
5           SARC-1       15.14 0.4467662 5 14.2 16.8       15.14000
6         UC15-01      3 16.80        NA 1 16.8 16.8       17.85000
7         UC15-02      4 16.40        NA 1 16.4 16.4       17.00000
8         UC15-03      1 20.00        NA 1 20.0 20.0       18.21667
9         UC15-04      5 14.10        NA 1 14.1 14.1       13.41667
10        UC15-05      3 18.60        NA 1 18.6 18.6       19.65000
11        UC15-06      2 21.60        NA 1 21.6 21.6       22.41667
12        UC15-07      3 16.60        NA 1 16.6 16.6       17.65000
13        UC15-09      2 17.60        NA 1 17.6 17.6       18.41667
14        UC15-10      4 16.80        NA 1 16.8 16.8       17.40000
15        UC15-11      3 22.70        NA 1 22.7 22.7       23.75000
16        UC15-12      2 17.80        NA 1 17.8 17.8       18.61667
17        UC15-13      3 17.70        NA 1 17.7 17.7       18.75000
18        UC15-14      3 18.40        NA 1 18.4 18.4       19.45000
19        UC15-15      2 19.50        NA 1 19.5 19.5       20.31667
20        UC15-16      1 23.10        NA 1 23.1 23.1       21.31667
21        UC15-17      3 22.60        NA 1 22.6 22.6       23.65000
22        UC15-18      4 24.40        NA 1 24.4 24.4       25.00000
23        UC15-19      3 23.30        NA 1 23.3 23.3       24.35000
24        UC15-20      1 15.80        NA 1 15.8 15.8       14.01667
25        UC15-21      2 18.20        NA 1 18.2 18.2       19.01667
```


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```
26          UC15-22      1 18.20          NA 1 18.2 18.2         16.41667
27          UC15-23      1 15.50          NA 1 15.5 15.5         13.71667
28          UC15-24      4 14.30          NA 1 14.3 14.3         14.90000
29          UC15-25      2 22.40          NA 1 22.4 22.4         23.21667
30          UC15-26      3 12.20          NA 1 12.2 12.2         13.25000
31          UC15-27      4 13.40          NA 1 13.4 13.4         14.00000
32          UC15-28      5 15.40          NA 1 15.4 15.4         14.71667
33          UC15-29      4 20.90          NA 1 20.9 20.9         21.50000
34          UC15-30      4 15.40          NA 1 15.4 15.4         16.00000
35          UC15-31      2 21.90          NA 1 21.9 21.9         22.71667
36          UC15-32      4 21.10          NA 1 21.1 21.1         21.70000
37          UC15-33      4 20.80          NA 1 20.8 20.8         21.40000
38          UC15-34      2 20.10          NA 1 20.1 20.1         20.91667
39          UC15-35      5 20.80          NA 1 20.8 20.8         20.11667
40          UC15-36      1 25.70          NA 1 25.7 25.7         23.91667
41          UC15-37      4 23.00          NA 1 23.0 23.0         23.60000
42          UC15-38      2 22.50          NA 1 22.5 22.5         23.31667
43          UC15-39      4 21.30          NA 1 21.3 21.3         21.90000
44          UC15-40      2 21.80          NA 1 21.8 21.8         22.61667
45          UC15-41      3 23.30          NA 1 23.3 23.3         24.35000
46          UC15-42      3 15.80          NA 1 15.8 15.8         16.85000
47          UC15-43      2 17.60          NA 1 17.6 17.6         18.41667
48          UC15-44      4 20.50          NA 1 20.5 20.5         21.10000
49          UC15-45      2 22.50          NA 1 22.5 22.5         23.31667
50          UC15-46      1 23.30          NA 1 23.3 23.3         21.51667
51          UC15-47      1 21.10          NA 1 21.1 21.1         19.31667
52          UC15-48      1 16.60          NA 1 16.6 16.6         14.81667
53          UC15-49      1 21.10          NA 1 21.1 21.1         19.31667
54          UC15-50      1 20.90          NA 1 20.9 20.9         19.11667
55          UC15-51      1 24.90          NA 1 24.9 24.9         23.11667
56            UCSO1        21.48 2.4255721 5 16.9 27.4         21.48000

Coefficient of Variation
========================
12.08275

Overall Adjusted Mean
=====================
19.40893

Standard Errors
===================
                                        Std. Error of Diff.  CD (5%)
Control Treatment Means                            1.436468 2.99642
0
Two Test Treatments (Same Block)                   3.212040 6.70019
8
Two Test Treatments (Different Blocks)             3.469400 7.23704
1
A Test Treatment and a Control Treatment           2.622620 5.47068
9

[1] 19.40893

$PV
[1] 10.71666

$GV
[1] 5.558057

$EV
[1] 5.1586

$GCV
[1] 12.14675

$`GCV category`
[1] "Medium"
```


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```
$PCV
[1] 16.86662

$`PCV category`
[1] "Medium"

$ECV
[1] 11.70212

$hBS
[1] 51.86372

$`hBS category`
[1] "Medium"

$GA
[1] 3.502616

$GAM
[1] 18.04642

$`GAM category`
[1] "Medium"

print(out2) SEED WEIGHT

Augmented Design Details
========================

Number of blocks           "5"
Number of treatments       "56"
Number of check treatments "6"
Number of test treatments  "50"
Check treatments           "GH3684, IT97k-499-35, KIRKHOUSE BENGA, P
ADI-TUYA, SARC-1, UCSO1"

ANOVA, Treatment Adjusted
=========================
                                     Df   Sum Sq Mean Sq F value Pr(
>F)
Block (ignoring Treatments)           4  4411515 1102879   2.276 0.0
970 .
Treatment (eliminating Blocks)       55 32607514  592864   1.223 0.3
173
  Treatment: Check                    5  9161070 1832214   3.780 0.0
143 *
  Treatment: Test and Test vs. Check 50 23446444  468929   0.968 0.5
563
Residuals                            20  9693494  484675
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

ANOVA, Block Adjusted
=====================
                               Df   Sum Sq Mean Sq F value Pr(>F)
Treatment (ignoring Blocks)    55 33581122  610566   1.260 0.2906
  Treatment: Check              5  9161070 1832214   3.780 0.0143 *
  Treatment: Test              49 24294029  495797   1.023 0.4972
  Treatment: Test vs. Check     1   126024  126024   0.260 0.6157
Block (eliminating Treatments)  4  3437907  859477   1.773 0.1738
Residuals                         20  9693494  484675

Augmented Design Details
========================

Number of blocks           "5"
Number of treatments       "56"
Number of check treatments "6"
Number of test treatments  "50"
```


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```
Check treatments          "GH3684, IT97k-499-35, KIRKHOUSE BENGA, P
ADI-TUYA, SARC-1, UCSO1"

ANOVA, Treatment Adjusted
=========================
                                      Df Sum Sq Mean Sq F value   Pr(
>F)
Block (ignoring Treatments)            4   61.4   15.36   4.570  0.00
875 **
Treatment (eliminating Blocks)        55 1215.6   22.10   6.578 1.28e
-05 ***
  Treatment: Check                     5  159.5   31.89   9.492 9.29e
-05 ***
  Treatment: Test and Test vs. Check 50 1056.1   21.12   6.286 2.03e
-05 ***
Residuals                             20   67.2    3.36
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ANOVA, Block Adjusted
=====================
                                Df Sum Sq Mean Sq F value   Pr(>F)
Treatment (ignoring Blocks)     55 1245.8   22.65   6.741 1.05e-05 **
*
  Treatment: Check               5  159.5   31.89   9.492 9.29e-05 **
*
  Treatment: Test               49 1048.9   21.41   6.371 1.86e-05 **
*
  Treatment: Test vs. Check      1   37.5   37.45  11.147  0.00327 **
Block (eliminating Treatments)  4   31.2    7.80   2.321  0.09204 .
Residuals                       20   67.2    3.36
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


print(out1)--------------- DAYS TO MATURITY

Augmented Design Details
========================

Number of blocks          "5"
Number of treatments      "56"
Number of check treatments "6"
Number of test treatments "50"
Check treatments          "GH3684, IT97k-499-35, KIRKHOUSE BENGA, P
ADI-TUYA, SARC-1, UCSO1"

ANOVA, Treatment Adjusted
=========================
                                      Df Sum Sq Mean Sq F value   Pr(
>F)
Block (ignoring Treatments)            4   83.1  20.768   9.017 0.000
248 ***
Treatment (eliminating Blocks)        55  909.8  16.543   7.182 6.18e
-06 ***
  Treatment: Check                     5   73.8  14.753   6.405 0.001
050 **
  Treatment: Test and Test vs. Check 50  836.1  16.722   7.260 6.19e
-06 ***
Residuals                             20   46.1   2.303
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ANOVA, Block Adjusted
=====================
                                Df Sum Sq Mean Sq F value   Pr(>F)
Treatment (ignoring Blocks)     55  967.8   17.60   7.639 3.67e-06 **
*
  Treatment: Check               5   73.8   14.75   6.405  0.00105 **
```


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```
  Treatment: Test                 49  797.1   16.27   7.063 7.95e-06 **
*
  Treatment: Test vs. Check        1   96.9   96.90  42.070 2.53e-06 **
*
Block (eliminating Treatments)  4   25.1    6.28   2.728  0.05823 .
Residuals                      20   46.1    2.30
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> describe.augmentedRCBD(out1)
$Count
[1] 56

$Mean
[1] 57.87143

$Std.Error
[1] 0.5328904

$Std.Deviation
[1] 3.987787

$Min
[1] 51.8

$Max
[1] 68.13333

$`Skewness(statistic)`
     skew         z
0.6352785 2.0164856

$`Skewness(p.value)`
[1] 0.04374922

$`Kurtosis(statistic)`
     kurt         z
3.0041703 0.4107603

$`Kurtosis(p.value)`
[1] 0.6812483

> gva.augmentedRCBD(out1)---
$Mean
[1] 57.87143

$PV
[1] 16.26776

$GV
[1] 13.96442

$EV
[1] 2.303333

$GCV
[1] 6.457245

$`GCV category`
[1] "Low"

$PCV
[1] 6.969468

$`PCV category`
[1] "Low"

$ECV
[1] 2.622492
```


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```
$hBS
[1] 85.84111

$`hBS category`
[1] "High"

$GA
[1] 7.142634

$GAM
[1] 12.34225

$`GAM category`
[1] "Medium"
```

> print(out2)---------NUMBER OF PLANTS WITH *STRIGA*

```
Augmented Design Details
========================

Number of blocks           "5"
Number of treatments       "56"
Number of check treatments "6"
Number of test treatments  "50"
Check treatments           "GH3684, IT97k-499-35, KIRKHOUSE BENGA, P
ADI-TUYA, SARC-1, UCSO1"

ANOVA, Treatment Adjusted
=========================
                                     Df Sum Sq Mean Sq F value Pr(>F
)
Block (ignoring Treatments)           4  4.561  1.1402   0.924  0.46
9
Treatment (eliminating Blocks)       55 30.660  0.5575   0.452  0.98
9
  Treatment: Check                    5  4.167  0.8333   0.676  0.64
7
  Treatment: Test and Test vs. Check 50 26.493  0.5299   0.430  0.99
2
Residuals                            20 24.667  1.2333

ANOVA, Block Adjusted
=====================
                               Df Sum Sq Mean Sq F value Pr(>F)
Treatment (ignoring Blocks)    55 27.087  0.4925   0.399  0.996
  Treatment: Check              5  4.167  0.8333   0.676  0.647
  Treatment: Test              49 22.880  0.4669   0.379  0.997
  Treatment: Test vs. Check     1  0.041  0.0408   0.033  0.857
Block (eliminating Treatments)  4  8.133  2.0333   1.649  0.201
Residuals                      20 24.667  1.2333

Treatment Means
```

> print(out1)------- *STRIGA* COUNT PER PLANT

```
Augmented Design Details
========================

Number of blocks           "5"
Number of treatments       "56"
Number of check treatments "6"
Number of test treatments  "50"
Check treatments           "GH3684, IT97k-499-35, KIRKHOUSE BENGA, P
ADI-TUYA, SARC-1, UCSO1"
```


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```
ANOVA, Treatment Adjusted
=========================
                                     Df Sum Sq Mean Sq F value   Pr(>F)
Block (ignoring Treatments)           4   42.9  10.717   5.390  0.00
412 **
Treatment (eliminating Blocks)       55 1214.9  22.089  11.109 1.41e
-07 ***
  Treatment: Check                    5    6.8   1.355   0.681  0.64
271
  Treatment: Test and Test vs. Check 50 1208.1  24.163  12.152 7.01e
-08 ***
Residuals                            20   39.8   1.988
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

ANOVA, Block Adjusted
=====================
                              Df Sum Sq Mean Sq F value   Pr(>F)
Treatment (ignoring Blocks)   55 1246.2   22.66  11.395 1.12e-07 **
*
  Treatment: Check             5    6.8    1.35   0.681    0.643
  Treatment: Test             49 1188.0   24.25  12.194 6.96e-08 **
*
  Treatment: Test vs. Check    1   51.3   51.34  25.819 5.70e-05 **
*
Block (eliminating Treatments)  4   11.6    2.91   1.463    0.251
Residuals                     20   39.8    1.99
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Coefficient of Variation
========================
95.00838

Overall Adjusted Mean
=====================
1.875298

Standard Errors
===================
                                         Std. Error of Diff.  CD (5%)
Control Treatment Means                            0.8918146 1.86029
3
Two Test Treatments (Same Block)                   1.9941581 4.15974
1
Two Test Treatments (Different Blocks)             2.1539370 4.49303
4
A Test Treatment and a Control Treatment           1.6282233 3.39641
4
```

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