

possible to develop new cultivars that resist pest injury yet still retain desirable horticultural characteristics (Eickhoff *et al.*, 2008).

Often, both a resistant and susceptible variety will have the same basic response to a pest, but the resistant variety will respond more quickly or more dramatically than the susceptible variety, reducing the amount of damage the pest causes. Plants that express antibiosis affect the biology of pests.

Dahms (1972) illustrated the antibiotic effect of resistant plant on differential rate of aphid development. Nymphs matured in five days (susceptible variety), 10 days (intermediate antibiosis) and 20 days (high antibiosis). Mortality of immature arthropods was one of the most important factors limiting the increase of arthropod population, which was also illustrated by Dahms (1972).

2.5.2.3 Tolerance

Plant tolerance is the degree to which a plant can support an insect population that under similar conditions would severely damage a susceptible plant (Cuartera *et al.*, 1999). When two cultivars are equally infested, the less tolerant one produces low yield. A tolerant plant may be colonized by a pest to the same extent as susceptible plants, but there is no reduction in yield both in quantity and quality (Eickhoff *et al.*, 2008). Plants with an ability to tolerate insect damage at times may produce more yield than the plants of a non-tolerant susceptible cultivar at the same level of insect infestation. Tolerance often occurs in combination with antixenosis and antibiosis components of resistance (Sharma, 2009).

2.5.2.4 Resistance of Cowpea to *Aphis craccivora* Koch

Antibiosis has been identified as the main mechanism responsible for aphid resistance in cowpea (Singh, 1977; Ansari, 1984; Ofuya, 1988) and is controlled by a single dominant gene (Singh and Ntare, 1985; Bata *et al.*, 1987; Ombakho *et al.*, 1987; Pathak, 1988).

A large number of aphid resistant lines have been developed and evaluated in international yield trials. These lines, which need no insecticide protection against aphids include, TVu36, TVu300, TVu310, TVu410, TVu2996, TVu3000, IT84S-2246, IT87S-1459, IT84S-2049 and IT93K-503-1 (Bata *et al.*, 1987; Ofuya, 1997). Others include IT8S-728-5, IT83S-728-13, IT83S-742-2, IT84E-1-108 (Obeng-Ofori, 2007). Other aphid resistant varieties include: IT90K-59, IT90K-76, IT97K-499-35 and IT00K-1251 (Singh, 2004). The resistance in genotype IT84S-2246 is the source of resistance in varieties IT90K-59, IT90K-76, IT97K-499-35 and IT00K-1251 (Singh, 2004).

Screening for aphid resistance has been conducted in IITA and several resistant lines have been identified (Singh and Jackai, 1985) and used in the breeding programmes to develop aphid resistant cultivars (Singh and Ntare, 1985). However, the resistance to *A. craccivora* of all the identified cowpea cultivars at IITA has recently broken down (Sharma, 2009).

In response to breakdown of resistance in several aphid resistant varieties, new resistant cultivars have been developed in recent studies. Souleymane *et al.* (2013) and Kusi *et al.* (2010a) conducted separate experiments to search for new sources of resistance in African cowpea. Souleymane *et al.* (2013) screened 109 cowpea cultivars and 92 wild cowpea accessions in Nigeria to search for new source of resistance to *A. craccivora*.

IT97K-556-6 showed the highest level of tolerance and was recommended for breeding programmes aimed at introgressing aphid resistance gene into susceptible cultivars.

SARC-1-57-2 was identified as resistant to *A. craccivora* among 22 cowpea varieties evaluated with seedling screening method (Kusi *et al.*, 2010a). The stability of SARC-1-57-2 was further tested in all major cowpea growing zones in Ghana. The study confirmed that the aphid resistance gene in SARC-1-57-2 is stable against *A. craccivora* in all major cowpea growing belts of Ghana. SARC-1-57-2 is an advanced line (F₆) developed from a cross between Apabgaala and UCR 01-11-52 (Kusi *et al.*, 2010a). Inheritance of aphid resistance gene follows the mendallian inheritance pattern.

2.6 Role of Characterization in crop improvement programmes

Characterization of crops is the first step in any crop improvement programme (De Vicente *et al.*, 2005). Characterization helps in diversity studies, identification, and in the selection of suitably diverse parents to obtain heterotic hybrids. It is also needed for germplasm conservation purposes. Various morphological, biochemical and molecular markers are used for the characterization of cowpea germplasm.

2.6.1 Morphological characterization

Morphological Characterization involves recording characters which are highly heritable and can easily be scored visually in most environmental conditions (IBPGR, 1993).

The main advantages of conducting morphological characterization are that: (i) published descriptor lists are readily available for most major crop species including *Vigna unguiculata* (L.) Walp, (ii) it can be carried out *in situ* (on-farm) and (iii) it is

relatively inexpensive and relatively easy to carry out (Hoogendijk and Williams, 2001).

Morphological markers are analysed at the phenotype level and comes along with the following disadvantages; they are influenced by environmental conditions, they are labour intensive and require large populations of plants in performing breeding experiments. Moreover, they need large plots of land and/or greenhouse space in which to be grown; but they have remained useful till now as a highly recommended first step that should be undertaken before more in-depth biochemical or molecular studies (e.g. DNA fingerprinting) are attempted (Smith & Smith, 1992).

The agro-morphological variability in several cowpea varieties have been studied across the world (Ghalmi *et al.*, 2010; Aminasaun *et al.*, 2015; Bennett-Lartey and Ofori, 2000). However, there is little information on the agro-morphological characteristics on newly improved cultivars. Recent agro-morphological characterization studies on cowpea varieties focus on parameters that benefits breeding for early maturity, pest and disease resistance and resistance to parasitic weeds (Singh *et al.*, 1997). On the other hand, other characteristics such as number of pods per plant, number of days to flowering, growth habit, flower colour, pod shape etc have been less researched (Cobbinah *et al.*, 2011).

2.6.2 Molecular marker-based characterization

The limitations in morphological characterization led to the development of molecular makers. Molecular marker-based characterization also known as DNA fingerprinting is a useful complement to morphological and physiological characterization of varieties. Molecular maker techniques are based on naturally occurring polymorphisms in DNA sequences.

Fingerprinting with molecular markers allows precise, objective and rapid variety identification of plant varieties (Beleke and Beleke, 2014). Molecular markers are stable and detectable in all tissues regardless of growth differentiation, development, pleiotropic effect, epistatic effects and not confounded to environment where they grow (Bhat, 1995; Milee *et al.*, 2008). There are several types of genetic markers and the choice of a particular marker depends on the level of diversity information needed. Some of the molecular markers used for molecular characterization are Restriction fragment length polymorphism (RFLP), Random amplified polymorphism DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple sequence repeats (SSRs) or short tandem repeats and Single Nucleotide Polymorphism (SNPs). One of the main uses of DNA markers in agricultural research has been in the construction of linkage maps for diverse crop species. Linkage maps have been utilised for identifying chromosomal regions that contain genes controlling simple traits (controlled by a single gene) and quantitative traits using QTL analysis (Mohan *et al.*, 1997).

2.6.2.1 Simple Sequence Repeats (SSR) markers

Simple Sequence Repeats (SSRs), also known as Microsatellites, are co-dominant markers that are routinely used to study genetic diversity in different crop species. These markers occur at high frequency and appear to be distributed throughout the genome of higher plants. These are DNA sequences that consist of two to five nucleotide core units such as (AT)_n, (CTT)_n and (ATGT)_n, which are tandem repeats. The regions flanking the microsatellites are generally conserved among varieties of the same species, allowing the selection of PCR primers that will amplify the intervening SSR in all varieties. Variation in the number of tandem repeats, *n*, results in different

PCR product lengths. Due to mutations these repeats are highly polymorphic even among closely related cultivars, causing variations in the number of repeating units. They can detect a large number of alleles; level of heterozygosity is high and follows Mendelian inheritance (Wu and Tanksley 1993). Microsatellites have become the molecular markers of choice for a wide range of applications in genetic mapping and genome analysis (Li *et al.*, 2001), genotype identification and variety protection (Senior *et al.* 1998), seed purity evaluation and germplasm conservation (Brown *et al.*, 1996), diversity studies (Xiao *et al.*, 1996), paternity determination and pedigree analysis (Ayres *et al.* 1997), gene and quantitative trait locus analysis (Blair and McCouch 1997) and marker-assisted breeding (Weising *et al.*, 2005). For identification of molecular markers linked to agronomically important genes, SSR is one of the best choices as compared to RAPD and AFLP for high polymorphic information and less costly (Young, 1999, Appiah-Kubi *et al.*, 2014). Doumbia *et al.* (2014) used 20 SSR markers to characterise 94 accessions of cowpea from Ghana and Mali and established the genetic relationship that exist among the accessions.

2.6.2.2 Marker-assisted selection (MAS)

A major breakthrough to plant breeding is the use of molecular markers to select varieties not only for qualitative traits but also for quantitative traits loci (QTLs). Many agriculturally important variations like productivity, quality, tolerance to environmental stresses, and some types of disease and pest resistance are controlled by QTLs and greatly depend on genetic x environmental interactions (Abdurakhmonov and Adukarimov, 2008).

A marker can either be located within the gene of interest or be linked to a gene determining a gene of interest (Brown *et al.*, 1996). Such markers can be detected early in the selection procedure thus the breeder can significantly reduce the number of seedlings grown and screened. This helps to reduce expenses and to increase efficiency of breeding (Kurt *et al.*, 2005). MAS can aid selecting for all target alleles that are difficult to assay phenotypically especially in the early generations. MAS also helps distinguish between heterozygote and homozygote varieties based on molecular data (Acquaah, 2012).

2.6.2.3 Marker assisted backcrossing (MABC)

Marker assisted backcrossing is a method in plant breeding to transfer favourable traits from a donor plant into an elite genotype (Acquaah, 2012). Markers can be used in MABC to either control the target gene or to accelerate the reconstruction of the recurrent parent genotype. Traditional backcross breeding requires 6 -8 backcrosses to fully reconstruct recurrent parent genotype, while MABC may reduce this to 3 – 5 generations. The theoretical proportion of the recurrent parent genome after n generations of backcrossing is given by $(2n+1-1)/2n+1$ (where n = number of backcrosses; assuming an infinite population size) (Acquaah, 2012). The percentages of recurrent parent recovery after each backcross generation are presented in Table 2.2.

Table 2.2 Percentage recurrent parent genome after backcrossing

Generation	Recurrent parent genome (%)
BC1	75.0
BC2	87.5
BC3	93.8
BC4	96.9
BC5	98.4
BC6	99.2

Source: Acquah, 2012

Although the initial cost of marker-assisted backcrossing would be more expensive compared to conventional breeding in the short term, the time savings could lead to economic benefits. This is an important consideration for plant breeders because the accelerated release of an improved variety may translate into more rapid profits by the release of new cultivars in the medium term to long-term (Morris *et al.*, 2003).

2.6.2.4 The Aphid resistance marker CP 171F/172R

Kusi (2014) screened 50 DNA markers (SSR primers) and identified the marker CP 171F/172R (left sequence: 5' – GTAGGGAGTTGGCCACGATA – 3'; Right sequence: 3'-CAACCGATGTAAAAAGTGGACA-5') to be tightly linked to the aphid resistance locus. The marker displayed a segregation pattern at band size of 176bp, consistent with the phenotypic scores obtained following aphid infestation of 128 lines in an earlier experiment conducted by the same author. CP 171F/172R was co-dominant and segregated in the expected 1:2:1 fashion following chi square analysis. The marker CP

171F/172R is about 50 cM away from the aphid resistance locus on linkage group 2 (Andargie *et al.*, 2011; Menendez *et al.*, 1997).

2.6.3 Description of some locally preferred cowpea varieties

2.6.3.1 Nhyira (IT87D-611-3)

Nhyira, which was released by CSIR-CRI in 2005, is one of the farmer preferred cowpea varieties in Ghana (Egbadzor *et al.*, 2013). The name Nhyira is the Akan word for blessing. This early maturing variety (65-68 days) is high yielding with average annual yield of 2,460 Kg/ha (CSIR-CRI, 2006). It has rough globose seeds, and grows to a height of 57cm without any pubescence on either stem or leaf. The leaf is broad, green in colour and obtuse in shape. The flower is purple in colour and mid vein is light green. The immature pods are green and the mature pods are brown in colour (Agyemang *et al.*, 2014). Nhyira has an erect growth habit and also drought tolerant. Nhyira is rich in energy, iron, phosphorus and protein (Agyemang *et al.*, 2014). It is also resistant to Anthracnose and Cercospora leaf spot disease. Nhyira is moderately resistant to many viruses and tolerant to leaf hoppers (CSIR-CRI, 2006; Kusi, 2014). The variety is cultivated in all the six cowpea growing regions in Ghana. Farmers prefer it for its resistance to pest, disease and drought while consumers also prefer it due to its early cooking and sweetness (CSIR-CRI, 2010).

2.6.3.2 Asontem (IT82D-32)

The name Asontem is an Akan word meaning early maturing (65 – 70days). This variety was developed by the International Institute of Tropical Agriculture (IITA) and

introduced in Ghana by CSIR-CRI in 2005 (Asafo-Adjei *et al.*, 2005). It produces red and medium sized smooth seeds weighing about 15 g/100 seeds (CSIR-CRI, 2006). Asontem has a semi-erect growth pattern. Though some consumers reject it for its red colour (Egbadzor *et al.*, 2013), others prefer the colour especially for the preparation of certain foods such as waakye in Ghana. Public perception ascribe red seed colour to high nutrients. This makes Asontem one of the consumer preferred varieties. Farmers also ascribe red seed colour to pest and disease resistance thus making Asontem one of the farmer preferred varieties.

2.6.3.3 Asetenapa (IT32D-1951)

The name Asetenapa is an Akan word which means good living. It is an early maturing cowpea variety released by CSIR-CRI in 1991. It bears smooth, ovoid seeds and grows to a height of 53 cm without any pubescence on either the stem or the leaf. The leaves are broad and are borne on long peduncles (CSIR-CRI, 2010). The leaf is green in colour and obtuse in shape. The flower is whitish cream and mid-vein colour is light green (Agyemang *et al.*, 2014). The immature pods are bright green and almost straight. Asetenapa seeds contain 11.2% moisture, 22.6% protein, 0.9% fat, 3.5% ash, 61.7% carbohydrate, 345.6% energy, 164.5 mg/100g calcium, 6.7 mg/100g iron and 828.0 mg/100g phosphorus (Agyemang *et al.*, 2014). Asetenapa is highly preferred in the local market for its seed colour, taste and early cooking. Farmers also prefer it for its high yield (2,500Kg/ha) (Egbadzor *et al.*, 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

Following the identification of SARC-1-57-2 as a credible source of resistance to the cowpea aphid, it is now possible to use this gene to improve upon the field resistance of existing cowpea cultivars (Kusi, 2014). While doing this, it is also important to maintain the identity of the cultivars.

3.1 Collection of Germplasm

The cowpea germplasm for the project was made up of 19 released varieties, 1 advanced line, 1 exotic line and 1 landrace collected from CSIR-CRI and CSIR-SARI (Table 3.1).

Table 3.1 Cowpea varieties evaluated for genetic diversity and aphid resistance

No.	Local Name	Accession Name	Source of material	Status	Year of Release
1	Hewale	IT93K-192-4	CSIR-CRI	Released variety	2012
2	Asomdwoe	IT94K-410-2	CSIR-CRI	Released variety	2012
3	Videza	IT95K-142-20	CSIR-CRI	Released variety	2012
4	Nhyira	IT87D-611-3	CSIR-CRI	Released variety	2005
5	Tona	IT87D-2075	CSIR-CRI	Released variety	2005
6	Asetenapa	IT32D-1951	CSIR-CRI	Released variety	1999
7	Adom	CR-06-07	CSIR-CRI	Released variety	1999
8	Ayiyi	IT83S-728-13	CSIR-CRI	Released variety	1992
9	Bengpla	IT83S-818	CSIR-CRI	Released variety	1992

Table 3.1 Continued

No.	Local Name	Accession Name	Source of material	Status	Year of Release
10	Asontem	IT82D-32	CSIR-CRI	Released variety	1999
11	Soronko	TVX2724-OIF	CSIR-CRI	Released variety	1999
12	Agyenkwa	11(8)-1	CSIR-CRI	Released variety	2015
13	Zamzam	11(9)-5	CSIR-CRI	Released variety	2015
14	Hansadua	11(9)-2	CSIR-CRI	Released variety	2015
15	Nketewade	11(9)-3	CSIR-CRI	Released variety	2015
16	Zaayura	SARC4-75	CSIR-SARI	Released variety	2008
17	Songotra	IT97K-499-35	CSIR-SARI	Released variety	2008
18	Padi-Tuya	SARC3-122-2	CSIR-SARI	Released variety	2008
19	Apabgaala	ITXP48-2	CSIR-SARI	Released variety	2003
20		SARC-1-57-2	CSIR-SARI	Advanced line	
21	Sanzi		Northern Ghana	Landrace	
22	Bra-01		Brazil	Exotic	

3.2 Agro-Morphological and Molecular Characterization of Cowpea Varieties

DNA fingerprinting in addition to agro-morphological characterization of plant varieties is an important stage in any breeding programme which involves artificial selection of materials. This project involved the selection of backcrossed materials which look exactly as the parent material except for the inclusion of the cowpea aphid resistant

gene. It was therefore important to take data on the molecular and agro-morphological traits of the parent materials before the start of the marker assisted backcross.

3.2.1 Agro-morphological Characterization of Cowpea Varieties

This study was conducted in the minor rainy season of Ghana on the experimental fields of CSIR-CRI, Fumesua. The varieties were planted on 4th August, 2015 and harvested from 3rd to 7th November, 2015. The weather condition within the period of the study is presented in Appendix 1. The experimental field was ploughed and harrowed with a tractor. The field was lined and pegged and divided into 3 blocks with the slope of the land being the source of variation. Ten seedlings of each variety (with 20 cm spacing) were sowed on a 100 cm x 60 cm ridge. In all, there were 22 ridges in each of the 3 blocks. The few rains were supplemented with manual irrigation when necessary. Two manual weedings were done on the experimental site. The first weeding was done in the third week after planting and the second weeding in the sixth week after planting. No fertilizer was applied since the objective was to characterise the varieties under farmer's field conditions. However, insects were controlled with the Lambda power® insecticide.

A total of 38 agro-morphological traits (Table 3.2) made up of 14 quantitative traits and 24 qualitative traits were measured using the IBPGR cowpea descriptors with slight modifications.

Table 3.2 List of Agro-morphological traits measured

	Qualitative Traits	Quantitative Traits
1	Pod pigmentation	Pod length (PL)
2	Leaf base pigmentation	Number of locules (NL)
3	Node pigmentation	Number of Seeds per pod (NSP)
4	Petiole pigmentation	Seed length (SL)
5	Branches pigmentation	Seed width (SW)
6	Stem pigmentation	Number of pods per peduncle (NPP)
7	Peduncle pigmentation	Number of peduncles (NP)
8	Sepal pigmentation	Leaf length (LL)
9	Twinning tendency	Leaf width (LW)
10	Raceme position	100 seed weight (SWg)
11	Petiole hairs	Number of pods per plant (NPPP)
12	Stem hairs	Plant height (PH)
13	Leaf texture	Leaf Area Index (LL/LW)
14	Leaf shape	Number of Days to 50% flowering (ND50F)
15	Leaf apex shape	
16	Leaf base shape	
17	Growth habit	
18	Seed colour	
19	Seed eye colour	
20	Flower colour	
21	Pod shape	
22	Pod colour at 40 days	
23	Pod tip shape	
24	Seed shape	

3.2.1.1 Qualitative traits

3.2.1.1.1 Growth habit

Growth habit was evaluated in the 6th week after planting. The varieties were classified as;

- 0 Erect (branching angle less acute than above)
- 1 Semi-erect (branches perpendicular to main stem, but not touch ground)
- 2 Intermediate (most lower branches touch ground)
- 3 Semi-prostrate (main stem reaches 20 or more centimetres)
- 4 Prostrate (plants flat on the ground; branches spread several metres)
- 5 Climbing

3.2.1.1.2 Plant pigmentation

Pigmentation status was recorded for stem, branches, petioles, peduncles and sepals in the 6th week after sowing. The level of pigmentation was classified as;

- 0 Not pigmented
- 1 Very slight
- 2 Moderate at the base and tips of petioles
- 3 Intermediate
- 4 Extensive
- 5 Solid

Node and Leaf base pigmentations were simply classified as;

0 Not pigmented

1 Pigmented

Pod pigmentation was also classified at three levels;

0 Not pigmented

1 Pod tip pigmented

2 Entire pod pigmented

3.2.1.1.3 Terminal leaflet shape

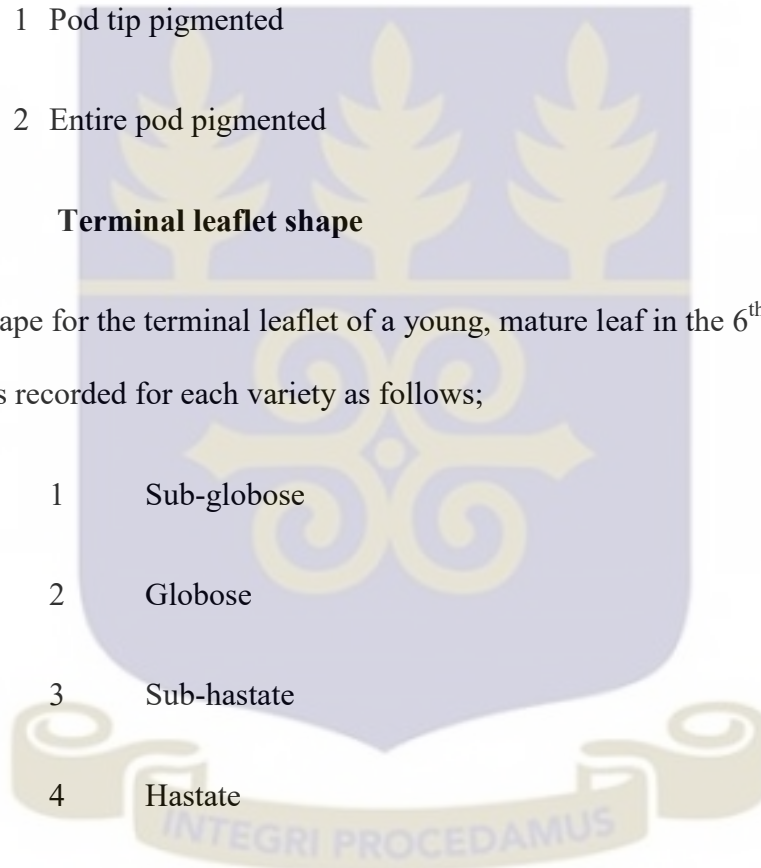
The leaf shape for the terminal leaflet of a young, mature leaf in the 6th week after sowing was recorded for each variety as follows;

1 Sub-globose

2 Globose

3 Sub-hastate

4 Hastate



The terminal leaf apex shape was also recorded for each variety as;

1 Acute

2 Oval

The shape of the leaf base of the terminal leaf was also recorded for each variety as;

1 Cuneate

2 Semi-hastate

3 Hastate

3.2.1.1.4 Twinning tendency

The twinning tendency for each variety was classified as;

0 None

1 Slight

2 Intermediate

3 Pronounced

3.2.1.1.5 Plant hairiness

Varieties were classified based on the plant hairiness on the stems, leaves and petioles in the 6th week as;

0 Smooth

1 Hairy

3.2.1.1.6 Raceme position

The raceme position for the varieties were recorded in the 6th week after sowing and classified as;

1 Mostly above canopy

2 In upper canopy

3 Throughout canopy

3.2.1.1.7 Immature pod colour

The colour for the immature pod of each variety was classified as;

- 1 Green
- 2 Green with purple tips
- 3 Purple-green

- 4 Purple

3.2.1.1.8 Pod shape

The pod shape for each variety was classified as;

- 1 Straight
- 2 Bent
- 3 S-Shaped
- 4 Sickle

The pod tip shape was also classified as;

- 1 Blunt
- 2 Pointed

3.2.1.1.9 Flower colour

The flower colour of each plant was recorded after comparison with a colour chart.

3.2.1.1.10 Seed shape

Seed shape was classified as;

- 1 Kidney
- 2 Ovoid
- 3 Crowder
- 4 Globose
- 5 Rhomboid

3.2.1.1.11 Seed colour

The seed colour of each plant was recorded after comparison with a colour chart.

3.2.1.1.12 Seed eye colour

The seed eye colour of each plant was recorded after comparison with a colour chart.

3.2.1.2 Quantitative traits

3.2.1.2.1 Plant height

The plant height was scored as the linear distance between the apex of the plant and the soil surface along the main axis of the plant with a thread and later stretched on a meter rule to ascertain the real numerical value. Values obtained were recorded to the nearest centimetre.

3.2.1.2.2 Leaf length

The leaf length of an individual cowpea plant was measured from the leaf base along the midrib to the leaf apex with a 15 cm rule. Leaf length was measured as the mean of three randomly selected terminal leaflets to the nearest centimetre.

3.2.1.2.3 Leaf width

Leaf width was scored by using a 15 cm rule to measure the widest width of the terminal leaflets used in sub section 3.2.1.2.2. The mean width was calculated and recorded to the nearest centimetre.

3.2.1.2.4 Leaf Area Index

The leaf area index was calculated as the ratio of leaf length to leaf width of each terminal leaflet measured in subsections 3.2.1.2.2 and 3.2.1.2.3.

3.2.1.2.5 Days to 50% flowering

This involved recording the number of days that elapsed from the day of sowing to the stage when 50% of plants had begun to flower for all varieties.

3.2.1.2.6 Pod length

The pod length was recorded as mean of the 10 longest pods measured from 5 randomly selected plants. The measurement was done with a 30 cm thread and later stretched on a 30 cm rule to ascertain the real numerical value.

3.2.1.2.7 Number of locules per pod

The mean number of locules per pod was counted for the 10 pods used in subsection 3.2.1.2.6.

3.2.1.2.8 Number of seeds per pod

The mean number of seeds per pod was counted for the 10 pods used in subsection 3.2.1.2.6 for consistency.

3.2.1.2.9 Number of pods per peduncle

This was recorded as the mean number of pods of 10 randomly selected peduncles for each variety.

3.2.1.2.10 Number of peduncles per plant

The mean number of peduncle per plant for 5 randomly selected plants was recorded for each variety.

3.2.1.2.11 Number of pods per plant

The mean number of pods per plant for 5 randomly selected plants was recorded for each variety.

3.2.1.2.12 Seed length and Seed width

The mean length and width of 10 randomly selected seeds of each variety were recorded with the aid of a micrometre screw gauge to the nearest centimetre.

3.2.1.2.13 Hundred Seed Weight (g)

After threshing, 100 seeds of each variety were weighted on an electronic balance to ascertain the mass to the nearest gramme.

3.2.2 Molecular characterization of cowpea varieties

The molecular work of this project was conducted in the Biotechnology laboratory of the CSIR-Crops Research Institute, Kumasi – Ghana.

3.2.2.1 DNA extraction

Leaf explants used for this study were harvested from two week old seedlings and kept in liquid nitrogen. The DNA was extracted using DNeasy™ Plant Mini extraction kit (Qiagen, Germany) following the methodology below:

For each cowpea variety, 100 mg of leaf tissue was weighed and ground in 2 ml eppendorf tubes with the aid of liquid nitrogen. Buffer AP1 (400 µl) and RNase A (100 mg/ml) were added and vortexed vigorously. The mixture was then incubated at 65°C for 10 minutes, mixed 2-3 times by inversion during incubation. A hundred and thirty microlitres of Buffer AP 2 was added to lysate, mixed and incubated on ice for 5 minutes. This was followed by centrifugation for 14000 rpm for 5 minutes. The lysate was pipetted into the QIA shredder min spin column and centrifuged at 14000 rpm for 2 minutes. The flow-through fraction was transferred into a new eppendorf tube without disturbing the debris pellet. 1.5 volumes of Buffer AP 3/E was added to lysate followed by mixing. A volume of 650 µl of the mixture including any precipitate was pipetted into the DNeasy mini spin column and then centrifuged at 8000 rpm for 1 minute. This step was repeated again after the flow-through was discarded. The DNeasy mini spin column was placed into a new 2 ml collection tube and 500 µl Buffer AW added. The mixture was centrifuged at 8000 rpm for 1 minute. The flow through was discarded and the collection tube reused. Five hundred microlitres of Buffer AW was added to the DNeasy min spin column and centrifuged at 14000 rpm for 2 minutes and then empty spanned for 2 minutes. The DNeasy min spin column was transferred to a new 1.5 ml eppendorf tube and 50 µl Buffer AE added. The mixture was incubated at room temperature for 10 minutes, centrifuged at 8000 rpm and 50 µl Buffer AE added again. This step was to elute the DNA finally from membrane.

3.2.2.2 DNA Quality check

Samples were assessed by electrophoresis through a 0.8% (w/v) agarose gel to check the quality of DNA extracted.

3.2.2.3 DNA concentration check and dilution

The concentration of the DNA was determined on a Nanodrop (spectrophotometer 2000C). The genomic DNA samples were diluted to a final concentration of 20 ng/ μ L with 1 \times TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA) and stored at -20 °C for further use.

3.2.2.4 Polymerase Chain Reaction using SSR markers

A total of 20 SSR primers were used to fingerprint the 22 cowpea varieties (Table 3.3). Information about the primers was obtained from the SSR panel in (Timko, 2009). The primers were synthesized by Inqaba Biotechnical Industries Ltd., Pretoria, South Africa. Polymerase chain reaction (PCR) amplification was conducted in 20 μ l volume tubes. Each PCR reaction contained 6 μ l “One Taq Quick-Load 2x Master Mix” (composed of 20mM Tris-HCl, 1.8mM MgCl₂, 22mM NH₄Cl, 22 mM KCl, 0.2 mM dNTPS and 25 units/ml One Taq DNA Polymerase), 2.0 μ l Molecular Grade Distilled Water (MGDW), 0.5 μ l of each primer pair and 1 μ l of genomic DNA sample to make a total volume of 10 μ l. The PCR amplifications were performed in a thermal cycler C1000 (Seegene, Korea). The thermal cycler was set to initial denaturation at 94°C for 1 minute followed by 35 cycles of denaturation for 30 seconds at 94°C, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute and ended with final extension at 72°C for 10 minutes.

Table 3.3 SSR primers used for molecular characterization and their sequences

NO.	SSR NAME	ORIGINAL NAME	LEFT SEQUENCE	RIGHT SEQUENCE	ANNEALING TEMPERATURE (°C)	PRODUCT SIZE (bp)
1	SSR-6265	CP215/CP216	CAGAAGCGGTGAAAATTCAAC	GCATGTTGCTTTGACAATGG	55	239
2	SSR-6258	CP201/CP202	GGTTTCCTAGTTGGGAAGGAA	ATTATGCCATGGAGGGTTCA	55	260
3	SSR-6243	CP171/CP172	GTAGGGAGTTGGCCACGATA	CAACCGATGTAAAAAGTGGACA	55	176
4	SSR-6218	CP117/CP118	GTGGAAGGAATGGGTCCAG	AGGAAATTTGCATTCCCTTGT	55	287
5	SSR-6217	CP115/CP116	GGGAGTGCTCCGAAAGT	TTCCCTATGAACTGGGAGATCTAT	55	294
6	SSR-6353	CP397/CP398	TCATGGGTAAATTTGCTTCAA	AAACCATGTGGTTGTTGCAC	50.9	109
7	SSR-6352	CP395/CP396	GTTGTGAGCTTCCCCAGATG	AATTTTGAACCCACCACCAG	55	127
8	SSR-6336	CP359/CP360	TGAAAAACAACGATATGCAGAAG	TCAGTCTTAGAATTGAGTTTTCTTCG	55	247
9	SSR-6323	CP333/CP334	CAAAGGGTCATCAGGATTGG	TTAAGCAGCCAAGCAGTTGT	55	218
10	SSR-6277	CP239/CP240	CACCCCGTACACACACAC	CACTTAAATTTTCACCAGGCATT	50.9	157
11	SSR-6436	CP573/CP574	GCAGAATCCTTGTGAACCTG	TTTCGCAATATGCCCTTTTC	50.9	280
12	SSR-6375	CP443/CP444	GCTCGGATATGGTCTGAAA	TCAGTGTGACACCATACCC	55	293
13	SSR-6371	CP435/CP436	TGCTCATCGTGCTTTGTCTT	CACTTCAGACTTAGAGCGAAGAAA	55	189
14	SSR-6370	CP433/CP434	CAACTTCACAGCCCTCACAA	TTGAAGGTATGGCCTTTTGTTT	55	253

Table 3.3 continued

NO.	SSR NAME	ORIGINAL NAME	LEFT SEQUENCE	RIGHT SEQUENCE	ANNEALING TEMPERATURE (°C)	PRODUCT SIZE
15	SSR-6356	CP403/CP404	TGCAATATGGACCAGAAGAAA	ATGCCCCAACAACAACATTT	55	158
16	SSR-6613	Y31	CTATTGGAATCTTGCCGTTG	CTTTACCTTTATGCAAACCAATTC	55	333
17	SSR-6608	Y26	CTAAATTATAATATTCGTCGGTC	GGTTAAGGAAAAGAGGGTAGG	55	299
18	SSR-6603	Y21	GAGAACTTCACGCACAATAG	CGCGGTAGCATGATTGAATTTTG	55	330
19	SSR-6587	Y1	GATATAGAATAGCATATTTAACATATTAG	GTTGAAAGTTTGATAGTAAAGTGG	55	319
20	SSR-6451	CP605/CP606	AAAGAGATACACATGCCTAACA	GACCAACAGCGACTTTGAGC	55	142
14	SSR-6370	CP433/CP434	CAACTTCACAGCCCTCACAA	TTGAAGGTATGGCCTTTTGTTT	55	253
15	SSR-6356	CP403/CP404	TGCAATATGGACCAGAAGAAA	ATGCCCCAACAACAACATTT	55	158
16	SSR-6613	Y31	CTATTGGAATCTTGCCGTTG	CTTTACCTTTATGCAAACCAATTC	55	333
17	SSR-6608	Y26	CTAAATTATAATATTCGTCGGTC	GGTTAAGGAAAAGAGGGTAGG	55	299
18	SSR-6603	Y21	GAGAACTTCACGCACAATAG	CGCGGTAGCATGATTGAATTTTG	55	330
19	SSR-6587	Y1	GATATAGAATAGCATATTTAACATATTAG	GTTGAAAGTTTGATAGTAAAGTGG	55	319
20	SSR-6451	CP605/CP606	AAAGAGATACACATGCCTAACA	GACCAACAGCGACTTTGAGC	55	142

3.2.2.5 Gel electrophoresis

The PCR products were run on vertical Polyacrylamide gel electrophoresis (v-PAGE) (Criterion cell model, vertical centron tank) to separate and resolve the bands. The 6% polyacrylamide gel was prepared as shown in Table 3.4.

Table 3.4 Preparation of 15ml of 6% Polyacrylamide gel

Reagent	Volume
Filtered Autoclaved Distilled Water (FADW)	11.335 ml
10x TBE	1.25 ml
40% acrylamide solution	2.25 ml
40% Ammonium per sulphate (APS)	150 μ l
TEMED	15 μ l
Total Volume	15 ml

The liquid polyacrylamide gel was quickly dispensed into a 10 ml cassette using a transfer pipette after preparation. A twenty-six tipped comb was used to create wells and allowed to polymerise. The tape covering the base of the cassettes was then removed before submerging the cassette into 1x TBE buffer contained in the vertical electrophoresis tank. The wells were then loaded with the PCR products after the comb was removed. A 100bp ladder (Invitrogen®) was loaded in the first well to determine band sizes of the PCR products. The gel was run to at least half way to the end of the tank and a spatula was used to prise off the cassette to release the gel gently. The gel

was stained with 0.5% Ethidium bromide and shook on an electric shaker for 1 hour. The gel was photographed under Ultraviolet light for further analysis.

3.2.2.6 Scoring of bands from v-PAGE

Scoring of bands was done with the alpha imager® scoring software version 3.4.0 along with a 100-bp DNA ladder (Invitrogen®) to identify the molecular-weight of the DNA samples.

3.3 Molecular Screening of Cowpea Varieties for Aphid resistance locus

A polymorphic test was conducted on the 22 cowpea varieties using the marker CP 171F/172R (left sequence: 5' – GTAGGGAGTTGGCCACGATA – 3'; Right sequence: 3' - CAACCGATGTAAAAAGTGGACA-5') as recommended by Kusi (2014) to distinguish resistant varieties from susceptible varieties at the genomic level. This included the SARC-1-57-2 and Apabgaala which were the resistant and susceptible checks respectively.

DNA samples were taken from the leaf primordial of the 22 varieties two weeks after planting. DNA was extracted using the Qiagen extraction kit as detailed in sub section 3.2.2.1.

PCR was run using the SSR marker CP 171F/172R according to the steps documented in sub section 3.2.2.4. The PCR products were run on a non-denaturing v-PAGE following the steps in subsection 3.2.2.5. The photographed bands of the samples along with the checks were analysed to determine the polymorphic state of the varieties at the aphid resistance loci.

3.4 Development of F₁ progenies

The F₁ generation was developed from a cross between the resistant line SARC-1-57-2 (Male) and three susceptible cultivars – Asontem, Nhyira and Asetenapa (Females) on the experimental field of CSIR-CRI, Kumasi. The parent materials were planted on 18th September, 2015. Each crossing block was made up of one row (2 m long) of the male variety interspersed in two rows (2 m long) of the female variety. Each row was made of 10 plants with 20 cm spacing. Artificial pollination was done between the hours of 06:00 hrs and 08:00 hrs when most petals are open. Artificially pollinated floral buds were well tagged with labels (Plate 3.1). The F₁ seeds were harvested on 13th November, 2015.



Plate 3.1: Artificial cross pollination (For each cross, the immature anthers in the floral bud of the female parent were emasculated with the aid of sterilized forceps and then manually pollinated with the pollen from the male variety)

