APHID RESISTANCE IN COWPEA *(VIGNA UNUICULATA* (L.) WALP.) AND ITS RELATIONSHIPS WITH MORPHOLOGICAL AND BIOCHEMICAL CHARACTERS.

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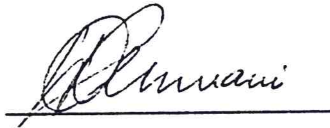
1994

DECLARATION

I, Githiri, Stephen Mwangi, hereby declare that the work presented in this thesis has not been presented for examination in any other university.

_______________ 18/10/94

We, (the undersigned), hereby declare that this thesis has been submitted for examination with our approval as supervisors.

Dr. P.M. Kimani  Date 17/11/94

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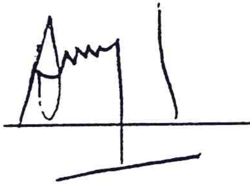
Dr. K. Ampong-Nyarko  Date 13 October 1994

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ABSTRACT

Aphid resistance in cowpea and its relationships with morphological and biochemical traits was studied in eight resistant (ICV 10, ICV 11, ICV 12, IT82E-25, Tvu 310, IT87S-1394, IT87S-1459, and IT84S-2246) and six susceptible (ICV 1, ICV 5, ICV 6, IT83D-237, Tvu 946 and Tvu 1509) cowpea cultivars, and their F_1, F_2, F_3, and backcross populations. The main aim of this work was to study the genetic variation for aphid resistance in some selected cowpea cultivars and identify heritable, easily identifiable morphological and/or biochemical markers that could be used for indirect selection of aphid resistant cultivars in breeding programmes. Both field and greenhouse experiments were conducted.

The results from these studies indicated that in cowpea, an antixenosis (non-preference) modality of resistance was important in the expression of resistance to aphids. Aphid resistance in each of the eight different sources of resistance studied was qualitatively inherited (monogenic) with resistance being dominant to susceptibility. All sources of resistance carried the same gene for resistance, *Rac,* whose expression in different cultivars was most probably influenced by modifiers.

Wide variations among cultivars were recorded with respect to most morphological traits studied, except for days to emergence and pubescence. All cultivars could be distinguished from one another morphologically, except ICV

10, ICV 11 and ICV 12. Based on dissimilarities in morphological traits, the cultivars in this study were grouped into three main clusters. Tests for association in a contingency table indicated that aphid resistance was associated ($P < 0.05$) with some morphological traits such as growth habit, immature pod colour, and seed colour.

Cultivars in this study were characterized by very low genetic diversity for biochemical traits studied. Out of 27 isoenzyme loci from 18 enzyme systems studied, only aspartate amino transferase was polymorphic among cultivars. The cultivars were polymorphic at two electrophoretic protein bands (62 kD and 23 kD). The biochemical (total protein and isoenzyme) trait variations were not associated with aphid resistance ($P < 0.05$).

This study identified four linkage groups among loci controlling morphological traits. The proposed linkage group I carried the loci Fbc and Sw controlling flower bud colour and swollen stem base, respectively. Linkage group II carried loci Pu^S, Pu^P, and Cbr controlling purple stems, purple pods (immature), and cocoa-brown pods (dry), respectively. Loci Pu^S and Pu^P were tightly linked as evidenced by low recombination frequency. Linkage group III carried the loci Pd, Ndt, Hg, and Bpd controlling peduncle colour, growth habit, plant type, and branching peduncle, respectively. The aphid resistance gene (*Rac*) was linked to locus Pd (30 ± 7.5%) and thus located in this group. Linkage group IV carried the loci Pt and Bk controlling purple-tip

pods and black pods (mature). Relationships among the four linkage groups is not known.

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GENERAL INTRODUCTION

Cowpea, *Vigna unguiculata,* is an important food legume in the tropics. It is mainly grown as a subsistence crop in various inter-cropping mixtures with maize, millet, sorghum, cassava and various other crops (Acland, 1971). It is a drought-tolerant crop with a high nitrogen-fixing capacity and can be grown in poor soils. Cowpea is consumed either as dry grain or as a vegetable in the form of leaves, green pods, and green peas. Dry cowpea grains contain about 24% protein thus making the crop an important protein source in the diet of the poor farmers of tropical Africa. In Kenya, cowpea is mainly grown in the Eastern, Western, and Nyanza provinces, and low acreages of the crop are sown in Central and Coast Provinces (Acland, 1971)..

Low productivity of cowpea in Africa is caused by many factors among which the most important include, use of unimproved seeds, poor soils, poor cultural and management practices, and high insect and disease infestation (Acland, 1971; Rachie, 1985). Although it is difficult to quantify losses caused by each factor, damage caused by insect pests both in the field and during storage contributes significantly to yield losses. Major pests of cowpea in Africa include aphids (*Aphis craccivora*) which attack the crop during the seedling stage; thrips (*Megalulothrips sjodtedji*), pod borers (Maruca testulalis) and pod sucking bugs (*Clavigralla tomentosicollis*) which attack during the

flowering and podding stages; and seed bruchids (*Callosobruchus maculatus*) which attack seeds in storage (Singh and Jackai, 1985).

The cowpea aphid, *Aphis craccivora* (Koch) is a major pest of cowpea in Africa and Asia (Singh and Jackai, 1985). It is a pest that is gaining increasing importance in Latin America (Daoust et al, 1985) and the USA (Chalfant, 1985). Aphids primarily infest seedlings, although large populations also infest flowers and pods (Singh and Jackai, 1985). Aphids damage the cowpea crop by sucking sap from the terminal shoots and petioles of new foliage. Low aphid population have no impact on the cowpea yields since the crop is able to compensate the attack. However, at high aphid population levels, plants of susceptible cultivars have reduced vigour, distorted leaves, and small, poorly nodulated root systems thereby resulting in low yields (Singh and Jackai, 1985). In extreme cases, the seedlings of susceptible plants are killed. An indirect and often more serious effect of this pest, even with low populations, is the transmission of cowpea mosaic viruses (Singh and Jackai, 1985).

Host plant resistance to insects offers an important means of pest control in crop plants either alone or as a component of an integrated pest management (Dent,1991). Germplasm screening for aphid resistance in cowpea has been conducted at the International Institute of Tropical Agriculture (IITA), and several resistant lines have been

identified (Singh and Jackai, 1985). Two sources of resistance (ICV 11 and ICV 12) were developed through induced mutations at the International Centre of Insect Physiology and Ecology (ICIPE) (ICIPE, 1986). Inheritance studies indicated that resistance to aphids was simply inherited with resistance being dominant to susceptibility (Fery, 1985; Bata et al, 1987; Pathak, 1988). Two non-allelic resistance genes, *Rac1* and *Rac2*, have so far been reported in cowpea (Bata et al, 1987; Pathak, 1988).

Statement of the problem

Aphid resistant cowpea cultivars have been identified. The mechanisms and genetics of resistance present in different cultivars is not well understood. Moreover, in screening cowpea for resistance to aphids, workers have relied mainly on natural infestation of plants with aphids in the field or on artificial aphid infestation in the greenhouse. Infestation levels in the field are, however, highly erratic and influenced by environmental conditions such that cultivars rated as resistant may in fact be susceptible or vice-versa. On the other hand, planting of large segregating populations may not be feasible in the greenhouse. Identification of markers (morphological and/or biochemical) for aphid resistance would be highly desirable in a breeding programme aimed at incorporating aphid resistance to high yielding cowpea cultivars. Knowledge on the mechanisms and inheritance systems of aphid resistance would facilitate

planning and choice of the breeding methods to use while the markers would be used as the basis for selecting aphid resistant plants from a population mixture even in the absence of aphids.

General objective

The general objective of this work was to study the genetic variation for aphid resistance in selected cowpea cultivars and identify heritable, easily identifiable markers that could be used for indirect selection of aphid resistant cultivars in breeding programmes.

Specific objectives

1. Study genetic variation for aphid resistance among selected cowpea cultivars;
2. Study genetic variation for some selected morpho-agronomical traits among selected cowpea cultivars and identify traits which are associated with aphid resistance, if any;
3. Study genetic variation for some biochemical (total protein and isoenzyme) characters among selected cowpea cultivars and identify traits which are associated with aphid resistance, if any;
4. Study linkage relationships among genes controlling morphological traits, biochemical traits and aphid resistance.

2.0. LITERATURE REVIEW

2.1. Cowpea

2.1.1. Taxonomy

According to the revision of *Vigna* by Marechal *et al.* (1978) (as quoted by Ng, 1990), the genus is divided into seven subgenera containing 81 species. Four subgenera (*Vigna, Haydonia, Plectotropis and Macrorhncha*) with a total of 54 species are indigenous in Africa, while three subgenera (*Sigmoidotropis, Lasiopron, and Ceratotropis*) are absent. The subgenus *Vigna* is divided into six sections (Catiang, Comosae, Liebrechtsia, Reticulatae, and Vigna). Section Catiang in which cowpea belongs has two recognized botanical species, *Vigna unguiculata* (L.) Walp. and *Vigna nervosa* Markotter. The species *Vigna unguiculata* ($2n = 2x = 22$) is differentiated into a cultivated subspecies, *unguiculata*, (cowpea) and three uncultivated subspecies, *dekindtiana, tenuis*, and *stenophylla* (wild relatives of cowpea). The cultivated and wild subspecies of *Vigna unguiculata* are interfertile but they cannot be crossed with *Vigna nervosa* (Ng, 1990). Marechal *et al.* (1978) (as quoted by Ng, (1990) divided the subspecies *unguiculata* into four cultigroups (cv-gr):

(i) *unguiculata*: the cowpea, most important group in Africa,

(ii) *biflora*: the catjang, a fodder and seed type mainly grown in South East Asia,

(iii) *sesquipedalis*: the yard-long bean or asparagus bean, a green pod vegetable in India, S.E. Asia, and China,

(iv) *sextilis*: grown for textile fibres of the long peduncles in Niger and Northern Nigeria.

2.1.2. Diversity and origin of cultivated cowpea

All cultivated crop species are usually variable in various traits because of artificial selection imposed on them by man under diverse environments and cowpea is no exception. Ng and Marechal (1985) reported that cowpea diversity has been recorded in several traits among which include: growth habit (prostrate, erect, semi-erect, climbing); pods (coiled, round, crescent, or linear); peduncles (5 cm to 50 cm long); maturity (53 - 120 days at IITA); flower and pod pigmentation (six patterns each); seed size (5 - 20 g/100 seed); and 62 eye colours and 42 eye patterns on the seed. Laghetti *et al.* (1990), using cowpea accessions collected from southern Italy, reported that cowpea cultivars are morphologically diverse with growth habit, seed size, maturity, and pigmentation of various plant parts being the most diverse traits. Once diversity has been created within the crop it is fixed in the cultivars through self-pollination.

The region of cowpea domestication is still uncertain, although, there is enough evidence to show that the crop was first domesticated in Africa. Based on present knowledge of the distribution of wild subspecies *unguiculata*, Baudoin and

Marechal (1985) suggested that East Africa, stretching from South Africa to as far north as Ethiopia, is the region of primary diversity for the wild forms of cowpea, and was probably the primary centre for cowpea domestication. They suggested that West Africa, with the largest number of primitive cultivars and weedy types, is a secondary centre of diversity. They also suggested that another centre of diversity lies in S.E. Asia, where people have intensively selected African domesticated varieties, prompting the development of specialized forms such as *Vigna biflora* and *Vigna sequipedalis*. Ng and Marechal (1985) reported that studies at IITA with over 10,000 accessions of cultivated and wild cowpea cultivars indicated more diversity among West African collections than among East African collections. They suggested that cowpea may have been domesticated in West Africa. Vaillancourt *et al.* (1993), working on isoenzyme diversity among wild and cultivated cowpea, observed very low isoenzyme variability among cultivated cowpea cultivars. They reported that out of 26 isoenzyme loci, only six were polymorphic. The polymorphic loci had rare and common alleles. The rare alleles were present in cultivars collected from all over Africa and the authors opined that there is no narrowly defined centre of cowpea domestication.

2.1.3. Cowpea pests

According to Singh and Jackai (1985), the cowpea crop is attacked by several insect species covering the main phytophagous taxa. The insects cause economic damage on the crop from seedling to harvest and storage stages. Major pests of cowpea include the following:

(i) Legume podborer (*Maruca testulalis*): larvae feed on flowers, flower buds, and green pods,

(ii) Cowpea aphid (*Aphis craccivora*): feeds on seedlings tender foliage, young pods, and can be a vector of viruses,

(iii) Legume bud thrips (*Megalulothrips sjodtedji*): feed on flower buds and flowers,

(iv) Coreid bugs (complex of nine species of which *Clavigralla tomentosicollis*, is most important in Africa): feed on green pods,

(v) Cowpea storage weevil (*Callosobruchus maculatus, C. chinensis*): feed on seeds in storage.

Several other insect species attack cowpea, but their effects are either below economic injury level or the pests are sporadic. Sporadic insects may become major pests in some years. The most important major pest varies with the regions and with the seasons. Several chemicals have been identified that could be used for controlling the insect pests. However, the high costs of the chemicals, coupled with public awareness of the pollution problems caused by most of the pesticides, have necessitated the advocacy of

alternative methods of insect pest control. Alternative methods of pest control include plant resistance, cultural control, biological control, and genetic control.

2.1.4. Cowpea genetics

Cowpea has been the subject of genetics research since the beginning of the 1900s. According to Fery (1985), this had been made possible by several characteristics inherent in the crop among which include: cowpea is a diploid with a relatively short life cycle, the crop has large flowers with untwisted keels which makes it one of the easiest legumes to emasculate and pollinate, and the crop is self-pollinating.

Fery (1985) reviewed genetics research conducted on cowpea by various authors since the 1900s. He reported that a total of 158 specific genes (for simply inherited characters) have been identified in the cowpea and more than 225 heritability estimates of economically important traits (quantitative traits) published. The literature has many examples of the same symbol being assigned to different genes, the same gene being assigned different symbols, or no symbol being assigned to a gene such that the total number of genes identified is definitely different from 158. Most of the genes identified are for morphological traits, and insect and disease resistance which are easily scorable in the cultivars. There are few linkage studies reported in cowpea and consequently the linkage map of cowpea is poorly

developed. Linkage studies in crops are important for indirect selection of traits that are difficult to score.

2.2. Host plant resistance

2.2.1. Mechanisms of resistance to insect pests

Resistance is the inherent ability of the host plant to prevent, restrict, retard or overcome pest infestation. The mechanisms of resistance currently widely recognized and originally proposed by Painter (1951) belong to three main categories:

1. Preference/non-preference, subsequently referred to as antixenosis by Kogan and Ortman (1978), for different plant types for food, shelter and oviposition;
2. Antibiosis, which affects insect survival, development, and reproduction; and,
3. Tolerance, which involves repair and regeneration of the damaged tissues.

Usually, combinations of more than one mechanism of resistance are employed by the resistant host plants against the insect pests.

2.2.2. Mechanisms of resistance to aphids

According to Auclair (1989), antibiosis is the principal mechanism of resistance to aphids by various plants. Antixenosis, however, has also been reported to occur in some plant species. For example, in peas, yellow-green

varieties are preferred by aphids over t
varieties; while in beans, specialized hooked
present on resistant varieties that impart
aphids (Auclair, 1989). It is, however, worth
aphid resistance, it is very difficult t
between antibiosis and acute antixenosis, whe
rather starve to death while on the non-prefer
than feed on it.

2.2.3. Mechanisms of aphid resistance in cowpe

MacFoy and Dabrowski (1984) studied d
chemical composition of stems of aphid
susceptible cowpea cultivars. They reported t
positive correlations between resistance and
and flavonoids contents. There were no correl
resistance and total sugars nor with tota
contents. Aphid growth rates were higher on t
than on the resistant cultivars. Aphids' fe
was longer with fewer probes/minute on th
cultivar compared with the resistant cultivar.

Aphid performance and behaviour on two re
lines (ICV 11 and ICV 12) were studied by G
(1988). The authors reported that aphid grow
much lower on the resistant cowpea lines
susceptible line (ICV 1). They suggested th
played an important role in the expression of
another experiment, the authors reported that

conditions, aphids invariably settled in larger numbers on the susceptible than on the resistant cultivars thus suggesting antixenosis. Once on a host, the aphids spent more time test probing on the resistant line than they did on the susceptible line. They also needed a longer time to decide whether to feed continously or to leave the resistant line, possibly indicating lack of an appropriate settling stimulus.

Firempong (1988) studied the components of resistance to aphids in six cowpea cultivars. He reported that resistance in the cultivar IT82D-812 was mainly due to antixenosis while both antixenosis and antibiosis were important in the expression of resistance in cultivar ICV 12.

Lattanzio *et al.* (1990) analyzed the qualitative and quantitative contents of pre-existing phenols in resistant and susceptible cowpea cultivars using high performance liquid chromatography (HPLC). The HPLC fingerprints of Vita 7 (susceptible) and Tvu 3000 (resistant) indicated that several phenolic compounds namely, p-coumeric acid, caffeic acid, ferulic acid, sinapic acid, kaempferol, quercetin, and isorhamnetin were present in both resistant and susceptible cultivars. The two cultivars differed from a quantitative viewpoint with the resistant cultivar having lower quantities of p-coumaric acid, and higher quantities of other phenolic compounds, especially quercetin, than the susceptible cultivar. The authors cautioned, however, that

the quantitative differences did not apply to all cultivars such that some resistant cultivars had lower phenolic contents than the susceptible cultivars.

2.3. Genetic diversity

2.3.1. Morpho-agronomical traits

Morpho-agronomical variation in germplasm collections has been widely used as a criteria for studying genetic diversity within the germplasm and for studying geographical patterns of variation among regions of origin in soybean, beans, and various other crops (Perry and McIntosh, 1991; Singh *et al.*, 1991b). Several morpho-agronomical traits are scored for in the germplasm accessions and the diversity analyzed using various statistical methods.

In cowpea, Laghetti *et al.* (1990) studied genetic diversity present among 147 germplasm accessions collected from southern Italy. The authors recorded data on 23 morpho-physiological traits during crop growth. The data indicated wide variations for all traits recorded with growth habit, seed size, maturity, and pigmentation of various plant parts being the most diverse traits.

The major disadvantage with morpho-agronomical traits is that their expressions are highly influenced by variations in the environmental conditions and by epistasis.

2.3.2. Biochemical traits

Studies in biochemical variation and linkage to various economically important traits are useful in expanding the current knowledge in crop genetics and breeding and are increasingly being used in many crops. Differences are easily identified after electrophoresis (a technique commonly used to separate proteins on the basis of charge, molecular weight and shape). On the supporting media, (gel) the zymograms for dominant and recessive genes are expressed in a co-dominant fashion such that homozygotes for dominant and recessive genes and heterozygotes can be distinguished. Expression of biochemical traits is not influenced by environmental conditions, dominance or epistasis.

2.3.2.1. Seed proteins

Seed protein profiles have been used in taxonomic and evolutionary studies as well as discriminating between wild and cultivated accessions of legume species (Brown *et al.*, 1981; Bliss and Brown, 1983; Gepts *et al.*, 1986), wheat (Sergio and Spagnoletti Zeul (1992), and other crops.

In cowpea, Pedalino *et al.* (1990) studied the seed protein patterns in 35 accessions using electrophoresis. The authors reported two protein pattern types, A and B, among the cultivars. Cultivars with protein pattern type A had polypeptide bands of 32 kD and 23 kD which were absent in cultivars with protein pattern type B. The authors observed

that two major globulins, CP1 and CP2 were present in all the accessions.

Oghiake *et al.* (1993) studied seed protein variation in 15 cowpea cultivars using electrophoresis. The authors did not observe variation among cultivars with respect to total proteins. They, however, detected variability at three polypeptide bands within the water-soluble fraction of proteins (albumins). They used this variability to distinguish cultivars.

2.3.2.2. Isoenzymes.

For many years, the dominant view was that each enzyme existed as a single molecular form and heterogeneity of enzymes was thought to be an artifact, caused by improper isolation and purification (Markert, 1977). Markert and Moller (1959), who suggested the word 'isozyme', were the first to prove that multiple forms of enzymes do occur naturally. Isozymes (or iso-enzymes) are defined according to the IUB (1984) recommendations as, multiple molecular forms of an enzyme occurring within a single species, as a result of the presence of more than one structural gene. Such multiple forms may, however, be due to multiple gene loci or multiple alleles at a single locus (= allozymes). In crop plants, isoenzyme variation has been used to characterize germplasm collections, delineate phylogenetic relationships, estimate population genetic parameters such as outcrossing rates, produce detailed linkage maps, and to

tag morphological and/or physiological characters of interest for convenient screening in breeding programmes (Kesseli and Michelmore, 1986; Cole *et al.*, 1991; Perry *et al.*, 1991; Singh *et al.*, 1991a).

Vaillancourt *et al.* (1993) studied isoenzyme variation among 112 collections of cultivated cowpea (*Vigna unguiculata* ssp. *unguiculata*) and 43 collections of wild cowpea (*Vigna unguiculata* ssp. *dekindtiana*) germplasm. They scored variation for 26 isoenzyme loci. The authors detected very low genetic diversity among cultivated cowpea with only six polymorphic loci. Four of these loci displayed one very common and one very rare allele. Accessions possessing these rare alleles were distributed all over Africa and thus a centre for isoenzyme diversity was not evident. Cultivated groups *biflora* (the catjang) and *sesquipedalis* (the yard-long bean) could not be distinguished from the cowpea. The authors, however, detected a lot of variation among wild cowpea with 19 out of 26 isoenzyme loci being polymorphic

Although biochemical variation has been used in various crops for phylogenetic studies and cultivar identification, and despite its proven advantages over the morphological traits traditionally used in gene-banks, there are few such studies that have been conducted in cowpea (Pedalino *et al.*, 1990; Oghiake *et al.*, 1993; Vaillancourt *et al.*, 1993).

2.3.3. Linkage

2.3.3.1. Introduction

Independent assortment between any two genes which are completely dominant should result in an F_2 phenotypic ratio of 9:3:3:1. Sometimes, however, different F_2 ratios have been observed. Linkage is said to occur if two or more genes co-segregate in the progeny thereby resulting in more parental-types and very few recombinants in the F_2 generation.

According to Strickberger (1990), the first departure from the 9:3:3:1 ratio was reported by Bateson and Punnet in 1909 in crosses between different varieties of sweet pea. These workers found that plants of different flower colour and pollen shape, when crossed together, gave rise to F_1 and F_2 offspring in which the genes for flower colour (A and a) and pollen shape (B and b) did not assort independently, but were "tied together" so that F_2 offspring appeared in ratios which contained too many of the original parental genotypes (A-B- and aabb) and too few of the newly recombined genotypes (eg A-bb and aaB-). They suggested that in such cases, parental-type gametes, AB and ab multiplied preferentially after meiosis, and hence, the higher numbers. There were, however, objections to this view by several workers. The true meaning of linkage was clarified by experimental data that began to appear from genetic studies of *Drosophila* by Morgan and his co-workers in the early 1900s (Strickberger, 1990). These workers detected numerous

hereditary characteristics on *Drosophila* many of which were associated (linked) together in groups. Thereafter, more cases of linkage were reported in various other organisms. Complete linkage between genes on the same chromosome, however, is a rarity in most sexually reproducing organisms. Recombination does occur between homologous chromosomes after crossing over during cell division.

Linkage between any two genes can be estimated by recombination frequencies obtained through either ratio of products or maximum likelihood methods (Immer, 1930; Allard, 1956). The ratio of products method is important when two genes whose linkage is being calculated are completely dominant and fully expressed. The fraction of recombinant types (eg. A-bb and aaB-) relative to the parental types (eg. AABB and aabb) gives the recombination estimate. The method is direct and easy to use when calculating recombination estimates between any two genes. This method, however, is not useful when the genes are partially dominant and more than four classes of genotypes can be distinguished. In such cases of partial dominance of one or both genes whose linkage relationships are being estimated, (heterozygotes can be distinguished from homozygotes), the maximum likelihood method becomes important. The maximum likelihood method can also be used for estimating linkage relationships among genes with inter-allelic interactions (epistasis). Estimates by each of these methods indicates no recombinants between any two or more tightly linked genes,

and recombination frequencies increase, as the genes are located farther apart from each other on the chromosomes, up to a maximum of 50% when the genes assort independently of each other. Independent assortment occurs when two genes are located far apart from each other on the same chromosome or when they are located on different chromosomes.

2.3.3.2. Linkage studies in other crops

Garvin *et al.* (1989) studied the genetics and linkage of 12 variable isoenzymes from eight enzyme systems in tepary beans (*Phaseolus acutifolius*). The authors reported that the isoenzymes were encoded by co-dominant alleles of simply inherited loci. Linkages were detected in four pairs of isoenzyme loci.

Griffing and Palmer (1987) studied inheritance and linkage among five isoenzyme (Aco-1, Aco-2, Aco-3, Aco-4, and Enp) and two morphological (Sp1 coding for leaf size, and T coding for pubescence colour) loci in soybean. The authors detected mobility variants for all the enzyme systems that they studied. They reported that isoenzyme locus Aco-3 was linked to both Sp1 and T loci. Other isoenzyme loci assorted independently of each other and of both Sp1 and T loci.

Shennoy *et al.* (1990) studied the inheritance and linkage of Shikimate dehydrogenase-I^2 (Sdh-I^2) allozyme with high seed protein content in rice. They reported an association between Sdh-1^2 and high seed protein in rice.

They suggested that Sdh-I^2 allozyme could be used as an effective marker in screening for high seed protein.

Kornegay *et al.* (1993) studied inheritance of five arcelin seed protein variants and resistance to Mexican bean weevils (MBW: *Zabotes subfasciatus* (Boheman)) in beans (*Phaseolus vulgaris* (L.)). Bioassay tests for insect response and biochemical tests to detect presence/absence of arcelin were performed on the same individual seed. The authors reported that arcelin variants arc-4 and arc-5 had the highest level of resistance to MBW with no weevils emerging from infested seed. Cultivars with other arcelin variants, arc-1, arc-2 and arc-3, had lower levels of resistance. Based on electrophoretic evaluation of presence/absence of protein band, each of the arcelin protein bands was found to be inherited as a single dominant gene. In bioassay tests, arc-1 and arc-5 were found to be inherited as completely dominant, with the presence of arcelin as a homozygote or heterozygote being associated with resistance to the insect. Other arcelin proteins were not highly associated with resistance and only intermediate to susceptible offspring were obtained when arcelin locus was a heterozygote.

Gaur and Slinkard (1991) studied inheritance and linkage relations among loci controlling 31 isoenzyme loci and two morphological traits, yellow vs green cotyledon and pinnate (normal) vs multipinnate leaves, in chickpea. They

identified seven linkage groups involving loci for 26 isozymes and the two morphological traits.

Kusmenoglu *et al.* (1992) studied isoenzyme variation in Ascochyta blight resistant chickpea lines with the objective of identifying linkages among loci for Ascochyta resistance morphological traits, and isoenzymes. The F_2 and/or F_3 families from crosses between resistant and susceptible lines were scored for four isoenzymes and for Ascochyta blight symptoms. The morphological traits growth habit and leaf type were investigated in some F_2 families. No linkages were detected among isoenzyme loci, or between isoenzyme and Ascochyta resistance loci. A loose linkage was, however, detected between the growth habit locus, Hg, and one isoenzyme locus, Pgd-c.

2.3.3.4. Linkage studies in cowpea

Qualitative-qualitative links among genes have been reported in cowpea by various authors. For example Harland (1920) reported that the genes controlling black seed coat, Bl, purple pod, P, and New Era seed-coat pattern were so tightly linked that these factors might be considered allelic rather than linked. Similarly, Spillman and Sando (1930) (as quoted by Fery, 1985) suggested linkage as the probable explanation for the association between genes conditioning the purple seed coat, Pr, brown seed coat, Br, dense speckling of the New Era seed- coat pattern, De, Taylor seed-coat pattern, T, blue seed-coat, F, and spotting

pattern, S. Sen and Bhowal (1961) reported that locus P^t, controlling purple-tipped pods, was linked to the loci Pb and Pbr controlling purple petiole base and purple branch base, respectively.

Brittingham (1950) reported the qualitative - quantitative links for several genes: Gene controlling vining tendency of cowpea, Vi-1, and the genes conditioning early maturity; general colour factor, C, and the genes conditioning pod length; buff seed coat gene, Bu, and the genes conditioning pod length; and C and the genes conditioning seed size. However, Saunders (1960a) suggested that the association between the colour factor C, pod length, and seed size reflects multiple effects of the C gene rather than linkage.

Associations among genes conditioning quantitative traits have also been reported in cowpea. For example, Saunders (1960b) suggested that the seed-coat colour and date of maturity are associated in a quantitative-quantitative linkage. Similarly, Roy and Riccharia (1948) suggested that the gene systems conditioning pod length and the fibre in the pod wall are linked.

Fery (1985) reviewed the world literature on cowpea genetics that have been conducted on various morpho-agronomical traits and pest resistance since the 1900s. He reported that there are very few linkage studies that have been conducted in cowpea compared with other crops and

suggested that in addition to new linkages being studied, the reported linkages needed to be confirmed.

3.0. GENERAL MATERIALS AND METHODS

3.1. Experimental site

The field and greenhouse experiments were conducted at Mbita Point Field Station (MPFS) of the International Centre for Insect Physiology and Ecology (ICIPE) located in Homa Bay district of Kenya. MPFS is located on the shores of lake Victoria, at latitude 0° 25'S, and longitude 34° 10'E and at an altitude of 1240 m above sea level. Annual diurnal mean temperature at Mbita is $22^{o}C$. Mbita receives a mean annual rainfall of 900 mm in two rainy seasons, long rainy season (March-May) and short rainy season (October-November). Farmers around Mbita grow their crops mainly during the long rainy season. The soils around Mbita are mainly the montmollironite clays with a high water holding capacity. The laboratory experiments were conducted at the Nairobi headquarters of the ICIPE.

3.2. Plant materials

The plant materials used in this study included eight cowpea cultivars that have been reported to be resistant to aphids, six susceptible cultivars, and the F_1, F_2, F_3, and backcross populations of 36 crosses among them.

3.2.1. Parent cultivars

Among the resistant lines, five were obtained from IITA (IT82E-25, IT87S-1394, IT84S-2246-4, IT87S-1459, Tvu 310) and three from ICIPE (ICV 10, ICV 11, and ICV 12) (Pathak and Olela, 1986; Pathak, Personal communication). Among the susceptible cultivars, three lines were obtained from IITA (IT83D-237, Tvu 946 and Tvu 1509) and three from ICIPE (ICV 1, ICV 5 and ICV 6). (Pathak and Olela, 1986; Pathak, personal communication). Some characteristics of these cultivars are given in Table 3.1. ICV 1, ICV 5, ICV 6, and ICV 10 were obtained from selections made from local collections. ICV 11 and ICV 12 were selected from mutagen-treated seeds of ICV 1. The rest of the cultivars are advanced breeding lines and cultivars obtained from IITA whose pedigree was not availableto us.

3.2.2. Crosses

Crosses were made between each of the eight resistant cultivars and susceptible cultivar, Tvu 946, to give F_1 seed. Techniques for crossing were adopted from Blackhurst and Miller Jr. (1980). F_1 plants were confirmed to be true crosses by the possession of small leaves and mottled seeds inherited from the pollen parent, Tvu 946. The F_1 plants were allowed to self pollinate to give F_2 seed. The F_1 plants were also crossed with the resistant and susceptible parents to give backcross (BC_1 and BC_2 respectively) seed.

Table 3.1. Cowpea cultivars used in inheritance and linkage studies, their origin and aphid resistance status.

		Characteristic	
Cultivar	Origin	Res/Sus[1]	Res. gene[2]
ICV 1	ICIPE	S	-
ICV 5	ICIPE	S	-
ICV 6	ICIPE	S	-
ICV 10	ICIPE	R	Rac1
1CV 11	ICIPE	R	Rac2
1CV 12	ICIPE	R	Rac2
Tvu 310	IITA	R	Rac1
Tvu 946	IITA	S	-
Tvu 1509	IITA	S	-
IT83D-237	IITA	S	-
IT82E-25	IITA	R	(?)
IT84S-2246	IITA	R	(?)
IT87S-1394	IITA	R	(?)
IT87S-1459	IITA	R	(?)

[1] Resistance/Susceptibility

[2] Reported gene for resistance present in the cultivar

Sources: ICIPE cowpea pedigree records; Pathak (1988).

The F_2 plants were grown in the field and after recording data on morphological traits, 40-73 plants from four crosses were selected and harvested individually to give F_3 seed. Crosses among the eight aphid resistant cultivars were also made following techniques described by Blackhurst and Miller Jr. (1980). The F_1 plants were grown and allowed to self pollinate to give F_2 seed.

3.3. Aphid culture

3.3.1. Initiation of the culture

Seedlings of susceptible cultivar, ICV 1, were sown in pots in the greenhouse. Two weeks after emergence, each seedling was infested with at least five aphids collected from a heavily infested farmer's field near Mbita.

3.3.2. Maintenance of the culture

Seedlings of ICV 1 were sown in pots at one month intervals. Aphids migrated on their own from older to younger seedlings whenever crowding occurred.

4.0. OBJECTIVE 1: APHID RESISTANCE IN COWPEA

4.1. Introduction

Resistant cultivars offers one of the best and often the cheapest means of insect pest control to the farmer since it does not involve use of expensive pesticides while the cost of developing resistant cultivars is borne by the government or a government agency. Resistant genotypes are normally present in nature and can easily be identified in well designed screening programmes. Once sources of resistance have been identified, they can be utilized either directly as cultivars, if they are good yielders and have other desirable traits, or the resistance trait can be incorporated into adapted cultivars in breeding programmes. Knowledge on the type of resistance present in different cultivars, its inheritance and the allelic relationships among different sources of resistance offers valuable information in planning a breeding programme aimed at incorporating insect pest resistance into adapted cultivars.

Cowpea germplasm screening for aphid resistance has been conducted at the International Institute of Tropical Agriculture (IITA) and several resistant lines have been identified (Singh and Jackai, 1985). Two sources of resistance (ICV 11 and ICV 12) were developed through induced mutations at the International Centre of Insect Physiology and Ecology (ICIPE) (ICIPE, 1986). Information on the mechanisms and inheritance of aphid resistance in some

of the identified sources of resistance is not known and, hence, the present studies. These studies were conducted with the aim of determining the reaction of some cowpea lines to aphid infestation, their mechanisms of resistance to aphids, its mode of inheritance, and the allelic relationships among different sources of resistance.

4.2. Materials and methods

4.2.1. Evaluation of cowpea cultivars for resistance to aphid infestation

Fourteen cowpea cultivars were sown in the greenhouse for preliminary evaluation of their reaction to aphids Table 3.1). The cultivars were sown in single rows, two metres long and 10 cm apart, and replicated three times. The plants were spaced 7 cm apart thus giving about 30 plants per row.

At the two-leaf stage (three to four days after seedling emergence), each test plant was infested with five fourth-instar aphids using a camel's hair brush. Damage due to aphid infestation was scored 14-16 days after infestation when all the susceptible check plants had been killed by the aphids. Depending on the proportion of seedlings dead, the cultivars were recorded as resistant (< 20 % dead), or susceptible (>70% dead) (Pathak, 1988).

4.2.2. Settling preference of aphids among cowpea cultivars

The settling preferences of aphids was studied under both choice and no-choice situations using procedures

adapted from Firempong (1988). Antixenosis in a choice situation was studied in trays (30 x 30 cm) filled with soil and placed inside the greenhouse using aphids reared on the susceptible cultivar, ICV 1. Fourteen cowpea cultivars (Table 3.1) were used in these experiments. Three seedlings of each cultivar were planted in hills spacings and later thinned to two plants/hill after germination. The hills were sown five cm apart in a single row in a circular arrangement in the tray. The experiment was conducted in a randomised complete block design (RCBD) and replicated eight times. Four days after seedling emergence, 100 fourth-instar aphids which had been collected in vials and kept overnight, were placed on a paper at the centre of the tray. The seedlings were searched the following day and the number of aphids settled on each seedling were counted. Square root transformation of the data was made. Analysis of variance was conducted on the transformed data and Duncan's multiple range test (DMRT) was used to separate the means using the MStat statistical package (MSTAT Institute, 1986).

Antixenosis under no-choice situation was studied in the greenhouse using 14 cowpea cultivars (Table 3.1) and aphids reared on ICV 1. Four seedlings of each of ten cultivars were sown in 10 litre volume buckets filled with soil collected from the field. Each bucket constituted a replicate. The experiment was conducted in a randomised complete block design with five replicates. Four days after seedling emergence, about 20 fourth-instar aphids were

placed on the soil at the centre of the bucket. On the following day, seedlings were searched and the number of aphids that had settled on each seedling counted. Analysis of variance was conducted on the data and Duncan's multiple range test used to separate the means using the Mstat statistical package (MSTAT Institute, 1986).

4.2.3. Aphid population growth on different cowpea cultivars

Aphid colony development on different cowpea cultivars was studied using procedures adapted from MacFoy and Dabrowski (1984). Ten cultivars (ICV 1, Tvu 946, ICV 10, ICV 11, ICV 12, IT82E-25, Tvu 310, IT87S-1394, IT87S-1459 and IT84S-2246) and aphids reared on ICV 1 were used in these studies. Four seedlings of each cultivar was sown in seven, 10 litre buckets filled with soil collected from the field. One bucket was sampled during each day of sampling. The experiment was conducted in a randomised complete block design (RCBD) with three replicates. Five days after seedling emergence, each seedling was infested with one fourth-instar aphid using a camel's hair brush. Aphid colony development on the cultivars was monitored by randomly selecting one pot for each cultivar and counting the number of progeny on the seedlings every day up to seven days after infestation. Analysis of variance was conducted on the data for each day separately, and Duncan's multiple range test was used to separate the means using the Mstat statistical package (MSTAT Institute, 1986).

4.2.4. Inheritance of aphid resistance

Inheritance of aphid resistance was studied in the parents, F_1, F_2, BC_1 (backcross of F_1 to resistant parent), and BC_2 (backcross of F_1 to susceptible parent) populations of crosses between eight resistant cultivars and susceptible cultivar, Tvu 946 (Table 3.1). One row each of P_1, P_2, F_1, BC_1 and BC_2, and three to five rows (depending on seed availability) of each F_2 population of each cross were sown in the greenhouse. Seedlings were sown in rows 10 cm apart, two metre long at intra-row spacings of 7 cm. Susceptible (ICV 1) and resistant (ICV 12) cultivars were planted as checks after every 10 rows of test material. Four days after emergence, each seedling was infested with five fourth-instar aphids to ensure that all seedlings were infested. Scoring for dead (susceptible) and alive (resistant) seedlings was done when all the seedlings of the susceptible check row were killed. Seedlings killed by factors other than aphid infestation were eliminated from this analysis. Data on the number of resistant/susceptible seedlings from aphid infestation from the F_2 and BC_2 generations of each cross were evaluated with "goodness of fit" tests for the 3:1 and 1:1 segregation ratios, respectively, for a single dominant gene. The difference between observed and expected values were adjusted with Yates' correction factor since only two genotypic classes (resistant and susceptible) were present (Strickberger, 1990).

The formula for calculating goodness of fit chi-square values was:

$$\sum [|O_i - E_i| - 0.5]^2 / E_i$$

Where: O_i = observed frequencies

E_i = expected frequencies

In order to confirm the segregation ratios, progeny rows of 50-67 F_3 lines from three randomly selected crosses (ICV 12 X Tvu 946, IT87S-1459 X Tvu 946, and IT84S-2246 x Tvu 946) were grown in the greenhouse. Seedlings of each F_3 line were sown in single rows at inter-and intra-row spacings of 10 and 7 cm respectively. Susceptible (ICV 1) and resistant (ICV 12) checks were planted after every 10 rows of test material. Seedlings were infested with aphids and each line scored if it had all resistant, all susceptible or both types of seedlings, when the susceptible check plants were dead. Data were tested by "goodness of fit" to test if segregation fit the expected 1:2:1 (non-segregating resistant : segregating : non-segregating susceptible) ratios expected from Mendelian segregation.

4.2.5. Allelism studies

The eight aphid resistant cultivars were crossed in a diallel to give F_1 seed. The F_1 plants were allowed to self-pollinate to give F_2 seed. One row each of P_1, P_2 and F_1, seven rows of each F_2, and check rows were sown in the

greenhouse as in the inheritance study above (4. 2.4). The seedlings were infested and scored for aphid resistance (alive) or susceptibility (killed) 14-16 days after emergence when all seedlings from the susceptible check row were killed. Data from each F_2 population were adjusted for seedlings that were recorded as dead for causes other than aphid infestation. This was done since preliminary results on aphid resistance had indicated that seedlings of resistant cultivars were attacked to various degrees by aphids.

Adjustment was made by:

1. Recording the proportion of dead seedlings of the parent cultivars (S_i), where i=1..n;
2. Using proportions of dead seedlings recorded for each cultivar, the proportion of F_2 seedlings of each cross that were expected to be dead and resistant was obtained by multiplication (S_1 x S_2);
3. The proportions of calculated F_2 seedlings that were expected to be dead and resistant for each cross were subtracted from the number of F_2 seedlings recorded as dead, and added to the number of F_2 seedlings recorded as alive.

Adjusted values from F_2 populations of each cross were evaluated for a 15:1 segregation ratio expected from a two completely dominant genes model. The difference between observed and expected values were adjusted with Yates'

correction factor (Strickberger, 1990). Crosses which had $\leq$ 5% dead F_2 seedlings were considered as not segregating.

4.3. Results

4.3.1. Cowpea resistance to aphid infestation

Resistance rating of different cowpea cultivars based on percentage of seedlings killed by aphid infestation is given in Table 4.1. The results indicated that less than 15% seedlings from cultivars ICV 10, ICV 11, ICV 12, Tvu 310, IT82E-25, IT87S-1394, IT87S-1459, and IT84S-2246, were killed by aphid infestation and thus, these cultivars were classified as resistant to aphids. Over 80% of seedlings from cultivars ICV 1, ICV 5, ICV 6, IT83D-237, and Tvu 946 were killed by aphid infestation and these cultivars were classified as susceptible. Aphids were found on the underside of leaves, leaf petioles, and young shoots. Some aphids (not quantified) were found leaving the resistant cultivars just a short time after infestation. When there was a high aphid population pressure on plants of the susceptible cultivar, alate aphids were formed which emigrated to the resistant cultivars. Seedlings of the susceptible cultivars died 16-20 days after aphid infestation.

Table 4.1. Resistance rating of cowpea cultivars following aphid infestation

Cultivar	No. seedlings alive	No. seedlings dead	Percent dead	Resistance rating[1]
ICV 1	0	90	100.0	Sus
ICV 5	14	77	84.6	Sus
ICV 6	8	79	90.8	Sus
IT83D-237	10	76	88.4	Sus
Tvu 946	12	78	86.7	Sus
Tvu 1509	7	78	91.8	Sus
ICV 10	79	6	7.1	Res
ICV 11	78	4	4.9	Res
ICV 12	81	3	3.6	Res
Tvu 310	80	3	3.6	Res
IT82E-25	77	12	13.5	Res
IT87S-1394	78	9	10.3	Res
IT87S-1459	78	5	6.0	Res
IT84S-2246	79	10	11.2	Res

[1] Res = Resistant, Sus = Susceptible

4.3.2. Settling preference of aphids among cowpea cultivars.

4.3.2.1. Choice situation

Results on the number of aphids settled on different cowpea cultivars under choice situation are presented in Table 4.2. The data indicated that significantly more aphids settled on the susceptible cultivar, ICV 1, than on the resistant cultivars. Among the susceptible cultivars, more aphids settled on ICV 1 than on Tvu 946. There were no significant differences between susceptible cultivar Tvu 946 and resistant cultivar IT82E-25. There were no significant differences among the resistant cultivars ICV 10, ICV 11, ICV 12, Tvu 310, IT87S-1394, IT87S-1459, and IT84S-2246. These data indicated that given a choice, the aphids moved to the susceptible cultivars, and more so to the cultivar they had been previously exposed to.

4.3.2.2. No-choice situation

The number of aphids settled on different cowpea cultivars under no-choice situation are presented in Table 4.2. The results indicated significant differences among cultivars. Significantly fewer aphids settled on the resistant cultivars ICV 12, IT84S-2246, ICV 10, IT87S-1459, and Tvu 310 than on both the susceptible cultivars. There were no significant differences among the cultivars ICV 1, Tvu 946, ICV 11, IT82E-25, and IT87S-1394.

Table 4.2. Mean number of aphids/plant recorded on different cultivars following infestation under choice and no-choice situations.

Cultivar	Choice no. settled	No-choice no. settled
ICV 1	14.25 a*	3.64 a*
Tvu 946	7.56 b	3.68 a
IT82E-25	3.88 bc	2.66 ab
IT87S-1394	2.63 cd	3.08 ab
ICV 10	2.63 cd	2.24 bc
ICV 11	2.50 de	2.90 ab
IT84S-2246	2.06 de	2.04 bc
IT87S-1459	1.63 de	2.30 bc
Tvu 310	1.50 e	2.32 bc
ICV 12	1.38 e	1.50 c

* Means in the same column followed by same letter are not significantly different by Duncan's multiple range test ($P \leq 0.05$).

4.3.3. Aphid colony development on different cowpea cultivars

The number of aphid progeny recorded on different cowpea cultivars over a seven-day period are presented in Table 4.3. The results indicated no significant differences in aphid population sizes among all the cultivars during the first three days of experimentation. Thereafter, significant differences in aphid population sizes among cultivars were recorded. Seven days after infestation, the susceptible cultivars (ICV 1 and Tvu 946) supported significantly larger populations than the resistant cultivars (ICV 10, ICV 11, ICV 12, IT82E-25, IT87S-1394, IT87S-1459, and IT84S-2246), although there were no significant differences in population sizes among the resistant cultivars or between the susceptible cultivars. These data indicated that, though not quite distinct in the early stages of experimentation, the fecundity of *Aphis craccivora* was high on the susceptible cultivars and low on the resistant cultivars. In some cultivars, lower population sizes were recorded on the fifth day than on the fourth day of experimentation which was probably due to emigration and sampling error.

Table 4.3. Mean number of aphids/plant on cowpea cultivars at different days following infestation with one aphid/plant

	Days after infestation				
Cultivar	2	3	4	5	7
ICV 1	3.10 a	7.33 a	10.33 abc	22.00 a	29.67 a
ICV 10	3.10 a	7.61 a	12.00 abc	7.30 c	14.00 b
ICV 11	3.57 a	8.30 a	14.30 ab	7.00 c	12.00 b
ICV 12	2.43 a	8.67 a	6.36 c	3.67 c	11.35 b
IT82E-25	3.90 a	8.00 a	7.68 bc	16.00 ab	16.00 b
Tvu 310	3.67 a	7.00 a	6.30 c	5.45 c	15.33 b
Tvu 946	4.57 a	9.69 a	15.33 a	19.68 a	35.02 a
IT87S-1394	3.10 a	7.00 a	7.66 bc	9.34 bc	11.70 b
IT87S-1459	2.67 a	8.65 a	8.00 bc	9.67 bc	11.75 b
IT84S-2246	2.67 a	6.17 a	7.67 bc	7.00 c	10.76 b

Means in each column (day) followed by the same letter are not significantly different by Duncan's multiple range test ($P \leq 0.05$).

4.3.4. Inheritance of aphid resistance

Results on seedling reaction to aphid infestation in the parents, F_1, and BC_1 (backcross between F_1 and resistant parent) generations where no segregation was expected to occur are presented in Table 4.4. Seedlings of the resistant parents, F_1, and BC_1 populations when infested with aphids indicated a resistant reaction. In each of these populations and for each cross, less than 10% of the seedlings were killed by aphid infestation. A similar proportion of seedlings from the resistant check cultivar (ICV 12) were also dead. Over 90% of the seedlings from susceptible parents died from aphid infestation 14-16 days after infestation.

Table 4.4. Seedling reaction to *Aphis craccivora* in parents, F_1 and BC_1 populations of cowpea crosses between eight aphid-resistant cultivars and susceptible cultivar, Tvu 946.

Code/ Parent	Parents		Cross[1]	F_1 population		BC_1 population	
	Res	Sus		Res	Sus	Res	Sus
	no			no		no	
1. ICV 1	0	29	3 x 2	18	2	13	1
2. Tvu 946	2	29	4 x 2	20	1	11	1
3. ICV 10	28	3	5 x 2	25	0	18	2
4. ICV 11	29	1	6 x 2	24	1	20	1
5. ICV 12	30	1	7 x 2	20	3	14	2
6. Tvu 310	29	1	8 x 2	26	3	15	3
7. IT82E-25	27	4	9 x 2	25	1	19	2
8. IT87S-1394	28	2	10 x 2	29	1	15	1
9. IT87S-1459	27	2					
10. IT84S-2246	28	1					

[1] Numbers refer to code of parents

BC_1 = cross between F_1 and the resistant parent

Results on segregation for aphid resistance in the F_2 and BC_2 (cross between F_1 and the susceptible parent) populations are presented in Table 4.5. Data from all the eight crosses showed good fits to the 3R:1R (resistant/ susceptible) and 1R:1S segregation ratios for the F_2 and BC_2 populations respectively thus indicating that each resistant cowpea line in this study carried a single dominant gene conferring resistance to aphids. The F_2 population from the cross IT82E-25 x Tvu 946 indicated a poor fit to the 3:1 ratio. This was because of a high number of dead plants resulting from seedling wilt which could not easily be distinguished from those dying from aphid infestation. The results of reciprocal crosses indicated no apparent maternal effect on the expression of resistance to aphids and data from such crosses were pooled during the analysis. The combined data indicated that the eight crosses were homogeneous ($P \mu 0.05$) and provided good fits to the 3R:1S and 1R:1S segregation ratios for F_2 and BC_2 populations respectively.

Single gene segregation of cowpea resistance to aphids was confirmed with data from selected F_3 lines of crosses ICV 12 x Tvu 946, IT87S-1459 x Tvu 946, and IT84S-2246 x Tvu 946 (Table 4.6). Segregation in the progenies of these lines fit the 1R:2RS:1S (resistant/segregating/susceptible) ratio expected from single gene inheritance.

Table 4.5. Segregation ratios and chi-square values for aphid resistance in F_2 and BC_2 populations of cowpea crosses between eight resistant cultivars and susceptible cultivar, Tvu 946.

Cross[1]	Pop.	Observed frequencies			Expected		
		Total	Res.	Sus.	ratio	X^2	P
ICV 10/Tvu 946	F_2	119	83	36	3:1	1.48	0.10-0.25
	BC_2	26	11	15	1:1	0.65	0.25-0.50
ICV 11/Tvu 946	F_2	81	60	21	3:1	0.03	0.75-0.90
	BC_2	23	10	13	1:1	0.43	0.50-0.75
ICV 12/Tvu 946	F_2	266	201	65	3:1	0.07	0.75-0.90
	BC_2	13	5	8	1:1	0.77	0.25-0.50
Tvu 310/Tvu 946	F_2	164	126	38	3:1	0.20	0.50-0.75
	BC_2	25	10	15	1:1	0.37	0.50-0.75
IT82E-25/Tvu 946	F_2	257	175	82	3:1	6.18	0.01-0.05
	BC_2	27	15	12	1:1	1.04	0.25-0.50
IT87S-1394/Tvu 946	F_2	147	113	34	3:1	0.33	0.50-0.75
	BC_2	21	12	9	1:1	0.48	0.25-0.50
IT87S-1459/Tvu 946	F_2	72	54	18	3:1	0.02	0.75-0.90
	BC_2	11	6	5	1:1	0.18	0.50-0.75
IT84S-2246/Tvu 946	F_2	105	77	28	3:1	0.08	0.75-0.90
	BC_2	19	11	8	1:1	0.53	0.25-0.50
All crosses	F_2	1211	889	322	3:1	1.55	0.10-0.25
	BC_2	165	80	85	1:1	0.22	0.50-0.75

[1] Progeny from reciprocal crosses were pooled.

Table 4.6. Segregation for aphid resistance in F_3 lines from cowpea crosses between three resistant cultivars and susceptible cultivar, Tvu 946.

Cross	Observed reaction			Expected		
	Res.	Seg.	Susc.	ratio	X^2	P
ICV 12/Tvu 946	17	30	8	1:2:1	3.40	0.05-0.10
IT87S-1459/Tvu 946	10	36	14	1:2:1	2.93	0.05-0.10
IT84S-2246/Tvu 946	10	30	10	1:2:1	2.00	0.05-0.10

Res = All progeny resistant, Seg. = Segregating into resistant and susceptible progeny, Susc. = all progeny susceptible.

4.3.5. Allelism of aphid resistance

Results on segregation for aphid resistance in the F_2 populations of crosses among the resistant cultivars indicated no segregation in the crosses ICV 10 x Tvu 310, ICV 10 x IT87S-1394, ICV 10 x IT84S-2246, ICV 11 x ICV 12, ICV 11 x Tvu 310, ICV 12 x IT87S-1394, IT82E-25 x Tvu 310, IT82E-25 x IT87S-1459, IT87S-1394 x IT87S-1459, IT87S-1394 x IT84S-2246, and IT87S-1459 x IT84S-2246 (Table 4.7). These data suggested that cultivar IT87S-1394 carried the same gene for aphid resistance as cultivars ICV 10, ICV 12, IT87S-1459, and IT84S-2246; Tvu 310 as cultivars ICV 10, ICV 11 and IT82E-25; cultivar ICV 11 as cultivar ICV 12; and cultivar IT82E-25 as cultivar IT87S-1459. These data, therefore, suggested that all the cultivars in this study carried the same gene for resistance to aphids.

Segregation for aphid resistance in F_2 populations was found in 15 crosses involving resistant cultivars and good fits to a 15R:1S (resistant:susceptible) ratio were obtained. The good fits to the 15:1 ratio suggested the involvement of two loci in the expression of resistance to aphids. There were far too many susceptible plants recorded in crosses ICV 12 x Tvu 310 and IT82E-25 x IT84S-2246 which could be attributed to early infestation of the test plants by aphids.

4.4. Discussion

4.4.1. Mechanisms of resistance

Results on aphid infestation of different cowpea cultivars indicated that cultivars ICV 10, ICV 11, ICV 12, Tvu 310, IT82E-25, IT87S-1394, IT87S-1459, and IT84S-2246 were resistant to aphid infestation while other cultivars were susceptible. Less than 15% of seedlings from these cultivars were killed by aphid infestation.

Results obtained from aphid settlement on different cowpea cultivars in a choice situation indicated that an antixenosis (non-preference) modality of resistance was prevalent in all the resistant cultivars. Few aphids settled on the resistant cultivars compared to those settling on the susceptible cultivars. These results confirm those of earlier workers (Karel and Malinga, 1980; Givovich *et al.*, 1988; ICIPE, 1993) who reported that antixenosis against aphid infestation is an important resistance mechanism in cowpea. Under field conditions, the resistant cultivars are free of aphids during most of the growth period and only a few aphids settle and reproduce on them (Pathak, 1988). Antixenosis under field conditions was well demonstrated by Muller (1958) who showed that whereas the number of aphids that land on both aphid-resistant and susceptible bean cultivars under field conditions are equal, there are more aphids that settle and reproduce on the susceptible cultivars compared to those that settle and reproduce on the resistant cultivars. He reported that the majority of the

aphids left the resistant host plants soon after landing. Dent (1991) proposed that antixenosis could be due to biophysical and/or biochemical properties of the host plant, for example, lack of stimulants or presence of deterrents and/or toxic metabolites. However, the factor(s) responsible for antixenosis against aphids in cowpea has not yet been identified.

Results of feeding under no-choice situation indicated that seedlings of all cultivars were infested by aphids and there was no apparent expression of antixenosis. There were no significant differences among susceptible cultivars ICV 1 and Tvu 946 and resistant cultivars ICV 11, IT82E-25, and IT87S-1394. These results were in agreement with those of MacFoy and Dabrowski (1984) who reported no apparent antixenosis in aphid feeding on cowpea cultivars under no-choice situations. MacFoy and Dabrowski (1984) attributed the lack of expression of antixenosis to the fact that aphids used in these experiments had been starved for a long time, and, hence, did not have an option but to feed on the available host even though it may have been undesirable. Moreover, in plant resistance to aphids, there is no immunity and both resistant and susceptible cultivars are attacked (Muller, 1958; Dent, 1991).

Results on aphid colony development indicated that aphids infested on susceptible cowpea cultivars had a higher fecundity than those infested on resistant cultivars. These results suggested that the susceptible cultivars were more

preferred for reproduction than the resistant cultivars. The differences in fecundity were not very clear in early stages of aphid colony development. During this period, aphid colony sizes on susceptible and resistant cultivars were not significantly different. Differences in colony sizes started appearing on the fourth day after infestation, when the aphids born soon after host invasion started to reproduce, and were quite clear on the seventh day. Firempong (1988) reported that the pre-reproductive period of aphids is about four days at MPFS and is not different among resistant and susceptible cowpea cultivars. Low aphid colony development on resistant cowpea cultivars compared to colonies developing on resistant cultivars were also reported by other workers (MacFoy and Dabrowski, 1984; and Givovich *et al.*, 1991). MacFoy and Dabrowski (1984) and Givovich *et al.* (1991) reported that aphids reared on resistant cultivars spent a considerably longer time probing the host plant than feeding compared with the periods spent by aphids reared on the susceptible cultivars. They attributed this feeding behaviour by the aphids to the presence of higher contents of phenolic compounds in the resistant cultivars compared with those in the susceptible cultivars and suggested that the reduced feeding may have contributed to low fecundity. Firempong (1988), on the other hand, attributed the lower rate of aphid colony development on resistant cultivars compared to that on susceptible cultivars to the presence

of, a shorter reproductive period by adult aphids, lower fecundity, and higher nymph mortality of aphids.

Results from experiments in this study indicated that, given a choice, aphids migrate towards the susceptible cowpea cultivars. Under no-choice situations, however, the aphids have no choice and they infest both susceptible and resistant cultivars. While on the plants, aphids have a higher fecundity on the susceptible cultivars than on the resistant ones. Under field conditions, therefore, when the two types of cultivars are exposed to the same level of aphid infestation, the susceptible cultivars will die from aphid infestation, unlike the resistant ones, since they will be preferred by aphids during the initial infestation, and once infested, aphids will have a higher fecundity on them than on the resistant cultivars.

4.4.2. Genetics of cowpea resistance to aphid infestation

Data in these studies indicated that resistance to aphids in each of the eight sources of resistance is controlled by a single dominant gene. These results confirmed those of earlier workers (Bata *et al.*, 1987; Pathak, 1988). Single (major) gene inheritance suggests that the aphid resistance trait can easily be incorporated into desired adapted cultivars through backcrossing. The major problem with single gene inheritance is that insects can easily develop new biotypes which might overcome the resistant cultivars. Biotype development can easily be

encountered with aphids which have a very short life cycle and reproduce parthenogenetically. Three biotypes of *Aphis craccivora* have been reported from West Africa where cowpea is widely grown (IITA, 1981). Fortunately no aphid biotypes have been reported from East Africa so far. Despite of this, however, identification of many sources of aphid resistance would be highly desirable to keep ahead of new biotype development in the aphids. Once other sources of resistance have been identified, pyramiding of two or more resistance genes in a single cultivar can be effected.

Results on allelism among eight sources of resistance to aphids in this study could not fully resolve whether one or two genes were involved in the expression of resistance. This was because cultivars earlier reported to carry the same gene for resistance (Pathak, 1988) behaved differently when crossed with a common cultivar (for example, ICV 10 and Tvu 310, both carrying *Rac1*, when crossed with IT87S-1394; also ICV 11 and ICV 12, both carrying *Rac2*, when crossed with IT87S-1394). It was suggested that linkage relationships of both *Rac1* and *Rac2* with other loci controlling morphological traits might resolve this, which they did, (see 7.3.3, this thesis). It can be inferred, therefore, that only one gene for resistance was present in all cultivars in this study and that expression of the resistance gene was under the influence of modifiers which were expressed only in some genetic backgrounds. This idea was further supported by observations that cultivars IT82E-25,

IT87S-1394 and IT84S-2246 suffered greater damage from aphid infestation in the greenhouse than other resistant cultivars (Table 4.1). Pathak (1991) reported that although resistance to some insect pests may be controlled by a single major gene, minor or modifier genes are often suspected to act together with the major gene, further enhancing or reducing the resistance level.

5.0. OBJECTIVE 2: MORPHOLOGICAL TRAITS VARIATIONS AND ASSOCIATIONS WITH APHID RESISTANCE

5.1. Introduction

Variation in morphological traits has been observed in all crops, including cowpea. This variation has been used in genebanks for studies in cultivar identification and phylogeny. Variation is also utilized by breeders and farmers when selecting for desired phenotypes. During selection, the workers hope that the phenotype they are selecting for is a direct representation of the genotype.

Cowpea has been the subject of genetics research since the beginning of the 1900s (Fery, 1985). This has been possible owing to the wide diversity in easily identifiable morphological traits such as pigmentation of various plant parts, maturity, and other traits, present in the crop (Fery, 1985; Lattanzio *et al.*, 1990). There has been no previous studies aimed at identifying traits associated with aphid resistance. These studies were conducted with the aim of identifying variability in cowpea with respect to various morphological traits, and clustering the cultivars together based on similarities in morphological traits to check if they formed natural groups that could distinguish between aphid resistant and susceptible cultivars. In addition, the genetics of some selected morphological traits was studied in order to understand how best these traits could be utilized in cowpea improvement.

5.2. Materials and Methods

5.2.1. Evaluation of parent cultivars

Fourteen cowpea cultivars were grown in the field at MPFS during the short rains (October-December) 1992 (Table 3.1). Each cultivar was sown in a single row, six metres long, at inter- and intra-row spacings of 60 cm and 30 cm, respectively. The experiment was conducted in RCBD and replicated three times. Data were recorded on five randomly selected plants of each cultivar on:

I. Vegetative stage:

(a) Days from sowing to seedling emergence,

(b) Stem colour of seedlings two weeks after emergence,

(c) Swollen stem base (Present/absent at flowering),

(d) Pale band next to major vein (Present/Absent at flowering),

(e) Pubescence on leaves and petioles (Present/Absent two weeks after emergence),

(f) Pubescence on stem (Present/Absent at flowering),

(g) Length of fully grown cotyledonous leaf,

(h) Width of fully grown cotyledonous leaf;

II. Flowering and podding stages:

(a) Days from emergence to first open flower,

(b) Outer corolla colour (Unopened flowers),

(c) Inner corolla colour (opened flowers),

(d) Colour of fully grown immature pod,

(e) Peduncle colour at pod maturity,

(f) Peduncle length at pod maturity,

(g) Branching peduncle (Present/Absent at pod maturity),

(h) Pod position in canopy (Above/Intermediate/Below canopy top at pod maturity),

(i) Erect versus drooping pods,

(j) Growth habit (Determinate/Non-determinate),

(k) Plant type (Compact/semi-spreading),

(l) Branch base colour (Purple/green at flowering),

(m) Petiole base colour (Purple/green at flowering);

III. Maturity traits:

(a) Days from emergence to first mature pod,

(b) Colour of dry mature pods,

(c) Seeds per pod of ten randomly selected pods,

(d) 100-seed weight,

(e) Seed-coat colour,

(f) Loose, wrinkled versus smooth seed-coat,

(g) Wrinkled versus smooth dry pod surface,

(h) Length of mature pods,

(i) Width of mature pods,

Data analysis

Variation in qualitative traits was recorded for all the cultivars. For each pair of cultivars, a measure of dissimilarity with respect to all morphological traits was calculated by counting the number of traits which were

different between the cultivars and dividing these with the total number of traits studied (Cole *et al.*, 1991). The dissimilarity matrix was subjected to hierarchical cluster analysis to determine whether the fourteen cultivars formed natural groups using the SAS Proc Cluster (Distance) software programme (SAS, 1988). Dendrograms were drawn based on the similarities and distance between clusters.

To determine whether there was association between various morphological traits and aphid resistance, 2 x 2 or 2 x 3 contingency tables were formed containing, for each morphological trait, the numbers of cultivars recorded as having the various forms of that trait, and resistance or susceptibility to aphid infestation as in Cole *et al.*, (1991). The contingency tables were subjected to chi-square analysis. Traits were considered to be associated with aphid resistance if the chi-square values were significant ($P \leq 0.05$).

Analysis of variance was conducted on the data for each quantitative trait to test the significance of differences among cultivars. Genotypic and phenotypic variances as well as heritability were estimated by analysis of variance (ANOVA) following the method of Nielsen *et al.* (1993) presented in Table 5.1. Simple correlation coefficients among different pairs of traits were computed using the mean values for each trait.

Table 5.1. Analysis of variance table used to evaluate quantitative trait variation in cowpea[#].

Source	df	Mean square	Expectations of mean square
Between lines	l-1	ML	$\sigma^2_E + r\sigma^2_G$
Within lines	l(r-1)	ME	σ^2_E
	$\sigma^2_G = (ML-ME)/r$		
	$\sigma^2_P = \sigma^2_G + \sigma^2_E$		
	Broad sense heritability $= \sigma^2_G/\sigma^2_P$		

Procedure adapted from Nielsen *et al.* (1993)

5.2.2. Segregation of qualitative traits

Segregation of selected morphological traits was studied in the parents, F_1, F_2, and F_2-derived F_3 populations of the following crosses:

1. IT87S-1459 X Tvu 946 for the traits: branching versus non-branching peduncle, green versus purple-tip pods, immature and mature pod colour, peduncle colour, plant type, and growth habit;
2. ICV 12 X Tvu 946 for the traits: pale band next to major vein (clear, pale, or green), green versus purple-tip pods, immature and mature pod colour, peduncle colour, plant type, growth habit, and erect versus drooping pods;
3. Tvu 310 X IT82E-25 for the traits: presence versus absence of swollen stem base, and unopened flower bud colour;
4. IT87S-1459 X ICV 5 for the traits: stem colour, and immature and mature pod colour;
5. IT84S-2246 X Tvu 946 for the traits: green versus purple-tip pod, green versus purple branch base, green versus purple petiole base, peduncle colour, and mature pod colour.

One row each of each parent and F_1, and five F_2 rows of each of the above crosses were sown in three replicates in the field at MPFS during the 1992 short rainy season. The experimental design used was RCBD. Individual plants were scored for each of the morphological traits that was

polymorphic between the parent cultivars involved in that cross. Data were analysed by "chi-square" to test if they fit expected ratios for Mendelian segregation. Yates correction factor was applied if only two classes of progeny were obtained (Strickberger, 1990).

In order to confirm the segregation ratios, progeny rows of 40-67 F_3 lines of the crosses ICV 12 X Tvu 946, IT87S-1459 X Tvu 946, and IT84S-2246 x Tvu 946 were grown in the field during the long rainy season, 1993. Seedlings of each F_3 line were sown in single rows at inter-and intra-row spacings of 60 and 30 cm respectively. During growth, the progeny of various crosses were scored for segregation in the polymorphic morphological traits. Data were analysed by chi-square to test if segregation fit the 1:2:1 (non-segregating dominant:segregating:non-segregating recessive) ratio expected from single gene segregation of Mendelian inheritance.

5.2.2. Genetics of quantitative traits

Genetic variation for quantitative traits was studied in parents and F_1 progenies of a diallel cross among 10 cultivars (ICV 1, ICV 10, ICV 11, ICV 12, Tvu 310, Tvu 946, IT82E-25, IT84S-2246, IT87S-1394, and IT87S-1459). The plants were sown in the field at MPFS during the short rainy season 1993 in single rows, 5 m long, and replicated three times. The plants were spaced at inter- and intra-row spacings of 60 cm and 30 cm respectively. Data were recorded

on five randomly selected plants of each entry on days to 50% flowering, days to maturity, peduncle length, pod length, seeds/pod, and 100 seed weight. Data on yield and yield-related traits could not be recorded due to heavy thrips and pod-sucking bugs infestations on the test materials. These pests could not be controlled using insecticides since ICIPE management prohibits the spraying of insecticides in the field. Heterosis (relative to better parent) and combining ability analyses were made on each trait.

Combining ability analysis was done according to method 2, and model I of Griffing (1956) based on the model:

$X_{ij} = \mu + g_i + g_j + s_{ij} + 1/bc \sum_k \sum_l e_{ijkl}$

where

X_{ij} = the mean value between crosses of i and j parents,

μ = population mean effect,

g_i = the general combining ability (GCA) effect for the ith parent,

g_j = the general combining ability (GCA) effect for the jth parent,

s_{ij} = the specific combining ability (SCA) effect for the cross between i^{th} and j^{th} parents such that s_{ij} = s_{ji} and,

e_{ijk} = the environmental effect associated with the ijk^{th} individual observations.

The methods for analysis of variance for general (GCA) and specific (SCA) combining ability are given in Table 5.2.

Table 5.2. Analysis of variance for Method 2, Model I of Griffing (1956) used for data analysis.

Source	Degrees of freedom	Sum of square*	Mean squares	Expectations of mean squares, Model I
GCA	p-1	S_g	M_g	$\sigma^2 + (p+2)(1/p-1) \Sigma g_i^2$
SCA	p(p-1)/	S_s	M_g	$\sigma^2 + 2/(p(p-1)) \Sigma_{i \le j} \Sigma s_{ij}$
Error	m	S_e	M_e	σ^2

* where

$$S_g = \frac{1}{p + 2} \left(\Sigma_i (X_{i.} + x_{ii})^2 - \frac{4}{p} X_{..}^2 \right)$$

$$S_s = \Sigma_{i \le j} \Sigma x_{ij}^2 - \frac{1}{p + 2i} \Sigma (X_{i.} + x_{ii})^2 + \frac{2}{(p+1) \cdot (p+2)} X_{..}^2$$

Testing for differences:

For differences among gca effects

$F(p-1,m) = M_g/M_e$

For differences among sca effects

$F(p(p-1)/2,m) = M_s/M_e$

Effects were estimated as follows:

$$g_i = \frac{1}{p + 2} \left(X_{i.} + x_{ii} - \frac{2}{p} X_{..} \right),$$

$$s_{ij} = x_{ij} - \frac{1}{(p+2)} X_{i.} + x_{ii} + x_{j.} + x_{jj} + \frac{2}{(p+1)\ (p+2)} X_{..}$$

Where

p = number of parents

m = error df of ANOVA in RCBD

M_e = mean square of error/number of replications

$(X_{i.} + x_{ii})$ = total value for rows + mean value of parents

$X_{..}$ = sum of individual value of parents and crosses.

Relative importance ratio (R) of GCA to SCA was calculated using the formula given by Baker (1978):

$R = 2S_g \ / \ (2S_g + S_s)$

Where,

S_g = sum of squares due to GCA (from ANOVA table)

S_s = sum of squares due to SCA (from ANOVA table)

The closer this ratio is to unity, the greater the predictability of progeny performance based on GCA alone.

Heterosis was estimated as:

$H = (F_1 - HP) \ / \ HP$

Where

F_1 = mean performance of the F_1 plants

HP = mean performance of the better parent

5.3. Results

5.3.1. Morphological trait variation

5.3.1.1. Vegetative and reproductive stages of crop growth

Data recorded on various morphological traits during the vegetative and reproductive stages of crop growth are presented in Table 5.3. Pubescence was monomorphic and found in all the cultivars. All other traits were polymorphic. Swollen stem base trait was found in the cultivar IT82E-25 only. This trait could easily be identified two weeks after seedling emergence and could not be confused with swelling of the stem as a result of nodulation. The cultivars could be grouped into two groups with respect to leaf width (wide and narrow) and leaf length (long and short).

Plant type

The fourteen cultivars were divided into two classes, non-determinate and determinate with respect to growth habit. Semi-spreading and compact (erect) plant types were recorded among the cultivars. The compact plant type was characterized by possession of branches held at an acute angle relative to the main stem, while the semi-spreading plant type was characterized by possession of branches held at wide angles (> 50°) relative to the main stem. Non-determinate and semi-spreading plant habits were more frequent among the cultivars in this study.

Table 5.3. Variation in morphological traits among cowpea cultivars during the vegetative and reproductive stages of crop growth.

Trait	Classification P/M[1]	Phenotypes	Frequency
Days emergence	M	1. 4-5 days	14
Swollen stem base	P	1. Normal	13
		2. Swollen	1
Pubescence	M	1. Pubescence	14
Leaf length	P	1. Long (> 5 cm)	12
		2. Short (< 5 cm)	2
Leaf width	P	1. Wide (> 3 cm)	11
		2. narrow (< 3 cm)	3
Stem colour	P	1. Green	13
		2. Purple	1
Clear leaf veins	P	1. Normal	11
		2. Clear	3
Flower bud colour	P	1. Green	11
		2. Cream-white	3
Flower colour	P	1. Purple	10
		2. Pale-purple	3
		3. White	1
Days flowering	P	1. Early (< 35 days)	3
		2. Medium (36-45 days)	10
		3. Late (> 46 days)	1
Pod colour (green)	P	1. Pale green	7
		2. Green	6
		3. Purple	1
Peduncle colour[2]	P	1. Light-purple	7
		2. Purple	3
		3. Green	4
Branching peduncle	P	1. Normal	13
		2. Branched	1
Purple-tip pod	P	1. Green	11
		2. Purple	3
Peduncle length	P	1. long (> 15 cm)	11
		2. Short (< 15 cm)	3
Erect pods	P	1. Drooping	11
		2. Erect	3
Pod position	P	1. Above canopy	2
		2. Intermediate	10
		3. Below canopy	2
Growth habit	P	1. Non-determinate	12
		2. Determinate	2
Plant type	P	1. Semi-spread	9
		2. Compact	5
Purple branch base	P	1. Purple	10
		2. Green	4
Purple petiole base	P	1. Purple	10
		2. Green	4

[1] M = monomorphic, P = polymorphic.
[2] Light purple = purple colour covering < 50% of peduncle length, Purple = > 50% peduncle coloured purple.

Pigmentation

According to Fery (1985), anthocyanin and a melanin-like substance are responsible for colour in the cowpea. The anthocyanins are responsible for all the colours in the flower petals, seeds, pods, peduncles, petioles, stems and leaves. The melanin-like pigment is found only in the seed coat and is responsible for a pale-yellow to deep copper-red basal colour.

Foliage

When the general colour factor C and the anthocyanin factor N are present, green plant organs other than pods can contain anthocyanin (Fery, 1985). Purple colour on some plant parts like the leaf petioles, pod tips, branch base, nodes or on the whole stem was recorded on some cultivars. Some cultivars were wholly green. Peduncles either contained anthocyanin or were wholly green. For the coloured peduncles, colour was found either all over the peduncle or only on a small part of the peduncle. A clear (pale green) band next to the major veins of the leaves was observed in cultivars ICV 10, ICV 11 and ICV 12.

Flowers

Flower bud colour was either green or creamy-white among the cultivars in this study. Colour of opened flowers indicated that cultivars could be distinguished into three groups, creamy-white, pale purple, and purple flowered.

Pods

The immature (green) pods of cowpea cultivars in this study were either green or purple. The intensity of the colour in each group varied with the cultivars such that the pods could be distinguished into green and light green, or purple and light purple. The trait pod position relative to canopy top was divided into three classes. One class formed pods high up above the canopy level, another group had pods below, while the third group was intermediate and had some pods below and some above the canopy level. The pods were either erect or drooping. The cultivars could be grouped into two groups with respect to peduncle length (long and short peduncles). Branching peduncles were recorded in cultivar IT87S-1459 only while all other cultivars had normal peduncles.

5.3.1.2. Maturity stages of crop growth

Data recorded on various morphological traits during the maturity stages of crop growth are presented in Table 5.4.

Maturity

The trait was measured by such criteria as days to flowering or days to maturity. The cultivars used in this study could be grouped into three maturity groups, early (< 35 days), medium (36-45 days) and late (> 45 days) with respect to days to flowering. The cultivars could also be grouped into three groups, early (< 60 days), medium (61-73

Table 5.4. Variation in morphological traits among cowpea cultivars during the maturity stage of crop growth.

Trait	Classification P/M[1]	Phenotypes	Frequency
Pod colour (dry)	P	1. Straw-yellow	12
		2. Cocoa-brown	1
		3. Greyish-brown	1
Wrinkled pod	P	1. Smooth	11
		2. Wrinkled	3
Loose testa	P	1. Normal	12
		2. Loose	2
Seed colour	P	1. Brown	9
		2. Creamy-white	1
		3. > one colour	4
Seeds per pod	P	1. > 15 seeds	1
		2. 11-15 seeds	11
		3 < 11 seeds	2
100-seed weight	P	1. Bold (> 15 g)	2
		2. Medium (10-15 g)	10
		3. Small (< 10 g)	2
Pod length	P	1. Long (> 11 cm)	11
		2. Short (< 11 cm)	3
Pod width	P	1. Broad (> 9 cm)	9
		2. Narrow (< 9 cm)	5
Days maturity	P	1. Early (< 60 days)	4
		2. Medium (61-73 days)	9
		3. Late (> 73 days)	1

[1] M = monomorphic, P = polymorphic.

days) and late (> 73 days) with respect to days to maturity. Cultivars classified as early or late in flowering were also early or late in maturity. ICV 5, classified as medium flowering, however, matured early.

Seed and pod traits

The seed coat colours of the cultivars in this study were highly diverse. Brown seeded types were the most common. Some cultivars were mottled or had shades of more than one colour. The cultivars could be grouped into three groups (bold, medium, and small seeded) with respect to 100-seed weight. Mature (dry) pod colour was straw-yellow, greyish-brown or cocoa-brown. Straw-yellow colour was the most common colour and was found in 12 cultivars in this study. Wrinkled and smooth mature pods could be distinguished among the cultivars. Cultivars were grouped into two groups based on pod width (wide and narrow pods) and pod length (long and short pods), and into three groups (few, medium, and many seeded) based on number of seeds per pod.

5.3.2. Variability and heritability estimates

The data indicated wide variability among cultivars for various morphological traits (Table 5.5). Days to maturity followed by peduncle length had the highest genotypic variances and were thus the highest variable. The leaf size traits had the least genotypic variances and were the least variable. Results on broad sense heritability estimates indicated that all traits studied were moderately to highly heritable (42-88%) (Table 5.5). Seeds per pod and leaf length had the lowest heritability estimates (42%) while 100-seed weight had the highest value (88%). Other quantitative traits had heritability estimates intermediate between these two extremes.

Table 5.5. Mean, variance, and heritability estimates for different quantitative traits recorded in 14 cowpea cultivars.

Trait	Range	Mean	Variance		H^1
			Genotypic	Phenotypic	
Days 1st flower	33.7-46.0	38.8	8.75	13.25	0.66
Days 1st mature pod	56.0-73.7	64.5	24.48	35.39	0.69
Leaf size (length) (cm)	4.5-6.7	6.0	0.35	0.83	0.42
Leaf size (width) (cm)	2.8-4.8	3.8	0.43	0.68	0.63
Peduncle length (cm)	11.1-25.7	18.7	16.52	21.64	0.76
Pod length (cm)	8.3-15.0	12.3	3.23	5.24	0.62
Pod width (cm)	6.7-11.7	9.9	2.04	3.55	0.57
Seeds/pod	10.2-16.5	12.4	1.86	4.42	0.42
100-seed weight (g)	7.2-17.5	12.2	6.32	7.35	0.88

[1] H = heritability estimate (broad sense).

5.3.3. Correlations among traits

Estimates of simple phenotypic correlation coefficients among various quantitative traits indicated that moderate to high positive correlation existed among most traits studied (Table 5.6). The parameters for measuring maturity (days to 50% flowering and days to maturity) were highly positively correlated (r = 0.93), as were the leaf size and pod size traits (leaf width and length (r = 0.81), pod width and length (r = 0.77), and leaf length and pod length (r = 0.76), respectively). Pod width was highly positively correlated with 100-seed weight (r = 0.80) and with days to maturity (r = 0.70). The data indicated no correlations among several traits, for example: between seeds per pod and maturity (r = -0.04), seed weight and seeds per pod (r = -0.20), and peduncle length and each of the maturity and leaf size traits. The values were estimated to be 0.01, 0.05, 0.05, and 0.10 for relationships between peduncle length and each of the traits days to flower, days to maturity, leaf width, and leaf length, respectively.

Table 5.6. Phenotypic correlation coefficients between different quantitative traits recorded in 14 cowpea cultivars.

	Traits							
Traits	Days maturity	Peduncle length	Leaf width	Leaf length	Pod length	Pod width	Seeds/ pod	100-seed weight
Days to flower	0.93**	0.01	0.37	0.53*	0.41	0.64*	0.10	0.55*
Days to maturity		0.05	0.17	0.46	0.40	0.70**	-0.04	0.62*
Peduncle length			0.04	0.10	0.61*	0.47	0.38	0.32
Leaf width				0.81**	0.59*	0.24	0.48	0.16
Leaf length					0.76**	0.56*	0.47	0.38
Pod length						0.77**	0.57*	0.55*
Pod width							0.20	0.80**
Seeds/pod								-0.20

*, ** Correlations significant at the 0.05 and 0.01 levels of probability, respectively

5.3.4. Cluster analysis

Results of average linkage cluster analysis of the 14 cultivars based on dissimilarities in morphological traits indicated that all the fourteen cultivars in this study could be distinguished from one another except for cultivars ICV 10, ICV 11, and ICV 12 which were identical morphologically (Figure 5.1). The results also indicated that cultivars in this study could be grouped into three main clusters. The first cluster composed of cultivars Tvu 946 and Tvu 1509 which were characterized by the possession of small leaves, a trait that was absent from other cultivars. The second cluster composed of cultivars IT82E-25, IT87S-1394, and IT84S-2246. These cultivars were characterized by the possession of white flower buds, a trait that was absent from other cultivars. The third cluster composed of the rest of the cultivars in this study, ICV 5, IT83D-237, Tvu 310, IT87S-1459, ICV 6, ICV 1, ICV 10, ICV 11, and ICV 12, with no character common among all of them.

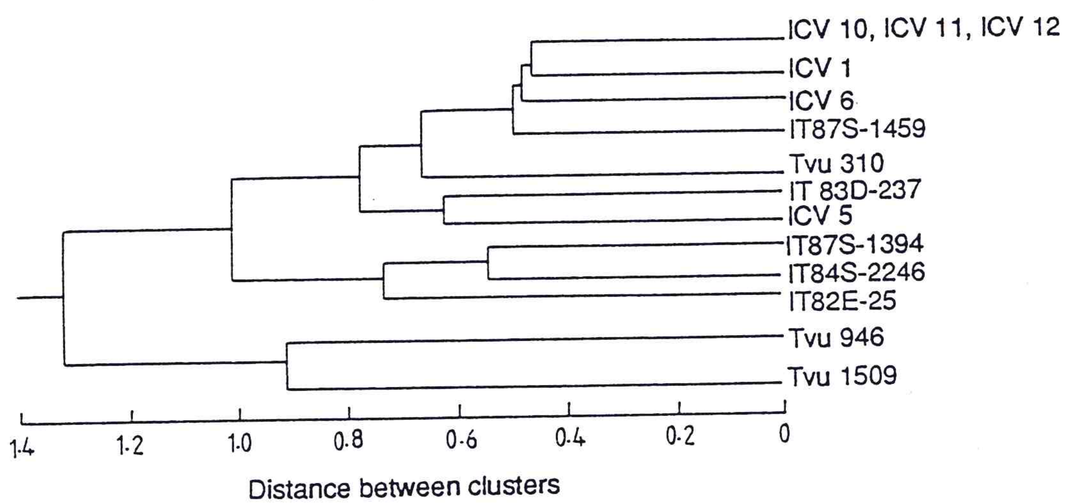

Figure 5.1 Dendogram of average linkage cluster analysis of 14 cowpea cultivars using variation in morphological traits as the measure of dissimilarity

5.3.5. Association of morphological traits with aphid resistance.

Results on tests for association between various morphological traits recorded from 14 cultivars and aphid resistance/susceptibility are presented in Table 5.7. Significant associations ($P \leq 0.05$) were found between aphid resistance/susceptibility and each of the traits growth habit, immature pod colour, and seed colour. Significant associations indicated that these morphological traits might be related to aphid resistance and possibly be used for distinguishing between resistant and susceptible cultivars in this study. No significant associations were found between aphid resistance and other traits studied.

Table 5.7. Relationship between morphological traits and aphid resistance.

Trait[2]	No. of cultivars in each genotypic class[1]						X^2
	Resistant cultivars			Susceptible cultivars			
	C-1	C-2	C-3	C-1	C-2	C-3	
Growth habit	8	0		3	3		5.09*
Swollen stem base	7	1		6	0		0.81
Stem colour	8	0		5	1		1.44
Flower bud colour	5	3		6	0		2.86
Flower colour	5	3	0	5	0	1	3.79
Peduncle colour	7	0	1	2	3	1	5.61
Clear band veins	5	3		6	0		2.86
Pod colour (green)	8	0	0	0	5	1	14.00**
Pod colour (dry)	8	0	0	4	1	1	3.11
Purple tip pods	8	0		4	2		3.11
Branching peduncle	7	1		6	0		0.81
Erect-drooping pods	6	2		4	2		3.11
Pods in canopy	7	0	1	3	2	1	3.38
Purple branch base	6	2		4	2		0.11
Purple petiole base	6	2		4	2		0.11
Wrinkled pod	5	3		6	0		2.86
Rough-smooth testa	6	2		6	0		1.75
Plant type	6	2		3	3		0.93
Peduncle length	7	1		4	2		0.88
Leaf size (width)	7	1		4	2		0.88
Leaf size (length)	7	1	0	4	0	2	3.61
Maturity	7	1	0	3	0	3	5.43
Seed colour	7	0	1	1	2	3	7.36**
100-seed weight	2	6	0	0	4	2	4.20
Pod length	5	3		4	2		0.03

[1] Morphological traits recorded on resistant and susceptible cultivars classified qualitatively into two or three classes; C-1, C-2, C-3

[2] See Table 5.3 for class determination of various traits.

*, ** Significant chi-square values at 0.05 and 0.01 levels of probability, respectively.

5.3.6. Inheritance of some morphological traits

5.3.6.1. Segregation of qualitative traits

Results of inheritance of some selected morphological traits from four crosses are presented in Table 5.8. The results indicated that all these traits were simply inherited (monogenic) and they segregated either in a 3:1 or 1:2:1 ratio. Results of pooled populations from different crosses, where applicable, also gave good fits for monogenic inheritance.

Single gene segregation of various morphological traits was confirmed with data from selected F_3 lines of crosses ICV 12 x Tvu 946 and IT87S-1459 x Tvu 946 (Table 5.9). Segregation of peduncle colour, purple-tip pods, plant type, dry pod colour, and erect versus drooping pods in the progenies of these lines fit the 1:2:1 (non-segregating dominant : segregating : non-segregating recessive) ratio expected from single gene inheritance both for individual crosses and for pooled progenies from the two crosses. Segregation of immature pod colour, branching peduncle, and growth habit did not fit the expected 1:2:1 ratio which was attributed to sampling errors in the F_2 populations.

Table 5.8. Segregation of some morphological traits in F_2 populations of cowpea crosses.

Character	Locus[1]	Cross	No. in each genotypic class[2] AA	A- or Aa	aa	Ratio tested	X^2	P
Growth habit	Ndt	1		71	24	3:1	0.004	0.95-0.99
		2		188	74	3:1	1.30	0.25-0.50
		1+2		259	98	3:1	1.02	0.25-0.50
Peduncle colour	Pd	1		70	25	3:1	0.03	0.75-0.90
		2		187	75	3:1	1.65	0.10-0.25
		1+2		257	100	3:1	1.57	0.10-0.25
Plant type	Gh	1		68	27	3:1	0.42	0.50-0.75
		2		202	60	3:1	0.51	0.25-0.50
		1+2		270	87	3:1	0.11	0.50-0.75
Purple-tip pods	P^t	1		77	18	3:1	1.55	0.10-0.25
		2		182	80	3:1	3.99	0.01-0.05
		1+2		259	98	3:1	1.02	0.25-0.50
Pod colour (dry)	Bk	1		77	18	3:1	1.55	0.10-0.25
		2		187	75	3:1	1.65	0.10-0.25
		1+2		264	93	3:1	0.16	0.50-0.75
Branching peduncle	Bpd	1		79	16	3:1	2.95	0.05-0.10
Erect/Drooping pods	Er	2		201	61	3:1	0.33	0.50-0.75
Swollen stem base	Sw	3		139	58	3:1	1.84	0.10-0.25
Flower bud colour	Fbc	3		147	50	3:1	0.002	0.90-0.95
Cocoa brown pods (dry)	Cbr	4		65	23	3:1	0.02	0.75-0.90
Clear band veins	Pal	2	56	136	70	1:2:1	1.88	0.10-0.25
Purple stem	Pu^S	4	24	37	27	1:2:1	2.43	0.10-0.25
Purple pods	Pu^P	4	26	40	22	1:2:1	1.09	0.25-0.50

[1] Loci symbols as per Fery (1985)
[2] AA, A-, = dominant phenotype, Aa = partially dominant phenotype, aa = recessive phenotype

Cross no. 1 = IT87S-1459 x Tvu 946, 2 = ICV 12 x Tvu 946, 3 = Tvu 310 x IT82E-25, 4 = IT87S-1459 x ICV 5.

Table 5.9. Segregation of some morphological traits in F_3 progenies of cowpea crosses.

Character	Locus[1]	Cross	No. in each genotypic class[2] AA	Aa	aa	Ratio tested	X^2	P
Growth habit	Ndt	1	15	37	21	1:2:1	1.00	0.05-0.75
		2	10	24	33	1:2:1	21.18	< 0.01
		1+2	25	61	54	1:2:1	14.33	< 0.01
Peduncle colour	Pd	1	15	35	23	1:2:1	1.88	0.25-0.50
		2	17	31	19	1:2:1	0.49	0.75-0.90
		1+2	32	66	42	1:2:1	1.89	0.25-0.50
Plant type	Gh	1	20	36	17	1:2:1	0.26	0.75-0.90
		2	18	31	18	1:2:1	0.37	0.75-0.90
		1+2	38	67	35	1:2:1	0.39	0.75-0.90
Purple-tip pods	P^t	1	20	40	13	1:2:1	2.01	0.25-0.50
		2	15	33	19	1:2:1	0.49	0.75-0.90
		1+2	35	73	32	1:2:1	0.39	0.75-0.90
Pod colour (dry)	Bk	1	20	47	6	1:2:1	11.41	< 0.01
		2	16	30	21	1:2:1	1.48	0.25-0.50
		1+2	36	77	27	1:2:1	2.56	0.25-0.50
Pod colour (immature)	Gn	1	20	45	8	1:2:1	7.90	< 0.01
		2	20	37	10	1:2:1	3.72	0.10-0.25
		1+2	40	82	18	1:2:1	14.33	< 0.01
Branching peduncle		1	20	35	5	1:2:1	9.17	< 0.01
Erect/Drooping pods	Er	2	15	30	22	1:2:1	2.19	0.25-0.50

1 Loci symbols as per Fery (1985)

2 AA, = dominant phenotype, Aa = segregating phenotype, aa = recessive phenotype

Cross no. 1 = IT87S-1459 x Tvu 946, 2 = ICV 12 x Tvu 946,

5.3.6.2. Combining ability estimates for quantitative traits

Table 5.10 shows combining ability estimates for four quantitative traits studied. There were significant differences among cultivars with respect to all traits studied except with number of seeds per pod where cultivars were similar. Significant GCA effects were obtained for the traits pod length and 100-seed weight. Non-significant SCA effects were obtained for all the traits studied which was an indication of the fact that the main effects were more important than dominance and gene interactions (epistasis) in the expression of these traits.

The GCA to SCA ratios were 0.74, 0.69, and 0.79 for the traits pod length, seeds per pod and 100-seed weight, respectively, thus suggesting that GCA effects were more important than SCA effects in the expression of progeny performance with respect to these traits. The GCA to SCA ratio for peduncle length was 0.47 thus suggesting that both SCA and GCA were equally important in the expression of this trait.

Table 5.10. Mean squares for analysis of variance for four quantitative traits of 10 cultivars and 45 F_1 hybrids of cowpea grown at MPFS, 1993 short rains.

		Mean squares			
Source[1]	Degrees of freedom	Pod length	Peduncle length	Seeds/ pod	100-seed weight
Replications	2	8.50	1050.23**	18.08	9.02**
Cultivars	54	8.87**	41.02**	12.57	16.53**
GCA+	9	10.71**	25.92	13.61	22.39**
SCA+	45	1.53	11.60	2.41	2.33
Error	108	2.90	17.77	14.86	1.31
R#		0.74	0.47	0.69	0.79

* ** Significant at the 0.05 and 0.01 level of probability, respectively

GCA+ = general combining ability, SCA = specific combining ability

R# Ratio that measures the relative importance of GCA and SCA

GCA effects in ten cowpea cultivars for four quantitative traits are presented in Table 5.11. The results indicated significantly positive GCA effects for the trait pod length in cultivar ICV 10, and for the trait 100-seed weight in cultivars IT87S-1459 and IT84S-2246. Cultivar ICV 10 was the best general combiner for the trait pod length while cultivar Tvu 946 was the worst. Cultivar Tvu 946 was the best general combiner for the trait peduncle length while cultivar IT82E-25 was the worst. Cultivars ICV 11 and ICV 12 were the best general combiners for the trait seeds per pod while cultivar IT87S-1394 was the worst general combiner for this trait. For the trait 100-seed weight, cultivar IT87S-1459 was the best general combiner while cultivar Tvu 946 was the worst. Overall the results indicated that cultivars IT82E-25 and IT87S-1394 were poor general combiners for all traits studied. Cultivar Tvu 946 was a poor general combiner for all traits studied except for the trait peduncle length where it was a good general combiner. There was no cultivar which was a good general combiner for all the traits studied. Data on days to 50% flowering and maturity were not reliable since the plants were heavily affected by thrips and pod sucking bugs infestations.

Table 5.11. General combining ability effects for four quantitative traits in 10 cowpea cultivars.

Cultivars	Traits			
	Pod length	Peduncle length	Seeds/ pod	100-seed weight
ICV 1	+0.07	+1.04	-0.30	-0.24
ICV 10	+1.33*	+0.92	+1.12	-0.67
ICV 11	+0.53	-0.54	+1.17	-0.09
ICV 12	+0.85	+0.08	+1.17	-0.02
IT82E-25	-1.22	-2.81	-1.23	-0.99
Tvu 310	+1.08	-1.80	+1.10	+0.78
Tvu 946	-1.42	+2.08	-0.15	-2.48
IT87S-1394	-0.66	-0.41	-1.49	-0.28
IT87S-1459	-0.30	+0.21	-0.41	+2.49**
IT84S-2246	-0.25	+1.24	-0.99	+1.51**
SE (gi)	0.70	1.72	1.57	0.47
LSD 5%	1.28	3.15	2.87	0.86
1%	1.69	4.15	3.78	1.13

*, ** Significantly greater than zero at 0.05 and 0.01 levels of probability, respectively.

Specific combining ability effects recorded for various traits are presented in Table 5.12. Of the 36 cross combinations, the number of combinations exhibiting positive SCA effects were 15 for the trait pod length, 27 for the trait peduncle length, 11 for the trait seeds per pod, and 25 for the trait 100-seed weight. Significantly positive SCA effects were recorded in nine crosses (ICV 1 x IT87S-1459, ICV 1 x IT84S-2246, ICV 10 x IT84S-2246, ICV 11 x IT87S-1394, ICV 12 x IT87S-1394, ICV 12 x IT87S-1459, IT82E-25 x IT87S-1459, Tvu 310 x IT87S-1459, and Tvu 310 x IT84S-2246) for the trait 100-seed weight only. There was no cross exhibiting positive SCA effects for all four traits.

For the trait pod length, the best three crosses with respect to positive SCA effects were Tvu 310 (good GCA) x IT87S-2246 (poor GCA), ICV 12 x Tvu 310 (both good GCA), and Tvu 310 (good GCA) x IT87S-1394 (poor GCA). For the trait peduncle length, several crosses indicated positive GCA effects. The best three crosses with respect to SCA effects were IT82E-25 (poor GCA) x IT87S-2246 (good GCA), ICV 10 x IT87S-2246 (both good GCA), and Tvu 946 (good GCA) x IT87S-1394 (poor GCA). For the trait seeds per pod, the best three crosses with respect to positive SCA effects were ICV 12 x Tvu 310 (both good GCA), ICV 10 (good GCA) x Tvu 946 (poor GCA), and ICV 11 x Tvu 310 (both good GCA). For the trait 100-seed weight, positive SCA effects were recorded in 25 crosses. The best three crosses with respect to this trait

Table 5.12. Specific combining ability effects for four quantitative traits recorded in cowpea crosses.

	Traits			
	Pod length	Peduncle length	Seeds/ pod	100-seed weight
ICV 1 x ICV 10	-2.98	-5.82	-2.53	-0.54
ICV 1 x ICV 11	-2.01	-5.15	+0.21	+0.18
ICV 1 x ICV 12	+1.36	-2.28	+1.64	+0.07
ICV 1 x IT82E-25	-1.88	+1.28	-4.09	-0.90
ICV 1 x Tvu 310	-0.21	+1.22	-0.13	+2.74
ICV 1 x Tvu 946	-3.51	+4.45	-2.13	-3.72
ICV 1 x IT87S-1394	-1.48	-0.18	-4.33	+0.28
ICV 1 x IT87S-1459	-2.14	+0.88	+0.57	+6.79**
ICV 1 x IT84S-2246	-0.24	+2.92	-2.13	+2.85*
ICV 10 x ICV 11	+0.13	+4.71	-1.10	+0.83
ICV 10 x ICV 12	+0.93	+1.64	+0.90	-0.22
ICV 10 x IT82E-25	-1.34	-2.79	-2.26	+0.06
ICV 10 x Tvu 310	+0.33	-5.29	-0.93	-0.02
ICV 10 x Tvu 946	-0.14	+0.47	+2.40	-1.22
ICV 10 x IT87S-1394	-1.97	+2.21	-2.26	-0.74
ICV 10 x IT87S-1459	-1.20	+3.84	-3.16	+1.35
ICV 10 x IT84S-2246	+1.06	+6.96	-2.30	+3.40*
ICV 11 x ICV 12	-0.82	+3.18	-2.38	-0.45
ICV 11 x IT82E-25	-1.16	-1.05	-1.15	-0.38
ICV 11 x Tvu 310	+1.68	-0.49	+2.05	-0.17
ICV 11 x Tvu 946	-1.66	+1.78	-0.31	-2.28
ICV 11 x IT87S-1394	-1.86	+0.58	-2.68	+3.00*
ICV 11 x IT87S-1459	+1.01	-1.02	-0.58	+1.39
ICV 11 x IT84S-2246	-3.09	-1.85	-2.61	+2.66
ICV 12 x IT82E-25	+0.76	-2.25	-0.18	+1.54
ICV 12 x Tvu 310	+2.76	+4.25	+3.02	-0.09
ICV 12 x Tvu 946	-0.41	+2.55	-2.25	-0.01
ICV 12 x IT87S-1394	-0.54	+1.08	-0.38	+3.15*
ICV 12 x IT87S-1459	-0.44	+2.15	+1.02	+3.83**
ICV 12 x IT84S-2246	-0.61	+4.92	-2.21	+2.10
IT82E-25 x Tvu 310	-0.14	+0.32	+0.36	+1.08
IT82E-25 x Tvu 946	-2.80	+4.09	-3.11	-3.09
IT82E-25 x IT87S-1394	-2.80	-0.68	-4.84	-2.74
IT82E-25 x IT87S-1459	-0.47	+2.52	-1.51	+3.18*
IT82E-25 x IT84S-2246	-1.77	+8.39	-1.58	-1.76
Tvu 310 x Tvu 946	+0.60	-4.50	-0.45	+2.64
Tvu 310 x IT87S-1394	+1.70	-4.67	-1.18	+2.24
Tvu 310 x IT87S-1459	-0.50	-4.90	-0.68	+4.19**
Tvu 310 x IT84S-2246	+2.86	-0.34	-1.25	+6.10**
Tvu 946 x IT87S-1394	+0.20	+6.82	+0.41	-1.37
Tvu 946 x IT87S-1459	-0.70	-3.08	+0.84	+1.68
Tvu 946 x IT84S-2246	-1.16	+4.15	-0.49	-0.22
IT87S-1394 x IT87S-1459	+0.12	+5.15	-0.21	-0.45
IT87S-1394 x IT84S-2246	-0.32	-3.95	-0.47	-1.50
IT87S-1459 x IT84S-2246	+0.04	+0.24	-0.04	+0.68
SE(sij)	2.20	5.44	4.98	1.48
LSD 5%	4.03	9.96	9.11	2.71
1%	5.30	13.11	12.60	3.57

*, ** Significantly greater than zero at 0.05 and 0.01 levels of probability, respectively

were ICV 1 (poor GCA) x IT87S-1459 (good GCA), Tvu 310 x IT84S-2246 (both good GCA), and Tvu 310 x IT87S-1459 (both good GCA). These results indicated that high SCA effects were obtained when crosses were made between either a parent with a high GCA and a parent with a low GCA or between two parents with high GCA effects.

The best general combiner for the trait pod length, cultivar ICV 10, had four positive SCA values in nine cross combinations; the second best general combiner, cultivar Tvu 310 had six positive SCA values; while the poorest general combiner, cultivar Tvu 946, had only two (Table 5.12). The best general combiner for the trait peduncle length, cultivar Tvu 946, had seven positive SCA values out of nine cross combinations; the second best general combiner, cultivar IT84S-2246, had six positive SCA values; while the poorest general combiner, cultivar Tvu 310, had only three (Table 5.12). The best general combiners for the trait seeds per pod, cultivars ICV 11 and ICV 12, had two and four positive SCA effects respectively, out of nine cross combinations; while the poorest general combiner, cultivar IT87S-1394, had only one positive SCA value (Table 5.12). The best general combiner for the trait 100-seed weight, cultivar IT87S-1459, had eight positive SCA values; the second best general combiner, cultivar IT84S-2246, had six positive SCA values; while the poorest general combiner, Tvu 946 had only two positive SCA values (Table 5.11).

5.3.6.3. Heterosis of various quantitative traits

Table 5.13 shows estimates of heterosis over the better parent for four quantitative traits in this study. Overall, the results indicated a positive mean heterosis value for peduncle length and negative values for all other traits studied. The mean values obtained were 1.57%, -7.81%, -16.26%, and -2.89%, for the peduncle length, pod length, seeds per pod and 100-seed weight, respectively. When individual crosses were considered, positive heterosis values of 15% and over were recorded for some traits (Table 5.13) and very low values for other traits (Appendix 2). For peduncle length, over 15% positive heterosis was obtained in crosses ICV 1 x IT82E-25, ICV 1 x Tvu 310, ICV 1 x IT87S-1394, ICV 1 x IT87S-1459, ICV 1 x IT84S-2246, ICV 10 x ICV 11, ICV 10 x IT87S-1459, ICV 10 x IT84S-2246, ICV 11 x IT87S-1394, IT82E-25 x IT84S-2246, Tvu 946 x IT87S-1394, and IT87S-1394 x IT87S-1459. For 100-seed weight, over 15% positive heterosis was obtained only in crosses ICV 10 x ICV 11, ICV 11 x IT87S-1394, and ICV 12 x IT87S-1394. For the two traits pod length and seeds per pod, over 15% positive heterosis was obtained in cross ICV 11 x Tvu 310 only. Cross ICV 1 x ICV 12 also recorded over 15% heterosis for pod length. Overall, these results indicated that, apart from a few cases, heterosis for all traits studied was quite low. Appreciable heterosis was expressed mainly in crosses between an ICIPE cultivar and an IITA cultivar.

Table 5.13. Heterosis of various quantitative traits in cowpea.

Traits	Cross	Populations			
		P_1 +	P_2	F_1	Heterosis*
Peduncle length (cm)	ICV 1 x IT82E-25	20.2	11.6	24.7	22.2
	ICV 1 x Tvu 310	20.2	20.1	24.7	21.9
	ICV 1 x IT87S-1394	20.2	17.8	23.3	15.0
	ICV 1 x IT87S-1459	20.2	21.0	24.3	16.0
	ICV 1 x IT84S-2246	20.2	19.3	26.4	30.4
	ICV 10 x ICV 11	21.6	18.3	26.6	23.2
	ICV 10 x IT87S-1459	21.6	21.0	25.7	19.1
	ICV 10 x IT84S-2246	21.6	19.3	28.8	33.7
	ICV 11 x IT87S-1394	18.3	17.8	21.6	17.8
	IT82E-25 x IT84S-2246	11.6	19.3	25.3	30.7
	Tvu 946 x IT87S-1394	25.7	17.8	30.0	16.8
	IT87S-1394 x IT87S-1459	17.8	21.0	26.4	26.0
Pod length (cm)	ICV 1 x ICV 12	13.3	15.0	15.4	15.8
	ICV 11 x Tvu 310	13.2	13.2	15.7	18.4
Seeds per pod (no.)	ICV 11 x Tvu 310	13.2	13.3	16.0	20.5
100-seed weight (g)	ICV 10 x ICV 11	11.3	11.5	13.3	15.7
	ICV 11 x IT87S-1394	11.5	12.2	16.2	32.6
	ICV 12 x IT87S-1394	12.1	12.2	15.4	26.4

+ P_1 = female parent, P_2 = male parent;

* Heterosis estimated as % improvement over better parent.

5.4. Discussion

5.4.1. Variability and cluster analysis

Variability among the cultivars in this study was recorded for all the morphological traits studied except for the trait leaf pubescence where all cultivars were found to be similar. Based on variability in the morphological traits, the cultivars could be distinguished from one another except for ICV 10, ICV 11, and ICV 12 which were morphologically similar. The most variable traits recorded in the cowpea cultivars in these studies were seed colour and flower colour whereby the cultivars could be grouped into three or more different classes. These results were in agreement with Lattanzio et al. (1990) who reported wide variability in cowpea and that the most variable traits in the cowpea samples they were working with were seed colour, growth habit, and pigmentation of various plant parts. Cowpea is a self-pollinated crop which undergoes only limited outcrossing (Fery, 1985). Any variability occurring within the cultivars either through mutation or out-crossing is effectively conserved through self-pollination present in the crop. Man, through preferential selection process has added to the diversity in morphological traits in cowpea.

Cluster analysis of the 14 cowpea cultivars based on dissimilarities in morphological traits gave three main clusters. The cultivars in the first cluster were characterized by the possession of small leaves, second cluster by the possession of creamy-white flower buds, and

cluster three comprised of the rest of the cultivars, and did not have any distinctive feature. These results suggested that after recording morphological trait variation among the cultivars, cluster analysis could be used to analyse interrelationships among cultivars and help identify traits that were peculiar to a given cluster. Cultivars in any given cluster are presumed to be very closely related.

5.4.2. Associations among various traits

Tests for association by chi-square in a contingency table indicated that some morphological traits, namely, growth habit, immature pod colour, and seed colour might be related to aphid resistance, a fact which suggested that these traits may be used for distinguishing between aphid resistant and susceptible cultivars in this study. This idea, however, had some flaws in that no given trait was found exclusively in the resistant cultivars or in the susceptible cultivars. The associations could have been a result of either mathematical computation or real linkage. It was suggested that linkage relationships between genes controlling aphid resistance and each one of these traits might be a better indicator of the relationships present.

Strong positive phenotypic correlations existed between days to flower and days to maturity, pod width and 100-seed weight; leaf length and leaf width; pod length and pod width; and leaf length and pod length. Theoretically, correlated traits can be improved simultaneously. The

observed positive correlations suggested that for example, improvement of a cultivar for early flowering could result in early maturity; and a cultivar with long leaves would also have wide leaves. Positive correlations are very important in plant breding for indirect selection of traits which are difficult to score. Non-significant correlations were recorded among other traits, for example seeds per pod and 100-seed weight; and seeds per pod and days to maturity. Theoretically, a non-significant correlation between any two traits implies that the two traits are not related and that improvement of one trait cannot influence the status of the other trait. Such relationships are not important for indirect selection.

5.4.3. Genetics of some morphological traits

Inheritance studies conducted for some of the morphological traits indicated that they were simply inherited (3:1 or 1:2:1 segregation ratios in the F_2 generation, and 1:2:1 segregation ratios in F_3 progenies). This was true for traits affecting pigmentation of various plant parts and plant habit. These results indicated that when either one of these traits is desired in an adapted cultivar, it can easily be incorporated through simple backcrossing. The results also indicated that maturity, leaf size, pod size, and seed size were quantitatively inherited. These results suggested that in a plant breeding programme,

these traits can be utilized through either pedigree or population improvement methods.

Heritability estimates of the quantitative traits studied indicated that these traits were moderately to highly heritable (H = 42-88%). These estimates were in agreement with the heritability estimates reported by various authors and summarized by Fery (1985). For example, Fery (1985) reported that published heritability estimates (narrow sense and broad sense combined) for days to flower and days to maturity were 48.3% and 47.8% respectively and ranged from 0% to 95.1% for days to flower and from 0% to 89.5% for days to maturity. The broad sense heritability estimates for days to flower and days to maturity in this study were 66% and 69% respectively. The heritability estimates in this study are within limits of the published estimates. Fery (1985) also reported that the heritability estimates (narrow sense and broad sense combined) for pod size, seeds per pod, and 100-seed weight were 75.2%, 52.8%, and 67.8%, respectively. The reported ranges for these estimates were 0-95% for pod size, 9.6-98.9 for seeds per pod, and 9.3-98.9% for 100-seed weight. The heritability estimates for these traits in the present studies were 57%, 42%, and 88% for pod size, seeds per pod, and 100-seed weight, respectively, values which were comparable with the published estimates. The medium to high heritability estimates suggest that these traits are controlled by many genes which could also be interacting among themselves. The

estimates on the lower and higher extremes suggested the involvement of dominant genes and/or additive x dominance interactions; the medium values suggested the involvement of many additive genes.

Significant GCA effects were obtained for the traits pod length and 100-seed weight. These results suggested that additive genes may be very important in the expression of these traits. Non-significant SCA effects were obtained for pod length, peduncle length, and seeds/pod thus indicating that main effects were important in the expression of these traits and that dominance and gene interactions were not very important in their expression. Significant SCA effects for 100-seed weight were recorded in some crosses thus suggesting the involvement of additive genes in the expression of this trait. The ratio of GCA to SCA suggested that GCA was more important than SCA in the expression of all quantitative traits studied, except for peduncle length where GCA and SCA effects were equally important. Significant SCA effects were obtained when crosses were made between parents at least one of which has a high GCA. In future studies, it is suggested that biometrical techniques be applied in order to elucidate the type of gene action involved in the inheritance of quantitative traits in cowpea.

Heterosis percentage values for four quantitative traits in this study were generally low, except in a few cases (crosses between an ICIPE and an IITA cultivar) where

an appreciable heterosis (15% and over) was recorded. Some of these traits (for example pod length and 100-seed weight) are positively correlated with yield (Fery, 1985). The low heterosis percentage, coupled with the fact that the crop is self-pollinated imply that heterosis breeding cannot be commercially exploited in this crop.

6.0. OBJECTIVE 3: BIOCHEMICAL TRAIT VARIATIONS AND ASSOCIATIONS WITH APHID RESISTANCE

6.1. Introduction

Biochemical (protein) markers are becoming increasingly important in genetics/breeding studies and are either fast replacing or being used together with morphological markers. Their importance in genetics/breeding research has been facilitated by several advantages that they have over morphological markers. Unlike morphological markers, biochemical markers are expressed in a codominant fashion such that they can be used for identifying heterozygotes, are not influenced by environmental conditions, dominance or epistasis, only a small tissue is necessary for analysis, and because of genetic constancy in an organism, very young tissues can be used for analysis thereby saving on time and resources (Kesseli and Michelmore, 1986). The biochemical markers most commonly used by various workers are isoenzymes and to a lesser extent, seed proteins.

Despite the importance of such markers, only a few studies have been conducted in cowpea on biochemical characterization of cultivars (Vaillancourt *et al.*, 1993; Oghiake *et al.*, 1993) and no studies have been conducted on the associations between biochemical markers and pest resistance and hence, the present studies. The purpose of this experiment was to study biochemical trait variation

among cowpea cultivars and to test if the recorded variation was related to aphid resistance.

6.2. Materials and methods

6.2.1. Total proteins variation

Techniques for total protein electrophoresis were adapted from Laemmli (1970) and Pedalino *et al.* (1990). The composition of various chemical solutions used in this experiment is given in Appendix 3.

6.2.1.1. Sample preparation

Seeds soaked for 24-30 hr in distilled water were used for protein extraction. Individual seeds of each cultivar were crushed in 0.5 ml of 20 mM borate buffer pH 8.9 containing 0.5 M NaCl (Pedalino *et al.* 1990). Extraction was made overnight at $4^{o}C$. Total protein extracts were collected by centrifugation (20,000 rpm, 20 min at $4^{o}C$). The supernatant solution was either used immediately or stored at $-20^{o}C$ for later use. During electrophoresis, each sample (2 μl containing about 200 ug protein) was mixed with sample buffer (10 μl). The mixture was boiled in a water bath for three minutes and allowed to cool before electrophoresis.

6.2.1.2. Gel preparation

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) was performed according to the method of Laemmli (1970) with a total monomer

concentration of 8% in the running gel and 3.13% in the stacking gel. Running gels were made by mixing 16.8 ml water, 9.6 ml acrylamide-bisacrylamide (30:0.8), 9.2 ml resolving gel buffer, 180 μl of 10% SDS, 180 μl of 10% ammonium persulphate, and 12 μl Temed, while 3.13% stacking gels were made by mixing 6 ml water, 1.0 ml acrylamide-bisacrylamide (30:0.8), 2.5 ml stacking gel buffer, 100 μl of 10% SDS, 50 μl of 10% ammonium persulphate, and 10 μl Temed.

6.2.1.3. Electrophoresis

The samples (10μl) were loaded onto the sample wells using a microsyringe. Molecular weight markers were loaded in one lane. Tris-glycine was used as the electrophoresis buffer. Electrophoresis was conducted at 20 mA, until the bromophenol-blue tracking dye reached the lower buffer solution.

6.2.1.4. Staining and De-staining

After electrophoresis, the gel was gently placed in a stain dish containing enough Coomasie brilliant blue stain to cover the gel. The stain dish was placed on a shaker for about one hour and the stain solution poured back into the stain bottle. A destain solution (125 ml Methanol, 175 ml Glacial Acetic Acid, and 2200 ml distilled water) was then added in the stain dish. Destaining was conducted until background colour of Coomasie brilliant Blue was removed and

clear bands of protein were visible on the gel. Protein bands were scored and photographs taken for later reference.

6.2.2. Isoenzyme variation

Techniques for isoenzyme analyses were adapted from Griffing and Palmer (1987), Glaszmann *et al.* (1988) and Wendel and Weeden (1989). The composition of various chemicals solutions used in these experiments are given in Appendix 4.

6.2.2.1. Gel and sample preparation

Starch gel was prepared using the appropriate buffer for the specific enzymes under investigation (Appendix 4). 4.5 ml of buffer solution, 45 ml of distilled water, and 4.0 g of hydrolyzed starch (14%) were carefully mixed in a 1-litre Erlenmeyer flask and heated with continuous swirling on a magnetic hot-plate until a clear, vigorously boiling solution was obtained. The solution was then de-aerated with a tap aspirator and poured onto a glass gel mold tray. Solid particles and air bubbles were quickly removed with forceps. The gel was allowed to cool for about 10 min at room temperature and then covered with a glass plate to prevent excessive evaporation and allowed to continue cooling for about one hour. When the gel was cold, horizontal slits were prepared approximately six cm from its cathodal end to facilitate comparison of migration distances among the bands.

Cowpea seeds soaked on wet filter paper for 24-30 hr at room temperature were used for enzyme extraction. The tissues were ground with 40 μl of extraction buffer (0.1 M Tris-Citrate, pH 7.5) in spot plates. Sewing-thread wicks were used to absorb the extracts. The wicks were inserted in the appropriate slits of starch gel ready for electrophoresis. Intermixing of extracts between adjacent wicks was avoided by removing excess extract with absorbent paper prior to insertion into the slits.

6.2.2.2. Electrophoresis

After loading the samples, the gel was mounted onto the electrode trays containing the appropriate tray buffer. Absorbent foam was used to connect the tray buffer and the gel. A glass plate was placed atop the foam plates to ensure contact between the tray buffer and the gel. Platinum wire in the anodal tray and ordinary stainless steel in the cathodal tray served as electrodes and were connected to a LKB continuous-current power supply. Electrophoresis was conducted at constant current (250 mA, 38 W) for about three hours at $4^{o}C$.

6.2.2.3. Staining

Zones of enzymatic activity were revealed by immersing the gel slices into different stain solutions (Appendix 4) for about one hr at room temperature. The zymograms were scored immediately and photographs taken for later analysis.

The enzymes assayed were: acid phosphatase (ACP), aconitase (ACO), alcohol dehydrogenase (ADH), aspartate amino transferase (AAT), diaphorase (DIA), esterase (EST), formic dehydrogenase (FDH), fumarase (FUM), glucose-6-phosphate dehydrogenase (G6PDH), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucomutase (PGM), phosphoglucose isomerase (PGI), phosphoglucose dehydrogenase (PGD), and shikimate dehydrogenase (SKD).

6.2.3. Association between aphid resistance and biochemical trait variation

To determine whether there was association between variations in biochemical traits and aphid resistance, 2 x 2 contingency tables were formed containing, for each biochemical trait, the number of cultivars recorded as having a fast/slow protein band or protein band present/ absent, and resistance or susceptibility to aphid infestation (Cole *et al.*, 1992). The contingency tables were subjected to Chi-square analysis. Traits were considered as associated if the Chi-square values were significant ($P < 0.05$).

6.2.4. Inheritance of biochemical trait variation

6.2.4.1. Segregation of protein band variation

Segregation of seed protein bands was studied in the crosses IT87S-1394 X Tvu 946 and IT87S-1459 X Tvu 946 where

polymorphism between the parent cultivars involved in a given cross had been observed. Electrophoresis was conducted following the procedure described above (6.2.1) and the protein bands scored for in the parents, F_1, and F_2 generations. Data on F_2 segregation were analysed by "goodness of fit" to test for the 1:2:1 or 3:1 ratios expected in single gene segregation of Mendelian inheritance.

6.2.4.2. Segregation of isoenzyme variation

The cross ICV 12 x Tvu 946 which indicated polymorphism between the parent cultivars for the AAT isozyme was used in these studies. The parents, F_1, and F_2, seeds were assayed for the AAT isoenzyme following the procedures outlined above (6.2.2) for the parent cultivars. Segregation for the marker isoenzyme was scored in the F_1 and F_2 generations. Segregation for the marker isoenzyme was analysed by "chi-square" to determine its inheritance.

6.3. Results

6.3.1. Protein bands variation

Results of the variation for total proteins are presented in Table 6.1. Variations among cultivars were recorded at the 62 kD and 23 kD protein bands. At the 62 kD band cultivars IT87S-1394 and IT84S-2246 had a slow band while all other cultivars had a fast band. At the 23 kD band, cultivars ICV 10, ICV 11, ICV 12 and Tvu 946 had an extra band that was absent in other cultivars. Variations at these bands indicated that the cowpea cultivars in this study could be divided into three groups: I. IT87S-1394 and IT84S-2246 (with slow band at 62 kD), II. ICV 10, ICV 11, ICV 12 and Tvu 946 (with extra band at 23 kD), and III. ICV 1, IT82E-25, Tvu 310 and IT87S-1459 (rest of the cultivars).

Table 6.1. Electrophoretic variation of total proteins among 10 cowpea cultivars.

Cultivar	Protein band[1]	
	62 kD	23 kD
ICV 1	F	A
ICV 10	F	P
ICV 11	F	P
ICV 12	F	P
Tvu 310	F	A
Tvu 946	F	P
IT82E-25	F	A
IT87S-1394	S	A
IT87S-1459	F	A
IT84S-2246	S	A

[1] F = fast band, S = slow band
A = band absent, P = band present.

kd 1 2 3 4 5 6 7 8 9 10

200

116

97·4

66·2 >—62

42·7

—23

21·5

6.3.2. Isoenzyme variation

The isoenzymes assayed and the number of loci identified in the cowpea cultivars are presented in Table 6.2. One zone of activity was noted when staining for AAT, ACO, ACP, DIA, IDH, G6PDH, LDH, ME, MPI, SDH and SKD enzyme systems. Therefore, one locus was named and scored in each of these enzyme systems. Two zones of activity were noted when staining for MDH, PGD, PGM, and TPI. Two loci were named and scored in each of these enzyme systems. Enzyme loci were numbered following the method of Vaillancourt *et al.* (1993) whereby the most anodal locus was called one, and numbering proceeded cathodally with increasing number. Three zones of activity were noted when staining for ADH, EST, and GPI. For the ADH enzyme, Vaillancourt *et al.* (1993) reported that the middle zone was a heterodimer between ADH-1 and ADH-2 and, thus, two loci were named and scored in this enzyme system. Three loci were, however, named and scored in each of the EST and GPI enzyme systems.

The results indicated that the cultivars were generally monomorphic at all enzyme loci assayed except at the AAT locus where cultivars ICV 6, ICV 10, ICV 11, IT83D-237, Tvu 946, and Tvu 1509 had a null allele. The proportion of polymorphic loci in these cowpea cultivars was 0.037 while the number of alleles per locus were 1.6. These data indicated that the cultivars used in these studies were not genetically diverse with respect to isoenzyme polymorphism.

Table 6.2. Zones of activity and enzyme loci scored in cowpea cultivars.

Enzymes[1]	EC[2] number	Migration direction	Zones of activity	Loci scored
AAT	2.6.1.1	anodal	1	1
ACO	4.2.1.3	anodal	1	1
ACP	3.1.3.2	anodal	1	1
IDH	1.1.1.42	anodal	1	1
ME	1.1.1.40	anodal	1	1
DIA	1.6.4.3	anodal	1	1
G6PDH	1.1.1.49	anodal	1	1
LDH	1.1.1.27	anodal	1	1
MPI	5.3.1.8	anodal	1	1
SDH	1.1.1.14	anodal	1	1
SKD	1.1.1.25	anodal	1	1
MDH	1.1.1.37	anodal	2	2
PGD	1.1.1.44	anodal	2	2
PGM	5.4.2.2	anodal	2	2
TPI	5.3.1.1	anodal	2	2
ADH	1.1.1.1	anodal	3	2
EST	3.1.1.1	cathodal	1	1
		anodal	2	2
GPI	5.3.1.9	anodal	3	3

[1] AAT = Aspartate amino transferase, ACO = Aconitase, ACP = Acid phosphatase, IDH = Isocitrate dehydrogenase, ME = Malic enzyme, DIA = Diaphorase, G6PDH = Glucose-6-phosphate dehydrogenase, LDH = Lactate dehydrogenase, MPI = Mannose phosphate isomerase, SDH = Sorbitol dehydrogenase, SKD = shikimate dehydrogenase, MDH = Malate dehydrogenase, PGD = Phosphogluconate dehydrogenase, PGM = Phosphoglucomutase, TPI = Triose phosphate isomerase, ADH = Alcohol dehydrogenase, EST = Esterase, GPI = Glucose-6-phosphate isomerase.

[2] EC = Enzyme commission number of the International union of biochemists

6.3.3. Association between aphid resistance and biochemical variation

Results of the test for association between aphid resistance and biochemical trait variation are presented in Table 6.3. Non-significant chi-square values ($P < 0.05$) were obtained in all cases. The results indicated that aphid resistance was not related to the biochemical variation recorded among cowpea cultivars in this study.

Table 6.3. Relationship between aphid resistance and biochemical trait variation in cowpea.

	No. of cultivars in each genotypic class				
	Resistant cultivars		Susceptible cultivars		
Trait	Present	Absent	Present	Absent	X^2
62 kD[1]	6	2	2	0	0.63
23 kD	4	4	1	1	0.00
AAT	6	2	2	4	2.43

[1] Fast and slow classes each for resistant and susceptible cultivars

6.3.4. Inheritance of protein bands variation

The results of the segregation of the mobility variants of electrophoretic total protein bands from two crosses are presented in Table 6.4. Segregation at the 62 kD protein band in cross IT87S-1394 x Tvu 946 indicated that all F_1 seedlings had both the fast and the slow band while F_2 seedlings segregated to give a good fit to a 1:2:1 (fast band only:fast and slow band:slow band only) ratio expected from single gene inheritance with codominant alleles ($P < 0.05$). Segregation at the 23 kD in crosses IT87S-1394 x Tvu 946 and IT87S-1459 x Tvu 946 indicated that all F_1 seedlings had one band while the F_2 population segregated to give a good fit to a 3:1 (present:absent) ratio expected from single gene inheritance with null alleles.

6.3.5. Inheritance of AAT isoenzyme

Results of inheritance of variation at the AAT locus are presented in Table 6.5. All F_1 seedlings had one band indicating that band presence was dominant over band absence. Segregation in the F_2 population indicated a good fit to the 3:1 (band present:absent) ratio expected from a single gene inheritance ($P \leq 0.05$).

Table 6.4. Segregation of mobility variants of electrophoretic protein bands in cowpea crosses.

Protein band	Cultivar/ crosses	No. in each genotypic class				$X^{2\#}$	$P^{\$}$
		Total	Fast	Fast/Slow	Slow		
62 kD	IT87S-1394	8	0	8	0	-	-
	Tvu 946	9	9	0	0	-	-
	F_1 pop.	8	0	8	0	-	-
	F_2 pop.	48	12	27	9	1.13	0.25-0.50
		Total	Present	Absent			
23 kD	IT87S-1394	8	0	8		-	-
	Tvu 946	9	9	0		-	-
	F_1 pop.	9	9	0		-	-
	F_2 pop.	44	29	16		2.21	0.10-0.25
23 kD	IT87S-1459	9	0	9		-	-
	Tvu 946	9	9	0		-	-
	F_1 pop.	6	6	0		-	-
	F_2 pop.	48	37	11		0.03	0.75-0.90

= Chi-square values for goodness of fit to a 1:2:1 or 3:1 ratio with 1 df

$ = Probability of a greater value of X^2

Table 6.5. Inheritance of mobility variants at the AAT locus in cross ICV 12 x Tvu 946 of cowpea.

Genotypes	N	Present	Absent	$X^{2\#}$	$P^{\$}$
	No. in each genotypic class				
ICV 12	6	6	0	-	-
Tvu 946	7	0	7	-	-
F_1	6	6	0	-	-
F_2	67	49	18	0.04	0.75-0.90

Chi-square values to test goodness of fit to a 3:1 (present/absent) ratio

$ Probability of a greater value of X^2

6.4. Discussion

Results on total protein variation indicated variation at two protein bands which could be used in distinguishing between cowpea cultivars. However, this variability is very limited and cannot be used for large scale separation of cultivars from one another. These results, though dealing with a few selected cultivars, confirmed earlier reports (Pedalino *et al.* (1990); Oghiake *et al.*, 1993). The report of Pedalino *et al* (1990) is especially important for it dealt with a large number of cultivars selected from among cowpea germplasm present at IITA. However, variability in protein bands, which could not be identified in the gels used, might still be present among cowpea cultivars in this study and it is suggested that additional methods to improve on protein resolution (for example silver staining) be used in future studies. In addition, quantification of protein variation should also be attempted.

Results on isoenzyme variation at 26 loci from 18 enzyme systems indicated that the cowpea samples in these studies were fairly similar with only one variable isozyme locus, AAT. Low genetic diversity in cowpea with respect to isoenzyme variability was reported earlier by Vaillancourt *et al.* (1993) who observed that out of 26 isozyme loci from 16 enzyme systems in their studies, only six loci showed polymorphism. Low genetic diversity in cowpea has also been reported by Young *et al.* (1992) using RFLPs (restriction fragment length polymorphisms) of cowpea DNA.

Overall, results from this and previous studies suggest that cowpea cultivars are very similar at least with respect to protein, isozyme and DNA polymorphism. Accessions of self-pollinated crops, like cowpea, often possess low within-accession variability because within-population variability is normally reduced during self-pollination (Doebley, 1989). Between accession variability, however, is expected to be high, which was not the case with these studies. Low genetic diversity in isoenzyme variation has also been reported in common beans, *Phaseolus vulgaris* (Koenig and Gepts, 1989).

Tests for association between aphid resistance and variation at each of the biochemical traits indicated no significant association. There are no previous studies of this nature in cowpea for comparison. However, in lettuce Cole *et al.* (1992) identified two allozyme bands which were related to the resistance of *Lactuca* species to the lettuce root aphid. Results from this study suggest that the observed variation in biochemical traits cannot be used as markers for aphid resistance in cowpea.

7.0. OBJECTIVE 4: LINKAGE ANALYSES AMONG GENES CONTROLLING VARIOUS TRAITS IN COWPEA.

7.1. Introduction

Linkage studies are important in marker-based selection in breeding whereby desirable traits which are difficult to score for are indirectly selected through the markers. Indirect selection becomes possible since it is known which genes are linked to one another. Linkage studies enable workers to construct linkage maps of various organisms and the locations of various marker genes on the chromosomes are known. Linkage maps, once developed, are important in cloning studies since the location on the chromosomes of a desired gene is known.

Linkage mapping in cowpea is still in a primitive stage and only a few studies on linkage have been conducted in the crop (Fery, 1985). Summarizing the genetic studies conducted in cowpea, Fery (1985) suggested that the few linkage studies conducted in the crop needed confirmation. The present studies were conducted with the aim of identifying linkages among loci controlling various morphological, biochemical and aphid resistance traits in cowpea.

7.2. Materials and Methods

7.2.1. Analysis of linkage relationships between two genes

Linkage analyses were conducted using data recorded on individual plants of the F_2 and F_2-derived F_3 progenies of

four crosses. Chi-square analysis was used to test for segregation of individual genes and co-segregation of genes controlling any two traits in the F_2 populations to test if they fit expected ratios from independent assortment of two genes following Mendelian inheritance. Individual genes were tested for 1:2:1 and 3:1 segregation ratios each with 1 df while joint segregation between any two genes was tested for 9:3:3:1, 3:6:3:1:2:1, and 1:2:1:2:4:2:1:2:1 ratios with 1 df, 2 df, and 3 df respectively. Significant chi-square values, if obtained, indicated that the two genes being tested were not independent of each other and linkage was suspected. Non-significant chi-square values for the joint segregation of any two genes suggested that the two genes being tested were independent of each other and that random assortment between them was taking place. Linkage between any two genes was estimated using recombination frequencies which were calculated using the Product method (Immer, 1930) and/or Maximum-likelihood method (Allard, 1956). Linkage was reported to be present if the estimated recombination frequency was less than 50%.

Formulae used to calculate segregation assuming a cross AABB x aabb (coupling phase) or AAbb x aaBB (repulsion phase) is made are given below:

F_1 genotype = AaBb

F_2 genotypes	: A-B-	: A-bb	: aaB-	: aabb
Expected frequencies	: 9	: 3	: 3	: 1

Class designation : a b c d

Note: classes **a** and **d** are the parental types while **b** and **c** are recombinants.

Chi-squares for segregation of various genes was calculated from the following equations:

Chi-square for gene A = $(a + b - 3c - 3d)^2/3n$

Chi-square for gene B = $(a + c - 3b - 3d)^2/3n$

Chi-square for co-segregation (AB) = $(a + 9d - 3b - 3c)^2/9n$

Recombination frequencies

Recombination frequencies were estimated by:

1. Product method

(a). Coupling phase: R = (bc/ad)

(b). Repulsion phase: R = (ad/bc)

Standard error of R = (1/´R)n

Where:

R is the recombination fraction estimate,

a,b,c,d, frequencies of various classes,

n = total number of individuals.

This method is not applicable in case of partial dominance of one or both genes, when more than four classes of genotypes are obtained. For such cases, the maximum-likelihood method becomes more applicable.

2. Maximum likelihood method

Method can be applied in case of dominance, partial dominance, and epistasis of one or both genes being tested. Assuming a cross is made between two cultivars whose two genes are partially dominant :

Coupling phase : AABB x aabb,

Repulsion phase: AAbb x aaBB,

The F_1 and F_2 genotypes will be:

F_1 genotype = AaBb

F_2	:AABB	AaBB	AABb	AaBb	AAbb	Aabb	aaBB	aaBb	aabb
Classes:	e	f	g	hi	j	k	l	m	n
Exp. Freq.:	1	2	2	4	1	2	1	2	1

The F_2 progeny can be distinguished into 9 classes.

If One gene is partially dominant while the other gene is completely dominant, the F_2 progeny can be distinguished into 6 classes as shown below for a case where gene A is completely dominant while gene B is partially dominant:

F_2	: A-BB	A-Bb	A-bb	aaBB	aaBb	aabb
Classes	: ef	ghi	jk	l	m	n
Exp. Freq.	: 3	6	3	1	2	1

Formulae and tables to be used when calculating recombination estimates for various segregation ratios were given by Allard (1956). For each segregation formula, a factor is given with which to multiply the observed class frequencies. The recombination estimate is the value at which the sum of the products between the tabulated values and the observed class frequencies is closest to zero.

7.2.1.1. Genes controlling morphological traits

Linkage among genes controlling various morphological traits was studied in the F_2 populations of the crosses Tvu 310 x IT82E-25, IT87S-1459 X Tvu 946, IT87S-1459 X ICV 5, and ICV 12 X Tvu 946. The parent cultivars involved in different crosses and their genotypes with respect to various morphological traits are given in Table 7.1.

Individual F_2 plants of each cross were scored for variation in the particular morphological traits which had been found to be polymorphic between the parent cultivars used in that cross. Co-segregation of any two loci was tested as described above (7.2.1).

Table 7.1. Traits studied, their symbols, and form in different cowpea cultivars used in linkage analyses.

Traits	Symbol	Description	Cultivars where trait studied
Growth habit	Ndt	non-determinate	ICV 12, IT87S-1459
	dt	determinate	Tvu 946
Swollen stem base	Sw	swollen stem base	IT82E-25
	sw	normal stem base	Tvu 310
Purple stem colour	Pu^S	purple stem	ICV 5
	pu^S	green stem	IT87S-1459
Plant type	Hg	semi-erect stems	ICV 12, IT87S-1459
	hg	erect stems	Tvu 946
Flower bud colour	Fbc	creamy-white	IT82E-25
	fbc	green	Tvu 310
Peduncle colour	Pd^P	purple	Tvu 946
	Pd^L	light purple	ICV 12, IT87S-1459
	pd	green	IT84S-2246
Purple branch base	Pbr	purple branch base	Tvu 946
	pbr	green branch base	IT84S-2246
Purple-tip pods	P^t	purple tips	Tvu 946
	p^t	green tips	ICV 12, IT87S-1459
Green immature pod colour	Gn	green pods	Tvu 946
	gn	pale green pods	ICV 12, IT87S-1459
Purple immature pod colour	Pu^P	purple pods	ICV 5
	pu^P	green	IT87S-1459
Greyish-brown dry pod colour	Bk	greyish-brown pods	Tvu 946
	bk	straw-yellow pods	ICV 12, IT87S-1459
Cocoa-brown dry pod colour	Cbr	cocoa-brown pods	ICV 5
	cbr	straw-yellow pods	IT87S-1459
Clear band veins	Pal	clear band	ICV 12
	pal	leaf wholly green	Tvu 946
Branching peduncle	bpd	branching peduncle	IT87S-1459
	Bpd	peduncle unbranched	Tvu 946
Erect pods	Er	Pods erect	Tvu 946
	er	drooping pods	ICV 12

7.2.1.2. Genes controlling biochemical traits

Linkage among loci controlling protein variation in young seedlings was studied in the F_2 populations of the crosses IT87S-1394 X Tvu 946 and IT87S-1459 x Tvu 946. Individual seedlings were assayed for the particular protein band (see 6.2 above) that was found to be polymorphic between the parents involved in a given cross. Co-segregation of protein bands was tested as described above (7.2.1).

7.2.1.3. Genes controlling morphological, biochemical, and aphid resistance traits

Linkage among genes controlling morphological traits, biochemical traits, and aphid resistance were studied in the F_2 and F_2-derived F_3 progenies of the crosses IT87S-1459 X Tvu 946, ICV 12 X Tvu 946, and IT84S-2246 X Tvu 946. For these studies, 40-73 F_2 plants from each cross were randomly selected. Variation in morphological traits were recorded on individual F_2 plants. At harvesting time, individual plants were harvested separately and seeds from each plant divided into three parts. A part of the seeds was analysed for the biochemical marker (four seedlings were used). Another part was grown in single F_3 progeny rows in the greenhouse and analysed for aphid resistance. The third part was grown in single unreplicated rows in the field and scored for the morphological traits of interest. For each morphological and biochemical trait, segregation among the F_3 progenies was

scored and used to deduce the F_2 genotype. Co-segregation among the genes controlling various traits (scored directly and deduced genotypes) was tested by chi-square as described above (7.2.1).

7.3. Results

Tests for independence between different gene pairs in this study indicated significant chi-square values for some traits and non-significant values for other traits. For those traits where chi-square values were significant, it was concluded that the loci were linked and recombination frequency between such gene pairs were calculated. For those traits where non-significant chi-square values were obtained, it was concluded that the loci were independent of each other.

7.3.1. Linkage among genes controlling morphological traits

Cross Tvu 310 x IT82E-25

Significant chi-square values ($P \leq 0.05$) were obtained for the joint segregation of the Sw (swollen stem base) and Fbc (flower bud colour) loci thus indicating that these loci were linked (Table 7.2). Estimates of recombination frequencies between these two loci were 41 ± 3.22% and 42 ± 4.8% by the ratio of products and maximum likelihood methods respectively.

Table 7.2. Recombination fractions among genes controlling morphological traits in F_2 populations of the crosses Tvu 310 x IT82E-25 and IT87S-1459 x ICV 5 of cowpea.

Loci tested	Phase#	No. in each genotypic class$									X^{2*}	$p^{\bar{o}}$ Product	$p^{\bar{o}}$ Maximum
		Cross Tvu 310 x IT82E-25 (N = 197)											
		a	b	c	d								
Sw-Fbc	C	109	30	38	20						6.16	41+3.2	41±4.8
		Cross IT87S-1459 x ICV 5 (N = 88)											
		ef	ghi	jk	l	m	n						
Pu^S-Cbr	C	24	35	6	0	2	21				64.31	-	31±5.7
Pu^P-Cbr	C	25	39	1	1	1	21				75.37	-	30±5.7
		e	g	j	f	hi	k	l	m	n			
Pu^S-Pu^P	C	15	8	1	10	26	1	1	6	20	72.48	-	4±1.5

C = coupling phase, R = repulsion phase

$ Class designations as per Allard (1956)

* Chi-square value for segregation in a 9:3:3:1 ratio with 1 df, 3:6:3:1:2:1 ratio with 2 df, or 1:2:1:2:4:2:1:2:1 ratio with 4 df

ō Recombination fraction ± standard error by the product (Immer, 1930) and maximum-likelihood method (Allard, 1956)

Cross IT87S-1459 x ICV 5

Significant chi-square values ($P \leq 0.05$) were obtained for the joint segregation of the loci Pu^S (purple stem colour) and Pu^P (immature pod colour), Pu^S and Cbr (dry pod colour), and Pu^P and Cbr (Table 7.2). Estimates of recombination frequencies by the maximum likelihood method gave 4 ± 1.5%, 31 ± 5.7%, and 30 ± 5.7% for the Pu^S-Pu^P, Pu^S-Cbr, and Pu^P-Cbr loci respectively. These data suggested that loci Pu^S and Pu^P in this cross were tightly linked with very few recombinants.

Cross IT87S-1459 x Tvu 946

Significant chi-square values ($P \leq 0.05$) were obtained for the joint segregation of most loci with one another thus suggesting that some of these loci might be linked (Table 7.3). Estimates of recombination fractions using both the product and maximum likelihood methods indicated that the loci: Ndt (growth habit) and Pd (peduncle colour), Ndt and Gh (plant type), Ndt and bpd (branching peduncle), Gh and bpd, and Pt (purple-tip pods) and Bk (dry pod colour) loci in this cross were linked with recombination frequencies of 25 ± 5.3%, 19 ± 4.6%, 37 ± 8.7%, 24 ± 9.5% and 20 ± 4.7% respectively by the maximum likelihood method (Table 7.3). These results indicated that loci Pd, Ndt, Bpd, and Gh were in the same linkage group, while loci Bk and Pt were in another linkage group.

Table 7.3. Recombination fractions among genes for morphological traits in F2 populations of the crosses IT87S-1459 x Tvu 946 and ICV 12 x Tvu 946 of cowpea

Loci tested	Phase[#]	Cross[$]	No in each genotypic class[&]				N	X^{2*}	p[õ]	
			a	b	c	d			Product	Maximum
Ndt-Pd	C	1	60	11	10	14	95	17.69	25±3.55	25±5.3
		2	156	32	31	43	262	53.15	26±2.19	26±3.2
		1+2	216	43	41	57	357	70.82	26±1.87	26±2.8
Ndt-Gh	C	1	61	10	7	17	95	31.07	19±3.08	19±4.6
		2	155	23	47	37	262	32.78	29±2.32	30±3.5
		1+2	216	33	54	54	357	60.53	26±1.87	26±2.8
Ndt-P^t	R	1	56	15	21	3	95	0.73	Independent	
		2	120	58	62	22	262	0.75	Independent	
		1+2	176	73	83	25	357	1.40	Independent	
Ndt-Bk	R	1	65	6	22	2	95	0.001	Independent	
		2	131	47	56	28	262	2.32	Independent	
		1+2	196	53	78	30	357	1.66	Independent	
Pd-P^t	R	1	57	13	20	5	95	0.01	Independent	
		2	132	55	50	25	262	0.75	Independent	
		1+2	189	68	70	30	357	0.63	Independent	
Pd-Bk	R	1	65	5	22	3	95	0.14	Independent	
		2	140	47	47	28	262	5.13	> 50	
		1+2	205	52	69	31	357	4.56	> 50	
Gh-Pd	R	1	53	17	15	10	95	2.58	Independent	
		2	149	38	53	22	262	2.32	Independent	
		1+2	202	55	68	32	357	4.56	42±2.4	43±3.7
Gh-P^t	R	1	57	11	20	7	95	0.86	Independent	
		2	140	62	42	18	262	0.04	Independent	
		1+2	197	73	62	25	357	0.09	Independent	
Gh-Bk	R	1	61	7	26	1	95	0.98	Independent	
		2	146	56	41	19	262	0.29	Independent	
		1+2	207	63	67	20	357	0.003	Independent	
P^t-Bk	C	1	75	2	12	6	95	8.85	17±2.9	20±4.7
		2	155	27	22	58	262	119.13	18±1.8	19±2.7
		1+2	230	29	34	64	357	118.48	18±1.5	19±2.4
Ndt-bpd	C	1	57	14	22	2	95	1.27	Independent	

Table 7.3 continued,

Pd-bpd	C	1	56	14	23	2			95	1.60	Independent	
Gh-bpd	C	1	53	15	26	1			95	4.35	24±6.5	24±9.5
P^t-bpd	R	1	63	14	16	2			95	0.09	Independent	
Bk-bpd	R	1	71	16	8	1			95	0.07	Independent	
Ndt-Er	R	2	134	44	67	17			262	0.90	Independent	
Pd-Er	R	2	156	31	45	30			262	16.63	> 50	
Gh-Er	R	2	150	52	51	9			262	2.58	Independent	
P^t-Er	C	2	139	62	43	18			262	0.08	Independent	
Bk-Er	C	2	148	53	39	22			262	2.09	Independent	
Gn-Er	C	2	178	23	52	9			262	0.49	Independent	
Ndt-Gn	R	2	157	21	73	11			262	0.29	Independent	
Pd-Gn	R	2	166	21	64	11			262	0.04	Independent	
Gh-Gn	R	2	177	25	53	7			262	0.02	Independent	
P^t-Gn	C	2	161	21	69	11			262	0.04	Independent	
Bk-Gn	C	2	164	66	23	9			262	0.21	Independent	
			ef	ghi	jk	l	m	n				
Pal-Ndt	C	2	38	77	37	44	43	23		59.42	> 50	
Pal-Pd	C	2	36	101	47	20	35	23		3.78	Independent	
Pal-P^t	R	2	39	98	44	17	38	26		3.28	Independent	
Pal-Gn	R	2	49	120	56	7	16	14		2.32	Independent	
Pal-Bk	R	2	36	93	48	20	43	22		6.23	> 50	
Pal-Gh	C	2	48	115	49	8	21	21		10.30	> 50	
Pal-Er	R	2	43	106	52	13	30	18		0.42	Independent	

C = coupling phase, R = repulsion phase
$ Cross 1 = IT87S-1459 x Tvu 946, Cross 2 = ICV 12 x Tvu 946
& Class designation as per Allard (1956)
* Chi-square value for segregation in a 9:3:3:1 ratio with 1 df or 3:6:3:1:2:1 ratio with 2 df
õ Recombination fraction ± standard error by the product (Immer, 1930) and maximum-likelihood method (Allard, 1956)

Cross ICV 12 x Tvu 946

Significant chi-square values (P μ 0.05) were obtained for the joint segregation of most loci with one another (Table 7.3). Estimates of recombination fractions using both the product and maximum likelihood methods indicated that the loci: Ndt and Pd, Ndt and Gh, and P^t and Bk were linked with recombination frequencies of 26 ± 3.2%, 30 ± 3.5%, 19 ± 2.7% respectively by the maximum likelihood method. The results from this cross confirmed those of cross IT87S-1459 x Tvu 946 above.

Estimates of recombination fractions from pooled F_2 populations of the crosses ICV 12 x Tvu 946 and IT87S-1459 x Tvu 946 using the product and maximum likelihood methods indicated that loci Ndt, Pd, and Gh were in one linkage group while Pt and Bk were in another linkage group (Table 7.3)

7.3.2. Linkage among genes controlling protein band variations

Results of the test for linkage among loci controlling variation in total protein bands in cross IT87S-1394 x Tvu 946 are presented in Table 7.4. A non-significant chi-square values (P μ 0.05) was obtained for the joint segregation of the 62 kD with the 23 kD. It was concluded, therefore, that the loci controlling these protein band variations were independent of each other.

Table 7.4. Recombination fractions among genes controlling total protein variation in cross IT87S-1394 x Tvu 946 of cowpea.

Bands	Phase#	No. in each genotypic class&						X^{2*}	P±SEō
		ef	jk	ghi	l	m	n		
62kD-23kD	R	8	4	15	9	5	3	0.15	Independe

R = coupling phase,

& Class designations as per Allard (1956)

* Chi-square values for a 3:6:3:1:2:1 ratio with 2 df

ō Recombination fraction estimates ± standard error by the maximum-likelihood method (Allard, 1956)

7.3.3. Linkage between aphid resistance gene (Rac) and genes controlling variations in morphological traits

Results of the tests for linkage between genes for aphid resistance *(Rac1* and/or *Rac2* (Pathak, 1988)) and thirteen morphological traits in four different crosses are presented in Tables 7.5 (for *Rac1*) and 7.6 (for *Rac2*). Significant chi-square values (P μ 0.05) were obtained for the joint segregation of Rac1 with each of the loci Pbr, Pd, Pt, Gn, Pu^S, and Pu^P (Table 7.5). The joint segregation of Rac1 with Pd in cross IT84S-2246 x Tvu 946 gave recombination estimates of 22 ± 5.1% and 26 ± 8.3% by the ratio of products and maximum likelihood methods respectively. These results suggested that, though not close together, loci Rac1 and Pd were in the same linkage group. Estimates of recombination fractions by the product and/or maximum likelihood methods for the joint segregation of Rac1 with each of the loci Pbr, Pt, Gn, Pu^S and Pu^P in three crosses gave values of over 50%. These results suggested that either Rac1 was not linked to any one of these loci or that it was on the same chromosome with these loci but located far apart from each one of them. Non-significant chi-square values (P μ 0.05) were obtained for the joint segregation of Rac1 with each of the loci Gh, Bk, Ndt, Bpd and Cbr (Table 7.5). These results suggested that Rac1 was not linked to any one of these loci.

Table 7.5. Recombination fractions between *Rac1* and 11 loci in three crosses of cowpea.

Loci tested	Phase#	Cross$	No. in each genotypic class&						N	X^{2}*	$p^{\tilde{o}}$ Product	$p^{\tilde{o}}$ Maximum
			a	b	c	d						
Pbr	R	1	24	7	2	7			40	10.00	> 50	
Pd	C	1	23	8	2	7			40	8.71	22±5.1	26±8.3
		2	42	17	8	6			73	0.67	Independent	
Gh	C	1	19	12	5	4			40	0.04	Independent	
		2	44	15	12	2			73	0.55	Independent	
P^{t}	R	1	24	7	3	6			40	6.40	> 50	
		2	51	8	9	5			73	3.08	Independent	
Bk	R	1	25	6	7	2			40	0.04	Independent	
		2	54	5	13	1			73	0.12	Independent	
Ndt	C	2	41	18	11	3			73	0.55	Independent	
bpd	R	2	48	4	7	1			60	1.07	Independent	
Gn	R	2	55	4	10	4			73	3.65	> 50	
Cbr	R	3	34	10	17	4			68	0.34	Independent	
			ef	ghi	jk	l	m	n				
Pu^{P}	C	3	13	21	10	8	7	6	68	4.71	> 50	
Pu^{S}	C	3	16	19	9	10	7	4	68	9.61	> 50	

C = coupling phase, R = repulsion phase
$ Cross 1 = IT84S-2246 x Tvu 946, Cross 2 = IT87S-1459 x Tvu 946, Cross 3 = IT87S-1459 x ICV 5
& Class designations as per Allard (1956)
* Chi-square value for segregation in a 9:3:3:1 ratio with 1 df or 3:6:3:1:2:1 ratio with 2 df
õ Recombination estimate ± standard error by the product (Immer, 1930) and

A significant chi-square value ($P \leq 0.05$) was obtained for the joint segregation of *Rac2* with locus Pd controlling peduncle colour (Table 7.6). Estimates of recombination fractions between these two loci were 35 ± 5.1% and 35 ± 7.5% by the ratio of products and maximum likelihood methods respectively. These results indicated that loci *Rac2* and Pd were in the same linkage group, though not close together. Linkage of *Rac1* to Pd in cross IT84S-2246 x Tvu 946 and *Rac2* to Pd in cross ICV 12 x Tvu 946 suggests that *Rac1* and *Rac2* are similar. Non-significant chi-square values ($P \leq 0.05$) were obtained for the joint segregation of the locus *Rac2* and each of the loci Ndt, Gh, Gn, P^t, Bk, Er, and Pal. These results indicated that locus *Rac2* was independent of all these loci.

Table 7.6. Recombination fractions between *Rac2* and eight loci in the cross ICV 12 x Tvu 946 of cowpea.

Loci tested	Phase#	No. in each genotypic class&						N	X^2*	p õ Product	p õ Maximum
		a	b	c	d						
Ndt	C	28	22	6	11			67	3.07	Independent	
Pd	C	39	11	9	8			67	4.31	35±5.1	35±7.5
Gh	C	38	12	11	6			67	0.88	Independent	
p^t	R	35	15	13	4			67	0.28	Independent	
Bk	R	34	16	12	5			67	0.04	Independent	
Gn	R	41	9	16	1			67	1.04	Independent	
Er	R	34	16	11	6			67	2.19	Independent	
		ef	ghi	jk	l	m	n				
Pal	C	13	25	12	6	10	1		2.35	Independent	

C = coupling phase, R = repulsion phase

& Class designations as per Allard (1956)

* Chi-square values calculated assuming a 9:3:3:1 ratio with 1 df, or 3:6:3:1:2:1 ratio with 2 df

õ Recombination fraction ± standard error by the product (Immer, 1930) and maximum-likelihood method (Allard, 1956)

7.3.4. Linkage between aphid resistance gene (Rac) and genes controlling variations in biochemical traits

7.3.4.1. Linkage between Rac and total protein loci

Results of the test for linkage between *Rac* and the loci controlling 23 kD protein band are presented in Table 7.7. A non-significant chi-square value (P μ 0.05) was obtained for the joint segregation of *Rac1* and the 23 kD protein band. It was concluded, therefore, that the two loci were independent of each other.

7.3.4.2. Linkage between Rac2 and isoenzyme loci

Results of the test of linkage between *Rac* and AAT loci are presented in Table 7.7. Non-significant chi-square values (P μ 0.05) were obtained for the joint segregation of *Rac2* and the AAT loci in the cross ICV 12 x Tvu 946. It was concluded that the two loci were independent of each other.

Table 7.7. Recombination fractions between *Rac* and three loci controlling biochemical variation.

Loci tested	Cross$	No. in each genotypic class&				N	X^{2*}	P^{õ}
		a	b	c	d			
Rac1-23 kD	1	32	10	7	1	48	2.22	Independent
Rac2-AAT	2	30	11	7	2	50	0.01	Independent

$ Cross 1 = IT87S-1459 x Tvu 946, Cross 2 = ICV 12 x Tvu 946

& Class designation as per Allard (1956)

* Chi-square value calculated assuming a 9:3:3:1 ratio with 1 df, or a 3:6:3:1:2:1 ratio with 2 df;

õ Recombination fraction ± standard error by maximum-likelihood method (Allard, 1956).

7.4. Discussion

Linkage analyses using genes controlling various simply inherited and easily identifiable morphological traits led to the identification of four linkage groups, viz, I, II, III and IV (Figure 7.1). Linkage group I contained loci Sw and Fbc; II contained loci Pu^S, Pu^P, and Cbr; III contained loci Pd, Ndt, Bpd, and Gh; while IV contained loci Pt and Bk. The relationships among these linkage groups could not be established in these studies. Genes controlling biochemical trait variations were not linked to any other gene nor among themselves.

Identification of markers for aphid resistance is highly desirable in breeding programmes. This study did not identify a closely linked marker for aphid resistance. A loose linkage was, however, identified between the aphid resistance gene (Rac) and the gene controlling peduncle colour (Pd). Co-segregation of these two genes in F_2 populations of three crosses gave a mean of 30 ± 7.5% recombinants. These values indicated that, although not close together, the genes controlling these two traits were located on the same chromosome (linkage group III) (Figure 7.1). During meiosis, however, the two loci may be separated by crossing-over. The loose linkage suggested that selection for aphid resistance based on peduncle colour might be more effective than random selection, although a close linkage would be more desirable.

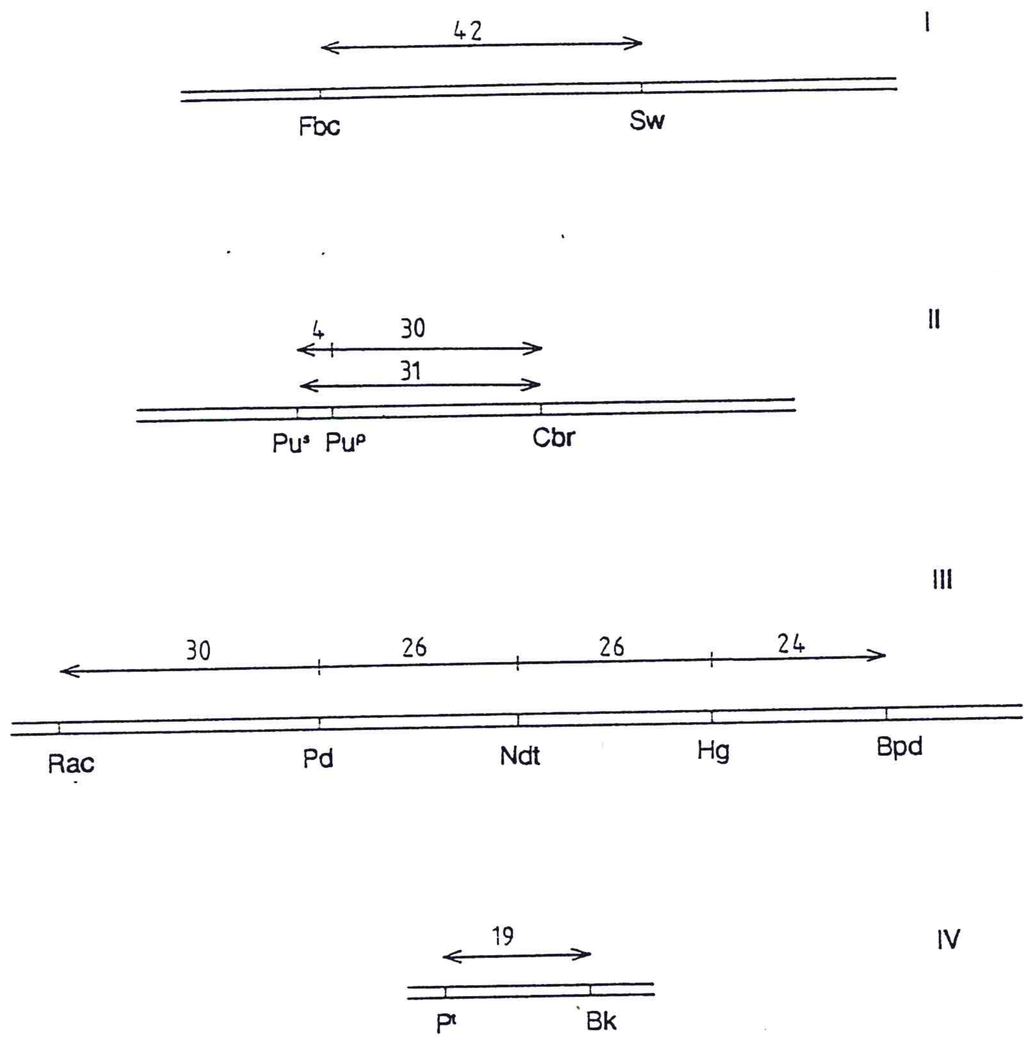

Figure 7.1 Linkage groups in cowpea

Linkage relationships among genes controlling various traits in an organism may be used in establishing a rudimentary gene map. Few linkage studies have been conducted in cowpea so far and consequently the crop has no linkage map such as those that have been established for various crops including maize (Helentjaris *et al.*, 1986), tomato (Bernatzky and Tanksley, 1986; Helentjaris *et al.*, 1986), lettuce (Landry *et al.*, 1987) and others. The proposed linkage groups in this study (Figure 7.1) form a starting point for developing a linkage map for cowpea.

8.0. GENERAL DISCUSSIONS AND SUGGESTIONS FOR FURTHER STUDIES

8.1. General discussions

Aphid resistance and its relationships with morphological and biochemical characters was studied in 14 cowpea cultivars and in the F_1, F_2, F_3, and backcross populations of crosses among them with the aim of identifying heritable markers for aphid resistance. These studies were proposed owing to the fact that workers trying to identify resistant cultivars normally rely on actual aphid infestation of plants either in the field or in the greenhouse. Aphid resistance in the field, however, is highly erratic and influenced by environmental conditions, while planting of large segregating populations as found in most breeding programmes, is not feasible in the greenhouse. Markers, if obtained, could be used for indirect selection of resistant/susceptible plants in the absence of aphids and with no worry of escapes even when environmental conditions become erratic. For these studies, a combination of field, greenhouse, and laboratory experiments were conducted.

The results from these studies indicated that in cowpea, an antixenosis modality of resistance was important in the expression of resistance to aphids. Given a choice between resistant and susceptible cultivars, more aphids settle and colonize the susceptible cultivars. Under no-choice situations hungry aphids have no choice but to feed on any cowpea cultivars present. While on the host plant,

however, aphids settled on susceptible cultivars spend more of their time feeding than probing (MacFoy and Dabrowski, 1984; Givovich *et al.*, 1991) and have a higher fecundity compared with aphids on resistant cultivars. The preferential settlement and fecundity by aphids while on different cowpea host plants shows one of the reasons why under field conditions, resistant cultivars are free of aphids during most of their growth period.

Resistance to aphid infestation was inherited as a monogenic dominant trait. Results of this study indicated that one or two genes may be involved in the expression of resistance to aphids. The first gene is the *Rac* gene present in all resistant cultivars in this study while the second gene is most probably a modifier which is present only in some cultivars. A modifier, when present, can either enhance or retard the expression of the major gene (Pathak, 1991).

Single gene inheritance of aphid resistance suggests that the trait can easily be incorporated into adapted cultivars through simple backcrossing. The major problem with single gene inheritance is that insects can easily develop biotypes which can easily overcome the resistant cultivars. Biotype development and their devastating effects on the crop has already been recorded with Hessian fly (*Hayetiola destructor*) infestation in wheat (Sosa, 1981), brown planthopper (*Nilaparvata lugens*) in rice (Saxena and Barion, 1985), and bean aphid (*Aphis fabae*) in beans (Pathak, 1970). In cowpea, three biotypes have been recorded

from West Africa (IITA, 1981). Since host plant resistant is the simplest and often cheapest method of aphid control, the identification of other sources of resistance would be highly desirable so as to keep ahead of biotype development. Once sources of resistance have been identified, they can be rapidly incorporated into materials with desirable agronomic and quality characteristics through either one of three strategies that have been suggested:

(i) Sequential release of cultivars with single genes for resistance. This strategy has been employed for resistance to brown planthopper at the International Rice Research Institute (IRRI) (Khush, 1979).

(ii) Pyramiding of resistance genes. This strategy aims at combining two or more major genes for resistance into the same cultivar. This strategy has been attempted at IRRI by pyramiding two genes for brown planthopper resistance into the same rice cultivar (Khush, 1979)

(iii) Development of multilines. This strategy, originally suggested by Borlaug (1958) aims at incorporating different genes into isogenic lines by backcrossing, mixing the lines in equal proportion, and releasing the result as a commercial multiline cultivar. This strategy was proposed as a way to control cereal rust, but its use in insect control has not been explored (Pathak, 1991).

Wide variations were recorded among cultivars in this study with respect to several morphological traits. These results confirmed earlier reports (Fery, 1985; Lattanzio *et*

al., 1990). The cultivars in these studies were, however, fairly alike with respect to biochemical trait variations that were studied. Once again these results confirmed earlier reports (Oghiake *et al.*, 1993; Vaillancourt *et al.*, 1993).

Cluster analysis of the cowpea cultivars using variations in morphological traits alone or morphological traits and isoenzymes indicated that for each method, three main clusters could be identified. The number of cultivars in each cluster, however, varied between the two methods and clustering of cultivars based on morphological traits alone (Figure 5.1) indicated that cluster one had three cultivars (Tvu 946, Tvu 1509, and IT83D-237 which were characterized by the possession of determinate growth habit), while clustering based on both morphological and isoenzymes traits (Figure 8.1) indicated that cluster one had only two cultivars (Tvu 946 and Tvu 1509 characterized by the possession of small leaves). It could be inferred that the use of both morphological and biochemical traits in germplasm characterization could be highly desirable, since the two types of information are complementary (Singh et al., 1991).

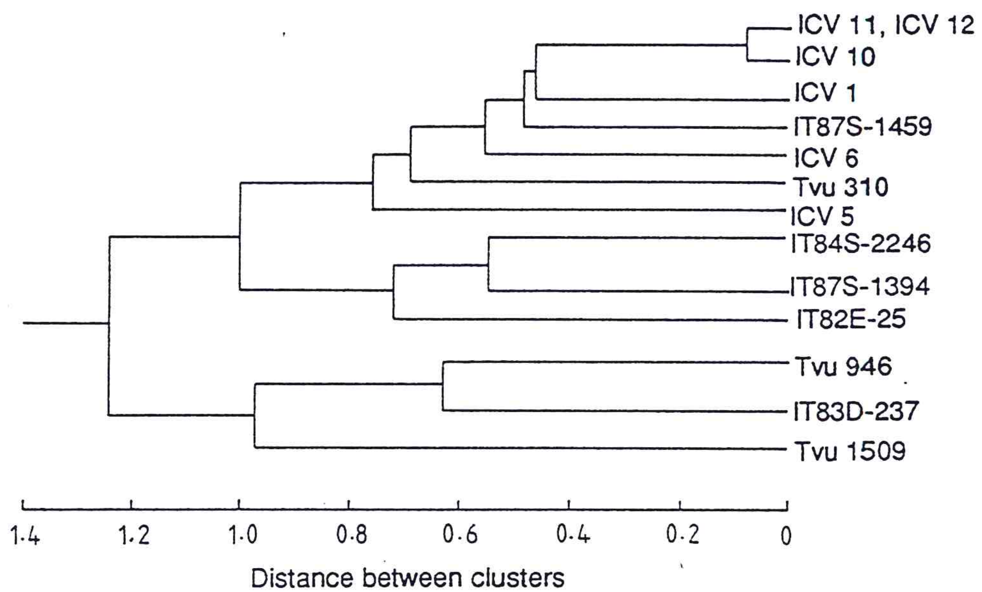

Figure 8.1 Dendogram of average linkage cluster analysis of 14 cowpea cultivars using variation in isoenzymes and morphological traits as the measure of dissimilarity

As many polymorphic traits as possible should be included for cluster analyses. However, cluster analyses in these studies did not reveal characters which could be used for distinguishing between aphid resistant and susceptible cowpea cultivars.

Tests for association between aphid resistance and various morphological traits in the parent cultivars indicated that some traits, namely, growth habit, immature pod colour and seed colour, might be related with aphid resistance and, thus, suggested that these traits might be used for indirect selection of aphid resistant plants in plant mixtures. These results, however, have to be taken with caution since no given form of these traits was found exclusively in the resistant cultivars or in the susceptibles and the observed associations may have been due to statistical associations and not real biological associations. To determine if these associations were biological or statistical, linkage studies were conducted which confirmed that the association was indeed biological at least as concerns growth habit. It was observed that the loci controlling growth habit and aphid resistance were located in the same linkage group (Figure 7.1).

Inheritance studies conducted on some morphological traits including pigmentation of various plant parts, plant habit, and others indicated that each one of these traits was simply inherited. These results confirmed earlier reports available in the literature on genetics research

that has been conducted with the crop since the early 1900s (Saunders, 1960a; Saunders, 1960b; Sen and Bhowal, 1960; Fawole and Afolabi, 1983; Fery, 1985). The results suggested that these traits, if economically important, can easily be incorporated into adapted cultivars through backcrossing. Other traits including maturity, leaf size, pod size, and seed size were quantitatively inherited. These results suggested that in a plant breeding programme, these traits can be utilized through a pedigree crop improvement method.

Heritability estimates of the quantitative traits studied indicated that these traits were moderately to highly heritable (H = 42-88%). These estimates were in agreement with the heritability estimates reported by various authors and summarized by Fery (1985). For example, Fery (1985) reported that published heritability estimates (narrow sense and broad sense combined) for days to flower and days to maturity were 48.3% and 47.8%. The broad sense heritability estimates for days to flower and days to maturity in this study were 66% and 69%, respectively. Fery (1985) also reported that the heritability estimates (narrow sense and broad sense combined) for pod size, seeds per pod, and 100-seed weight were 75.2%, 52.8%, and 67.8%, respectively. The heritability estimates for these traits in the present studies were 57%, 42%, and 88% for pod size, seeds per pod, and 100-seed weight, respectively, values which were comparable with the published estimates. The medium to high heritability estimates suggests that these

traits are controlled by many genes which could also be interacting among themselves. The estimates on the lower and higher extremes suggested the involvement of dominant genes and/or additive x dominance interactions; the medium values suggested the involvement of many additive genes.

Significant GCA effects were obtained for the traits pod length and seeds per pod thus indicating that dominance and gene interactions were not important in their expression. Gene interactions could be additive x additive (aa), additive x dominance (ad) or dominance x dominance (dd). Non-significant SCA effects were obtained for all traits studied. The type of gene action present in different cultivars and for the various traits was not investigated in this study and it is proposed that in future studies, biometrical techniques for studying type of gene action for various traits should be undertaken.

According to Fery (1985) a number of studies have shown that cowpea hybrids can exhibit considerable heterosis for many traits including seed yield, plant height, stem diameter, leaf width and length, pod length and earliness. Several crosses in this study exhibited heterosis for peduncle length, pod length, seed size and seeds per pod. Utilisation of heterosis in cowpea, however, would be faced with many difficulties since the crop is self-pollinated with limited outcrossing (Acland, 1971; Fery, 1985).

Linkage analyses in this study using genes controlling various simply inherited and easily identifiable

morphological traits led to the identification of four linkage groups, viz, I, II, III and IV (Figure 7.1). Linkage group I contained loci Sw and Fbc; II contained loci Pu^S, Pu^P, and Cbr; III contained loci Pd, Ndt, Bpd, and Gh; while IV contained loci Pt and Bk. The relationships among these linkage groups could not be established in these studies. Genes controlling biochemical trait variations were not linked to any other gene nor among themselves. According to Fery (1985) research on gene linkage in cowpea has been minimal, and much of the reported linkages need further study and verification. Unfortunately, of the few linkages reported by Fery (1985), none were present in this study.

Linkage analyses using data from two crosses in these studies indicated that the reported genes for aphid resistance, *Rac1* and *Rac2*, were both loosely linked to the locus Pd, controlling peduncle colour located on the proposed linkage group III (Figure 7.1). These data suggested that *Rac1* and *Rac2* were not different from one another. The involvement of modifiers may have contributed to the occurrence of segregants in the F_2 populations of crosses between some resistant cultivars in these studies. Similar results may have been recorded by previous workers (Pathak, 1988) which may have led them to report the occurrence of two non-allelic loci in the expression of resistance to aphids.

8.2. Suggestions for further studies

The results from these studies indicated that resistance to *Aphis craccivora* in cowpea is controlled by a single dominant gene, *Rac*. Locus *Rac* is linked to locus Pd, controlling peduncle colour. Both *Rac* and Pd can easily be incorporated into desired adapted cultivars through simple backcrossing and selection of aphid resistance plants based on peduncle colour, which is better than random selection, could be undertaken. Loci Pd and *Rac*, however, are located far apart from one another on the same chromosome and can easily be separated by crossing-over during meiosis. Identification of closely related markers for aphid resistance would be highly desirable.

Future studies aimed at identifying markers for aphid resistance could attempt any one or all of the following suggested approaches:

1. Aphid resistance: Attempts should be made to identify other genes for aphid resistance. The new genes, if identified, could be tested for linkage to genes controlling morphological and/or biochemical trait variations.
2. Morphological trait variations: Attempts should be made to identify other traits polymorphic among resistant and susceptible cultivars and test for their co-segregation with aphid resistance gene, *Rac*.
3. Protein variations: Attempts should be made on improving the resolution of proteins in the gels. This might be

achieved by using two dimension gels. Quantification of various protein bands present in both aphid resistant and susceptible cowpea cultivars should also be attempted. Any protein band variations (qualitative or quantitative) among resistant and susceptible cultivars should then be tested for linkage with aphid resistance genes.

4. Isoenzyme variations: Attempts should be made to stain for more enzyme systems and check for polymorphism among aphid resistant and susceptible cultivars.
5. Chemical variations among cowpea cultivars and bioassays: Attempts should be made to identify variations in the chemical composition of resistant and susceptible cowpea cultivars. Any chemicals found to be polymorphic among the cultivars could then be tested for their effects on eliciting aphid orientation (settling), feeding, and/or reproduction behaviour. When undertaking these studies, however, one has to note that settling behaviour will be most influenced by surface factors, feeding by phloem factors, and reproduction by both surface and phloem factors.
6. DNA patterns: Identification of variations among cowpea cultivars with respect to DNA patterns might be a better approach for future studies for identifying markers. This is especially so considering that not all variations at DNA level are expressed as variations in the protein or phenotype of the individual. Variations at the DNA level could be identified through RFLP or PCR analyses. As far as

costs, time, and technical know-how are considered, however, other markers are to be preferred over DNA analyses especially in the tropics.

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APPENDICES

Appendix 1. Quantitative trait variation in cowpea cultivars

Cultivar	Traits								
	DF	DM	Pl	Pdl	LL	LW	PW	SP	SWT
ICV 1	34 c	57 c	13.3 ab	20.2 bcd	6.7 a	4.6 a	9.0 bcd	12.6 bc	12.1 de
ICV 5	37 bc	59 c	12.8 ab	16.8 cd	6.1 ab	4.5 a	8.7 cde	12.8 bc	12.0 de
ICV 6	40 b	66 b	11.1 bcd	13.6 ef	6.3 a	4.6 a	9.7 abcd	11.3 bc	12.2 de
ICV 10	40 b	66 b	15.0 a	21.6 abc	6.7 a	4.2 abc	10.7 abc	14.4 ab	11.3 e
ICV 11	40 b	65 b	13.2 ab	18.3 cd	6.7 a	4.3 a	11.0 abc	13.2 bc	11.5 e
ICV 12	40 b	67 b	15.0 a	21.6 abc	6.7 a	4.3 a	11.3 ab	16.5 a	12.1 de
IT82E-25	40 b	65 b	9.3 de	11.6 f	5.5 abc	3.2 d	8.7 cde	11.1 c	10.7 e
IT83D-237	39 b	65 b	13.5 ab	23.6 ab	5.7 abc	3.4 bcd	12.0 a	12.2 bc	14.1 bc
Tvu 310	46 a	74 a	13.1 ab	20.1 bcd	6.7 a	4.8 a	10.3 abc	13.3 bc	13.5 cd
Tvu 946	34 c	56 c	10.1 cde	25.7 a	4.6 c	2.8 d	7.7 de	12.6 bc	8.6 f
IT87S-1394	39 b	69 ab	12.3 bc	17.8 cd	5.9 abc	3.3 cd	9.7 abcd	10.3 c	12.2 de
IT87S-1459	40 b	67 b	12.9 ab	21.0 bcd	5.5 abc	3.5 bcd	11.3 ab	10.4 c	17.5 a
Tvu 1509	34 c	58 c	8.3 e	11.1 f	4.9 bc	2.9 d	6.7 e	11.8 bc	7.2 f
IT84S-2246	40 b	70 ab	12.6 abc	19.3 bcd	6.3 a	3.0 d	11.7 a	11.6 bc	15.8 b

Means in the same column followed by same letter are not significantly different by Duncan's multiple range test ($P < 0.05$)

Appendix 2. Heterosis of various quantitative traits in cowpea.

Cross	Traits							
	Peduncle length		Pod length		Seeds/pod		100-seed weight	
	F_1	Heterosis	F_1	Heterosis	F_1	Heterosis	F_1	Heterosis
ICV 1 x ICV 10	17.6	-18.3	11.0	-26.6	9.9	-31.0	11.8	-2.1
ICV 1 x ICV 11	18.3	- 9.5	12.0	- 9.6	12.7	-3.8	12.5	3.8
ICV 1 x ICV 12	21.2	-2.0	15.4	15.8	14.1	-14.7	12.4	2.5
ICV 1 x IT82E-25	24.7	22.2	12.1	-8.6	8.4	-33.6	11.4	-5.1
ICV 1 x Tvu 310	24.7	21.9	13.8	4.0	12.3	-7.3	15.1	11.9
ICV 1 x Tvu 946	27.9	8.7	10.5	-20.9	10.3	-18.2	8.6	-28.5
ICV 1 x IT87S-1394	23.3	15.0	12.5	-5.6	8.1	-35.5	12.6	3.6
ICV 1 x IT87S-1459	24.3	16.0	11.9	-10.6	13.0	3.3	19.1	9.4
ICV 1 x IT84S-2246	26.4	30.4	13.8	3.8	10.3	-18.0	15.2	-3.6
ICV 10 x ICV 11	26.6	23.1	14.6	-2.9	12.8	-10.9	13.3	15.7
ICV 10 x ICV 12	23.5	8.8	15.4	2.5	14.8	-10.3	12.3	1.3
ICV 10 x IT82E-25	19.1	-11.6	13.1	-12.6	11.7	-19.0	12.6	11.1
ICV 10 x Tvu 310	16.6	-23.2	14.8	-1.5	13.0	-9.7	12.5	-7.5
ICV 10 x Tvu 946	22.3	-13.0	14.3	-4.9	16.3	13.4	11.3	-0.3
ICV 10 x IT87S-1394	24.1	11.6	12.5	-16.8	11.7	-19.0	11.8	-3.5
ICV 10 x IT87S-1459	25.7	19.1	13.3	-11.7	10.8	-25.2	13.8	-20.8
ICV 10 x IT84S-2246	28.8	33.7	15.5	3.3	11.6	-19.2	15.9	0.8
ICV 11 x ICV 12	24.2	12.0	13.2	-12.2	11.6	-29.8	12.7	4.9
ICV 11 x IT82E-25	20.0	8.9	12.8	-3.0	12.8	-2.6	12.8	10.9
ICV 11 x Tvu 310	20.5	2.3	15.7	18.4	16.0	20.5	13.0	-3.7
ICV 11 x Tvu 946	22.8	-11.2	12.3	-6.8	13.7	3.8	10.9	-5.6
ICV 11 x IT87S-1394	21.6	17.8	12.1	-8.3	11.3	-14.2	16.2	32.6
ICV 11 x IT87S-1459	20.0	-4.6	15.0	13.3	13.4	1.7	14.5	-16.8
ICV 11 x IT84S-2246	19.2	-0.8	10.9	-17.6	11.4	-13.8	15.8	0.3
ICV 12 x IT82E-25	16.5	-23.6	13.0	-13.4	11.4	-31.0	13.8	13.9
ICV 12 x Tvu 310	23.0	6.5	15.0	0.0	14.6	-11.7	12.2	-9.8
ICV 12 x Tvu 946	21.3	-16.9	11.8	-21.1	9.3	-43.6	12.2	1.1
ICV 12 x IT87S-1394	19.8	-8.1	11.7	-22.0	11.2	-32.2	15.4	26.4
ICV 12 x IT87S-1459	20.9	-3.2	11.8	-21.3	12.6	-23.8	16.1	-8.0
ICV 12 x IT84S-2246	23.7	9.6	11.6	-22.5	9.4	-43.3	14.4	-8.9
IT82E-25 x Tvu 310	17.2	-14.3	12.3	-6.4	11.9	-10.8	14.1	4.8
IT82E-25 x Tvu 946	21.0	-18.3	9.7	-4.3	8.4	-33.4	10.0	-7.1
IT82E-25 x IT87S-1394	16.2	-9.0	9.7	-20.9	6.7	-39.7	10.3	-15.4

Appendix 2 continued,

Cross								
IT82E-25 x IT87S-1459	19.4	-7.5	12.0	-6.8	10.0	-9.7	16.2	-7.2
IT82E-25 x IT84S-2246	25.3	30.7	10.7	-15.3	9.9	-14.2	11.3	-28.4
Tvu 310 x Tvu 946	17.3	-32.7	12.9	-2.3	12.1	-8.8	14.2	5.3
Tvu 310 x IT87S-1394	17.1	-14.8	14.0	6.1	11.4	-14.3	13.8	2.4
Tvu 310 x IT87S-1459	16.9	-19.6	11.8	-10.6	11.9	-10.5	15.8	-9.9
Tvu 310 x IT84S-2246	21.4	6.8	15.1	14.9	11.3	-14.8	17.7	12.1
Tvu 946 x IT87S-1394	30.0	16.8	10.7	-12.3	10.4	-17.7	9.1	-25.0
Tvu 946 x IT87S-1459	20.1	-21.8	9.8	-23.6	10.8	-14.3	12.2	-30.3
Tvu 946 x IT84S-2246	27.3	6.3	9.4	-25.8	9.5	-24.8	10.3	-34.8
IT87S-1394 x IT87S-1459	26.4	26.0	11.8	-8.5	9.5	-8.6	15.0	-14.1
IT87S-1394 x IT84S-2246	17.3	-10.3	11.3	-10.3	9.3	-19.9	14.0	-11.4
IT87S-1459 x IT84S-2246	23.2	10.5	12.1	-6.0	10.2	-11.8	17.9	2.6
Mean		1.6		-7.8		-16.3		-2.9

APPENDIX 3

I. CHEMICAL SOLUTIONS FOR SDS-PAGE

Solution 1. Tris-HCl 1.5 M, pH 8.8 (Separating gel buffer)

Tris - 18.15 g

HCl - 1 N 30 ml

Adjust pH with 1 N HCl and raise volume with distilled water to 100 ml.

Solution 2. Acrylamide : Bisacrylamide (30:0.8%) (Monomer)

Acrylamide - 30 g

Bisacrylamide - 0.8 g

Dissolve in 100 ml of distilled water and filter with Whatmann paper.

Store in a darkened bottle at $4^{o}C$.

Solution 3. Tris-HCl 0.5 M, pH 6.8 (Stacking gel buffer)

Tris - 1.5 g

HCl - 1 N 10 ml

Adjust pH to 6.8 with 1 N HCl and raise volume to 25 ml with distilled water.

Solution 4. Sample buffer

250 ul stacking gel buffer

250 ul 50% glycerine

400 ul 10% SDS

100 ul mercaptoethanol

10 ul Bromophenol Blue (0.1 g in 10 ml ethanol)

Solution 5. Tris-glycine buffer (Electrophoresis buffer)

Tris - 30 g

Glycine - 144 g

SDS - 5 g

Dissolve in 5 l distilled water.

Solution 6. Staining solution

Brilliant Blue Coomasie R 250 - 2.5 g

Methanol (Technical grade) - 450 ml

Glacial acetic acid - 100 ml

Distilled water - 450 ml

Solution 7. Destaining solution 1.

Methanol (Technical grade) - 500 ml

Glacial acetic acid - 92 ml

Distilled water 408 ml

Solution 8. Destaining solution 2

Methanol (Technical grade) - 125 ml

Glacial acetic acid - 175 ml

Distilled water - 2200 ml

APPENDIX 4

CHEMICAL SOLUTIONS USED IN ISOENZYME ANALYSIS

A. Gel and electrode buffers

1. Tris-Citrate, pH 6.3

Used for AAT, ACO, ADH, ACP, DIA, G6PDH, EST, LDH, ME, MDH, MPI, TPI enzyme systems.

Electrode buffer : 16.35 g Tris
9.04 g Citric acid, Monohydrate
Dissolve in 1 l water and adjust pH to 7.0 with citric acid.

Gel buffer : Dilute 1 part electrode buffer with 14 parts distilled water.

2. Histidine-Citrate, pH 5.7.

Used for GPI, IDH, PGM, PGD, SDH, SKD enzyme systems.

Electrode buffer: 10.09 g L-Histidine
3.0 g citric acid
Dissolve in 1 l water and adjust pH to 5.7 with citric acid.

Gel buffer : Dilute 1 part electrode buffer with 6 parts distilled water.

B. Enzyme stains

Stock solutions

(a). Tris-HCl - pH 7.4, 0.3 M

(b). NAD - 10 mg/1 ml water

(c). NADP - 10 mg/1 ml water

(d). $MgCl_2$ - 1 mg/10 ml water

(e). MTT - 10 mg/1 ml water

(f). PMS - 10 mg/1 ml water

(g). Tris-E

1. Acid phosphatase (ACP, EC No. 3.1.3.2)

50 mM Na-acetate buffer, pH 5.0	- 50 ml
Na-Á-naphthyl acid phosphate	50 mg
$MgCl_2$ (10mg/10ml water)	1 ml
Fast Garnet GBG salt	50 mg

2. Alcohol dehydrogenase (ADH, EC No. 1.1.1.1)

Tris-HCl buffer pH 8.5	6 ml
Ethanol	1 ml
$MgCl_2$	1 ml
NAD	0.5 ml
NBT (10 mg/1 ml water)	0.5 ml
MTT	0.5 ml
PMS	0.5 ml

Add equal amount of agarose.

3. Esterases (EST, EC No. 3.1.1.1)

Na-Phosphate buffer 15 ml

-Dissolve about 15 mg Á-naphthyl acetate in 0.5 ml acetone and about 15 mg ß-naphthyl acetate in 0.5 ml acetone.

-Add these to a tube containing Na-phosphate buffer drop by drop.

-Pour solution onto the gel, shake regularly, and incubate for 20-30 min.

-Add about 10 mg GBC salt and shake until bands appear.

4. Isocitrate dehydrogenase (IDH, EC. No. 1.1.1.42)

Tris-HCl	3 ml
DL-Isocitric acid	30 mg
Water	3 ml
NADP	0.5 ml
$MgCl_2$	2 ml
MTT	0.5 ml
PMS	0.5 ml

5. Malate dehydrogenase (MDH, EC. No. 1.1.1.37)

Tris-HCl	3 ml
DL-Malic acid substrate	3 ml
Water	3 ml
$MgCl_2$	1 ml
NAD	0.5 ml
MTT	0.5 ml
PMS	0.5 ml

6. Lactate dehydrogenase (LDH, EC. No. 1.1.1.27)

Tris-HCl buffer, pH 8.5	3 ml
Lactic acid	3 ml
NAD	0.5 ml
NBT	0.5 ml
PMS	0.5 ml

7. Glucosephosphate isomerase (GPI EC No. 5.3.1.9)

Tris-HCl	3 ml
Water	3 ml
Fruictose-6-Phosphate (10 mg/1 ml)	10 ml

$MgCl_2$	2 ml
NADP	0.5 ml
MTT	0.5 ml
PMS	0.5 ml

Glucose-6-Phosphate dehydrogenase (100 mg/ml) 8 ul (Add last).

8. Phosphoglucomutase (PGM, EC No. 5.4.2.2)

Tris-HCl	3 ml
Water	3 ml
Glucose-1-Phosphate (10 mg/1 ml)	10 ml
$MgCl_2$	2 ml
NADP	0.5 ml
MTT	0.5 ml
PMS	0.5 ml

Glucose-6-Phosphate dehydrogenase (100 mg/ml) 20 ul (Add last).

9. Malic enzyme (ME, EC No. 1.1.1.40)

Tris-HCl	3 ml
L-Malic acid	0.4 ml
$MgCl_2$	2 ml
NADP	0.5 ml
MTT	0.5 ml
PMS	0.5 ml

10. Shikimate dehydrogenase (SKD, EC No. 1.1.1.25)

Tris-HCl	3 ml
Water	3 ml
Shikimic acid	10 mg

NADP	0.5 ml
MTT	0.5 ml
PMS	0.5 ml

11. Phosphogluconate dehydrogenase (PGD, EC No. 1.1.1.44)

Tris-HCl	3 ml
Water	3 ml
6-phosphogluconic acid (Na or Ba salt)	10 mg
$MgCl_2$	2 ml
NADP	0.5 ml
MTT	0.5 ml
PMS	0.5 ml

12. Glucose-6-phosphate dehydrogenase (G6PDH, EC No. 1.1.1.49)

Tris-HCl	3 ml
Water	3 ml
Glucose-6-phosphate, (Na salt)	10 mg
$MgCl_2$	2 ml
NADP	0.5 ml
MTT	0.5 ml
PMS	0.5 ml

13. Aconitase (ACO, EC No. 4.2.1.3)

Soln A:	Tris-HCl	6 ml
	Agar solution	6 ml
Soln B:	Tris-HCl	3 ml
	Cis-Aconitic acid	10 mg
	Isocitrate dehydrogenase	6 units
	$MgCl_2$	1 ml

NADP	0.5 ml
MTT	0.5 ml
PMS	0.5 ml

- Mix the two solutions and pour onto the gel

14. Diaphorase (DIA, EC No. 1.6.4.3)

NADH	5 mg
Tris-E buffer	20 ml
DCIP (1% in water freshly filtered)	0.5 ml
MTT	0.5 ml

15. Aspartate amino transferase (AAT) EC No. 2.6.1.1)

L-Aspartic acid	20 mg
Á-ketoglutaric acid	10 mg
Fast Blue BB salt	15 mg
Pyridoxal-5′-phosphate	0.2 mg
Electrode buffer	15 ml

Adjust pH to be close to 8.5

16. Mannose phosphate isomerase (MPI, EC No. 5.3.1.8)

Solution A:	Tris-HCl	3 ml
	Agarose	6 ml
Solution B:	Tris-HCl	3 ml
	$MgCl_2$	1 ml
	NAD	0.5 ml
	Mannose-6-phosphate	15 mg
	Glucosephosphate isomerase	8 units
	Glucose-6-phosphate dehydrogenase	8 units
	MTT	0.5 ml
	PMS	0.5 ml

Bring solution A to a boil. Cool to 60°C. Gently mix in solution B and pour on gel. Once agar has solidified, incubate until blue bands appear.

17. Sorbitol dehydrogenase (SDH, EC No. 1.1.1.14)

Tris-HCl	6 ml
Sorbitol	25 mg
$MgCl_2$	0.5 ml
NAD	0.5 ml
NBT	0.5 ml
PMS	0.5 ml
MTT	0.5 ml

18. Triose-phosphate isomerase (TPI, EC No. 5.3.1.1)

Tris-HCl	6 ml
Arsenic acid, Na salt	20 mg
Dihydroxyacetone phosphate	5 mg
NAD	1 ml
Glyceraldehyde-3-phosphate	20 units
MTT	0.5 ml
PMS	0.5 ml