FACULTY OF LIFE SCIENCES
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# PhD thesis

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# Enhancing Cowpea (*Vigna unguiculata* L.) Production Through Insect Pest Resistant Line in East Africa

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## PREFACE

This thesis comprises various multidisciplinary studies done over a period of three years. The work started by collecting cowpea landraces from various regions of Tanzania and multiplication of these landraces from October 2007 to January 2008 in Tanzania. Fields and storage experiments were also conducted in Tanzania from August 2009 to January 2010. The initial part of the molecular experiments was conducted from February 2008 to August 2008 together with PhD courses at the University of Copenhagen. The final part of the molecular experiments together with the participation in further PhD courses and the writing of the articles that compose the thesis were done from August 2009 to December 2010.

The thesis is composed of the summaries in English and Danish, an introduction into several issues relevant for the content of this thesis and three papers that present the results of the different experiments that have been conducted in this thesis. These papers have been submitted to different journals

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## SUMMARY

Cowpea [*Vigna unguiculata* (L)Walp] is an important grain legume in East Africa. In Tanzania this crop is mainly grown by small scale farmers, often women. By its specific characteristics, cowpea is especially suitable for resource limited communities and marginal areas. It is a multifunctional crop that provides food to human being and feed to livestock, it fixes nitrogen, is a protein rich, drought tolerant and early maturing crop. Beside drought, the most important problems in cowpea production are insect pests, both pre- and post-harvest. A loss of up to 100% due to insect pest infestation can be realized in absence of management of these pests. At present, the most effective management practice for these pests is by application of insecticides. However, insecticides are rarely an option for resource limited farmers, due to low availability and high cost, notwithstanding the environmental and health hazards posed by these chemicals, especially when applied by poorly educated farmers. The best alternative to the insecticide use is host plant resistance.

This multidisciplinary study included the evaluation of various cowpea accessions in field and storage and diversity studies. It aimed at characterization of the genetic pool of Tanzanian landraces in relation to the structure of the genetic variation, in relation to traits against insect pests both in field and storage and in relation to other important agronomic traits which might be in relation to the resistance to major insect pests.

We collected 300 accessions from farmers in various regions of Tanzania as follows: Dodoma, Tabora, Singida, and Rukwa from July to August 2007. Further 100 additional accessions were previously collected from different regions other than those mentioned above and preserved at the National Plant Genetic Resources Institute (TPRI) in Arusha, Tanzania. Together, they added up to 400 accessions that were multiplied at Miwaleni (Moshi, Tanzania) from September 2007 to January 2008 during dry season. DNA was isolated from the 400 accessions at Copenhagen University, Department of Agriculture and Ecology. The accessions' DNA was PCR amplified using 12 SSR primer pairs and analyzed. Basing on the results of their genetic distances, 200 accessions covering the widest possible spectrum of genetic diversity were selected for the purpose of field and storage evaluation experiments. The DNA from 340 accessions was further used for a diversity analysis using 26 SSR primer pairs.

For morphological traits evaluation, a two-season field experiment was established at Miwaleni (Moshi) and Tengeru (Arusha) in Tanzania from October 2008 to January 2009 during dry season and from March 2009 to August 2009 during wet season. Two hundred accessions were evaluated in a Completely Randomized Block Design (CRBD) with three replications. Results showed that temperature was the major factor that to a great extent influenced flowering time and both aphids and thrips infestation. Leaf color, growth habit, pod hairiness and seed size showed the highest heritability and were not affected by the environment, Pod hairiness was strongly positively correlated to seed weight and seed size in all environments. A multiple regression analysis showed that flowering time was the major determinant of grain yield in three out of four environments of the study. Early flowering was associated with high grain yield. One accession showed no aphids infestation in all environments; and was superior in terms of grain yield, yield stability and resistance against thrips.

In order to study the extent and structure of the genetic diversity within the collection, 26 SSR makers were employed on a total of 340 accessions that included 288 cultivated cowpea landraces and 24 wild *cowpea genotypes*. The genetic distance matrix was visualized by non-metric Multi-Dimensional Scaling (nmMDS) and the genetic distances were also calculated between the groups of accessions divided according to their domestication status (wild/cultivated) and country of origins. Further, Bayesian structure analysis was applied and a Mantel test of the matrix of the genetic vs. the matrix of geographic distances was carried out. An AMOVA analysed the explained variance of the groups from the structure analysis and the groups of the domestication status and origin. In general, a high genetic diversity was observed among the accessions in the analysis. Geographic distances showed no correlation with the genetic distances, thus indicating a high degree of geographic mixture of the genotypes by trading. The most important genetic grouping indicated by several of the applied analysis parted one group including the majority of the Tanzanian landraces together with the wild accessions from Kenya from a smaller group of Tanzanian landraces with the wild accessions from Tanzania and Uganda. Thus it could be concluded, that most of the cultivated accessions in Tanzania go back to wild cowpeas as they occur in Kenya. The smaller group that is closer related to the Tanzanian wild cowpeas either developed through an independent cultivation event or by hybridization of cultivated with wild accessions. This group constitutes an important genetic resource that might contribute useful alleles to the rest of the cultivated cowpeas.

For the storage experiment, 200 cowpea accessions were evaluated by enhanced infestations of cowpea weevil (*Callosobruchus maculates*) in a free choice design at the TPRI laboratory (Arusha, Tanzania) from February 2009 to January 2010. The experimental design was a Completely Randomized Design (CRD) with three replications. The temperature, humidity and light were maintained at optimum for the weevils' activity. Infestation was initiated by placing five kg of heavily infested cowpea at four corner of the laboratory room. The accessions were scored for the number of undamaged seeds, the weight loss, the exit holes and the dead larvae and adults. In order to partition the resistance, the extent of infestation and the percentage of failure in completing the full weevil development were calculated. Weight loss was highly correlated with the extent of infestation, but only poorly with the failure rate of the weevil. The percentage of dead adults was negatively correlated with the thrips infestation from the field experiment measured above. The infestation related component of resistance was independent from the development failure related component of resistance and therefore, only one accession was superior in both components.

The observed agronomic and resistance-related traits that influence cowpea production in field, the storage-resistance components we found, as well as our results in relation to the structure of the genetic diversity of cowpea accessions from Tanzania constitute steps towards the improvement of cowpea as a crop. The best and most promising accessions can now be crossed and the understanding gained on the character of those important traits can be used in the subsequent selection procedure. It is our hope and conviction that the implementation of the knowledge acquainted in the present study will result in a crop that is better suited for the coming challenges due to necessary increase of the food production together with shifts of growing conditions due to climatic changes.

## RESUMÉ

Vignabønnen [*Vigna unguiculata* (L) Walp] er en vigtig bælgplante i Østafrika. I Tanzania bliver denne afgrøde primært dyrket af små landmænd, ofte kvinder. Med sit særlige karakteristika er vignabønnen specielt velegnet til ressource-begrænsede samfund og marginale områder. Den er en multifunktionel afgrøde, der giver mad til mennesker og foder til husdyr; den binder kvælstof; den er en proteinrig, tørke-tolerant og tidlig modnet afgrøde. Udover tørke er skadedyr, både før og efter høst de største problemer i vignabønne-produktioner. Hvis disse skadedyr ikke kontrolleres, kan der ske tab på op til 100% af det mulige høstudbytte. På nuværende tidspunkt er anvendelsen af insekticider den mest effektive kontrolmetode. Dog er deres anvendelse kun sjældent muligt for de ressource-begrænsede landmænd, der typisk dyrker vignabønner, dels pga. manglende tilgængelighed og dels pga. høje omkostninger. Derudover er der fare for miljø-og sundhedsproblemer ved brug af disse kemikalier, især når de anvendes af dårligt uddannede landmænd. Det bedste alternativ til anvendelse af insekticider er værtsplante-resistens.

Denne tværfaglige undersøgelse omfattede evaluering af forskellige vignabønne-accessioner i marken, i forrådslageret og mht. deres genetiske variation. Undersøgelsens mål var en karakterisering af den genetiske pulje af vignabønne-landracer fra Tanzania i forhold til strukturen af den genetiske variation, i forhold til deres resistens mod skadedyr både i marken og i lager og i forhold til andre vigtige agronomiske egenskaber, der er relaterede til resistens over for skadedyrene.

Vi indsamlede 300 accessioner fra landmænd i forskellige regioner i Tanzania: Dodoma, Tabora, Singida og Rukwa fra juli til august 2007. Yderligere 100 accessioner, var tidligere indsamlet fra forskellige andre regioner og opbevaret på 'National Plant Genetic Resources Institute' (TPRI) i Arusha, Tanzania. Alt i alt var det 400 accessioner, der blev opformeret på marken i Miwaleni (Moshi, Tanzania) fra september 2007 til januar 2008 i den tørke årstid. DNA fra 400 accessioner blev isoleret på Københavns Universitet, Institut for Jordbrug og Økologi. DNA'et fra disse accessioner blev PCR-amplificeret ved brug af 12 SSR-primerpar og den genetiske afstand mellem accessionerne blev beregnet ud fra forskel i båndstørrelsen. Resultaterne blev brug til at udvælge 200 genotyper, der dækker det bredest mulige spektrum af genetiske diversitet, både til markforsøgene og til lagereksperimentet Derudover blev DNA fra 340 accessioner analyseret med 26 SSR-primerpar til diversitetsanalyse.

Til evaluering af morfologiske egenskaber med relation til resistens mod bladlus og trips blev der gennemført et markforsøg i fire forskellige miljøer: to steder (Miwaleni ved Moshi og Tengeru ved Arusha) og to forskellige sæsoner (fra oktober 2008 til januar 2009 i den tørre årstid og fra marts 2009 til august 2009 under regntiden). To hundrede accessioner blev undersøgt i et fuldstændigt randomiseret blok design med tre gentagelser. Resultaterne viste, at temperaturen var den vigtigste faktor, der i vid udstrækning påvirkede blomstringstidspunkt og angreb fra både bladlus og trips. Bladfarve, vækstype, bælgens behåring og frøstørrelse viste den højeste heritabilitet og blev ikke påvirket af miljøet. Bælgens behåring var stærkt positivt korreleret med frøvægt og frøstørrelse i alle fire miljøer. En multipel regressionsanalyse viste, at blomstringstid var den vigtigste faktor for kerneudbytte i tre ud af de fire miljøer af undersøgelsen. Tidligere blomstring gav højere kerneudbytte. En enkelt vignabønne-accession viste ingen bladlus angreb i alle miljøer og var derudover bedre i forhold til kerneudbyttets højde og stabilitet og i forhold til resistens mod trips.

I alt 340 accessioner, deriblandt 288 accessioner fra dyrkede landracer og 24 vilde vignabønner, blev undersøgt med 25 SSR markører for at analysere omfang og struktur af den genetiske diversitet. Den resulterende afstands-matrix blev visualiseret med hjælp af 'non-metric Multi-Dimensional Scaling' (nmMDS) og den genetiske afstand blev også beregnet mellem de grupper af accessioner der var defineret gennem deres domesticeringsstatus (vildt/dyrket) og oprindelsesland. Derudover blev der gennemført en Bayes'iansk struktur analyse og en Mantel-test af matrixen af den genetiske vs. matrixen af den geografiske afstand. En AMOVA-analyse forklarede varians for grupper fra strukturanalyse og for de grupper der var defineret pga. domesticeringsstatus og oprindelse. Generelt var der høj genetisk diversitet blandt de accessioner der var inkluderet i analysen. Der var ingen sammenhæng mellem geografiske og genetiske afstande, hvilket tyder på en høj grad af geografisk blanding af genotyper ved handel i Tanzania. Den vigtigste genetiske opdeling adskilte en gruppe, der omfattede hovedparten af de tanzaniske landracer sammen med de vilde accessioner fra Kenya, fra en mindre gruppe af Tanzanias landracer sammen med de vilde accessioner fra Tanzania og Uganda. Således kunne det konkluderes, at de fleste af de dyrkede accessioner i Tanzania går tilbage til de vilde vignabønner der forekommer i Kenya. Den mindre gruppe, der er tættere relateret til Tanzanias vilde vignabønner er enten opstået gennem en uafhængig domesticeringsbegivenhed eller ved hybridisering af dyrkede med vilde genotyper. Denne gruppe udgør en vigtig genetisk ressource, der kan bidrage nyttige alleller til resten af de dyrkede vignabønner i fremtidigt planteforædling.

I lagringsforsøget blev 200 accessioner af vignabønner (de samme der har været i marken) vurderet ved kunstigt forøget angreb af vignabønne-billen (*Callosobruchus maculates*) i et 'frit-valg-design' i TPRI-laboratoriet ( Arusha, Tanzania) fra februar 2009 til januar 2010. Det eksperimentelle design var et fuldstændigt randomiseret design med tre gentagelser. Temperatur, luftfugtighed og lys blev stabiliseret sådan at det var optimalt for snudebillen. Angreb blev indledt ved at placere fem kg af stærkt angrebne vignabønner i de fire hjørne af laboratoriet. Accessionerne blev bedømt for antallet af ubeskadigede frø, vægttab, udgangshuller, og for døde larver og døde voksne biller i frøene. For at opdele resistensen i dens komponenter blev der lavet beregning på omfanget af angreb på den ene side og den procentvise andel af billens svigt i at afslutte den fuldstændige udvikling (døde larver og biller i frøen) på den anden side. Vægttabet var stærkt korreleret med angrebets omfang, men kun dårligt korreletert med billens fejlslagen udvikling. Den procentvise andel af døde voksne biller var negativt korreleret med tripsangrebet fra markforsøge. Resistenskomponenter var uafhængige af hinanden og derfor fandtes der kun en enkel accession der viste god angrebsresistens og samtidigt gode evner til at forhindre snudebillens fuldstændige udvikling.

De agronomiske resistens-relaterede egenskaber vi har fundet, som har indflydelse på vignabønne-produktionen i marken, de lagrings-resistens komponenter vi har karakteriseret, samt vores resultater i forhold til strukturen af den genetiske variation af vignabønne landracer i Tanzania er de første skridt mod en forbedring af vignabønne som afgrøde. De bedste og mest lovende accessioner kan nu krydses og den viden mht. vigtige egenskaber vi har opnået i denne undersøgelse kan bruges i den efterfølgende selektionsprocedure. Det er vores håb og overbevisning, at anvendelsen af den viden, vi har opnået i den foreliggende analyse vil resultere i en afgrøde, der er bedre egnet til de kommende udfordringer på grund af den nødvendige forøgelse af fødevareproduktionen sammen med en forventet skift af vækstbetingelser på grund af klimaforændringer.

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# 1 INTRODUCTION

## 1.1 Taxonomy of Cowpea

Cowpea [*Vigna unguiculata* (L) Walp.] is a dicotyledonous crop in the order *Fabaceae*, subfamily *Faboideae* (Syn. *Papillionoideae*), tribe *Phaseoleae*, subtribe *Phaseolinae*, genus *Vigna* and section Catiang. Is a diploid plant containing 22 chromosomes Timko and Singh (2008) and its nuclear genome size is estimated to cover 620 million base pairs (Mbp) (Timko et al. 2008). The genus was divided into subgenera based upon morphological characteristics, the extent of genetic hybridization and geographical distribution of the species. The major groups consist of the African sub-genera *Vigna* and *Haydonia*, the Asian sub-genus *Ceratotropis,* and the American subgenera *Sigmoidotropis* and *Lasiopron* (Timko and Singh 2008). *V. unguiculata* sub-species *unguiculata* includes four cultivated groups: *unguiculata biflora* (or *cylindrical*), *sesquipedalis*, and *textilis* (Ng and Maréchal 1985). *V. unguiculata* subspecies *dekindiana*, *stenophylla*, and *tenuis* are intermediate wild progenitors of cultivated cowpea and form the major portion of the primary gene pool of cowpea. Fatokun and Singh (1987) pointed out that, wild subspecies like *pubescence* that do not readily hybridize and show some degree of pollen sterility form a secondary gene pool.

## 1.2 Origin, Domestication and Diversity

The precise origin of cultivated cowpea is not known. However, Asia and Africa were discussed as domestication sites of this crop. Recently, Asia has being questioned as a center of domestication due to the lack of wild ancestors. By reason of the highest genetic diversity of the crop and the presence of the most primitive form of wild cowpea, (Padulosi 1987; 1993), Southern Africa is the most probable center of domestication. The determination of the origin and domestication of cowpea had been based on morphological and cytological evidence, information on its geographical distribution and cultural practices (Ng 1995; Ng and Maréchal 1985). Padulosi and Ng (1997) suggested Southern Africa to be the center of origin, while domestication occurred in West Africa. The cultivated cowpea (*V. unguiculata*) evolved through domestication and selection from the annual wild cowpea (ssp. *dekindtiana*), and during this process seed dormancy and pod dehiscence was lost (Ng 1995). The distribution of diverse wild cowpea from Ethiopia to South Africa lead to the proposition that East and Southern Africa are primary centers of diversity, while West and Central Africa are secondary centers of diversity (Baudouin and Mere'chal 1985).

## 1.3 Morphological Description

Based on the investigation conducted by Padulosi and Ng (1997) and supported by (Baudouin and Mere'chal 1985; Padulosi 1997), about the range of variation and number of varieties found in wild cowpeas as well as their primitive characteristics, such as perenniality, hairiness, small size of pods and seeds, pod shattering with pronounced exine on the surface of the pollen, out-breeding and bearded stigma, the highest genetic diversity and most primitive forms of wild *V.unguiculata* occur in southern Africa.

Variability in morphology of different cowpea accession is very high. There are three types according to their uses: for grain, forage or dual purpose. *Vigna unguiculata* is a herbaceous, prostrate, climbing or sub erect annual plant, growing 15-80 cm high. Leaves are alternate trifoliate with petiole 5-25 cm long. The lateral leaflet is opposite and asymmetrical, while the central leaflet is symmetrical and ovate. The inflorescence are racemose, flowers are white, cream, yellow or purple. Growth habit is either determinate or indeterminate Seeds are variable in size and shape: kidney, ovoid, crowder, globose and rhomboid (IBPGR 1983). Seeds are of various colors: white, brown, black, cream or gray, dotted (black, brown), purple, red. Pods length ranges from 8-22 cm with 10-20 seeds per pod (Chavalier 1944).

## 1.4 Cowpea Population Structure

The development and use of molecular markers has enabled the analysis of structure of plant genomes and their evolution including the relationships among cowpea accessions (Choi et al. 2004; Fatokun et al. 1993; Yang et al. 1994). Fatokun et al (1993), using RFLP markers, reported high level of genetic variation within *Vigna* species. Using RAPD analysis, Kaga et al. (1996)., separated cowpea accession into two main groups that differed by 70% at molecular level and five sub groups whose composition were in accordance to taxonomic species classifications. A study on genetic relationship among *Vigna* species conducted by Ajibade et al (2000) using Inter Simple Sequence Repeat (ISSR) markers showed that closely related species within each subgenera clustered together; thereby the cultivated cowpea grouped together with the wild subspecies of *V. unguiculata.*

## 1.5 Germplasm Collection and Conservation

Cowpea germplasm is maintained in collections in different international centers, universities as well as regional and country centers. The largest collections are held by the International

Institute for Tropical Agriculture (IITA) with more than 14,000 accessions (Timko and Singh 2008). Other collections are held by the are United States Department of Agriculture (USDA), the University of California-Riverside, the 'Istituto di Genetica Vegetale' (IGV) in Bari, Italy, the Agricultural University Wageningen (The Netherlands), the Botanical Research Institute (Pretoria, South Africa) and the International Plant Genetic Resource Institute (IPGRI) in Harare (Zimbabwe). In Tanzania there is a collection of more than 400 cowpea accessions with TZA code, conserved at National Plant Genetic Resources Center (NPGRC) TPRI-Arusha. The collection mission is done yearly and the number of accessions is expected to rise with time.

### 1.6 Social-economic Importance of Cowpea

Cowpea is a multipurpose crop, providing food for human and feed for livestock and it is a cash generating commodity for farmers, small and medium-size entrepreneurs. It can also be used as cover crop (Langyintuo et al. 2003; Singh 2002; Timko et al. 2008). The very early maturity characteristics of some cowpea varieties provide the first harvest earlier than most other crops during production period. This is an important component in hunger fighting strategy, especially in the Sub-Saharan Africa where the peasant farmers can experience food shortage a few months before the maturity of the new crop. Its drought tolerance, relatively early maturity and nitrogen fixation characteristics fit very well to the tropical soils where moisture and low soil fertility is the major limiting factor in crop production (Hall 2004; Hall et al. 2002). This crop is grown worldwide with an estimated cultivation area of about 12.5 million hectares annually and an annual worldwide production of over 3 million metric tons (Li et al. 2001). About 70% of the cowpea production occurs in marginal areas of West Central, East and Southern Africa. Nigeria is the largest producer and consumer of cowpea at estimated annual yields of 2 million metric tons (Singh et al. 2002; Timko et al. 2008). In Tanzania, cowpea is regarded as a ‘women’s crop, because, contrary to other crops, the production process to marketing is often handled by women. Thus, it is among the crops that are generating income to female farmers and traders. Cowpea is among the dominating grains legumes traded almost in all local markets especially in the central, southern and western part of Tanzania.

Significant amount of cowpea is also produced in Peru, northern Brazil, parts of India and the southeastern and southwestern regions of North America. The United States are estimated to

produce about 80,000 mt. The states involved in this production include Tennessee, Missouri, Louisiana, Alabama, Georgia, Texas, California and Arkansas (Fery 2002).

## 1.7 Nutritional Value of Cowpea

The protein found in cowpea is, similar as the one from other legumes, rich in the essential amino acids lysine and tryptophan (Timko and Singh 2008). However, the protein nutritive value of these legumes is lower than that of animal proteins because they are deficient of sulfur amino acids and contain a non-nutritional factors (phytates and polyphenols), enzymes inhibitors (against trypsin, chymotrypsin and R-amylase) and hemagglutinins (Jackson 2009). Minerals and vitamins are the other nutritional important constituents of the cowpea seeds. It has been reported that folic acid, a vitamin B necessary during pregnancy to prevent birth defect in the brain and spine content is found in higher quantity in cowpea compared to other plants (Hall et al. 2003; Timko and Singh 2008). Total seed protein content in seed ranges from 23% - 32% of the seed weight (Nielsen et al. 1993). The total crude protein in foliage ranges from 14-21% and in crop residues, it is 6-8%. This crop has no toxicity effect to ruminants, however for the monogastrics, trypsin inhibitors and some tannin need to be considered. Diet containing 20-25% untreated grain pose no problem, further more heat treatment reduces trypsin inhibitors (Cook et al. 2005). The presence the high protein content in all cowpea parts consumable by human and animal (leaves, stems, pods and seeds), is the key factor in alleviating the malnutrition among women and children and improvement of healthy status of the livestock in resource limited households where regular access to animal protein is limited due to low economic status.

## 1.8 Various Forms of Cowpea Dishes

Different dishes can be prepared from cowpea. The young tender leaves can be cooked and eaten as vegetable, the green pods can be cooked and eaten just like green beans, the seeds can be cooked when fresh (semi-ripe) and, when full matured and dry, eaten as pulses. In Tanzania and other African countries, cowpea is used for preparation of stew that is either used with together with cereal dishes or directly mixed with the cereals as maize, wheat, sorghum and rice. This kind of food is very popular within the community and preferred to be used in a large gathering for example in school and hospitals, due to its simplicity of preparation and handling.

## 1.9 Production Constraints

Both abiotic and biotic stresses can result in a significant yield reduction in cowpea. Despite cowpea being more drought tolerant than many other crops, still moisture availability is the major constraints to growth and development, especially during germination and flower setting. Erratic rainfall affects adversely both plant population and flowering ability, resulting into tremendous reduction of grain yield and total biomass in general (Timko and Singh 2008). Under these conditions, early maturing varieties could be the coping strategy. Insect pests, a wide range of bacterial diseases, fungal and viral diseases are further causative factor for yields losses experienced by cowpea growers. Under proper insect pest management the yields are as high as 2.0 t/ha compared to the low average yields (1.0 t/ha) normally experienced in subsistence farming in West and Eastern Africa (Quin 1997; Timko and Singh 2008).

## 1.10 Biotic Stresses of Cowpea

Insect pests belong to the major biotic stresses in cowpea growing regions in both developing and developed counties (Dauost et al. 1985). The major insect pests in East Africa are aphids [*Aphis craccivora* Koch (Homoptera:Aphididae)], thrips (*Megalothrips sjostedti*), cowpea weevil [*Collosobruchus maculatus* Fabricius (Coleoptera:Bruchidae)] and a multiple of sucking bugs and leaf eating beetles. In Tanzania, aphids are the major causing factor for significant yield losses. Early infestation, especially during seedling stage, often results in total crop failure. Also due to thrips infestation, a tremendous yield losses have been reported in Tanzania, Ghana, Cameroon and Nigeria (Ezueh 1981; Price et al. 1983; Ta'Ama 1983). Omo-Ikerodah et al (2009) reported that yield loss due to thrips infestation ranged between 20 to 80%. Under severe infestation, a 100% yield loss has been observed (Singh and Allen 1980). Abdel-Aal (1982) found up to 50% weight losses within a period of 3 months of storage due to weevil damage.

The parasitic weed (striga) also poses a major threat to cowpea production in Africa. Two striga species and its distribution in Africa have been reported. *Striga gesneriodes* is mostly found in Sudan and West Africa, while *Alectra vogelii* is found in Guinea, Sudan, West and Central Africa and part of Eastern and Southern Africa (Timko and Singh 2008). *Alectra vogelii* is more widely distributed than S*triga gesneriodes*.

To be able to design a proper method for identifying plant genotype resistance to a particular insect pest, a proper understanding of the pest in question is of vital importance. Therefore, major insect pests in East Africa are described below.

### *1.10.1 Aphids*

Cowpea aphid, (*Aphis craccivora* Koch) is an important pest of cowpea in most tropical areas where cowpea is grown (Obopile and Ositile 2010). The adult aphid is relatively small (1.5 - 2.5 mm long) and usually shiny black, while nymphs are smoky gray and waxy. The adult may be winged (alate) or wingless (apterious) and when present, the wings are large and transparent, bearing few veins. Apterae and alate forms are always females that in asexual reproduction give birth to live young aphids. Alate adult are produced whenever the aphids are subjected to stress, for example overcrowding, limited food supply and fluctuating temperature (Dixon 1985; Obopile and Ositile 2010; Whitworth and Ahmad 2009). Cowpea aphids feed on tender young leaves, shoots, succulent green stems and pods. The damage is caused by both adults and nymphs and is either direct through depleting plants assimilates through sucking and through injection of its toxic saliva to the plant or through transmission of virus particles that in turn cause disease to the plant. They also secrete honeydew that usually forms sooty mold which compromises plant photosynthesis (Whitworth and Ahmad 2009).

Various screening methods have been developed for major insect pests of cowpea, including aphids. Field and laboratory evaluation are among the screening methods that have been employed, and came up with some accessions with good source of resistance to aphids (Ehlers and Hall 1997; Jackai and Daoust 1986; Obopile and Ositile 2010). Subjecting large number of genotypes to insect pressure and observing the insect feeding behavior at vulnerable host plant parts, might be the proper method to discriminate among existing gene pools of crop plants for aphid, thrips and bruchid tolerance or resistance.

### *1.10.2 Flower Thrips*

Cowpea crop has been reported to be infested with two species of thrips, *Sericothrips occipitalis* and *Megalurothrips sjostedti* (*Thripidae*) (Ezueh 1981). Thrips (*Megalurothrips sjostedti*).are small, opportunistic and ubiquitous insects of often only a few millimeters length and generally yellow, brown or black in color (Morse and Hoddle 2006). Singh and Taylor (1978) pointed out that plant parts mainly attacked by thrips are flower buds and later the flower themselves. Flower abortion is of normal magnitude in plants that are infested with

thrips. Flower damage by thrips is characterized by a distortion, malformation and discoloration of the floral parts. Thrips also feed on the terminal leaf bud and bracts/stipules and cause deformation (Ezueh 1981). Apart from the direct damage caused by thrips, it has been reported that they are vector for a number of pathogens that they transmit mechanically from plant to plant (Ullman et al. 1997).

### *1.10.3 Storage Weevil*

The cowpea weevil [*Callosobruchus maculatus* (Fabricius)] is the most important post-harvest storage pest of cowpea. The weevils occur wherever the cowpea is grown. The adult beetle are small (3 mm long) and orange-brown with dark markings. The adult lays eggs on the pods that are at maturity stage in the field, and on hatching the larvae bore the pod wall and seed coat and enter the seed. Messina (1984) reported high mortality of larvae in the field due to failure of larvae to penetrate the seed after drilling through the pod wall. The adult emergence occurs after harvest Booker (1967) in the store where real destruction happens due to re-infestations and easiness of larvae penetration into the seed because usually the seeds are stored after shelling.

Re-infestation occurs repeatedly during storage period. In store, each female lays 40-60 white flat eggs and glues it on the seeds surface; on hatching the larva bore into the seed, where it feed, grow and pupate before emerging as adult out of the seed after about 3-4 weeks. A single seed can be infested with multiple larvae (Fox 1993; Giga and Smith 1983; Messina 1993). It is reported that about 8-10 or more larvae can be found in a single seed. Thus, heavily damaged seeds show many exit holes (Ofuya and Agele 1990). Both sexes can mate soon after emergence and they require neither food nor water to reproduce and can mate several times during their life time. The beetle longevity is slightly affected by relative humidity (Giga and Smith 1983). Both sexes live an average of 7 days (Fox 1993; Messina 1993). The complete life cycle takes about five weeks; this means that a new generation rises every month during storage. An infestation of up to 100% of the stored seeds has been reported within 3 to 5 months under farmer's storage conditions (Redden et al. 1984; Singh 1980). The reduction in seed weight is directly proportional to the number of exit holes on the seeds, thus the yield losses can be easily estimated for different accession (Singh et al. 1983). A single beetle is able to cause a weight loss of grain of up to 3.5% (Booker 1967).

Different cowpea accessions are not equally damaged during pest infestations due to the preference of the pest connected to certain characteristics of the plant or due to defense mechanisms of the plant.

## 1.11 Countermeasures against These Biotic Stresses

There are several methods suggested for managing biotic stresses, such as: chemical, biological and agronomic control, IPM (Integrated Pest Management) and host-plant resistance. Adoption of chemical control has been variable largely due to problems with availability and cost of inputs and the required changes in cropping strategy (Jackai and Daoust 1986). Further, the health risks and environmental pollution potentially caused by the unscrupulous use of pesticides, demand for skilled application which rarely be expected by resource-limited farmers. Host-plant resistance to insect pest damage is the most economically and environmentally sound method of pest management for both large scale and subsistence cowpea production. This approach is less labor intensive and more secure compared to other methods, thus very appropriate for resource-limited farmers. Due to these merits, developing varieties with sustainable resistance to these insect pests and other biotic stresses is a major goal of national and international cowpea breeding programs.

### *1.11.1 Searching for Host-plant Resistance.*

Several screening methods to identify genotypes with resistance to major cowpea insect pests have been developed (Ehlers and Hall 1997). However, despite of the evaluation of many cowpeas accessions, plants with high levels of resistance to most of the major insect pests have not yet been released to farmers. Nevertheless though, Singh (2005) reported the identification of accessions with a satisfying level of resistance to aphids and moderate level of resistance to flower thrips, pod bugs and pod borer. Traditionally, morphological and agronomic traits coupled with statistical methods have been successfully used in various agronomic and breeding programs for the identification of accessions resistant to biotic and abiotic stresses. However, progress in breeding work has been very slow based on the field screening methods currently available. The identification of molecular markers for insect resistance would greatly facilitate and hasten the development of resistant genotypes to these biotic stresses.

Molecular techniques such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and

microsatellites or simple sequence repeat (SSR) provide additional and useful tools for the study of variations in many organisms (Choi et al. 2004; Gepts et al. 2005; Timko et al. 2008).. To develop plants with host-plant resistance/tolerance to insect pests requires methods to determine whether genetic variation exists among the plant population of the species in question. Experience gained in the use of molecular techniques in major crops in genetic variation studies provides the opportunity to apply similar techniques in cowpea. The combined use of morphological and molecular methods for the study of genetic variations among cowpea in Tanzania would provide useful information for the improvement of this crop particularly in relation to insect pest resistance.

### *1.11.2 Plant Defense Mechanisms*

Through co-evolution of pests and plants, the plant-hosts naturally developed protective mechanisms that help them to successfully survive insect pest attack. One example are protease-inhibitors that prevents the insects to feed effectively on the such protected plants. This mechanism was first reported by Green and Ryan (1972). The defensive function of inhibitors is attributed to their ability to suppress insect digestive enzymes, depriving vital body organs from nutrients with the death of the insect as consequence (Zhu-Salzman and Zeng 2008). About eight or more protease-inhibitor families have been reported (Garcia-Olmedo et al. 2001). Ryan (1990) reported inhibitor families specific to the following four proteolitic enzymes: serine, cystene, aspartic and metallo-protease Additional valuable plant compounds involved in host plant resistance mechanisms are enzymes such as β-1,3-glucanases, chitinases and α-amylases (Fritig et al. 1998; Garcia-Olmedo et al. 1998).

Cowpea possesses a protease-inhibitor called Cowpea Tripsin Inhibitor (CpTI). This compound was found to be responsible for resistance to major storage insect pest in some lines of cowpea. Elevated level of trypsin inhibitor was reported to be the key player in protective role in these lines (Gatehouse and Boulter 1983). Trypsin inhibitors are also found in soybean and barley (Ryan 1990). The gene responsible to confer this kind of resistance has been reported to successful transferred to other crop species through genetic engineering and performed to the expectation (Ismail et al. 2010.

## 1.12 Molecular Approaches in Genetic Diversity Studies of Cowpea

The traditional methods for estimating the genetic diversity has been the use of morphological markers. However the low availability of morphological markers, their poorly known genetic

control and environmental influence on phenotypic expression at different stages of growth has been the major limitation for using these as a reliable tool for diversity studies. The development and use of molecular markers technologies, such as Restriction Fragment Length Polymorphisms (RFLP) (Lambrides et al. 2000),, Random Amplified Polymorphic DNAs (RAPD) (Betal et al. 2004; Lakhanpaul et al. 2000; Santalla et al. 1998),, Amplified Fragment Length Polymorphisms (AFLPs) (Zong et al. 2003) and microsatellites or Simple Sequence Repeats (SSR) (Li et al. 2001; Wang et al. 2004), have greatly facilitated the analysis of the structure of plant genomes and their evolution including the genetic structure and variations among cowpeas accessions (cultivated and wild). An analysis of *Vigna* species done by Fatokun et al (1993) using RFLP markers revealed the existence of a high level of genetic variations within the genus from African origin relative to those from Asian origin.

In a study of the structure of 23 accessions of five species within the subgenus *ceratotropics* using RAPD markers, Kaga et al (1996) reported the existence of two main groups differing by 70% at molecular level. A study conducted by Ajibade et al (2000) using Inter Simple Sequence Repeat (ISSR) DNA polymorphism for analysis of genetic relationships among 18 *Vigna* species found that closely related species within each sub-general clustered together, and cultivated cowpea grouped closely with the wild sub-species of *Vigna unguiculata*. Ba et al (2004) studied the characterization of genetic variation in domesticated cowpea and its wild progenitor, and their relationship using RAPD. They found high diversity in cultivated cowpea, but only weak structure. Further, their study revealed high diversity in wild cowpea from East Africa compared to those from West and Southern Africa.

## 1.13 Conclusion

The multi-functionality and wide adaptation to various ecological conditions, especially water scarcity, of cultivated cowpea, may ascribe this crop an important role in the future. The current climatic changes, this globe is facing, pose extinction risk to many plant species that fail to adapt to these changes especially high temperature and moisture deficit. Drought tolerance, high temperature tolerance, low-input adaptation and high protein content put this crop at a stage of being among the priority crops to be considered in order to cope with the current world climate change accompanied by food shortages and nutrient deficiencies especially to children and particularly in developing countries. Fortunately the gene diversity within the primary gen pool of this crop is ample and thereby provides the opportunity to even better adapt the characteristics of this crop to human needs. One of the fields where

improvement will be most beneficial is the resistance/tolerance against biotic stresses, and especially against pests, which are hampering the yield of the crop considerably. For any attempt to realize a profitable output from cowpea production, a sustainable method for managing the insect pest should have first priority. One of most affordable and sustainable means of controlling the major insect pest is through host-plant resistance.

Naturally, resistance against pests cannot be the only breeding target, but needs to be combined with other important traits such as high yields, early maturity and drought tolerance in a single genotype. Therefore, the first step is to identify genotypes having one or several of these desired traits and then combine them by breeding.

With the current available and efficient molecular marker tools, breeding work has been shortened and reached a more reliable and efficient level. A first step is to clarify the genetic structure within the primary (and secondary) pool of the crop in order to get to know, which crosses will likely have a strong effect on the diversity in the resulting segregating population which forms the base for the subsequent selection. A next step would then be to identify markers which are linked or functional for the traits in focus, especially insect resistance. Fortunately, cowpea is known to possess an important insect-inhibiting compound called Cowpea Tripsin Inhibitor (CpTI). The use of combined morphological and molecular methods to obtain cowpea genotypes that will be resistant to major insect pests should therefore be feasible. The abundance of genetic diversity in cowpea provides a great opportunity for the improvement of this crop in the current ongoing and future breeding programs. The currently available molecular tools for studying plant genome will certainly assist in the future expansion of marker-assisted selection and breeding to efficiently achieve this goal.

### 1.14 Aim of this study

1 To study genetic diversity of cowpea landraces collected from farmers' field using 26 SSR markers so that the information can be used both to design proper conservation approach for preventing further genetic erosion of cowpea and design the best crosses for crop improvement in breeding.
2 To investigate both genetic and environmental factors influencing cowpea production with emphasis on thrips and aphids under natural infestation, for the purpose of the development of appropriate methods for managing these pests and to select genotypes with superior resistance/tolerance against these pests.

3 To investigate the behavior of seed grain of cowpea landraces in storage towards enhanced infestations by *Callosobruchus macullatus* (F) the major cowpea storage weevil, in order to identify landraces that are tolerant/resistant to this pest and to gain knowledge on the function of this tolerance/resistance.

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# 2 PAPER 1: INTRINSIC AND EXTRINSIC FACTORS INFLUENCING IMPORTANT TRAITS IN COWPEA (*VIGNA UNGUICULATA* (L.) WALP.).

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## 2.1 Abstract

Cowpea (*Vigna unguiculata* L. Walp) is an important grain legume in East Africa and is mainly grown by small-scale farmers. Drought tolerance, early maturity, nitrogen fixation, and low fertility requirement are important characteristics for adaptation to the dry regions of Sub-Saharan Africa. Cowpea is a multifunctional crop providing food to both humans and animals. Because it is rich in protein, cowpea is a cheap source of protein for resource-limited families in Sub-Saharan Africa. Insect pests are the major constraint of cowpea production in East Africa. The major insect pests are aphids (*Aphis craccivora*) and flower thrips (*Megalurothrips sjostedti*). Farmers employ different management practices

including intercropping, time of sowing, the manipulation of plant density, and the use of insecticides to reduce yield loss caused by these pests. The development of cultivars with multiple resistances to biotic and abiotic stresses is the best alternative for the pest management practices. Yield, yield component, and host plant resistance against aphids and thrips of 200 genetically different cowpea accessions were studied in field experiments conducted in 2 contrasting seasons in 4 different environments during the 2008 -2009 cropping season in Tanzania. The results indicated that temperature was the major factor that greatly influenced flowering time, aphid infestation, and thrip infestation. Leaf color, growth habit, pod hairiness, and seed size had the highest heritability and were mostly unaffected by the environment. Pod hairiness was strongly and positively correlated to seed weight and seed size in all environments. Moreover, the growth habit and flowering time were strongly and positively correlated across all environments. A multiple regression analysis result showed that flowering time was the major determinant of grain yield in three of the four environments studied. Early flowering was associated with high grain yield. One accession showed no aphid

infestation in all environments, superior grain yields, good yield stability, and resistance against thrips. The results of this study provide a foundation for the improvement of cowpeas in East Africa based on genotypes that are superior for certain key characteristics and the knowledge of their relationships under specific climatic conditions.

Keywords: *Vigna unguiculata*; *Aphis craccivora*; *Megalurothrips sjostidti*; Trait; Breeding; Tanzania

## 2.2 Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an important grain legume in East Africa and is mainly grown by small-scale farmers. The crop flourishes well in areas where the minimal and maximal temperatures range between 18.2°C and 27.6°C, respectively, during the growing season. Cowpea grows in a wide range of environments covering 40°N to 30°S (Richie, 1985). When compared to other crop species, cowpea has considerable adaptation to high temperature and drought (Ehlers and Hall, 1997). Whereas other crops fail due to a shortage of soil moisture, cowpea survives. Hall and Patel,(1985) reported cowpea dry grain yield as high as 1000 kg $ha^{-1}$, which was obtained in the Sahelian environment with low humidity and only 181 mm of rainfall. Furthermore, due to its high ability to fix atmospheric nitrogen, cowpea is a valuable part of farming systems in areas where soil fertility is limited by enriching the soil through residues (Elowad and Hall, 1987). Due to the early maturity and drought tolerance characteristics of cowpeas, farmers in paddy growing areas usually grow cowpea in the same field after harvesting paddy to efficiently utilize the residual moisture that is usually available after harvesting. In addition, early maturity is an important property for the cowpea crop to escape the peak insect population density, especially during the vulnerable developmental stage. The cowpea crop is also tolerant of low soil fertility because of its ability to fix atmospheric nitrogen (Elowad and Hall, 1987). All of these characteristics allow cowpea crops to fit well in the dry regions of Sub-Saharan Africa.

Cowpea is a multifunctional crop because it provides food to both humans and animals. The leaves, green pods, and beans form portions of the human diet where it is grown. In addition, it is a cash-generating crop for both small-scale farmers (especially women at the farm level) and large-scale grain traders (Singh, 2005; Timko and Singh, 2008).. The crop has a high protein content that ranges between 20% and 26%, and it has a starch content that ranges between 50% and 67% (Singh et al., 1997). All of the edible parts of cowpea are rich in protein. For this reason, it is a cheap source of protein for resource-limited populations in both

rural and urban areas. The relatively early maturity characteristics of some cowpea varieties provide peasant farmers with vegetables within a short period of time after the onset of the cropping season. In general, farmers in Tanzania and East Africa intercrop cowpea with maize, sorghum, millets, cotton, and cassava (Timko and Singh, 2008). The intercropping farming system is a type of risk distribution because of the unpredictability of rainfall.

Insect pests are the major constraint of cowpea production in East Africa (Singh and van Emden, 1979; Singh and Allen, 1980; Muleba and Ezumah, 1985; Jackai and Daoust, 1986). The major insect pests are aphids (*Aphis craccivora* Koch), flower thrips (*Megalurothrips sjostedti* Trybom), and cowpea weevils (*Callosobruchus maculatus* F). Resource-limited farmers in East Africa employ different management practices to minimize insect pest damage. The following management practices are used by the farmers: crop rotation, intercropping (Kitch et al., 1997; Nabirye et al., 2003), time of planting, and the manipulation of plant density (Nabirye et al., 2003). 2003). Farmers with higher incomes also apply pesticides for control measures. This method of pest management, however, is not readily adopted due to the required changes in cropping strategy, cost of input, and unreliable availability of pesticides (Jackai and Daoust, 1986). Furthermore, the concerns of environmental pollution and applicant security demand ecological and economical viable alternatives for insect pest management, such as host plant resistance (McNamara and Morse, 1996). The development of cultivars with multiple resistances to biotic and abiotic stresses is a current and future focus in breeding (Ehlers and Hall, 1997). Studies concerning host plant resistance against pest and/or diseases must be carried out in the context of the interaction between host, pest/disease, and environment. Several researchers have reported that aphid population dynamics are significantly influenced by environmental factors, such as temperature (Ruggle and Gutierrez, 1995; Diaz and Fereres, 2005). Therefore, they proposed a study to test the interaction between the environment and the genotypic resistance of cowpea varieties against aphids (*A. craccivora*).

In nature, plants have different protective mechanisms against insect pest damage and diseases (Kogan, 1986). These mechanisms may be mechanical barriers in which high concentrations of lignin or biochemical compounds, such as protease inhibitors, are produced to debilitate insect proteolysis (Boulter et al., 1989; Ji-Eun, 2009). In cowpeas, the trypsin inhibitor, CpTI, has been reported to have insecticidal properties against a wide range of insects (Ismail et al., 2010). The function of protease inhibitors in plant protection against

insects has been studied by several researchers as reviewed by Valueva and Mosolov (2004), using genetic engineering, CpTI has been transferred to other crops to improve their insect resistance (Boulter et al., 1989; Gatehouse et al., 1997). In a study on the effect of protease inhibitors on *Callosobruchus maculatus* (cowpea grain weevil), however, Amirhusin et al., (2007) suggested that targeting multiple digestive proteases may be more effective in insect pest control than the inhibition of a single enzyme class.

The low grain yield of cowpeas in East Africa is caused not only by biotic and abiotic stresses but also by suboptimal genotypes. Most of the small-scale farmers in East Africa use unimproved cowpea landraces. The average yield obtained in this region is approximately 250 kg ha$^{-}$1, which is approximately five times less than the yield obtained in well managed experiments using improved seeds (Whitbread and Lawrence, 2006; Omo-Ikerodah et al., 2009). To respond to this challenge, a need for identifying and developing cowpea genotypes containing important traits, such as high yield, tolerance to biotic stresses, and tolerance to abiotic stresses, should be given a high priority in this region.

Studying relationships among different traits are important for decision making to simultaneously select two or more traits. Two desirable traits that are positively related can be easily selected and improved together, but two desirable traits that are negatively correlated are difficult to simultaneously improve. Therefore, the aims of this study were to analyze yield, yield components, resistance against aphids, resistance against thrips, and several phenotypic factors that may further influence the yield and resistance in the contrasting environments where cowpeas are grown. The interactions of these traits were studied and set in the context of environmental factors. Finally, based on the observations mentioned above, superior genotypes that may help to improve the resistance and yield level of cowpeas were selected.

## 2.3 Materials and Methods

### *2.3.1 Plant Material*

Among the 413 cowpea landraces used in this study, 300 were directly collected from farmers and 113 were obtained from the National Plant Genetic Resources Centre (NPGRC) in Tanzania. These 413 cowpea landraces were multiplied at the Miwaleni experimental field to obtain enough seed for use in field, storage and diversity studies. Each landrace was separately planted in a single row three meters long. Maximum yield from each plant was

insured by adopting wide spacing (60 cm intra-row and 90 cm inter-row) and regular insect control through pesticide application. During harvesting, a single plant was randomly chosen from each landrace, harvested separately and placed in a labelled cloth bag. The seeds harvested from this single plant of each landrace were later used for field, storage and diversity studies. Five seeds from the single plant of each landrace were sampled for genetic analysis. Genetic distances between the landraces were determined using microsatellites. Based on these genetic distances, 200 genetically distant landraces were selected for use in field and storage experiments.

### *2.3.2 Field Experiment*

Two experimental sites representing the climatic conditions from which the cowpea accessions were collected were chosen for this study. The Miwaleni site, located in Moshi district at 3º 25´ 22´´S 37º 27´ 5´´ E, represents low- to medium-altitude agro-ecological zones with altitudes ranging from 0 to 500 meters above sea level (m.a.s.l). This site is characterised by relatively low annual precipitation (500–700 mm/year), low to medium relative humidity (56–71%), and relatively high temperatures (20–27˚C). The Tengeru site, located in Arusha district at 03º22´29.3´´ S 036º48´30´´ E, represents high-altitude agro-ecological zones with altitudes ranging from 1200 to 1324 m.a.s.l. This site is characterised by relatively high annual precipitation (1400–2000 mm/year), high relative humidity (>71%) and low temperatures (17–25˚C). These two sites (Miwaleni and Tengeru) belong to the Tropical Pesticide Research Institute (TPRI) and are used solely for experimental field research. Due to the continuous presence of various crops (especially pigeon peas, green peas, beans, cowpeas and cereal crops), insect-pest populations (especially aphids and thrips) have built up over time, making these sites hot spots for these and other pests.

Two cycles of field experiments were conducted for this study. The first cycle was conducted under irrigation during the dry period, covering the months of September 2008 to January 2009. The second cycle was conducted during the rainy season, from February to May 2009. The experimental fields were ploughed, harrowed and ridged at 75-cm spacing. The experimental design was a completely randomised block design (CRBD) with three replicates (Gomez and Gomez, 1984). The experimental unit (plot) was a single 3-m-long row. The total number of plots per experiment was 600. Before sowing, the plots were watered thoroughly to ensure even germination. Following watering, the first cycle was established on 16 September and 25 September 2008 at the Miwaleni and Tengeru sites, respectively; the second cycle was

sown on 28 January and 30 January 2009 for Miwaleni and Tengeru, respectively. Two hundred (200) cowpea landraces were randomly assigned to 600 plots. A single 3-m-long row of each landrace was sown at a spacing of 90 cm between rows and 30 cm within rows. Four seeds were hand sown in each hole; the plants were thinned to two per hill after germination. Weeding and irrigation were done according to appropriate local praxis. No pesticide was applied for insect-pest control. Maize was planted around the trial field for two reasons. First, maize is taller than cowpea and thus shielded the trial field against strong winds. Second, maize is an alternate host for aphids and was used to attract more aphids toward the cowpeas. Pigeon peas which attract thrips, had been previously planted near the trial field and were flowering when the cowpea trial was established, thus increasing the pressure of thrips on the cowpeas.

### *2.3.3 Trait evaluation*

Several traits, including vegetative and reproductive characteristics, were recorded according to the standards of the International Board for Plant Genetic Resources (IBPGR) cowpea descriptor IBPGR, (1983), with modification in the number of items to be scored per trait. Sampling procedures were non-destructive. Twenty-one parameters were recorded in this study. The traits, their specifications and the acronyms used for each trait in the text are shown in Table 2-1. The method adopted for aphid scoring was similar to that of Ombakho et al., (1987), with some modifications. Subjective scoring was performed on a scale from 0 to 7, where 0 indicated no infestation and 3, 5 and 7 indicated low, medium and high infestation, respectively. Scoring was performed at an interval of 14 days. Three plants in every fifth hill in each plot, starting from the first hill, were inspected and scored for infestation. Each plot contained eleven hills. Five of these hills were sampled. All scoring was performed visually with minimal disruption to the sampled plants. Due to the severity of infestation, differentiation between colonies was difficult; therefore, it was more appropriate to score the number of infested plants in each plot than to count the number of aphids or aphid colonies. The same sampling procedures and scoring scale adopted for aphids were employed to assess thrips. Thrips populations were scored visually using a modification of the method of Ezueh, (1981). The sampled plants were bent and shaken on a circular white plastic tray (45-cm diameter). The thrips that were dislodged from the flowers were scored by assigning values on a scale based on the estimated thrips population.

Table 2-1: Parameter description, acronyms, scales of recording and unit of measurement.

| Parameter | Acronym | Phenotypic scale/score/unit of measure |
|---|---|---|
| Flowering time | *FwT* | Days from germination to 50% flowering |
| Growth habit | *GrH* | 1= Determinate, 2= indeterminate |
| Aphids infestation | *Aph* | 0= no infestation, 3= low infestation, 5=medium |
| Thrips infestation | *Thr* | infestation, 7=High infestation |
| Plant count at germination | *Pp1* | Amount in number |
| Plant count at harvest | *Pp2* | Amount in number |
| Seed weight | *GrWt* | Weight of 100 seeds in (g) |
| Seed size | *SdSz* | 3= small, 5=medium, 7=large |
| Grain Yield/plot | *Yld* | Weight in (g) |
| Grain Yield per plant | *YldPl* | Weight in (g) |
| Leaf colour | *Lfc* | 3= pale, 5= light green, 7 = dark green |
| Pod hairs | *PdHr* | 3 = glabrescent, 5 = short appressed hairs (pubescent), 7= pubescent hirsute |
| Pod length | *PdL* | cm |
| Seed/pod | *Sdpd* | Amount in number |

The mean values of precipitation, temperature and relative humidity during experimental establishment in the two seasons at each site are shown in Table 2-2 and Table 2-3.

Table 2-2: Rainfall, average temperature (Temp.) and relative humidity (RH) for Miwaleni.

| | Cropping season 2008 | | | | | | Cropping season 2009 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jul | Aug | **Sept** | **Oct** | **Nov** | **Dec** | **Jan** | **Feb** | **Mar** | **Apr** | **May** | Jun |
| Rain (mm) | 12.1 | 0 | 0 | 0 | 0 | 19.8 | 0 | 0 | 0 | 10 | 20 | 97.3 |
| Temp. (ºC) | 21.1 | 22.1 | 23.6 | 25.7 | 25.7 | 26.6 | 27.3 | 26.9 | 27.6 | 25.9 | 24.5 | 23.1 |
| RH (%) | 71 | 67 | 59 | 56 | 58 | 64 | 51 | 55 | 53 | 65 | 70 | 69 |

Months in **bold** indicate the vegetation period for the crop from sowing to harvest.

Table 2-3: Rainfall, average temperature (Temp.) and relative humidity (RH) for Tengeru.

| | Cropping season 2008 | | | | | | Cropping season 2009 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jul | Aug | **Sept** | **Oct** | **Nov** | **Dec** | **Jan** | **Feb** | **Mar** | **Apr** | **May** | Jun |
| Rain (mm) | 2.4 | 0 | 1.4 | 0 | 14.4 | 26.4 | 46 | 42 | 36.1 | 123.8 | 224.5 | 3.6 |
| Temp. (ºC) | 18.2 | 18.1 | 19.5 | 21.7 | 22.1 | 21 | 21.7 | 21.8 | 22.9 | 21.5 | 20 | 19.1 |
| RH (%) | 60 | 60 | 56 | 43 | 53 | 52 | 46 | 45 | 62 | 45 | 66 | 64 |

Months in **bold** indicate the vegetation period for the crop from sowing to harvest.

## 2.4 Data analysis

Data were analysed using Microsoft Excel (Microsoft, Redmond) for basic calculations and R v. 2.10 statistical software, (R-Develoment core team., 2008) for advanced calculations. A visual test for outliers was performed based on the data distribution for each trait. For each trait, basic statistics were calculated at different levels. To estimate differences in static stability, the environmental variance $S^2$ (Lin et al., 1986; Becker and Leon, 1988) of each accession *i* was calculated for each trait using the following formula: $S_i^2 = \sum (m_{ij} - m_i)^2 / (e-1)$, where $m_{ij}$ is the accession mean in the environment, $m_i$ is the accession mean across environments and *e* is the number of environments. To improve readability, *S²'* (= *S²*/1000) is presented instead of *S²* in the tables.

The structure of the data variance was analysed using ANOVA with the genotype G, the season S (dry vs. humid) and the altitude of the site L (high vs. low) as non-random main factors. Thus, the trait response $R_{ijkr}$ of genotype *i* at location *j* in season *k* and block *r* was analysed using the following statistical model: $R_{ijkr} = m + G_i + L_j + S_k + B_r(L_jS_k) + GL_{ij} + GS_{jk} + LS_{jk} + GLS_{ijk} + e_{ijkr}$, where *m* is the grand mean, *B* is the block effect and *e* is a random error. In addition, spatial inhomogeneity was analysed by statistically testing the significance of the block effect and visually observing the distribution of the residuals after ANOVA at the field level. Further, the heritability of all traits was analysed using the following formula:

$$h^2 = \frac{\hat{\sigma}_g^2}{\frac{\hat{\sigma}^2}{r_e} + \frac{\hat{\sigma}_{ge}^2}{e} + \hat{\sigma}_g^2}$$ (Schön et al., 1993),

where $\hat{\sigma}^2$ is the estimated total variance, $\hat{\sigma}_g^2$ is the estimated genetic variance, $\hat{\sigma}_{ge}^2$ is the estimated genetic * environmental variance, $r_e$ is the number of replications and *e* is the number of environments.

Relationships between traits were analysed using Pearson's product-moment correlation. For insect-infestation scores, yield components and yield as a response variable, a step-wise multiple regression (stMR) was applied using the functions *stepAIC* from the 'MASS' package (Venables and Ripley, 2002) and *calc.relimp* from the 'relaimpo' package (Groemping, 2006) within the R software to obtain the main influential factors. The choice of the variables included in the full model before optimisation was determined using the results of the correlation analyses and literature references.

## 2.5 Results

### *2.5.1 Single-trait observations*

The means for the various traits within each environment and the heritability ($h^2$) for each trait are shown Table 2-4. Traits with relative high heritability were leaf colour (*LfC*, 0.991), seed size (*SdSz*, 0.983), growth habit (*GrH*, 0.967), grain weight (*GrWt*, 0.967) and pod hairiness (*PdHr*, 0.831). Consequently, the means of these traits were similar between environments. In contrast, aphid infestation (*Aph*) and thrips infestation (*Thr*) showed very low heritabilities (0.056 and 0.142, respectively). For the remaining traits, namely flowering time (*FwT*), yield (*Yld*) and yield per plant (*YldPl*), heritability of 0.640, 0.526 and 0.267 were observed, respectively. At Miwaleni, the lower-altitude, drier and warmer location (Table 2-2), flowering was consistently earlier and yield was higher than at Tengeru, the higher-altitude, cooler and more humid location Table 2-3). The fully irrigated season in 2008 produced lower *YldPl* values (26.28 g in Miwaleni and 16.51 g in Tengeru) than the rainy season in 2009 (42.87 g in Miwaleni and 33.78 g in Tengeru). No aphids or thrips were observed at Miwaleni during the 2009 season. During both seasons at Tengeru, *Aph* was higher than during the 2008 season at Miwaleni. *Thr* was highest during the 2009 season at Miwaleni (5.31 scaling points), followed by the 2009 season at Tengeru (4.39) and the 2008 season at Tengeru (3.73). The environmental variance of yield as a measurement of yield (in-) stability varied from 2,566 to 209,451 and showed only a weak correlation (0.1722) with the plot yield (Fig. 2-1).

Table 2-4: Estimated heritability and traits mean for the different environments

| Traits | Miwaleni | | Tengeru | | $h^2$ |
|---|---|---|---|---|---|
| | 2008 | 2009 | 2008 | 2009 | |
| *FwT* | 53.32 | 51.28 | 62.43 | 57.95 | 0.640 |
| *GrH* | 2.80 | 2.80 | 2.81 | 2.82 | 0.967 |
| *Aph* | 3.31 | | 5.43 | 5.00 | 0.056 |
| *Thr* | 5.31 | | 3.73 | 4.39 | 0.142 |
| *GrWt* | 12.08 | 13.77 | 12.77 | 12.78 | 0.943 |
| *SdSz* | 4.94 | 4.94 | 4.92 | 4.96 | 0.983 |
| *Yld* | 389.44 | 689.41 | 181.61 | 298.71 | 0.526 |
| *YldPl* | 26.28 | 42.87 | 16.51 | 33.78 | 0.267 |
| *LfC* | 5.96 | 5.98 | 5.97 | 5.98 | 0.991 |
| *PdHr* | 3.89 | 3.85 | 3.88 | 3.88 | 0.831 |

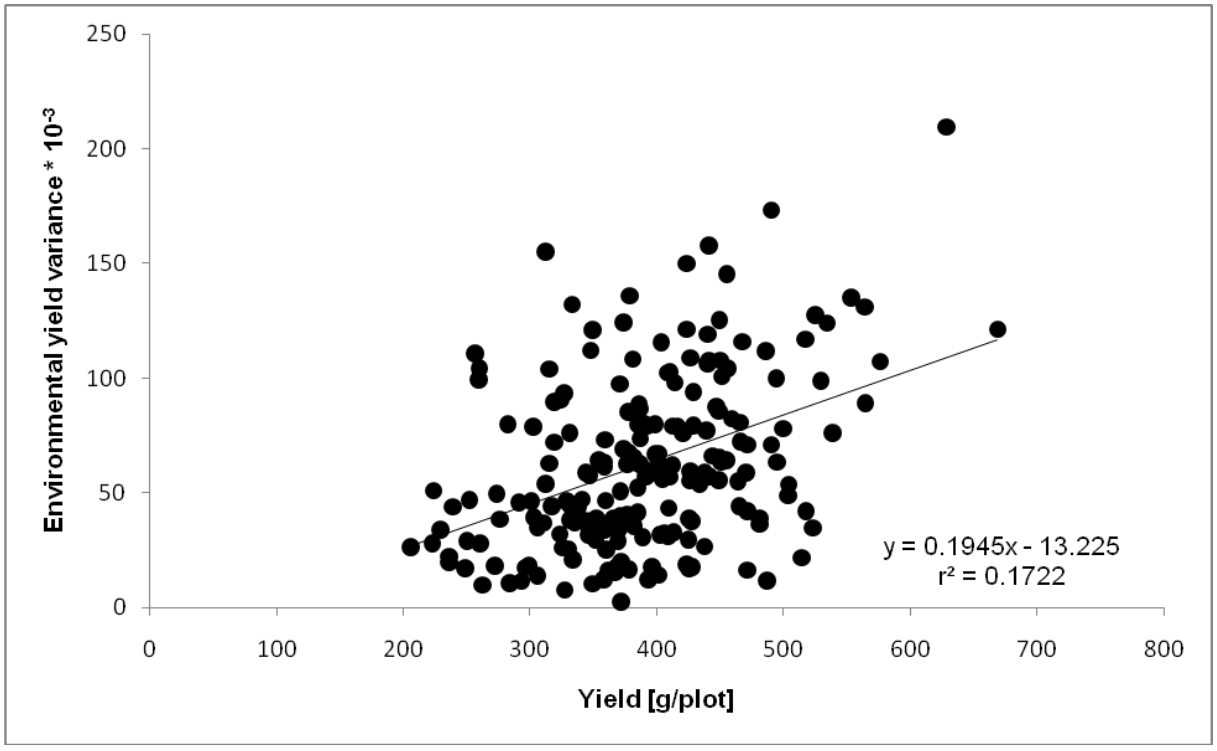


Fig. 2-1: The accession's Yld in relation to its yield variance at different environments.

Table 2-5: ANOVA results (F-values and significances for main factors and interactions) for different traits.

| **Source** | Accession | Location | Season | Acc. x Loc. | Acc. x Seas. | Loc. x Seas. |
|---|---|---|---|---|---|---|
| **Df** | 1 | 1 | 1 | 199 | 199 | 1 |
| *FwT* | 3.426 *** | 730.146 *** | 113.989 *** | 1.552 *** | 1.101 | 19.268 *** |
| *GrH* | 7.918 *** | 1.678 | 0.116 | 0.247 | 0.282 | 0.089 |
| *Aph* | 1.265 * | 333.983 *** | 13.927 *** | 1.449 *** | 0.937 | |
| *Thr* | 1.226 * | 155.903 *** | 32.635 *** | 0.951 | 1.154 | |
| *Pp1* | 4.149 *** | 941.430 *** | 26.848 *** | 1007 | 3.182 *** | 7.682 ** |
| *Pp2* | 5.108 *** | 1065.476 *** | 8.49 ** | 0.734 | 3.532 *** | 43.086 *** |
| *GrWt* | 15.382 *** | 9.439 ** | 122.317 *** | 1.360 ** | 0.743 | 114.774 *** |
| *SdSz* | 9.918 *** | 0.124 | 0.012 | 0.289 | 0.107 | 0.026 |
| *Yld* | 1.962 *** | 1217.283 *** | 582.521 *** | 0.987 | 0.904 | 100.653 *** |
| *YldPl* | 1.991 *** | 71.202 *** | 222.293 *** | 0.978 | 2.383 *** | 0.112 |
| *LfC* | 10.486 *** | 0.092 | 0.011 | 0.094 | 0.096 | 0.007 |
| *PdHr* | 3.680 *** | 0.099 | 0.050 | 1.474 *** | 0.189 | 0.036 |

Df = Degrees of freedom

*, **, ***: significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

In the analysis of variance (ANOVA, Table 2-5), significant differences between the accessions were detected for all traits studied. Nevertheless, *Aph* and *Thr* showed the weakest significances, with p-values of 0.0123 and 0.0258, respectively. For the traits with the highest heritabilities (*GrH*, *SdSz*, and *LfC*), no other factor in the model had a significant influence on the variance. *PdHr* showed further significance only in the accession × location interaction. For the other traits (*FwT*, *Aph*, *Thr*, *Pp1*, *Pp2*, *GrWt*, *SdSz*, *Yld* and *YldpPl*), significant effects were detected for both the location and the season, while more inconsistent results were obtained for the three possible interactions (Table 2-5). Of the traits studied, only *Aph* and *Thr* were not significant influenced by any interaction. *Pdl* and *Sdpd* were recorded at only one location; therefore, these two traits were excluded from the analysis.

### *2.5.2 Trait interactions*

There was a relatively strong, consistent linear correlation between *GrH* and *FwT* in all environments, resulting in r-values of +0.525, +0.327, +0.245 and +0.400 for Miwaleni 2008 (Mi.08), Miwaleni 2009 (Mi.09), Tengeru 2008 and Tengeru 2009, respectively (Table 2-6 and Table 2-7). Thus, early flowering was preferentially observed together with the determinate growth type and late flowering with the indeterminate growth type. Also, *LfC* was positively correlated with *GrH* in all environments, showing r-values between +0.441 and +0.348 (all with error probabilities < 0.000). The mean value of leaf colour was lower (i.e., the leaves were brighter green on average) in the group of accessions with determinate growth (4.96 on a scale from 1 to 7) than in the group of accessions with indeterminate growth (6.09, data not shown). Despite these differences, the full range of colour values (from 3 to 7) was found in both groups. Further, except in the Te.09 environment, *Grh* showed a significant negative correlation with *Pp1* and *Pp2*, indicating fewer plants per plot for indeterminate accessions than for determinate accessions. Other than *GrH*, *FwT* was consistently correlated across all four environments with only one other trait, *LfC*. The correlation values for this relationship ranged from +0.164 (Mi.09) to +0.336 (Mi.08) and were always smaller and less significant than the r-values between *GrH* and *LfC*. On average, later-flowering plants had darker-coloured leaves.

Further, in all environments but Te.08, *FwT* showed a significant negative correlation with both *Pp1* and *Pp2*, indicating that relatively earlier flowering occurred in plots with higher plant density and *vice versa*. This effect was stronger in Miwaleni (r-values between –0.256 and –0.395 for *Pp1* and between –0.346 and –0.424 for *Pp2*) than in Te.09 (–0.200 and –

0.201 for *Pp1* and *Pp2*, respectively). In Mi.08 and Te.09, *FwT* was also negatively correlated with *Yld*, with correlation coefficients of –0.403 and –0.250, respectively.

Table 2-6: Pearson's r values and its respective significance for correlations between traits at Miwaleni (upper right half: 2008 and lower left half: 2009).

| | *FwT* | *GrH* | *Aph* | *Thr* | *Pp1* | *Pp2* | *GrWt* | *SdSz* | *Yld* | *YldPl* | *LfC* | *PdHr* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *FwT* | | +0.525*** | +0.177* | +0.160* | –0.256*** | –0.346*** | –0.029 | +0.006 | –0.403*** | +0.062 | +0.336*** | +0.185** |
| *GrH* | +0.327*** | | +0.012 | +0.258*** | –0.218** | –0.244** | +0.051 | +0.076 | –0.089 | +0.118 | +0.391*** | +0.096 |
| *Aph* | | | | +0.048 | +0.136 | +0.085 | –0.134 | –0.129 | –0.122 | –0.116 | –0.079 | –0.062 |
| *Thr* | | | | | +0.053 | +0.167* | –0.104 | –0.101 | +0.143 | –0.032 | +0.241** | –0.085 |
| *Pp1* | –0.424*** | –0.163* | | | | +0.885*** | –0.262*** | –0.092 | +0.199** | –0.476*** | –0.084 | –0.121 |
| *Pp2* | –0.424*** | –.163* | | | +0.956*** | | –0.304*** | –0.118 | +0.300*** | –0.585*** | –0.051 | –0.131 |
| *GrWt* | +0.233** | +0.109 | | | –0.370*** | –0.389*** | | +0.664*** | –0.029 | +0.323*** | –0.044 | +0.309*** |
| *SdSz* | +0.190** | +0.008 | | | –0.307*** | –0.335*** | +0.825*** | | –0.128 | +0.055 | –0.035 | +0.233** |
| *Yld* | –0.083 | +0.024 | | | +0.263*** | +0.277*** | –0.169* | –0.103 | | +0.192** | +0.063 | +0.108 |
| *YldPl* | +0.245*** | +0.144* | | | –0.599*** | –0.653*** | +0.193** | +0.131 | +0.221** | | +0.017 | +0.041 |
| *LfC* | +0.164* | +0.348*** | | | –0.094 | –0.114 | +0.026 | –0.036 | +0.198* | +0.198* | | +0.088 |
| *PdHr* | +0.035 | +0.084 | | | +0.176* | –0.160* | +0.261*** | +0.214** | +0.133* | +0.162* | +0.083 | |

*, **, ***: significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

Table 2-7: Pearson's r values and its respective significance for correlations between traits at Tengeru.(upper right half: 2008 and lower left half: 2009).

| | *FwT* | *GrH* | *Aph* | *Thr* | *Pp1* | *Pp2* | *GrWt* | *SdSz* | *Yld* | *YldPl* | *LfC* | *PdHr* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *FwT* | | +0.245** | +0.180* | +0.033 | –0.004 | +0.008 | –0.101 | +0.043 | +0.044 | –0.006 | +0.261*** | +0.088 |
| *GrH* | +0.400*** | | +0.033 | +0.185* | –0.198* | –0.223** | –0.036 | +0.031 | +0.048 | +0.127 | +0.441*** | +0.064 |
| *Aph* | +0.193** | –0.017 | | +0.142 | –0.039 | –0.051 | +0.054 | +0.110 | –0.210** | –0.090 | +0.052 | +0.051 |
| *Thr* | +0.046 | +0.057 | +0.012 | | –0.018 | –0.060 | +0.033 | +0.085 | –0.298*** | –0.242** | +0.214** | –0.060 |
| *Pp1* | –0.200** | –0.092 | –0.044 | +0.376*** | | +0.972*** | –0.103 | –0.147 | +0.417*** | –0.355*** | –0.053 | –0.182* |
| *Pp2* | –0.201** | –0.090 | –0.037 | +0.378*** | +0.998*** | | –0.122 | –0.151 | +0.421*** | –0.374*** | –0.077 | –0.164* |
| *GrWt* | –0.061 | –0.045 | +0.075 | –0.195 | –0.232** | –0.230** | | +0.671*** | –0.299*** | –0.054 | –0.011 | +0.343*** |
| *SdSz* | +0.135 | +0.067 | +0.129 | –0.098 | –0.224** | –0.220** | +0.658*** | | –0.304*** | –0.109 | –0.080 | +0.277*** |
| *Yld* | –0.250** | +0.015 | –0.195** | –0.305*** | +0.383*** | +0.379*** | –0.008 | –0.130 | | +0.371*** | +0.103 | +0.108 |
| *YldPl* | +0.084 | +0.093 | –0.163* | –0.569*** | –0.413*** | –0.413*** | +0.213** | +0.145 | +0.441*** | | +0.084 | –0.085 |
| *LfC* | +0.324*** | +0.399*** | +0.106 | +0.085 | –0.077 | –0.080 | –0.039 | –0.029 | –0.019 | +0.142 | | +0.055 |
| *PdHr* | +0.024 | +0.093 | –0.025 | –0.167 | –0.136 | –0.138 | +0.321*** | +0.253*** | +0.106 | +0.136 | +0.153 | |

*, **, ***: significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

In addition to its correlations with *GrH* and *Fwt*, *LfC* was positively correlated with *Thr* at both sites during the 2008 season, indicating that plots with darker-coloured leaves had higher thrips infestations and plots with brighter-coloured leaves had lower thrips infestations. The correlation coefficients were +0.241 in Mi.09 and +0.214 in Te.09. In addition to this correlation, *Thr* was highly negatively correlated with *Yld* and *YldPl* during both seasons at

Tengeru only, showing correlation coefficients of –0.298 and –0.242 in Te.08 and –0.305 and –0.569 in Te.09 for *Yld* and *YldPl*, respectively.

Further, *Pp1* and *Pp2* were positively correlated with *Thr* in Te.09, with r-values of +0.376 and +0.378, respectively. In Mi.08, a relatively weaker positive correlation was observed between *Thr* and *Pp2*, with an r-value of +0.167. In these environments, higher thrips infestations were observed in plots with higher plant densities and *vice versa*.

Finally, a weaker positive correlation (r = +0.160) was observed between *Thr* and *FwT* in Mi.08. The positive correlation between *Aph* and *FwT* was more consistent than that between *Thr* and *Fwt*; the former relationship was observed in all three environments where aphids occurred (r-values of +0.177, + 0.189 and +0.193 in Mi.08, Te.08 and Te.09, respectively).

Like *Thr*, *Aph* was negatively correlated with yield during both seasons at Tengeru. However, the r-values (–0.210 and –0.195 for Te.08 and Te.09, respectively) were lower than those for *Thr*. Further, a correlation with *YldPl* was observed only in Te.09 (r = –0.163) and was only marginally significant. As expected, the yield components *Pp2* and *Pp*1 were always highly positively correlated with yield in all environments, with correlation coefficients between +0.885 (Mi.08) and +0.998 (Te.09). The difference between the correlations of *Pp1* and *Pp2* with *Yld* was highest in Mi.08 (r-values of +0.199 and +0.300, respectively) and much lower in the other three environments. The second yield component, *YldPl*, was also always positively correlated with yield, but showed higher r-values (+0.371 and +0.441 for Te.08 and Te.09, respectively) in Tengeru than in Miwaleni (+0.192 and +0.221 for Mi.08 and Mi.09, respectively). *YldPl* was highly negatively correlated with *Pp1* and *Pp2* in all environments. Higher correlation coefficients were observed in Miwaleni than in Tengeru, with larger differences between *Pp1* and *Pp2* (Table 2-6 and Table 2-7). Significant correlations between *GrWt* and *Yld* were observed only in Mi.09 and Te.08, with correlation coefficients of –0.169 and –0.299, respectively. In these two environments, accessions bearing larger grains tended to have lower yields. In all environments, *GrWt* was negatively correlated with *Pp1* and *Pp2*. Except during the 2009 season at Tengeru, *GrWt* was positively correlated with *YldPl* in all environments. As expected, *GrWt* was highly positively correlated with *SdSz* in all environments, with r-values ranging from +0.825 in Mi.08 to +0.658 in Te.09. Both *GrWt* and *SdSz* were positively correlated with *PdHr* in all environments. The correlation was always stronger for *GrWt* than for *SdSz*. The r-values for the correlation between *GrWt* and *PdHr*

were +0.309, +0.261, +0.343 and +0.321 in Mi.08, Mi.09, Te.08 and Te.09, respectively. In Mi.09, *PdHr* was also positively correlated with *Yld*, with an r-value of +0.133.

Table 2-8: Different traits and their corresponding r2 values and effect on Yld, GrWt and FwT at different environment.

| | *Yld* | | | *GrWt* | | | *FwT* | | |
|---|---|---|---|---|---|---|---|---|---|
| Env. | factors | effect | r² (%) | factors | effect | r² (%) | factors | effect | r² (%) |
| Mi.08 | *FwT* | –3.89 | 4.6 *** | *PdHr* | +0.30 | 1.2 *** | *GrH* | +6.68 | 10.4 *** |
| | *LfC* | +26.28 | 2.2 *** | | | | *Aph* | +1.60 | 8.4 *** |
| | | | | | | | *Pp1* | –0.19 | 3.5 *** |
| Mi.09 | *FwT* | –7.90 | 2.0 *** | *FwT* | +0.09 | 3.1 *** | *Pp1* | –0.15 | 4.8 *** |
| | | | | *PdHr* | +0.34 | 2.1 *** | *GrH* | +1.65 | 2.8 *** |
| Te.08 | *Aph* | –20.79 | 8.1 *** | *PdHr* | +0.42 | 3.6 *** | *GrH* | +2.33 | 2.2 *** |
| | *Thr* | –10.32 | 3.0 *** | | | | | | |
| Te.09 | *Thr* | – 27.65 | 7.8 *** | *Thr* | –0.28 | 4.0 *** | *GrH* | +1.55 | 5.0 *** |
| | *FwT* | –8.08 | 3.0 ** | *PdHr* | +0.31 | 2.1 *** | *Aph* | +0.25 | 2.4 *** |
| | | | | | | | *Pp1* | –0.12 | 1.8 *** |
| | | | | | | | *Thr* | +0.28 | 1.3 *** |

Env. = environment (Mi. = Miwaleni, Te. = Tengeru, .08 = 2008, .09 = 2009)
effect = estimated effect, r² = partial r² with corresponding significance
(*, **, ***: significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively).

The results of the step-wise multiple-regression analysis of various factors related to yield, grain weight and flowering time are presented in Table 2-8. For *Yld*, *Fwt* was included in the final model for three out of four environments. The effect was always negative, meaning that early-flowering plants had higher yields. This factor explained 2.0-4.6% of the phenotypic variation. At Tengeru, *Thr* influenced *Yld* during both seasons, but with a higher explained variance (7.8%) in Te.09 than in Te.08 (3.0%). In Te.08, *Aph* had the strongest effect on *Yld* (–20.8 with an explained variance of 8.1%). In Mi.08, *LfC* showed a strong positive estimated effect but explained only 2.0% of the phenotypic variance. In all four environments, *PdHr* showed a significant positive effect on *GrWt*, explaining 1.2-3.6% of the phenotypic variation. In Mi.09, *FwT* had a positive effect on *GrWt*, explaining 3.1% of the variation. In Te.09, *Thr* explained 4% of the genetic variation of *GrWt*. In all four environments, *FwT* was significantly influenced by *GrH*, which explained up to 10.4% of the phenotypic variation (Mi.08). In Mi.08, Te.08 and Mi.09, *Ppl* contributed significantly to the variance in *FwT*. The estimated effect was always negative; thus, sparse stands tended to show delayed flowering compared to dense stands. In addition, *Aph* (in Mi.08 and Te.09) and *Thr* (in Te.09 only) were

significant factors in the regression model. Their effect was always positive, indicating that late flowering occurred in plots with stronger infestations. The estimated effect of *Aph* was largest in Mi.08, delaying flowering by 1.6 days (8.4% explained variance).

### *2.5.3 Selection of the best accessions*

Among the 200 accessions that were evaluated, the accessions with the lowest and most stable infestation levels for *Aph* (Table 2-9) and *Thr* (Table 2-10) together with above-average yields and yield-stability values were selected. Table 2-9 and Table 2-10 show additional traits characterising these accessions.

Table 2-9: Best 10 accessions in respect to means and stability measure ($S^2$) for *Aph* and *Yld* in relation to other selected important traits.

| Acc. | *Aph* | | *Yld* | | *Thr* | *GrH* | *FwT* | *GrWt* | *LfC* | *PdH* |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | $S^{2\prime}$ | Mean | $S^{2\prime}$ | Mean | Mean | Mean | Mean | Mean | Mean |
| 001 | 1.0 | 0.0 | 514 | 21.9 | 2.7 | 2.8 | 52.5 | 13.4 | 5.7 | 4.2 |
| 163 | 1.0 | 0.0 | 223 | 28.1 | 2.3 | 3.0 | 62.5 | 14.5 | 3.0 | 3.7 |
| 024 | 3.0 | 3.1 | 504 | 53.8 | 3.7 | 3.0 | 58.0 | 9.9 | 6.8 | 4.7 |
| 022 | 3.2 | 1.0 | 433 | 53.8 | 4.1 | 3.0 | 58.4 | 11.0 | 5.7 | 3.3 |
| 041 | 3.2 | 1.0 | 564 | 131.1 | 4.8 | 2.7 | 53.4 | 12.7 | 4.3 | 3.3 |
| 129 | 3.2 | 1.4 | 327 | 7.7 | 4.8 | 3.0 | 57.8 | 13.1 | 7.0 | 3.9 |
| 016 | 3.2 | 1.9 | 518 | 42.0 | 3.4 | 2.8 | 53.7 | 15.9 | 6.3 | 4.5 |
| 066 | 3.2 | 1.9 | 366 | 38.7 | 5.2 | 3.0 | 59.1 | 14.2 | 5.8 | 3.7 |
| 142 | 3.2 | 2.4 | 361 | 77.9 | 5.2 | 3.0 | 57.1 | 11.4 | 6.3 | 3.3 |
| 019 | 3.3 | 0.2 | 315 | 62.9 | 4.1 | 3.0 | 64.6 | 11.0 | 6.4 | 3.8 |
| μ | 4.6 | 3.1 | 391 | 63.8 | 4.8 | 2.8 | 56.3 | 12.8 | 6.0 | 3.9 |

μ = mean for all 200 accessions, $S^{2\prime} = S^2/1000$

Accession 001 had the lowest aphid infestation (1.0) and also produced a good yield level (514 g/plot) and yield stability ($S^{2\prime}$ = 21.9) relative to the trial means (391 g/plot and $S^{2\prime}$ = 63.8). This accession is listed in the third position in Table 2-10 because of its low infestation level and high stability for *Thr*. This accession is characterised by indeterminate growth and relatively late flowering; its values of *GrWt*, *LfC* and *PdH* are close to the overall average values. The second accession listed in Table 2-9 (163) occupies the first position in Table 2-10 because of its low overall mean value for *Thr* (2.3), even though its environmental variance for *Thr* was relatively high ($S^{2\prime}$ = 5.3) and its yield was relatively low (223 g/plot). Like all other accessions in Table 2-9, it exhibited indeterminate growth. This accession also

flowered relatively late and had a very pale colour (*LfC* = 3.0). These two accessions were the only ones with consistently lowest levels of *Aph* in all environments and replicates.

Table 2-10: Best 7 accessions in respect to means and stability measure ($S^2$) for *Trh* and *Yld* in relation to other selected important traits.

| Acc. | *Trh* | | *Yld* | | *Aph* | *GrH* | *FwT* | *GrWt* | *LfC* | *PdH* |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | $S^{2\prime}$ | Mean | $S^{2\prime}$ | Mean | Mean | Mean | Mean | Mean | Mean |
| 163 | 2.3 | 5.3 | 223 | 28.1 | 1.0 | 3.0 | 62.5 | 14.5 | 3.0 | 3.7 |
| 117 | 2.5 | 0.1 | 303 | 78.9 | 5.4 | 3.0 | 63.2 | 14.8 | 5.0 | 4.1 |
| 001 | 2.7 | 1.0 | 514 | 21.9 | 2.7 | 2.8 | 52.5 | 13.4 | 5.7 | 4.2 |
| 043 | 3.2 | 0.6 | 391 | 57.3 | 4.3 | 2.7 | 53.3 | 10.9 | 6.3 | 3.7 |
| 028 | 3.2 | 0.6 | 346 | 31.8 | 3.4 | 1.7 | 49.2 | 15.1 | 7.0 | 4.0 |
| 136 | 3.3 | 1.0 | 331 | 76.3 | 4.3 | 3.0 | 65.5 | 8.7 | 7.0 | 3.7 |
| 065 | 3.7 | 0.4 | 376 | 62.7 | 4.8 | 2.8 | 56.2 | 15.2 | 7.0 | 5.1 |
| μ | 4.8 | 2.2 | 391 | 63.8 | 4.6 | 2.8 | 56.3 | 12.8 | 6.0 | 3.9 |

μ = mean for all 200 accessions, $S^{2\prime} = S^2/1000$

Excluding these two accessions, there was not even a spurious correlation between *Aph* and *Thr* (data not shown). The other eight selected accessions listed in Table 2-9 only showed infestation levels between 3.0 and 3.3. Accession 041 produced the highest yield (564 g/plot) in this group, but also had the highest environmental variation for yield ($S^{2\prime}$ = 131.1). The best accessions chosen on the basis of *Thr* infestation levels (Table 2-9) did not include any accessions that were infestation free, as 117 and 001 were for *Aph*. Nevertheless, the accessions shown in this table combined low infestation levels and environmental stability for *Thr*, with the exception of accession 163. The ranges of *GrH*, *FwT*, *GrWt*, *LfC* and *PdH* values among these lines were representative of the whole set of 200 accessions.

## 2.6 Discussion

Four environments with contrasting temperature, humidity and rainfall levels were chosen for the experiment (Table 2-2 and Table 2-3). In general, the Miwaleni site was characterised by higher temperatures (average across both seasons: 25.9ºC), higher relative humidity (average across both seasons: 59%) and lower rainfall (sum across both seasons: 50 mm) compared to Tengeru (21.3ºC, 52% and 515 mm, respectively). At both locations, less rainfall occurred during the off-season period in late 2008 compared to early 2009. This difference was larger at Tengeru (42 vs. 472 mm in 2008 and 2009, respectively) than at Miwaleni (20 vs. 30 mm

in 2008 and 2009, respectively). These climatic differences affected the traits observed in the four environments (Table 2-4).

Flowering time is influenced by both intrinsic and extrinsic factors. In particular, its onset is modulated by temperature and photoperiod in cowpea and other annual crops (Njoku, 1958; Vince-Prue, 1975; Hadley et al., 1983). Thus, the differences in flowering time among the cowpea genotypes in our study may be partly associated with the differences in temperature between the four environments. We found a strong association between the temperature during the first two months of cowpea growth and the date of flowering (Table 2-2, Table 2-3 and Table 2-4), resulting in earlier flowering at Miwaleni than at Tengeru and in the 2009 season compared to the 2008 season. Even though photoperiod is an important factor affecting flowering time, as mentioned above, we did not consider it in this study because the experiments were conducted in the equatorial region, where the difference between day and night lengths at any particular time of the year is small.

Climatic factors, such as extremely high and low temperatures, high precipitation and low humidity, decreased the abundance of thrips and aphids. Except at Miwaleni in 2009, where we found no infestation of thrips or aphids, the infestation intensity of thrips was associated with temperature, showing the highest infestation levels at Miwaleni in 2008 and the lowest infestation levels at Tengeru in 2008, which was the coolest environment. The absence of infestation by both pest species at Miwaleni in 2009 may be due to the excessively high temperature (27.6°C) and low humidity (53%) coupled with high wind velocity (data not provided) experienced in this environment. These climatic factors probably exacerbated desiccation, leading to pest population crashes. On the other hand, our results showed that in a situation of low rainfall (36.1 mm) and moderate temperatures (19.5–25.7°C) and humidity (56%), infestation was inevitable under the conditions of our experiment, especially during the first two months of crop establishment, when thrips populations build up.

Further, the results of our trial showed that relatively lower temperatures coupled with slightly wet conditions favoured aphid infestations (Table 2-2, Table 2-3 and Table 2-4). In this point, our results agree with the findings of Hasan et al., (2009) and Aheer et al., (2007). According to Hasan et al.,(2009), high cloudiness and relatively high humidity and dew point favoured aphid populations on mustard plants, while slight rainfall quickly decimated the aphid population. They further reported that high maximum temperatures, high dew points and longer periods of sunshine positively affected aphid numbers, while high minimum

temperatures, high relative humidity and high wind speeds negatively affected aphid numbers. According to Aheer et al., (2007), relatively high humidity (65%), minimum temperature (9.57°C) and maximum temperature (28.3°C) are optimum conditions for the development of aphid populations. Thus, we found that thrips react differently to environmental factors than aphids. While the magnitude of thrips infestation can mostly be explained by temperature, especially in the first months of crop development, aphid infestations are determined by both temperature and atmospheric humidity. Therefore, thrips infestations were highest at Miwaleni during the 2008 season, which was characterised by low rainfall and relatively high temperatures and humidity, while aphid infestations were highest at Tengeru during both seasons, where we observed relatively low humidity and temperatures coupled with light dew during the first three months of crop growth.

Growth type, pod hairiness and leaf colour are highly heritable traits of cowpea plants (Table 2-4) and are therefore independent of climatic influences. Even though leaf colour can also be influenced by the nutrient status of the plant, our results show that a darker colour is characteristic of indeterminate plants and also shows high heritability (Table 2-6 and Table 2-7). One explanation may be genetic linkage between genes influencing these two traits. Githiri et al.,(1996) have found a recombination level of 26% between the indeterminate growth habit and peduncle colour in cowpea. The correlation between leaf colour and growth type is used by farmers in East Africa to select cowpea genotypes for vegetable use because indeterminate cowpeas continuously provide fresh leaves and pods, thus ensuring a long period of harvesting. Growth habit also influences flowering time (Table 2-6, Table 2-7 and Table 2-8); determinate types enter into the reproductive phase earlier than indeterminate ones. On the other hand, growth habit showed no direct effect on grain yield, even though we would expect at least an indirect correlation due to the strong correlations between flowering time and yield and between growth habit and yield. This lack of correlation may be due to the fact that growth habit also influenced other factors that in turn influenced yield-related traits in the opposite direction. Further, our results showed that in both off-season environments, thrips preferred indeterminate plants, likely because of the continuous production of flower buds over a long period of time (overlap between the vegetative and reproductive phases). Flower buds are the preferred plant part for by thrips. Pod hairiness, another highly heritable phenotypic trait of the cowpea accessions, positively influenced both seed weight and seed size (Table 2-6, Table 2-7 and Table 2-8). In the multiple-regression model, pod hairiness was the only factor that had a significant positive influence on grain weight in all environments,

especially during the dry season in 2008 (Table 2-8). This relationship has not been reported previously for cowpea. Pod hairs probably play a role in minimising evapo-transpiration through the pod surface, resulting in proper grain filling due to adequate moisture-retention time to achieve the important physiological processes. Our results suggest that pod hairiness is an important trait contributing to drought-stress tolerance in cowpea and therefore should be selected for when seeking cowpea accessions that are adaptable to dry environments.

In three out of four environments, multiple-regression analyses identified flowering time as a factor that influenced yield (Table 2-8). In these environments, earlier flowering times significantly contributed to increased grain yields. Similarly, Umar et al., (2010) have reported significant negative correlations between flowering time and pods per plant and between flowering time and seeds per pod ($r = -0.6011$ and $r = -0.6159$, respectively). At Tengeru in 2008, where aphids significantly affected yields, we detected no effect of flowering time, likely because the severe aphid damage caused stunted growth. Altogether, under conditions of either absence or moderate levels of insect-pest infestation, early-flowering genotypes produced relatively higher yields. Because flowering time and not growth type was more strongly correlated to grain yield (Table 2-6 and Table 2-7) and was selected in the multiple regression (Table 2-8), we conclude that this effect was not caused indirectly by growth habit. Nevertheless, indeterminate accessions usually also exhibit late flowering. On the other hand, these types offer multiple simultaneous uses (leaves, fresh pods and dried beans) and are therefore preferable for small-scale farmers (Asiwe, 2007).

As expected, plant density at harvest (a yield component) was positively related to yield. Furthermore, this yield component was negatively related to grain weight and seed size, suggesting that higher plant-population density leads to high grain yields at the expense of relatively small seeds compared to low-density conditions. This effect is probably caused by stronger competition for resources, such as light, water and nutrients, in a denser stand. This conclusion is also supported by the fact that plant density before thinning was strongly negatively correlated to flowering time, suggesting that the onset of flowering occurred earlier in plots containing dense plant populations and therefore lower resource availability per plant. This result is similar to that of Samih, (2008), who has reported that early flowering in beans (*Phaseolus vulgaris* L.) is significantly associated with high population density and vice versa. Also, Willenborg et al., (2009) have reported that dense plant populations in spring wheat tend to accelerate flowering time. Further, the earlier flowering in denser stands that

results in higher yields may also indirectly influence the above-mentioned correlation between flowering time and yield. Interestingly, grain weight (the second yield component analysed) was negatively correlated with yield. This correlation may be attributable to the above-mentioned negative correlation between plant density and grain weight.

We also observed positive relationships between plant density and thrips populations but negative relationships between plant density and aphid populations. Dense stands disfavour aphids and favour thrips (Karungi et al., 2000a). Gethi and Khaemba, (1991) have also reported a preference of thrips for dense cowpea stands. Several other studies have reported that low plant density favours aphids (Edema and Adipala, 1996; Edema et al., 1997; Karungi et al., 2000a; Karungi et al., 2000b). The reason for the different behaviour of these two pest species with respect to cowpea plant-population density might be that thrips, whose feeding is limited to tender shoots and flowers, prefer higher plant densities that provide sufficient refuge. Aphid colonisation and fecundity, in contrast, are reduced by high plant density through interference with their visual systems (Kennedy et al., 1961; A'Brook, 1964; Naidu et al., 1998). This finding is important for the appropriate planning of experiments involving these two pests, especially when high infestations are required, and for managing these pests in a production scheme by means of manipulating sowing density.

We found limited correlations between yield and yield stability among the 200 cowpea accessions studied (Fig. 2-1). Even though the highest-yielding accessions (about 620 g/plot) were the most unstable, with an environmental variance of about 0.225, the correlation between yield and yield stability was generally rather weak. Thus, many above-average-yielding genotypes in our collection showed high environmental yield stability. The highest-yielding landrace in our collection produced about 2.97 t $ha^{-1}$ (average of all four environments, extrapolated from the plot size), while the lowest-yielding landrace produced 0.92 t $ha^{-1}$. The yields normally obtained by farmers in East Africa and Nigeria are only about 200-400 kg $ha^{-1}$ and 200-300 kg $ha^{-1}$, respectively (Nabirye et al., 2002). Although research-managed and farmer-managed cowpea production is difficult to compare, these values show the range of potential yields for these landraces even under infestation by thrips, aphids and other field pests.

Among the large number of accessions that we tested across four environments, we selected some genotypes that exhibited promising resistance to either aphids (Table 2-9) or thrips (Table 2-10).

Accessions with consistently lower aphid and thrips infestations across locations and over seasons suggested possible host-plant resistance to these two pests, while accessions that showed high environmental variance of infestations might have simply escaped infestation. Accession 001 has shown to have a combined stable resistance to both aphids and thrips and was also among the highest yielders (514 g/plot) compared to the trial mean of (391 g/plot). It is an indeterminate, relatively early-flowering accession with relatively heavy beans and a dark leaf colour. In contrast, accession 163, which showed the lowest thrips and aphid infestations among all accessions, had below-average yields under infestations of both pests. However, the stability of its resistance against thrips was low compared to the experimental mean. This indeterminate accession is late flowering and has large beans and a rather light leaf colour. Other accessions showed high yields but relatively low pest tolerance. For example, accession 041 produced the highest yields (564 g/plot) but was not among the best in terms of aphid resistance and was the least stable. We found two lines that remained free of aphids across all replicates and environments (the above-mentioned lines 001 and 163). For resistance against thrips, only quantitative differences could be detected. The mean of the selected accessions did not differ from the overall mean for any of the physiological traits shown in Table 2-9 and Table 2-10. Consequently, none of these characteristics alone can be used to identify superior genotypes.

The lines that we selected are a starting point for a breeding program to improve cowpea yields under both abiotic- and biotic-stress conditions in Eastern Africa. We have characterised these lines and shown which traits are advantageous under the climatic and agronomic conditions of our experiment and which desired traits conflict with each other. Our results show that, at least at the actual yield level of landraces, it is possible to combine high yield with high yield stability. Further, our results show which traits and conditions favour two important cowpea pests, aphids and thrips. These results can be used to improve and adapt the best of these lines to a given environment through directed crosses and selection and to identify conditions that will maximise their yield potential.

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# 3 PAPER 2: HIGH GENETIC DIVERSITY WITHIN EASTERN AFRICAN COWPEAS [*VIGNA UNGUICULATA* (L.) WALP.] AS REVEALED BY SSR MARKERS

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## 3.1 Abstract

The cowpea [*Vigna unguiculata* (L) Walp.] is a diploid crop that grows in a wide range of environments between 40°N and 30°S. This species has considerable ability to adapt to high temperatures and drought compared to most crop species. Cowpea is used for both food and feed. All edible parts of the plant are rich in protein, thereby providing a cheap and reliable source of protein for resource-limited people in both rural and urban areas. Three hundred twelve cowpea accessions were used in this study. Of these, 288 were cultivated landraces that were collected from farmers' fields across 14 regions of mainland Tanzania, and 24 were wild accessions obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Twenty-six nuclear microsatellite markers were used to explore the genetic diversity of these landraces and wild accessions. A high genetic diversity level maintained in the Tanzanian landraces was observed, compared to the wild accessions included in the study. The geographic distance between the collection sites of the accessions did not correlate with their genetic distance. Structural analysis revealed a clear genetic structure in the analysed 312 cowpea accessions from East Africa. The structure data suggest that Tanzanian cultivated cowpeas are divided into two groups, of which one originates from a Kenyan wild species, which is related to spp. *dekindtiana,* and the second is either a product of hybridisation between cultivated cowpeas and Tanzanian wild cowpeas, ssp. *pubescens*, or a product of an independent domestication event. The genetic diversity richness in cowpea landraces found in Tanzania could be a reliable source for important traits that will help to improve this crop in national and international breeding programs.

**Key words:** *Vigna unguiculata* (L.) Walp, Genetic diversity, domestication, Tanzania

## 3.2 Introduction

The cowpea [*Vigna unguiculata* (L.) Walp.] is a diploid crop containing 22 chromosomes, and its nuclear genome size is estimated to be 620 million base pairs (Mbp) (Timko et al. 2008). The crop is presumed to have originated in Africa, as wild cowpea only exists in Africa (Steele 1976). Based on the studies conducted by Coulibaly et al. (2002), the domestication occurred in Northeastern Africa. Timko et al. (2008) stated that the genus *Vigna* is divided into subgenera based upon morphological characteristics, the extent of genetic hybridisation and the geographical distribution of the species. The major groups consist of African sub-genera *Vigna* and *Haydonia*, Asian sub-genus *Ceratotropis*, and the American sub-genera *Sigmoidotropis* and *Lasiopron. V. unguiculata* sub-species *unguiculata* includes four cultivated groups: *unguiculata*, *biflora* (or *cylindrical*), *sesquipedalis*, and *textilis*. *V. unguiculata* subspecies *dekindtiana*, *stenophylla*, and *tenuis* are intermediate wild progenitors of the cultivated cowpea that form a major portion of the primary cowpea gene pool (Ng and Maréchal 1985).

The classification and nomenclature of wild cowpea taxa within *Vigna unguiculata* is complicated and subject to discussions amongst taxonomists (Singh 1997). Padulosi (1993) classified wild *V. unguiculata* species into three subspecies (spp.): spp. *dekindtiana* that according to Ng (1995) is very similar to the cultivated *V. unguiculata*, spp. *protracta* and spp. *pubescens*. The former spp. *Burundiensis* that is found in Kenya, Burundi, Zaire and Uganda merged with spp. *dekindtiana* according to Pasquet (1993a). Spp. *pubescens* is primarily found in Tanzania (Padulosi and Ng 1997). In contrast to ssp. *dekindtiana*, which is part of the primary gene pool of cowpea, this subspecies does not readily hybridise and shows some degree of pollen sterility (Fatokun and Singh 1987).

Cowpea grows in a wide range of environments covering 40°N to 30°S (Richie 1985), and it has considerable ability to adapt to high temperatures and drought compared to most crop species (Ehlers and Hall 1997). In an environment where other crops fail due to a shortage of soil moisture, cowpea survives. Hall (1985) reported a cowpea dry grain yield as high as 1000 kg $ha^{-1}$ that was obtained in the Sahelian environment with low humidity and only 181 mm of rainfall. Cowpea is a multi-functional crop because it provides both food for human beings and feed for animals. The young tender leaves, green pods and green beans form part of the human diet where it is grown. The dried beans are traded and eaten far beyond the limits of its cultivation area. The crop is a source of income to both small-scale farmers (especially women who farm) and larger scale grain traders (Singh 2005; Timko and Singh 2008). The

protein content ranges from 29 to 43% based on dry weight, with the highest values in younger leaves (Nielsen et al. 1997). Like other grain legumes, the protein found in cowpeas is rich in the essential amino acids lysine and tryptophan (Timko and Singh 2008). The protein nutritive value of these legumes, however, is lower than that of animal proteins because of their relatively low content of sulphur amino acids and anti-nutritional factors (phytates and polyphenols), enzyme inhibitors (trypsin, chymotrypsin and R-amylase) and haemagglutinins (Jackson 2009). Minerals and vitamins are other important constituents of the cowpea seeds. It has been reported that the content of folic acid, a B vitamin necessary during pregnancy to prevent birth defects in the brain and spine, is present in high quantities in cowpeas compared to other plants (Hall et al. 2003; Timko and Singh 2008). All of the edible parts of the cowpea are rich in protein. For this reason, it is an inexpensive source of protein for resource-limited people in both rural and urban areas.

Maintenance of genetic diversity of this crop is of paramount importance to ensure the adaptability of the crop to adverse and changing ecological conditions. The understanding of the genetic diversity and the genetic structure among the existing genotypes is a crucial initial step toward planning a comprehensive conservation strategy. The conventional methods for estimating genetic diversity have been based on the use of morphological markers. However, the low availability of those markers, the lack of knowledge about how genes are controlled, and the environmental influence on phenotypic expression at different stages of growth have been the major limitations for using these markers as reliable tools in diversity studies (Dikshit et al. 2007)

DNA polymorphisms have been extensively employed as a means of assessing genetic diversity in various organisms (Xiao et al. 1996). The use of molecular marker tools, such as Random Amplified Polymorphic DNAs (RAPDs) (Ba et al. 2004; Lakhanpaul et al. 2000; Santalla et al. 1998), Amplified Fragment Length Polymorphisms (AFLPs) (Zong et al. 2003), and microsatellites or Simple Sequence Repeats (SSRs) (Flajoulot et al. 2005; Wang et al. 2004), have greatly facilitated the analysis of the structure of plant genomes and their evolution, including the genetic structure and variations among cowpeas accessions (cultivated and wild). Microsatellite or Simple Sequence Repeat (SSR) markers have valuable properties, such as a high level of polymorphism and information content, unambiguous designation of alleles, even dispersal, selective neutrality, high reproducibility, high throughput applicability, co-dominance, and a rapid and simple genotyping assay (Timko et al. 2008; Wang 2002). The SSR markers are widely used in genotype identification, variety

protection, genetic mapping, genome analysis, seed purity evaluation, germplasm conservation (Brown et al. 1996; Nielsen et al. 1997; Senior et al. 1998), qualitative and quantitative trait locus analysis (Koh et al. 1996), marker assisted breeding (Ayres et al. 1997 and Weising 1998), diversity studies (Xiao et al. 1996), paternity determination, and pedigree analysis (Ayres et al. 1997; Bowers et al. 1999; Ven and McNicol 1996). Smith et al. (1997) and Senior et al. (1998) concluded that for measuring genetic diversity, assigning lines to heterotic groups, and fingerprinting, the discriminative power of SSRs is equal to or greater than that of RFLPs and is cost effective. SSRs have been used to investigate genetic diversity in various crops, including cowpea (Gillaspie et al. 2005; Li et al. 2001), maize (Inghelandt 2010; Senior et al. 1998) rice (Xiao et al. 1996; Yang et al. 1994), soybean (Rongwen et al. 1995), and wheat (Plaschke et al. 1995).

To provide the future selection and breeding of cowpeas in Tanzania and East Africa with the necessary knowledge about the genetic richness and composition of the available genetic resources, the present work focuses on the analysis of the genetic diversity and genetic structure of cultivated and wild cowpea accessions collected within East Africa. Further, the consequences of the respective findings, for both conservation strategies and crop improvement, are discussed.

## 3.3 Material and Methods

### *3.3.1 Plant Material*

Three hundred twelve cowpea accessions were used in this study. Out of these, 288 were domesticated accessions that were collected from farmer's fields across 14 regions of mainland Tanzania, and 24 were wild accessions obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The origin of the accessions and collection place (country, agricultural zone and region) are presented in Table 3-1. All domesticated accessions were multiplied from single seeds to acquire genetically uniform accessions. Two seeds from each accession were sampled and sown in a glass house at the University of Copenhagen to obtain young leaves from the seedlings for DNA extraction.

Table 3-1: Countries and different, regions and agro-ecological zones of Tanzania with amount of collected, domesticated and wild cowpea.

| Accession | Country | Agro-ecological zone | Region | Amount collected |
|---|---|---|---|---|
| Domesticated | Tanzania | Southern highlands | Mbeya (MB) | 1 |
| | | | Sumbawanga (SB) | 2 |
| | | Western | Kigoma (UJ) | 4 |
| | | | Tabora (TB) | 104 |
| | | Lake | Mwanza (MZ) | 23 |
| | | | Bukoba (BK) | 2 |
| | | | Mara (MR) | 6 |
| | | Central | Singida (SD) | 42 |
| | | | Dodoma (DO) | 51 |
| | | Eastern | Morogoro (MG) | 14 |
| | | | Tanga (TA) | 2 |
| | | Southern | Mtwara (MT) | 15 |
| | | | Lindi (LD) | 14 |
| | | | Ruvuma (RV) | 8 |
| Wild | Tanzania | | | 15 |
| | Kenya | | | 8 |
| | Uganda | | | 1 |

### *3.3.2 Genomic DNA Extraction*

A single leaf from a 15-day-old cowpea seedling from each accession was cut, put in a 1.5 mL Eppendorf tube and freeze-dried for 48 hours. DNA isolation using Cetyl Trimethyl Ammonium Bromide (CTAB) (100 mM Tris-HCl, pH 7.5, 660 mM NaCl, 10 mM EDTA, 140 mM ß-mercaptoethanol, and 10% w/v CTAB) from the milled, freeze-dried leaves was performed according to the protocol described by Saghai Maroof et al. (1984) with minor modifications.

### *3.3.3 SSR Assay and PCR Amplification*

All of the samples were tested for 26 nuclear microsatellite markers. Their names, linkage groups, sequences, sources of the marker, references, numbers of alleles and polymorphic information contents (PICs) are listed in Table 3-2. Of these markers, 14 originated from *V. unguiculata,* and 12 originated from either *V. umbelata* or *V. nakashimae*. Sequence-specific

forward primers with an M13 tail at the 5´ end of the marker and reverse primers, together with universal fluorescently labelled M13 primers, were used for labelling (Schuelke 2000). The universal primer was labelled with FAM (blue, Tetrachloro-6-carboxyfluorscein), NED (green, 5-fluoroscein phosphormidite) or VIC (yellow, Hexachloro-6-carboxyfluorescein) fluorescent dyes. PCRs for nuclear microsatellites were performed in Thermo-Fast 96-well plates from ABgene in a final reaction volume of 10 µl, containing 100 ng template DNA, ammonium buffer (1.5 mM $MgCl_2$), 1.5 mM $MgCl_2$, 200 µM dNTP-mix, 50 nM forward primer with M13 tail, 200 nM reverse primer, 250 nM M13 primer and 0.5 U Taq DNA Polymerase. PCR reactions were carried out in a GeneAmp® PCR system 2700 thermal cycler from Applied Biosystems using a touchdown program with the following amplification profile: one initial cycle at 94°C for a 3 min. denaturation; followed by 18 touch-down cycles at 94°C for 1 min., 64°C down to 56°C for 1 min. and 72°C for 1 min.; then 20 cycles at 94°C for 1 min., 55°C for 1 min., and 72°C for 1 min.; and finally, a 1 cycle extension at 72°C for 5 min.

### *3.3.4 Fragment Detection and Genotyping*

The amplified fragments were detected using an AB 3130XL DNA analyser (sequencer). The PCR products from the same accession but from the three reactions with primers labelled with different colours were mixed into one micro-well plate. Fifteen microliters of a mixture of a ROX-labelled size standard and formamide were aliquoted into each micro-well together with 5 µl of the PCR product mixture of the three different primers. The mixture was denatured for 2 min. at 94°C and transferred to the sequencer.

### *3.3.5 Statistical analysis*

DNA fragment analysis and genotyping was performed using the GeneMarker Genotyping, Software version 1.75 (Soft genetics, State College, PA, USA). All genetic analyses were performed in an Excel (Microsoft Excel v. 2007, Redmond, USA) VBA application package programmed at the Institute of Agriculture and Ecology in Copenhagen, except for the AMOVA, which was calculated in GenAlEx (Peakall and Smouse 2006) using 999 permutations. Nei's gene diversity (Nei 1973) for each marker was calculated according to the following formula where $p_i$ is the frequency of the single allele of $i^{th}$ individual and n is the number of individuals. The PIC was calculated using the formula as follows: PIC = 1-. (Botstein et al. 1980), where $p_i$ is the frequency of the allele in the $i^{th}$ individual and $n$ is the number of alleles per marker. The genetic distance was calculated using modified Roger's

distance (Wright 1978) according to the following formula: where *p* and *q* are allele frequencies of the two accessions, *n* is the number of alleles and *m* is the number of loci. The resulting matrix of genetic distances was used in a non-parametric multi-dimensional scaling (MDS) by applying the R Statistics Package (R development Core Team 2008) with the MASS package (Venables and Ripley 2002). A matrix of geographic distances was calculated based on the GPS positions of the collection sites assuming the Earth to be a perfect globe with a radius of 6371 km. A Mantel test of the matrix of the genetic vs. the matrix of geographic distances was performed using the mentioned VBA application with 999 permutations. A Bayesian structure analysis was carried out by calling the software STRUCTURE (Pritchard et al. 2000) from the VBA application 20 times for each group number k. For the determination of the most likely number of groups, the method of Evanno et al. (2005) was used.

## 3.4 Results

### *3.4.1 Marker Statistics and Differences between Groups of Domestication and Origin*

Twenty-six primer pairs that amplified clearly distinguishable polymorphic bands in our detection system were used to analyse 312 cowpea accessions, where 288 were cultivated landraces and 24 wild accessions. The names of these primers, their sequences, sources and references, the number of alleles per primer and the PICs that we found in the present analysis are listed in Table 3-2. The allele number per primer ranged from two to eighteen. The primer pairs for the marker VM24 amplified the largest number (22) of alleles, while VM13 yielded only two alleles.

Table 3-2: Linkage group for localized markers, name, sequence and source of the primer pairs, statistic information and references.

| Linkage group | Name | Primer sequence | Source | Reference | No of alleles | PIC |
|---|---|---|---|---|---|---|
| | VM05 | AGCGACGGCAACAACGAT<br>TTCCCTGCAACAAAAATACA | *V.ung* | Li et al 2001 | 4 | 0.332 |
| | VM12 | TTGTCAGCGAAATAAGCAGAGA<br>CAACAGACGCAGCCCAACT | | | 5 | 0.435 |
| | VM13 | CACCCGTGATTGCTTGTTG<br>GTCCCCTCCCTCCCACTG | | | 2 | 0.331 |
| | VM28 | GAATGAGAGAAGTTACGGTG<br>GAGCACGATAATATTTGGAG | | | 4 | 0.502 |
| | VM31 | CGCTCTTCGTTGATGGTTATG<br>GTGTTCTAGAGGGTGTGATGGTA | | | 6 | 0.433 |
| | VM36 | ACTTTCTGTTTTACTCGACAACTC<br>GTCGCTGGGGGTGGCTTATT | | | 4 | 0.373 |
| | VM39 | GATGGTTGTAATGGGAGAGTC<br>AAAAGGATGAAATTAGGAGAGCA | | | 6 | 0.038 |
| | VM40 | TATTACGAGAGGCTATTTATTGCA<br>CTCTAACACCTCAAGTTAGTGATC | | | 8 | 0.233 |
| | VM71 | TCGTGGCAGAGAATCAAAGACAC<br>TGGGTGGAGGCAAAAACAAAAC | | | 5 | 0.471 |
| | VM17 | GGCCTATAAATTACCCCAGTCT<br>TGTGTCTTTGAGTTTTTGTTCTAC | | | 6 | 0.105 |
| | VM23 | AGACATGTGGGCGCATCTG<br>AGACGCGTGGTACCCATGTT | | | 4 | 0.364 |
| | VM27 | GTCCAAAGCAAATGAGTCAA<br>TGAATGACAATGAGGGTGC | | | 17 | 0.167 |
| 8 | VM24 | TCGTGACCTAGTGCCCACC<br>TCAACAACACCTAGGAGCCAA | | Somta et al 2006 | 22 | 0.354 |
| 8 | VM37 | TGTCCGCGTTCTATAAATCAGC<br>CGAGGATGAAGTAACAGATGATC | | | 10 | 0.416 |
| 3 | CEDG043 | ACTATTTCCAACCTGCTGGG<br>AGGATTGTGGTTGGTGCATG | *V. umb/V. naka* | Somta et al 2006 | 13 | 0.304 |
| 10 | CEDG068 | TGGGATCAGTGAATTCGCCAG<br>TCTCCATAGGAACCCCTGAAAG | | | 4 | 0.380 |
| 4 | CEDG088 | TTGTTGTTTACTAAGAGCCCGTGT<br>TCTTGTCATTTAGCACTTAGCACG | | | 10 | 0.566 |
| 6 | CEDG118 | GCTGGAATCATAATACCGCCTTGT<br>AACCCAACCAACCCTTGTGGTAAG | | | 18 | 0.433 |
| 7 | CEDG143 | CTGGACGCGTCTACTCAGAC<br>GATGAACTCGTCTCGCTCATCG | | | 9 | 0.582 |
| 1 | CEDG149 | GGCACTGGTTTTCTAAGGTTGTTG<br>GGCTGAAGGTGATGACAGAAG | | | 11 | 0.171 |
| 10 | CEDG180 | GTGCGTGAAGTTGTCTTATC<br>GGTATGGAGCAAAACAATC | | | 10 | 0.157 |
| 1 | CEDG214 | CTACCTATCTGAGGGACAC<br>CACTCACTGCAAAGAGCAAC | | | 9 | 0.280 |
| 6 | CEDG248 | GTGGATTCACTCGCTTCC<br>CAGAACACAAAAGGGTTCTCG | | | 15 | 0.161 |
| 5 | CEDG268 | GCTATCAATCGAGTGCAG<br>CATCTCCCTGAAACTTGTG | | | 7 | 0.171 |
| 8 | CEDG271 | CACTCCCACTGCCAAACAAGG<br>GCACTAAAGTTAGACGTGGTTC | | | 11 | 0.551 |
| 9 | CEDG304 | GTTGCATGCTATATTTTGGTTCAC<br>ACCACTTCATAATCCCTGAG | | | 16 | 0.642 |
| Average for markers from | | *V. ung* | | | 7.6 | 0.325 |
| | | *V. umb*/*V.naka* | | | 11.1 | 0.367 |

*V.ung = Vigna unguiculata, V.umb = Vigna umbelata, V.naka = Vigna nakashimae,*

PIC = Polymorphic Information Content.

The PIC varied from 0.038 to 0.642, with an average of 0.344. Table 3-3 shows an overview of the average number of alleles, the average number of unique alleles and the gene diversity for the 288 cultivated accessions from Tanzania and the 24 wild accessions. These wild accessions were distributed in the collected countries as shown in brackets, from Tanzania (15), Kenya (8) and Uganda (1).

Table 3-3: Origin, respective total number of accessions and marker analysis summary.

| Accession | Origin | Total number | Avg. allele number | Avg. private allele number | Avg. Gene diversity (GD) | Std. dev. GD |
|---|---|---|---|---|---|---|
| Domesticated | Tanzania | 288 | 7.77 | 4.46 | 0.358 | 0.019 |
| Wild | Tanzania | 15 | 3.81 | 1.08 | 0.518 | 0.115 |
| | Kenya | 8 | 1.88 | 0.08 | 0.285 | 0.415 |
| | Uganda | 1 | 0.58 | 0.00 | 0.038 | 0.519 |
| All wild | | 24 | 4.50 | 1.23 | 0.540 | 0.096 |
| All accessions | | 320 | 9.08 | | 0.385 | 0.337 |

The average number of alleles obtained from the cultivated accessions was 7.77, and the average from the wild accessions was 4.50. The average number of unique alleles was 4.46 for cultivated accessions and 1.23 for wild accessions. In total, an average of 9.08 alleles per marker was obtained. As both the average number of alleles and the average number of unique alleles are affected by the sample size, which differed noticeably (Table 3-3), these values have to be compared carefully. In contrast, the average gene diversity is relatively robust against differences in sample size. All 24 wild accessions revealed a gene diversity of 0.540, which was significantly larger than the gene diversity of the cultivated accessions (0.358). Within the group of the wild accessions, the Kenyan accessions showed a lower gene diversity (0.285) than the Tanzanian accessions (0.518).

### *3.4.2 Genetic distance*

A distance matrix of all of the accessions was calculated using a modified Roger's distance. Out of 312 individuals analysed, 296 had unique genotypes based on their marker pattern with the 26 SSR markers. Five genotypes occurred twice, and one genotype occurred six times. The collection sites of the latter are indicated by a star in Fig. 3-1.

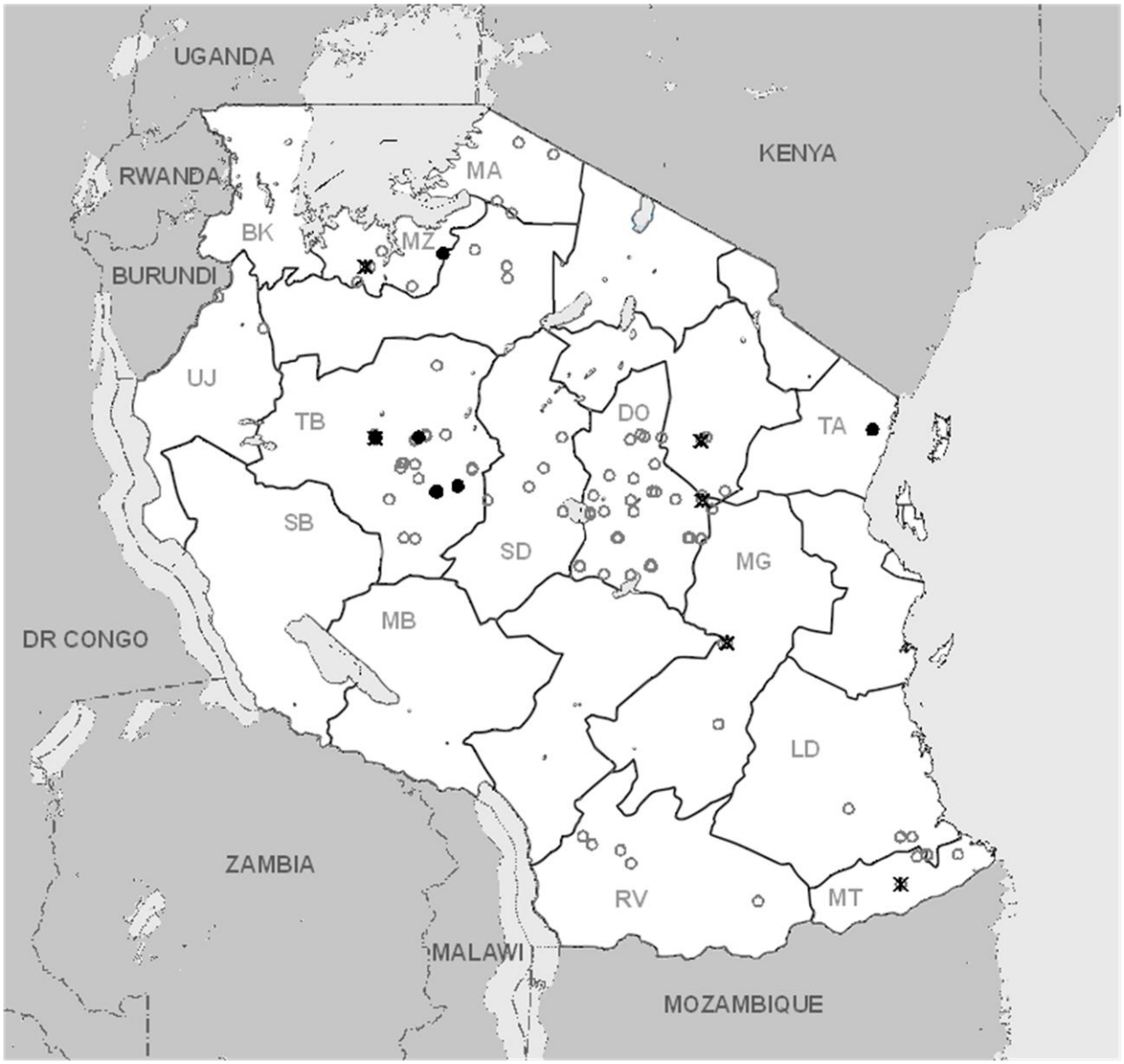


Fig. 3-1: Map of Tanzania showing precise location of collection of cultivated accessions. Filled dots = groups of domesticated cowpea with genetic similarity to wild accession, stars: abundant accession from Tanzania.

The diversity results were visualised using a non-metrical multi-dimensional scaling (MDS) on two dimensions as shown in Fig. 3-2, with cultivated accessions as open circles and wild accessions with different filled symbols according to their origin. The stress statistic of this analysis was 22.2%. Most of the cultivated accessions from Tanzania clustered together with the wild cowpeas from Kenya in the lower left part of Fig. 3-2. A smaller portion of the cultivated cowpeas from Tanzania clustered together with the wild Tanzanian cowpea accessions in the upper right part of the graph. Additionally, the single wild accession from

Uganda was found in this group. The area occupied by the Tanzanian wild cowpeas is larger than the one occupied by the Kenyan cowpeas, indicating higher gene diversity in the wild cowpeas from Tanzania. The modified Roger's distance was also calculated between the cultivated groups and their origin and is shown in Table 3-4. The lowest distance in this comparison is the one between the group of Tanzanian cultivated accessions and the wild cowpeas from Kenya (0.1175), while the genetic distance between the wild and the cultivated accessions from Tanzania was 0.4336.

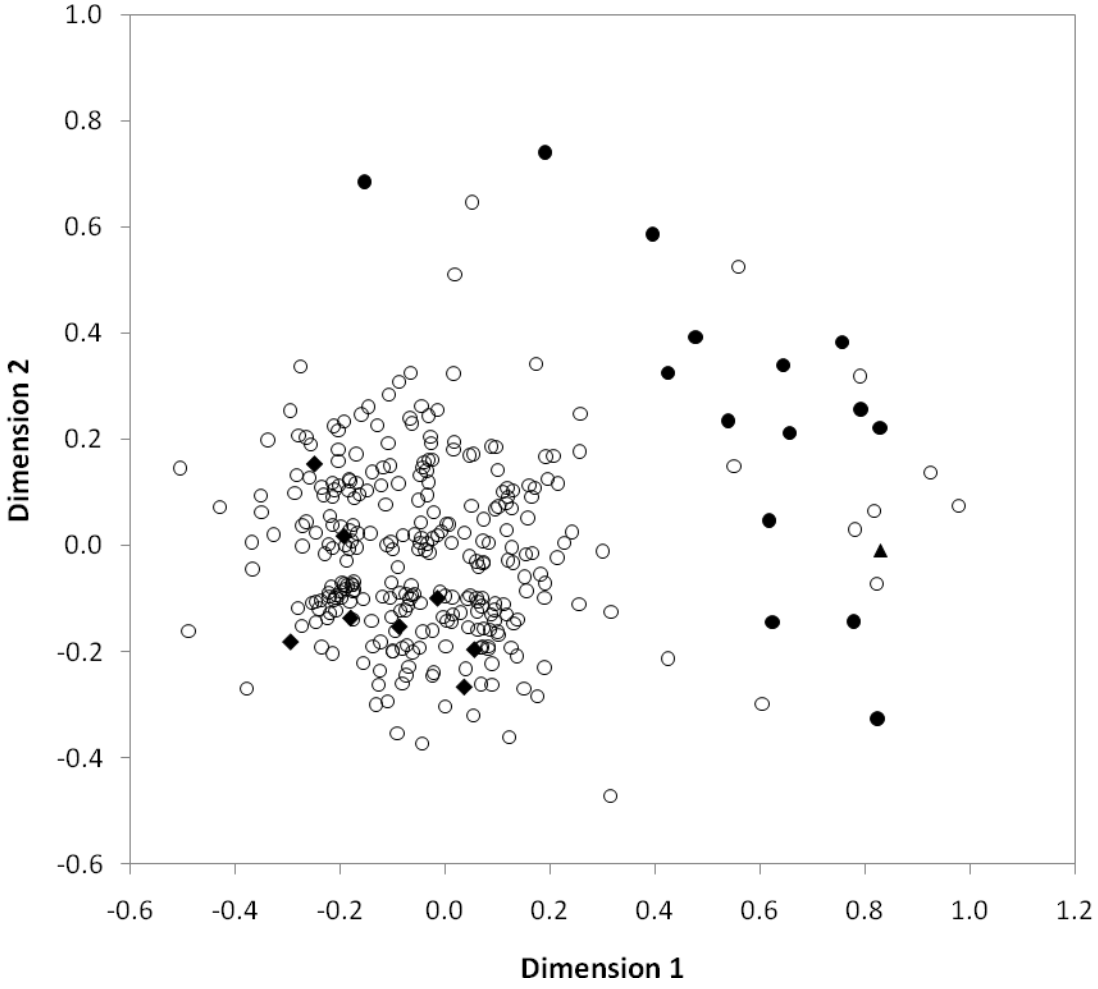


Fig. 3-2: Multiple dimensions (MDS) for 312 cowpea accessions based on 26 SSR loci. Solid symbol = wild accessions, open symbol = domesticated accessions, circles = Accessions from Tanzania, Diamond = accessions from Kenya and Triangle = accession from Uganda

Table 3-4: Modified Roger's distance between different countries accession groups.

| Country/group | | Domesticated | wild | | |
|---|---|---|---|---|---|
| | | Tanzania | Tanzania | Kenya | Uganda |
| Domesticated | Tanzania | 0.0000 | 0.4336 | 0.1175 | 0.6604 |
| Wild | Tanzania | 0.4336 | 0.0000 | 0.4793 | 0.4602 |
| | Kenya | 0.1175 | 0.4793 | 0.0000 | 0.6908 |
| | Uganda | 0.6604 | 0.4602 | 0.6908 | 0.0000 |

### *3.4.3 Genetic structure*

The structure analysis results are shown in Table 3-5. We found an optimum number of three groups. Group 2 was the largest group that contained 154 individuals, and group 3 was the second largest. Of the 288 cultivated accessions, 274 were found in these two groups, together with the 8 wild accessions from Kenya. In group 3, accessions from the central zone of Tanzania are present in a relatively higher number (71 out of 126) compared to the distribution in group 2 (47 out of 148), while accessions from the other zones showed a lower frequency in group 3 compared to group 2. Group 1 was the smallest of the structure-derived groups and contained only 30 individuals. All 15 wild accessions from Tanzania and the single wild accession from Uganda fell in this group. In addition, 14 of the 288 Tanzanian landraces were members of this group.

Table 3-5: Groups as resulting from structure analysis vs. domestication and zone of collection.

| Accession and country | Collection site | Structure groups | | | Total |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| Wild | Tanzania | 15 | | | 15 |
| | Kenya | | 6 | 2 | 8 |
| | Uganda | 1 | | | 1 |
| | Total | 16 | 6 | 2 | 24 |
| Tanzania domesticated | Lake zone | 2 | 20 | 13 | 35 |
| | Central zone | 5 | 47 | 71 | 123 |
| | Eastern zone | 3 | 10 | 3 | 16 |
| | Western zone | 4 | 45 | 27 | 76 |
| | Southern highland zone | 0 | 0 | 1 | 1 |
| | South zone | 0 | 26 | 11 | 37 |
| | Total | 14 | 148 | 126 | 288 |
| Total | | 30 | 154 | 128 | 312 |

A comparison between the results of the structure analysis and the MDS showed a high level of consistency between the results of these two methods (Appendix A: Fig. 5-1). The cluster in the lower left part of Fig. 3-2 represents groups 2 and 3 of the structure results. Group 2 appears in the lower half of this cluster and group 3 in the upper half. The accessions in the upper right part of the graph represent group 3. The collection sites of the 14 cultivated individuals from Tanzanian accessions that grouped together with the Tanzanian wild accessions are shown as filled circles in Fig. 3-1. None of the accessions were found in the southern zone. A significant difference between these plants and the rest of the cultivated accessions was found in the aforementioned field experiments (Sariah et al 2010, submitted, Appendix A: Table 5-1). The average number of days to flowering for these accessions was three days earlier than the rest. They also showed a lighter green leaf colour and reduced leaf, stem and pod hairiness. A higher survival rate of the plants, as indicated by 20% more plants per plot, was also observed in this field experiment and resulted in 20% higher yields.

An AMOVA of the cultivated accessions, where the zones and regions of collections were known, was calculated based on the SSR data with two different grouping strategies: by geographic zones and sites of collection within the respective regions (Table 3-6) and by the groups formed by structure (Table 3-7). For the geographic data, only the regions within the zones were significant, and they explained 3% of the genetic variance and had a ϕ-value of 0.033. The groups found by structure explained a much higher percentage of the genetic variance (17%), and consequently, the ϕ-value was considerably higher (0.196).

Table 3-6: AMOVA with grouping by geographical zones and regions.

| Source | df | SS | MS | Est. Var. | Expl.Var | ϕ-value | sign. |
|---|---|---|---|---|---|---|---|
| Among zones | 4 | 141.7 | 35.43 | 0.00 | 0% | -0.008 | 0.947 |
| Among region | 9 | 298.0 | 33.11 | 0.76 | 3% | 0.033 | 0.001 |
| Within region | 273 | 6097.6 | 22.34 | 22.34 | 97% | | |
| Total | 286 | 6537.3 | | 23.10 | 100% | 0.025 | 0.001 |

Df = degree of freedom, SS = Sum of square, MS = Mean square, Est.var.= Estimated variance, Expl.var = Percentage of variance explained by factor, sign. = error probability

Table 3-7: AMOVA with grouping based on structure derived groups.

| Source | df | SS | MS | Est.var | Exp.var. | ϕ - value | sign. |
|---|---|---|---|---|---|---|---|
| Among groups | 2 | 692.6 | 346.31 | 4.188 | 17% | 0.196 | 0.001 |
| Within groups | 284 | 5844.7 | 20.58 | 20.580 | 83% | | |
| Total | 286 | 6537.3 | | 24.768 | 100% | 0.196 | 0.001 |

Df = degree of freedom, SS = Sum of square, MS = Mean square, Est.var.= Estimated variance, Expl.var = Percentage of variance explained by factor, sign. = error probability.

### *3.4.4 Comparison of genetic and geographic distance*

A Mantel test for genetic distance (modified Roger's distance) versus geographic distance in the collection site resulted in a non-significant correlation coefficient (Pearson R) of only -0.019 (Appendix A: Fig. 5-2). Further, a visual inspection of the structure-derived groups versus their position on the collection site map (shown for group 1 in Fig. 3-1) and of the MDS-position with symbols representing zones and regions (Appendix A: Fig. 5-3) showed no distinguishable structure. In addition, we tested any peculiar distribution of the traits that we had analysed in the aforementioned field experiment, including seed colour, growth habits, the plant's hairiness, flowering date, seed size and yield, on the MDS-plot and on the map (data not shown). We did not find any evidence for tendencies of these traits in relation to the collection site of the respective accessions or the structure-derived groups, aside from those described above for group 1.DiscussionIn the present study on cowpeas (*Vigna unguiculata*), we used microsatellite markers with primer pairs derived from cowpeas (prefix 'VM', Table 3-2) and with primers pairs derived from azuki beans (*Vigna angularis* (Willd.) Ohwi and Ohashi) and rice beans (*Vigna umbellata* (Thunb.) Ohwi and Ohash) (both with the prefix 'CEDG'). Our results showed a successful interspecies application of the SSR markers used, which resulted in a high degree of polymorphism in both cultivated landraces and wild accessions of cowpea. On average, the 'alien' CEDG markers resulted in 11.1 alleles, while the VM markers yielded 7.6 alleles per marker. Similarly, the average polymorphic information content (PIC) of the CEDG markers was 0.367 but was 0.325 for the VM markers. Another example of the successful application of 'alien' SSRs was presented by Diouf and Hilu (2005), who used SSR primer pairs developed in *Vigna acunitifolia* together with markers developed in *Vigna unguculata* for SSR amplification of *Vigna unguiculata* lines. In addition, Chaitieng et al. (2006) reported the successful PCR amplification of black

gram (*Vigna mungo*) SSRs, using SSR primers developed for cowpeas and the common bean. The same authors reported the effective application of RFLP markers derived from cowpeas, *phaseolus* beans and soybeans to discriminate *Vigna mungo* varieties. The reason that the SSR markers derived from *V. angularis* and *V. umbellata* showed a higher average PIC value might be explained by the fact that the respective markers had already been mapped, which was not the case for most of the *V. unguiculata*-derived SSRs. However, the PIC values we detected for both CEDG and VM markers were in agreement with the PIC value detected previously in cowpea. In a study of 141 cowpea accessions collected from Ghana and tested with 25 SSR markers, Asare et al. (2010) detected an average PIC value of 0.38. The number of alleles amplified by various SSR markers used in our study, varied greatly and ranged from two alleles per marker to a maximum of 22 alleles per marker. As the number of alleles is dependent on the number of accessions, it is difficult to compare different studies with each other. Nevertheless, the number of alleles we detected was in the same range as that found in other studies done on other crops. For example, SSR amplification in rice yielded 3 to 11 alleles (Yang et al. 1994); in soybean, the number of alleles ranged from 11 to 26 (Rongwen et al. 1995), in wheat, from 3 to 16 (Plaschke et al. 1995), in maize, from 2 to 23 (Pejic et al. 1998) and in cowpea, from 2 to 7 (Li et al. 2001) and from 2 to 6 allele per locus (Asare et al. 2010). The possible reason for a relatively higher number of alleles amplified by SSRs in our study compared to the results obtained by Li et al. (2001) and Asare et al. (2010) might be a result of the larger number of lines and wider genetic diversity used in our experiment compared to the 90 breeding lines and one wild line used by Li et al. (2001).

Both the genetic distances between the groups of accessions from different countries (Table 3-4), as well as the representation of the genetic distances between the individual accessions in MDS, Fig. 3-2) revealed that most of the cultivated Tanzanian cowpea accessions are closer to the Kenyan wild accessions than to the Tanzanian wild accessions (Table 3-4). In the MDS, the wild Kenyan cowpeas cluster together with the largest portion of the cultivated cowpeas from Tanzania, while a smaller subset of the cultivated Tanzanian cowpeas clustered together with the Tanzanian wild cowpeas and the single wild accession from Uganda (Fig. 3-2). The results of the structure analysis also confirmed this observation, where the Kenyan wild cowpeas group together with the majority of the Tanzanian cultivated cowpeas in groups 2 and 3, while group 1 consists of the wild accessions from Tanzania and Uganda and a minority of the Tanzanian cultivated cowpeas (Table 3-5). From this result, we might therefore deduce that most of the cultivated accessions found in Tanzania were not

domesticated from the 'domestic' wild accessions but rather were domesticated elsewhere, e.g., in Kenya, and then were adopted for use in Tanzania. Further, the wild accessions from Tanzania and Uganda might belong to another subspecies other than the wild accessions from Kenya. The subspecies *dekindtiana* is found in Kenya (Pasquet. 1993a), whereas ssp. *pubescens*, which today is found in Tanzania, immigrated from South Africa (Padulosi and Ng 1997), and these two wild subspecies form two different gene pools (Fatokun and Singh 1987). The group of cultivated Tanzanian cowpeas that is more closely related to the wild Tanzanian cowpeas could then either be the result of an independent domestication of wild cowpeas from the *pubescens* gene pool or the result of a hybridisation of wild cowpeas from Tanzania (from the *pubescens* gene pool) with the cultivated cowpeas. In this context, the differences between the two groups of cultivated cowpeas are interesting (Appendix A: Table 5-1). The better survival rate of Tanzanian cultivated cowpeas that were closer to the Tanzanian wild cowpea group and the earlier flowering could be either the result of a better adaptation to conditions in Tanzania or the result of the enrichment of the gene pool by the additional diversity from the *pubescens* pool through hybridisation. The fact that the collection sites of these 'wild-like' cultivated Tanzanian cowpeas is not spread over the whole area of cowpea cultivation but is rather 'island-like' in the Mwanza (MZ) region, the Tanga (TA) region and the Tabora (TB) region (Fig. 3-2), might lead to the assumption that the events that resulted in these accessions might have happened several times and caused isolation from each other. The presence of these two gene pools in the accessions used in this analysis resulted in a clear structure as revealed by the structure analysis (Table 3-5) and leads to a considerable effect on the groups in AMOVA (Table 3-7). This result is in contrast to the lack of structure reported by Ba et al. (2004), who analysed 46 cowpea lines using RAPD. The reason for these contradictory results could be due to the higher number of accessions used in this study, the sampling strategy and/or the high discrimination power of the microsatellite markers (Senior et al. 1998; Smith et al. 1997).

While we observed a high genetic diversity maintained in the Tanzanian landraces, the geographic distance between the collections sites did not correlate with the genetic distance between the respective Tanzanian landraces. In agreement with Asare et al. (2010) that found only loose correlation between the genetic distribution of cowpea accessions in Ghana and their geographical region from which the samples were obtained, we observed no correlation between the genetic and geographical distance. This observation was directly shown by the Mantel test but was also supported by the results of the AMOVA, where the grouping by

zones and regions only explained 3% of the genetic variance an as further illustrated in Fig. 3-1, where the distribution of the most abundant accession is shown with a star. The reason for this observation might be due to the crisscrossing cowpea grain trading from all over Tanzania done by small entrepreneurs. The most dependable seed sources for small scale farmers in Tanzania are the grain markets because the availability of improved seed in the rural areas is very limited. Our results underscore less cowpea population differentiation among regions than within them, as reported by Nzuki (2001), who studied the diversity of different cowpea accessions sampled from different origins.

In conclusion, we observed a clear genetic structure in the analysed 312 cowpea accessions from East Africa. Although the collection area covered was vast (about 1.3 million square km), the geographic distance was not reflected in the genetic distance of the accessions analysed. The wild accessions seem to be divided into two gene pools, one from Kenya and one from Tanzania, and the majority of the Tanzanian landrace accessions are more closely related to the Kenyan than to the Tanzanian gene pool of wild cowpea accessions. These observations led us to the following recommendations for conservation and utilisation of the cowpea material: (a). The distance between collections sites has only very limited influence on the diversity of the collected material. Therefore, a sampling for conservation purposes will not benefit from a strategy with widespread and evenly distributed collection sites. (b) In contrast to the first statement, the discovery of the genetic diversity between the collected accessions is important, as the Tanzanian landraces, which are more closely related to the Tanzanian wild accessions and which add considerable genetic variation, are difficult to distinguish from the more common landraces, which are closely related to the Kenyan gene pool of wild cowpeas. (c) The relatively better survival rate in the field from the smaller group of landraces close to the Tanzanian wild pool shows that, assuming they arrived by hybridisation, there is an advantage to gain from crossing landraces from both pools. Obviously, this potential is only poorly used at present and could lead to noticeable improvements in the yield and yield stability of cowpeas and thereby to a higher security of food and feed supply for farmers working under marginal conditions.

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# 4 PAPER 3: PROMISING RESISTANCE TO *CALLOSOBRUCHUS MACULATUS* (F) IN COWPEA LANDRACES FOUND IN TANZANIA

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## 4.1 Abstract

The cowpea seed beetle or cowpea weevil (*Callosobruchus maculates* (F.)) is the most important post-harvest pest of cowpeas. This pest frequently infests up to 100% of the stored seeds within 3 to 5 months of storage in absence of control measures, causing value deterioration of up to 100%. Identification of host plant resistance would be the breakthrough in the sustainable control of this pest. An enhanced infestation experiment using a 'free choice' design and involving 200 landraces was conducted over a time period of 10 months in Tanzania. Indicators of resistance such as weight loss, exit holes and dead larvae and adults within the seed were investigated. Further, data from a large field trial that assessed agronomic traits and resistance to aphids and thrips for the same accessions were correlated to the traits in the present study. The resistance against the cowpea weevil was portioned into the prevention of infestation and the hindrance of the completion of the beetle's life cycle. For both types of resistance, superior genotypes of landrace were found. One genotype with a white seed coat displayed both types of resistance. The resistance component related to the degree of infestation had the highest impact on the loss of weight and the number of exit holes. The level of thrip infestation measured in the field experiment was correlated with the storage resistance component associated with the premature death of the weevils in the seeds. Both components of resistance influenced the slope of the weight loss in the experiment. The landraces with superior resistance identified in our study are a valuable resource for the improvement of cowpea storage losses.

## 4.2 Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an important grain legume in east Africa that is primarily grown by small-scale farmers. It has a considerable ability to adapt to high

temperatures and drought compared to other crop species (Ehlers and Hall, 1997). Hall and Patel (1985) reported cowpea dry grain yields as high as 1000 kg $ha^{-1}$ obtained in a Sahelian environment with low humidity and only 181 mm of rainfall. Furthermore, due to its high ability to fix atmospheric nitrogen, cowpea form a valuable part of farming systems in areas in which soil fertility is limiting by enriching the soil through harvest residues (Elowad and Hall, 1987). Cowpea is a multifunctional crop in the sense that it provides food and feed. The crop has a high protein content that ranges between 20% and 26% and starch content between 50% and 67% (Singh et al., 1997). The availability of protein content in all of the edible parts of this crop, such as the leaves, green pods and beans, make this crop a good source of this vital nutrient at all stages of growth and development. Economically, cowpea is an income-generating crop for both small-scale farmers (especially women at the farm level) and larger scale grain traders (Singh, 2005; Timko and Singh, 2008). Insect pests are the major constraint to cowpea production. This constraint becomes more pronounced for small-scale farms due to their limited resources for controlling this pest (Jackai and Daoust, 1986; Singh and Allen, 1980; Singh and van Emden, 1979).

The cowpea weevil, or the cowpea seed beetle as it is more accurately known, *Callosobruchus maculatus* (F.), is the principal post-harvest pest of cowpeas (Jackai and Daoust, 1986). The adult female lays eggs on the seeds. These eggs are white, and despite being small (0.6 mm long), they are readily visible on the surface of the seed. The eggs hatch within 5-7 days. The larvae bore into the seeds, feed and complete their development inside the seed. At the end of their development, the insects emerge as adult weevils, leaving behind a hole at the exit point (Dick and Credland, 1986; Singh et al., 1984). The duration of the complete life cycle of this weevil ranges between 22 and 30 days (Fox, 1993; Messina, 1993). Thus, each month there is a new generation that is infesting the stored beans. This pest frequently infests up to 100% of the stored seeds within 3 to 5 months of storage in the absence of control measures (Singh, 1980; Southgate, 1978). Cowpea weevil infestation causes reductions in the weight, nutritional value, viability and, naturally, saleability of cowpeas (Swella and Mushobozy, 2007). The pest is distributed worldwide. It is found in Africa, Australia, Central and South America, Europe, Northern Asia, the Mediterranean area, South and South-east Asia, the USA and Canada (http://www.padil.gov.au/pbt 2010). In Tanzania, bruchids appear wherever cowpea is grown.

Currently, there are only a limited number of technologies available for resource-limited farmers to combat cowpea bruchids. Control methods such as the use of insecticides and fumigation are very effective; however, these methods are either not available or are too expensive for most small-scale farmers (Tarver et al., 2007). Therefore, non-chemical approaches to control this pest have been adopted. These methods include triple bagging using plastic bags (Tarver et al., 2007), mixing seeds with ash in the storage containers (Songa and Rono, 1998; Wolfson et al., 1991), solar treatment (Kitch et al., 1992), the use of various botanical insecticides, e.g., neem (Bottenberg and Singh, 1996), and storage in sealed containers (Singh, 1977). Some of these methods, however, are not always effective, especially when a large quantity of seed is involved. Therefore, there is a need for better alternative control methods. Host plant resistance against insects is the most appropriate alternative/complementation for both small-scale and commercial cowpea producers.

In nature, plants have different protective mechanisms against insect pest damage and against diseases (Kogan, 1986). These mechanisms include, for example, mechanical barriers in which high concentration of lignin is present or biochemical compounds such as protease inhibitors to debilitate insect proteolysis (Ahn and Zhu-Salzman, 2009; Boulter et al., 1989). In cowpea, the trypsin inhibitor (CpTI) has been reported to have insecticidal properties against a wide range of insects (Ismail et al., 2010). Studies have shown that different cowpea cultivars have different abilities to either deter the development of bruchid larva inside the seed (Dick and Credland, 1986; Singh et al., 1984) or to influence the extent of oviposition by their seed coat (Credland and Wright, 1990). The most important indicator of the resistance conferred by these intrinsic factors, especially CpTI, is the deterrence of the survival of the bruchid larva inside the seed, thus reducing seed deterioration.

Identification of cowpea accessions with a certain degree of natural resistance to the weevils and the characterisation of this resistance is a major step towards the crossbreeding of cowpea lines resistant to cowpea grain storage weevils. Thus, the aim of this study was to analyse the characteristics of 200 accessions for post-harvest resistance against *Callosobruchus maculatus* (F), specifically focusing on reducing both the infestation and by decreasing the survival rate of the larvae in the beans. The post-harvest resistance we detected here could be related to results related to the diversity structure and the field performance of these accessions discussed in previous papers.

### 4.3 Materials and Methods

An enhanced cowpea weevil infestation experiment was conducted at the Tropical Pesticide Research Institute (TPRI), Plant Protection Department Laboratory, Arusha, Tanzania, using a free weevil choice design. The experiment started in February 2009 and ended in December 2009. Among the 413 cowpea landraces collected for this study, 300 were directly acquired from farmers. The collection area covered 21 districts of Tanzania. Another 113 landraces were obtained from the National Plant Genetic Resources Centre (NPGRC) of Tanzania. Multiplication of the 413 cowpea landraces was done at the Miwaleni experimental field, near Arusha, to obtain sufficient genetically uniform seed for use in field and storage experiments. During harvesting, a single plant was randomly chosen from each landrace, harvested separately and placed in labelled cloth bag. Five seeds from the single plant of each landrace were sampled for genetic analysis. The genetic distances between the landraces were determined using microsatellites (Sariah et al., submitted). Based on these genetic distances, 200 genetically distant landraces were chosen and grown in a field experiment covering two seasons and two environments (Sariah et al., submitted). Selected data from this experiment were also used in the present analysis. Seed material from each of the 200 accessions was harvested after the first season of this field experiment and dried to 13% moisture. One hundred grams from each accession was weighed into 5 × 10 × 10-cm hard paper bags, which formed the experimental units for a Completely Randomised Design (CRD) experiment with three replications. These experimental units were randomly placed on shelves and left uncovered for the weevil to freely move in and out of during the process of choosing their preferred host. The sources of infestation were the previously reared weevils in a cowpea grain sample that were placed in the four corners of the lab about 1.5 m from the experimental units. The relative humidity in the lab was kept at 70%, and the temperature was maintained at 26°C. Sampling for data recording was done four times with an interval of approximately 60 days between the first three samplings and an interval of 120 days between the third and the fourth samplings. During all samplings, the weight of the whole bag and the number of undamaged seeds in a random subsample of 100 beans was taken. In the last two samplings, the number of exit holes and the number of dead larva and dead adult weevils within the seed were counted from 100 seeds subsample random taken from each experimental unit. The effective infestation was calculated as the sum of the number of exit holes, dead larvae and dead adults. The number of dead larvae, the number of dead adults and the sum of these two values (as emergence failure) were calculated as percentage of effective infestation. In the

present paper, only those data from the last observation were used (Table 4-1). Further, the accumulated weight loss (‘*Loss*’) over time was calculated from the bag weight at the different sampling dates as the area under the loss progress curve.

Table 4-1: Trait acronyms and description

| Acronym | Description |
|---|---|
| Infest | Effective weevils infestation per 100 seeds |
| fWgt | Final weight of the container |
| Loss | Area under the weight loss curve |
| UndSd | Undamaged seeds per 100 seeds |
| ExHol | Exit holes per 100 seeds |
| DdLv | Dead larva inside the seed per infestation (%) |
| DdAd | Dead adult weevils inside the seed per infestation (%) |
| Fail | Failure to complete life cycle per infestation (%) |

Data were analysed using R-Statistical software v. 2.10 (R.Development.Core.Team, 2006) for the data shown in Table 4-2 and using Microsoft Excel (Microsoft, Redmond) for the other calculations and graphs including Pearson’s product moment correlation.

### 4.4 Results

The traits 'Final weight of the sample’ (*fWgt*), 'Undamaged seeds per 100 seeds' (*UndSd*), ‘Number of exit-holes per 100 seeds’ (*ExHol*) and the numbers of dead adult weevils and larvae were observed during the experiment. Subsequently, the effective weevil infestation (*Infest*), the area under the weight loss curve (*Loss*), the percentage of dead larvae (*DdLv*), the percentage of dead adult weevils (*DdAd*) and the percentage of failure to complete the life cycle relative to the level of infestation (*Fail*) were calculated as described above (see also Table 4-1). The reason behind the calculation of *Loss* was to include variations in the progress of the weight loss over time into the analysis. The calculation of *Infest* and the relative numbers of *DdLv*, *DdAd* and *Fail* should allow the separation of the effects of the prevention of egg deposition and penetration of the larvae on the one side (expressed as variation in *Infest*) and the prohibition of successful completion of the life cycle (expressed as *Fail* and subdivided into *DdLv* and *DdAd*) on the other side. With the exception of E*xHol* and *DdAd*, the cowpea accessions explained a significant part of the variance observed for the trait (Table

4-2). Table 4-2 further shows the trait means for the experiment and for the accession means for the three repeats, as well as the standard deviation and the range.

Table 4-2: Traits statistics: mean, standard deviation (SD) and range of the accession means and average standard error of the individual accession means, F-value and significance of one-way ANOVA with the accession as factor

| Trait | *Mean* | *SD* | *Min* | *Max* | *SE.acc* | *F(ANOVA)* | *p(ANOVA)* |
|---|---|---|---|---|---|---|---|
| *Infest* | 221.0 | 36.55 | 42.0 | 331.7 | 43.5 | 1.360 | 0.0058 |
| *fWgt* | 81.45 | 4.42 | 69.50 | 95.63 | 4.65 | 1.809 | 0.0000 |
| *Loss* | 1338 | 331.0 | 434.3 | 2361 | 225.4 | 1.340 | 0.0082 |
| *UndSd* | 15.05 | 8.10 | 3.67 | 80.00 | 7.23 | 1.821 | 0.0000 |
| *ExHol* | 160.7 | 26.71 | 24.00 | 235.7 | 15.42 | 1.119 | 0.1783 |
| *DdLv* | 14.81 | 3.30 | 7.01 | 28.60 | 4.40 | 1.421 | 0.0019 |
| *DdAd* | 12.25 | 2.86 | 4.66 | 22.9 | 3.56 | 1.198 | 0.0690 |
| *Fail* | 27.06 | 5.92 | 13.1 | 48.3 | 7.69 | 1.280 | 0.0214 |

The latter statistics were sufficiently high to indicate an ample genetic potential for trait improvement. In addition, Table 4-2 also shows the average standard error for the means, which indicate that many of the differences between the accession means were highly significant. The amount of egg disposition was not measured directly but was reflected by the effective infestation level (*Infest*). Figure 1 shows examples of low (Fig. 4-1a), medium (Fig. 4-1b) and high (Fig. 4-1c) infestation.


Fig. 4-1:Different levels of eggs deposition (small white dots) and damage on different colored, bright white (a, acc.164), dull white (b, acc.195) and gray (c) cowpea seeds.

The correlation between the traits studied and calculated in the present experiment and traits measured in the field experiment mentioned above was analysed, and the results are presented in Table 4-3. From the field experiment, the only traits showing a significant correlation with the storage experiment traits were included. In addition to the expected correlations revealed by calculations, e.g., between *fWgt* and *Loss* and between *Infest* and its components *ExHol*, other interesting correlations or absence of correlations were found.

Table 4-3: .Pearson's r values (upper right part) and its respective significance (lower left part) for correlations between traits based on accession means (non-significant values in gray).

| | fWgt | Loss | UndSd | ExHol | Infest | DdAd | DdLv | Fail | Thrips | SdWgt |
|---|---|---|---|---|---|---|---|---|---|---|
| fWgt | | -0.8603 | 0.3288 | -0.3373 | -0.3358 | 0.0618 | 0.0005 | 0.0301 | 0.1044 | 0.4629 |
| Loss | 0.0000 | | -0.2675 | 0.2611 | 0.2483 | -0.0627 | -0.0364 | -0.0505 | -0.1054 | -0.4103 |
| UndSd | 0.0000 | 0.0001 | | -0.6434 | -0.6249 | 0.1387 | 0.0370 | 0.0874 | 0.0949 | 0.1105 |
| ExHol | 0.0000 | 0.0002 | 0.0000 | | 0.8892 | -0.3365 | -0.1652 | -0.2542 | -0.0607 | -0.0338 |
| Infest | 0.0000 | 0.0004 | 0.0000 | 0.0000 | | 0.0871 | 0.2760 | 0.1955 | -0.1134 | 0.0302 |
| DdAd | 0.3885 | 0.3816 | 0.0516 | 0.0000 | 0.2240 | | 0.8536 | 0.9571 | -0.1489 | 0.1300 |
| DdLv | 0.9945 | 0.6121 | 0.6066 | 0.0200 | 0.0001 | 0.0000 | | 0.9679 | -0.1303 | 0.1249 |
| Fail | 0.6751 | 0.4815 | 0.2219 | 0.0003 | 0.0057 | 0.0000 | 0.0000 | | -0.1443 | 0.1321 |
| Thrips | 0.1442 | 0.1404 | 0.1848 | 0.3971 | 0.1126 | 0.0364 | 0.0676 | 0.0427 | | -0.0459 |
| SdWgt | 0.0000 | 0.0000 | 0.1222 | 0.6378 | 0.6740 | 0.0684 | 0.0801 | 0.0638 | 0.5228 | |

The final weight (*fWgt*) and the accumulated weight loss (*Loss*) were highly correlated with effective infestation (*Infest*), with correlation coefficients of -0.3373 and 0.2483, respectively. In contrast, *DdAd*, *DdLv* and *Fail*, which measure the suppression of the development of the weevil in the bean, showed no significant correlation with *fWgt* or *Loss*. *DdAd* and *DdLv* showed a very high correlation (r = 0.8536), and the effective infestation level (*Infest*) was correlated with *DdLv* but not with *DdAd*. The level of thrip infestation measured in the field experiment (*Thrips*) showed a weak correlation with *DdAd* and *Fail* (r values of -0.1489 and -0.1443, respectively) but not with *DdLv* or *Infest*. The degree of aphid infestation in the field experiment was not correlated with any of the storage experiment traits (data not shown). The seed weight measured in the field experiment (*SdWgt*) showed a high correlation with *fWgt* and *Loss* (r values of 0.4629 and -0.4102, respectively), meaning that heavier (larger) seeds were less affected by weight loss resulting from weevils. The correlation between the two traits representing two aspects of weevil resistance, *Infest* and *Fail*, was significant and positive but was only moderate in size (r = 0.1955). Figure 2 shows the scatter plot of the

relationship between *Infest* and *Fail*. According to the effective infestation, three different groups ('Low', 'Medium' and 'Severe') were formed as indicated by the vertical lines in the plot. Acc.164 had the lowest effective infestation and also had a very high emergence failure. Acc.005 and Acc.099 had the highest emergence failure levels and had medium infestation levels.

Table 4-4: Means and rankings for the accessions with the best ranks for the respective trait (bold) and yield data for the all accessions.

| Acc | SdCol | Infest | fWgt | DdAd | DdLv | Fail | Yield |
|---|---|---|---|---|---|---|---|
| Acc.005 | Brown | 190.3 (34) | 80.58 (114) | **22.9 (1)** | **25.3 (3)** | **48.3 (1)** | 423.5 (68) |
| Acc.017 | Gray | 273.7 (186) | 70.30 (194) | 18.5 (7) | **25.6 (2)** | **44.1 (3)** | 395.5 (93) |
| Acc.034 | Brown w/dots | 146.0 (6) | 79.27 (136) | 8.7 (177) | 9.0 (163) | 17.7 (185) | 436.6 (62) |
| Acc.050 | Red | 169.0 (13) | **89.62 (3)** | 15.7 (23) | 14.1 (118) | 29.8 (60) | 282.3 (182) |
| Acc.066 | Brown w/dots | **121.3 (2)** | 89.30 (5) | 9.0 (172) | 9.6 (184) | 18.7 (181) | 356.7 (127) |
| Acc.099 | Gray | 224.0 (100) | 80.13 (124) | 19.2 (5) | **28.6 (1)** | **47.8 (2)** | 238.8 (194) |
| Acc.195 | White | 147.5 (7) | 84.29 (56) | 11.9 (107) | 14.0 (121) | 25.9 (82) | 205.7 (200) |
| Acc.161 | Gray | 248.0 (149) | **91.58 (2)** | 16.5 (14) | 18.4 (28) | 18.4 (16) | 409.1 (196) |
| Acc.164 | White | **42.0 (1)** | **95.63 (1)** | **21.4 (2)** | 21.4 (5) | 42.9 (4) | 318.9 (165) |
| Acc.173 | Brown w/dots | **132.8 (3)** | 86.93 (18) | 7.8 (185) | 8.0 (194) | 13.4 (194) | 439.2 (54) |

The best ranking accessions for the traits *Infest*, *fWgt*, *DdAd*, *DdLv* and *Fail* are presented in Table 4-4 together with the accession means and the accession ranks (in brackets) for these traits. Further, the yield of these accessions in the field experiment and the seed colour is indicated in the table. Both white-seeded accessions and all three accessions with brown seeds with black dots are represented in the table. These two groups showed relatively low infestations compared to accessions with other seed colours. On the other hand, the three accessions with brown and black dot seeds had very low rankings for *Fail*. In general, a high ranking for one of the traits *Infest*, *fWgt* or *Fail* rarely coincided with high rankings in the two other traits. Accession 164, with white seeds, is the exception, as it ranked high in all of the storage resistance related traits. In relation to yield, none of the accessions excelled, only showing medium to low ranks for this trait. The positions of most of these best ranking accessions are also shown in Figure 2.

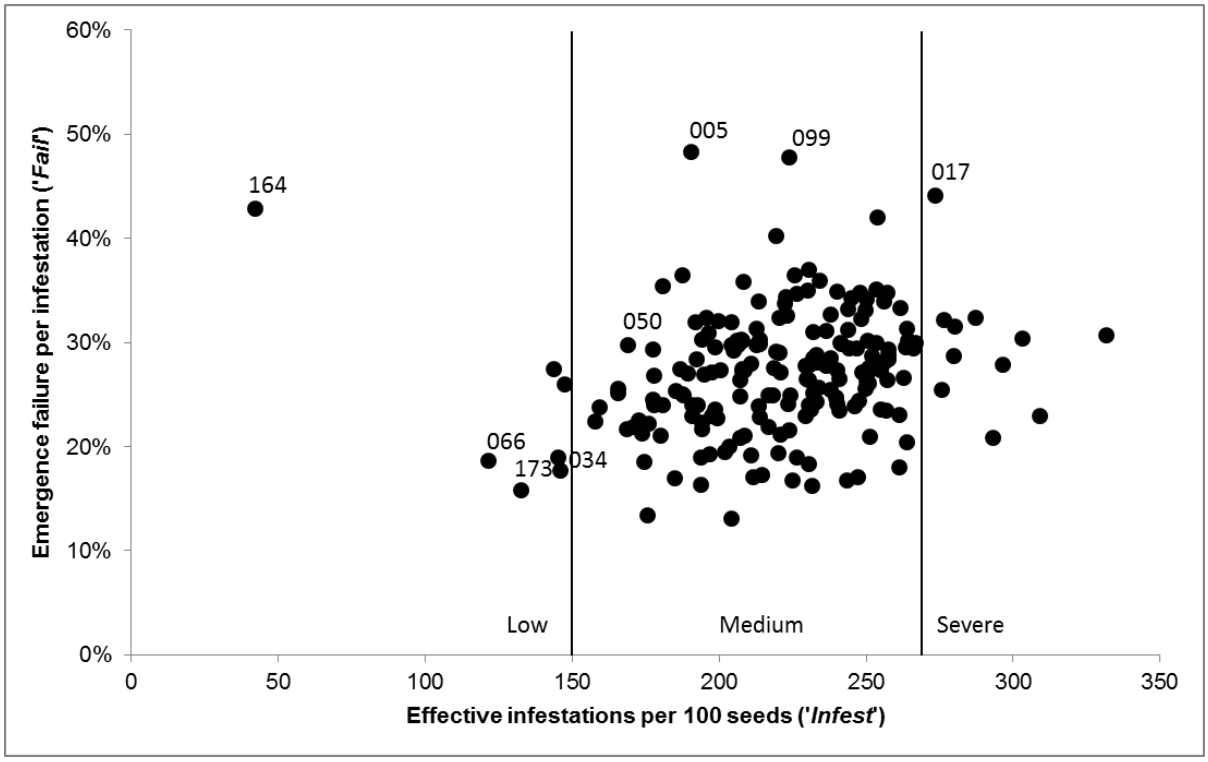


Fig. 4-2: Level of effective infestation of weevils on 200 cowpea accessions and their percentage suppression of weevils emergence.
Position of most of the accessions shown in Table 4 are indicated.

The trends of weight loss over time during the experiment of the five representative accessions from Table 4-4 are shown in Fig 3. Accession 164 had a very low loss of weight over time. The final weight of this accession at 340 days was just below 96 g. Accessions 050 and 099 that had medium to high values for *Fail* (Table 4-4, Fig. 4-2). The weight loss was initially rapid but stabilised in the end. In contrast, accession 034 and accession 173, which low values for *Fail*, showed weaker flattening of the weight reduction curve and thus crossed the curves of 050 and 099, respectively.

## 4.5 Discussion

In the present study, various important traits of dry cowpea seed with respect to storage weevil *Callosobruchus maculates* infestations were analysed. There were considerable and significant differences in the trait means between accessions, indicating that in the gene pool considered here, there was a potential for improvement by selection and crossbreeding for resistance against this storage pest.

As reported above, some of the originally reported traits were influenced both by the extent of infestation and by the ability of the different cowpea accessions to prevent the larvae from successfully feeding on the beans, which lead us to calculate traits that were mainly affected by only one of the two components of weevil resistance. Thus, the trait '*Infest*' represents the degree of infestations, while '*Fail*', '*DdLv*' and '*DdAd*' represent the countermeasures of the crop. With the exception of accession 164, a good rank in '*Infest'* did not coincide with good ranks in the three traits related to weevil development failure and *vice versa* (Table 4-4, Fig. 4-2). This result shows that these two trait complexes are largely independent. This conclusion was further substantiated by the fact that '*DdLv*' and '*DdAd*' were highly correlated with each other but were only relatively weakly correlated or not at all correlated, respectively, with '*Infest*' (Table 4-3). The final weight ('*fWgt*') and the accumulated weight loss ('*Loss*'), which represent the measurable damage to the seeds, were not significantly correlated with the traits representing development failure but were highly correlated with the extent of infestation (Table 4-3). Obviously, the preference of the weevil for a certain cowpea accessions, which is reflected by '*Infest*', had the primary effect on the weight damage. Interestingly, the best accessions for '*Infest*' had a white seed coat or a brown seed coat with dark spots (Table 4-4). The white accessions also showed lower egg deposition compared to other genotypes (Fig. 4-1). As the present study was a 'free choice design', the lower infestation level of the white and brown accessions was most likely due to relative avoidance of these types of seeds by the weevil. The reason for this avoidance might be directly related the colour of the seed coat. Alternatively, other traits that are either pleiotropic or linked with the seed colour influenced the choice of the weevils. These traits might be olfactory traits or traits that prevented the larvae from entering the seeds. Singh et al. (1984) also found resistance against *Callosobruchus maculatus* in a white cowpea accession, although in their method of evaluation, no choice was given to the weevil, and the damage was related to the egg deposition. Further, one of the white accessions in our experiment (164) showed both a low level of infestation and a high failure rate of the weevil. The other white accession that we examined (195) had a mediocre failure rate.

Even though the failure percentage ('*Fail*') and the other two traits; the percentage of dead larvae ('*DdLv*') and the percentage of dead adults ('*DdAd*'), did not significantly influence the loss of weight in the present experiment. These traits are important for the progress of epidemics of a weevil infestation. In Figure 3, the weight loss curves of accessions 099 and 050, which both had high failure rates but had different levels of infestation, showed a more

pronounced flattening of the weight curve during the time of the experiment compared to accessions 173 and 034, which had lower failure rates. This decrease in slope corresponds to the relative suppression of the reproduction of the weevil. The cause of the higher mortality of larvae and adults is most likely the presence of compounds in the seed that are either toxic or prevent the larvae from feeding efficiently. This could be, e.g., a protease inhibitor such as the Cowpea Trypsin Inhibitor (CPTI), which had been found in this crop (Xu et al., 1996). This compound interferes with the metabolic activities of insects belonging to Coleoptera, Lepidoptera and Orthoptera (Boulter et al., 1989). Furthermore, the observed correlation between thrip infestation in the field experiment and adult weevil emergence failure in the storage experiment might imply that both types of resistance are partly influenced by the same components of the crop.

Even though we measured the weight loss of the cowpeas as indicator of damage, the most important economic damage caused by infestation is the reduction of the quality of the grain through oviposition and larvae development within the seed (Giga, 1981) rather than grain weight loss. The exit holes and eggs of the weevils reduce the market value of the beans considerably. An economic evaluation done in Brazil indicated that seed with only 5% bruchid damage were devaluated by 53% in an open market (Bastos, 1973). Further, seed germination is heavily reduced in an infested seed stock, rendering the beans unsuitable for the next season's sowing. Santos (1971) reported 100% seed germination failure observed for seeds with only four holes of weevil damage. Therefore, both the prevention of infestation and the obstruction of the weevil development are important components of the resistance of cowpea against this important storage pest.

In our experiment, we found accessions that exhibited either low infestation rates or high suppression of the weevil development. Only accession 164 combined these two resistance components. Further, all of the best accessions for one of the two resistance components showed low yields in the field experiment (Table 4-4). These findings emphasise the need not only for the selection of the best genotypes but also for subsequent breeding programs in which the best accessions for the different storage resistance components, as identified in this study, can be crossed with cowpea genotypes contributing advantageous agronomic traits. It can be expected that the best offspring lines from these crosses will combine high and stable yields with low losses during storage, thereby improving the livelihood of cowpea growers.

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# 5 APPENDIX A: SUPPLEMENTARY MATERIAL (PAPER 2)

Table 5-1: Trait comparison of the 14 cultivated accessions grouped into STRUCTURE group 1 vs. the accessions in group 2 and 3: means and results of t-Test for different of means

| | Mean of domesticated accessions | | Results of t-test | |
|---|---|---|---|---|
| Trait | group 1 | group 2 & 3 | t-value | significance |
| Aphids infestation | 4.28 | 4.61 | 1.34 | 0.182 |
| Thrips infestation | 4.66 | 4.47 | -0.85 | 0.398 |
| Flowering time | 54.15 | 56.45 | 3.05 | 0.009 |
| Growth habit | 2.72 | 2.81 | 0.85 | 0.397 |
| Leaf colur | 5.28 | 6.01 | 2.37 | 0.019 |
| Leaf hairiness | 3.63 | 4.06 | 2.06 | 0.040 |
| Stem hairiness | 3.66 | 4.05 | 1.81 | 0.072 |
| Pod hairiness | 3.56 | 3.90 | 2.90 | 0.011 |
| Pod length | 15.01 | 15.28 | 0.82 | 0.412 |
| Pod shape | 2.04 | 2.41 | 1.15 | 0.250 |
| Plant per plot | 19.29 | 15.97 | -2.56 | 0.011 |
| Seed size | 4.74 | 4.97 | 0.83 | 0.405 |
| Seed weight | 12.60 | 12.85 | 0.37 | 0.713 |
| Seed per pod | 15.01 | 15.28 | 0.82 | 0.412 |
| Grain yield | 465.89 | 386.39 | -3.23 | 0.001 |

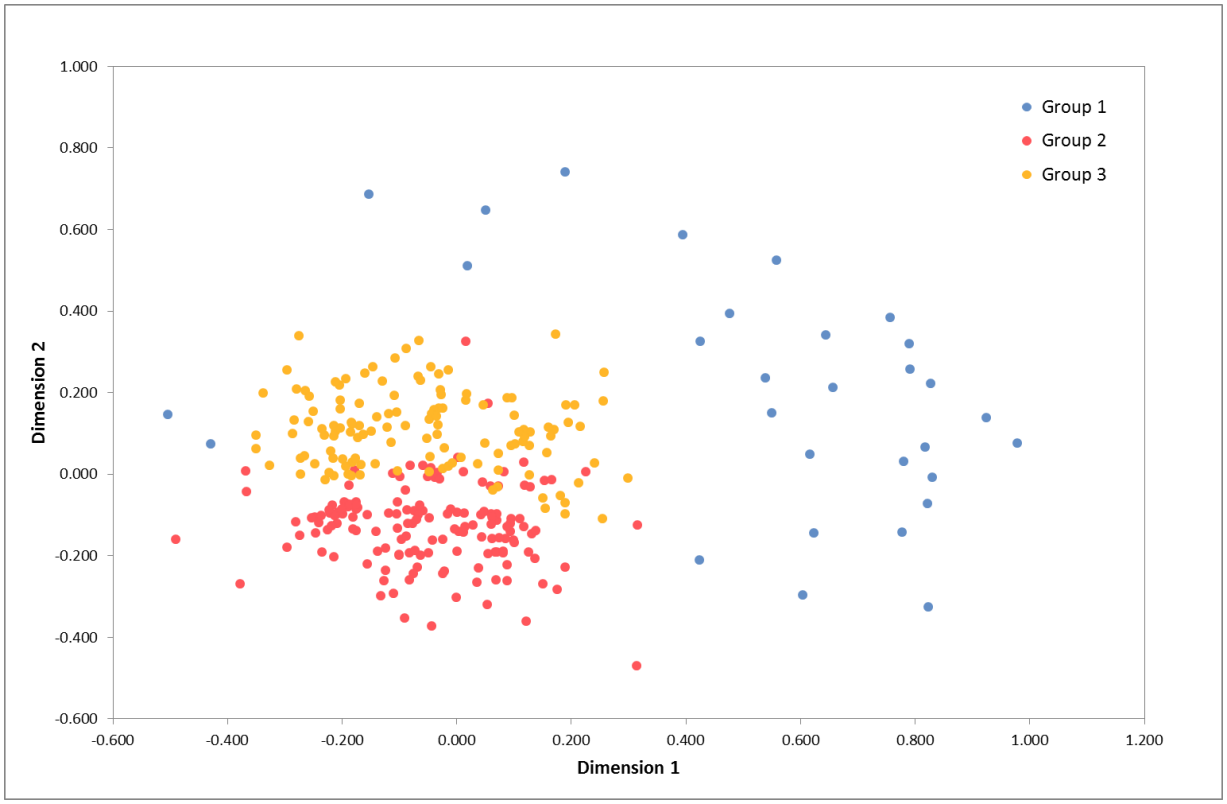


Fig. 5-1: MDS results with groups from STRUCTURE calculation indicated by symbol

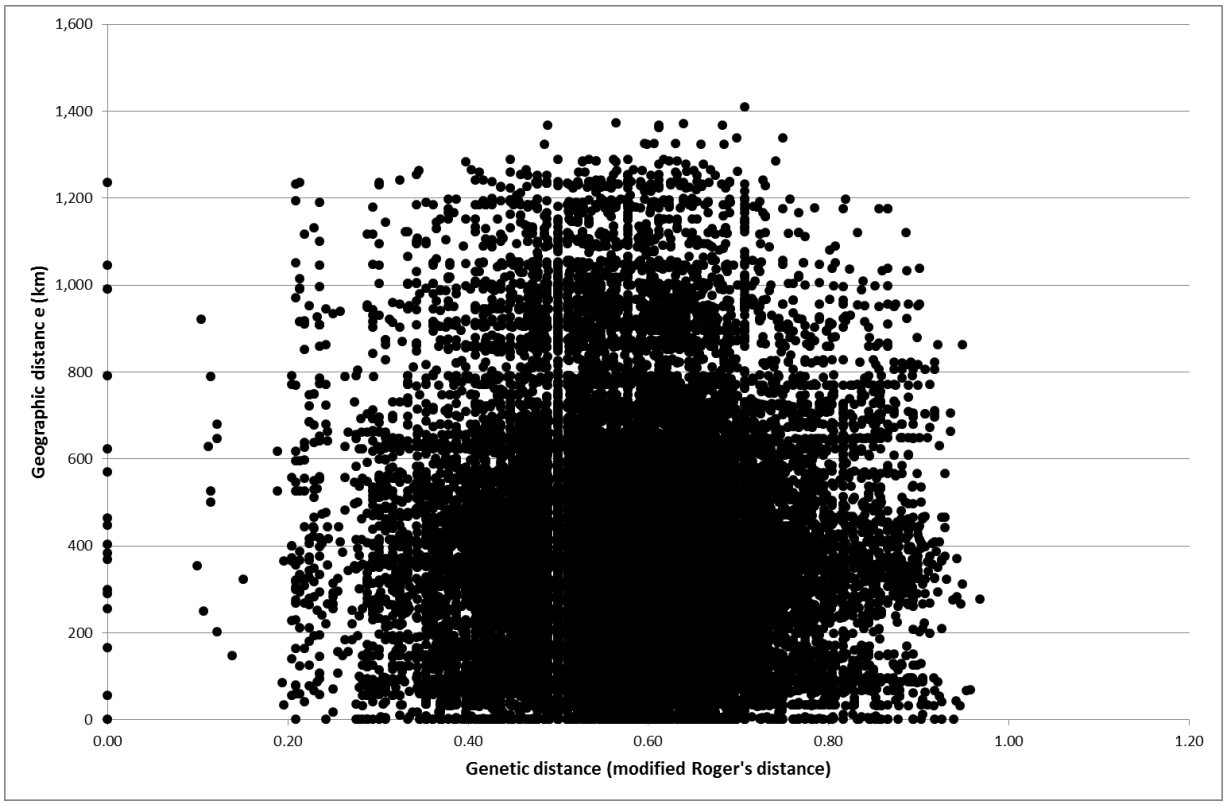


Fig. 5-2: Genetic distance of accessions vs. geographic distance of collections sites

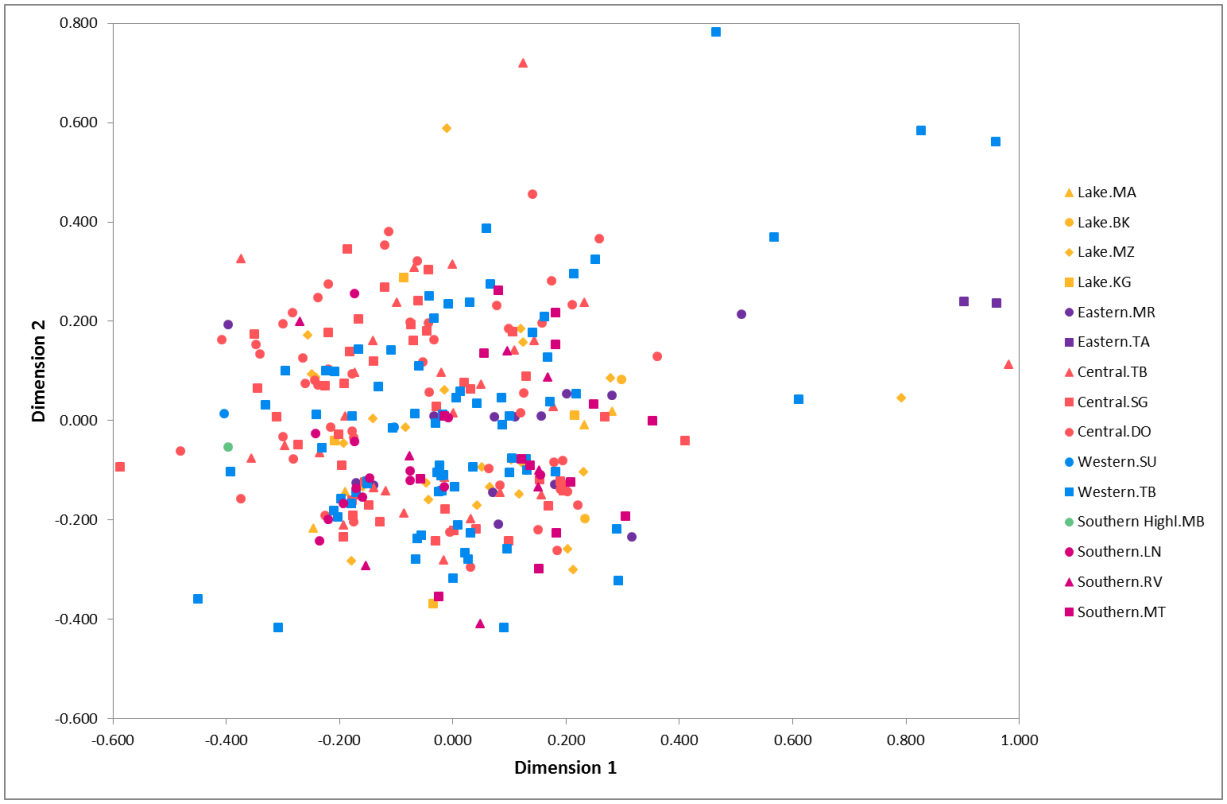


Fig. 5-3: MDS results with the Zone and Region indicated as symbol