THE GENETIC, MORPHOLOGICAL AND PHYSIOLOGICAL EVALUATION OF AFRICAN COWPEA GENOTYPES

BY

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Thesis presented in accordance with the requirements for the degree *Magister Scientiae Agriculturae* in the Faculty of Natural and Agricultural Sciences, Department of Plant Sciences (Plant Breeding) at the University of the Free State

University of the Free State

Bloemfontein

2006

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DEDICATION

I dedicate this piece of work to:

- *my late parents Nzogne Bathelemy and Ngandjou Julienne*
- *my wife Dr. N. Mayasi*
- *and to my brothers and sisters: Dominique, Jeanne, Marie, Emmanuel, Philippe, Maurice and Joseph*

ACKNOWLEDGEMENTS

I would like to convey my sincere gratitude and thanks to all those who have been instrumental in the course of my studies at the University of the Free State and during the course of my stay in South Africa. It is not possible to mention the names of all the individuals who contributed to this piece of work but I fully recognise and appreciate your valuable contributions.

The Department of Plant Sciences (Plant Breeding) is thanked for their financial support, which has made it possible for me to undertake these studies and accomplish this research work.

My deep gratitude goes to my supervisors Prof. R.L. Verhoeven and Prof. M.T. Labuschagne and also Dr. Maartens for their unanimous support, guidance and encouragement during my entire postgraduate study.

I am also grateful to thank Mrs Sadie Geldenhuys for her help and valuable assistance during my study period.

It gives me great pleasure to thank all the graduate students in the Department of Plant Sciences for their help and encouragement. Particularly, the assistance of Ibrahim Benesi is greatfully acknowledged for his help, friendship and collaboration I enjoyed during my studies. Special thanks go to Elizma Koen for her expertise, patience, determination and encouragement.

Special thanks are also extended to Fr. Emmanuel Mosoehu, Kosana, Prospere, Malefa Tsolo and Dr. L. Mohase for their love and encouragement.

Above all, I would like to thank God, for his protection and provision in all my needs.

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LIST OF ABBREVIATIONS

µm	= micrometer
ABA	= Absicisic acid
AFLP	= Amplified fragment length polymorphism
ANOVA	= Analysis of variance
cm	= centimeter
CMS	= Cell membrane stability
CRSP	= Collaboration research support programme
CV	= Coefficients of variations
DF	= Degree of freedom
DNA	= Deoxyribonucleic acid
FAO	= Food and agricultural organisation
FC	= flower colour
g	= gram
GP	= growth pattern
hr	= hour
IBPGR	= International Board of Plant Genetic Resources
IITA	= International Institute of Tropical Agriculture
IPP	= immature pod pigmentation
kg	= kilogram
LC	= leaf colour
LM	= leaf marking
MAS	= Markers Assisted Selection
MB	= number of main branches
mg	= milligram
mm	= millimeter
NCSS	= Number Cruncher Statistical System
NMS	= number of nodes on main stem
PA	= pod attachment to peduncle
PAGE	= Polyacrylamide gel electrophoresis
PC	= plant curvature

PEG = Polyethylene glycol

PH = plant hairiness

PH = plant height

PL = peduncle length

PL = pod length

PP = number of pods per peduncle

PP = plant pigmentation

PPt = number of pods per plant

PW = pod weight

QTL = Quantitative traits loci

RAPD = Random amplified polymorphic

RFLP = Restriction Fragment length polymorphism

RP = raceme position

SP = number of seeds per pod

SS = seed shape

ST = splitting of testa

SW = seed weight

TLL = terminal leaflet length

TLS = terminal leaflet shape

TLW = terminal leaflet width

TT = testa texture

TT = twinning tendency

UPGMA = Unweighted pair group method of arithmetic average

Chapter 1

INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp.) is a grain legume grown in savanna regions of the tropics and subtropics. Its value lies in its high protein content (23-29%, with potential up to 35%); and its ability to fix atmospheric nitrogen, which allows it to grow on, and improve poor soils (Steele, 1972).

It is cultivated for its seed (shelled green or dried), pods and/or leaves, which are consumed in fresh form as green vegetables, while snacks and main meal dishes are prepared from the dried grain. All the plant parts used for food are nutritious, making it extremely valuable where many people cannot afford protein foods such as meat and fish. The rest of the cowpea plant, after pods are harvested, is also used as a nutritious livestock fodder. Cowpea also has the ability to be intercropped with cereals such as millet and sorghum. Its diversity of uses, nutritive content and storage qualities have made cowpeas an integral part of the farming system in the West African region (Eaglesham *et al.*, 1992). However, most of the world's cowpeas are grown primarily in dry regions where drought is prevalent among several yield-reducing factors (Watanabe *et al.*, 1997).

Drought is one of the most important constraints threatening the food security of the world (Barters and Nelson, 1994). The economies of most of African nations rely heavily on exports of rain dependent agricultural products, which are often seriously affected during periods of severe drought. This makes drought a serious natural disaster in Africa, as it is associated with many socioeconomic miseries. Drought on the African continent often causes large scale water and food deficits, hunger, famine, exodus of people and animal, diseases, deaths, and many other severe, chronic societal problems (Ogallo, 1993).

William (1989), reported that about 26% (17 225 700 square miles) of the world's cultivated land falls in arid and semi-arid areas, where water is the major limiting factor to crop production. The remaining land also experiences occasional droughts during the crop season and timely and sustainable irrigation must be assured. However, it is just not possible to irrigate all the land, as sufficient

irrigation water is not available. The only alternative left, therefore, is to breed crops tolerant to drought stress. The development of drought tolerant varieties has become an important objective in many plant breeding programmes.

Selecting appropriate genotypes for environmental stress is, however, limited by inadequate screening techniques and the lack of genotypes showing clear differences in response to well defined environmental stresses (Bruckner and Frohberg, 1987). Selection for drought tolerance, while maintaining maximum productivity under optimal conditions, has also been difficult (Barters and Nelson, 1994), due to the low heritability of yield in such conditions.

Germplasm screening for tolerance to drought under naturally occurring drought stress does not seem to be reliable. Lack of uniform drought stress in the field will render screening for drought tolerance ineffective and thus limits progress for selection. Selection must occur under controlled environments, where drought can be reliably induced to distinguish between tolerant and susceptible genotypes, particularly at flowering or grain filling stages in seed crops (Rodomiro *et al.*, 1998).

Moustafa *et al.* (1996) also stated that there is a limitation in selecting for drought tolerance and a need to identify drought tolerant screening techniques that are repeatable and that can be used in a population of high genetic variation, because of the multitude of factors involved in drought tolerant mechanisms.

When plants are subjected to drought stress, a number of physiological and morphological responses have been observed and the magnitude of the response varies among species and between varieties within a crop species (Kramer, 1980). Morphological and physiological traits that might enhance drought tolerance have been proposed, but only a few of these mechanisms have been demonstrated in the expression of tolerance under field conditions (Ludlow and Muchow, 1990). In some cultivated cereals, osmotic adjustment has been found to be one of the most effective physiological mechanisms underlying plant resistance to water deficit (Turner and Jones, 1980; Morgan, 1984; Blum, 1988). Osmotic adjustment, as a process of active accumulation of compatible osmolytes in plant cells exposed to water deficit may enable (1) a continuation of leaf elongation, though at reduced rates (Turner, 1986); (2) stomatal and

photosynthetic adjustments (Morgan, 1984); (3) delayed leaf senescence (Hsiao *et al.*, 1984); (4) better dry matter accumulation and yield production for crops in stressful environments (Boyer, 1982).

A better understanding of both the morphological, physiological and biochemical mechanisms involved in plant response to water deficit could therefore help improve cowpea productivity in dry land areas. Different mechanisms may make a drought tolerant plant. It may be by drought avoidance or drought tolerance (Blum and Ebercon, 1981). Drought avoidance is the ability of a plant to escape periods of drought, particularly during the most sensitive periods of its development (Visser, 1994). Drought tolerance is the ability of the plant to endure or withstand a dry period by maintaining a favourable internal water balance under drought conditions.

Genetic diversity in the available gene pool is the foundation of all plant improvement programmes. It is a source of variation, which is raw material for the improvement work. This genetic diversity is essential to decrease crop vulnerability to abiotic and biotic stress, ensure long-term selection gain in genetic improvement, and promote rational use of genetic resources (Martin *et al.*, 1991; Tesemma *et al.*, 1991; Messmer *et al.*, 1993; Barrett and Kidwell, 1998).

Assessment of genetic diversity in cowpea genotypes would facilitate development of cultivars for specific production constraints by providing an index of parental lines to be used in breeding programmes.

The general objectives of this study were:

1. to assess genetic diversity of cowpea accessions from Cameroon, South Africa, and Kenya, by morphological markers

2. to discriminate between drought tolerant and susceptible cowpea accessions at flowering stage using the pot test screening method

3. to determine the varietal difference of cowpea in response to water stress under laboratory conditions using the cell membrane stability (CMS) test

4. to determine the varietal difference of cowpea in response to water stress under laboratory conditions based on stomatal behaviour (sensitivity) and density.

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Chapter 2

LITERATURE REVIEW

2.1 Cowpea

2.1.1 Origin, domestication and distribution

Cowpea (*Vigna unguiculata*) is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times (Summerfield *et al.*, 1974). A lack of archaeological evidence has resulted in contradicting views supporting Africa, Asia, and South America as origin (Johnson, 1970; Summerfield *et al.*, 1974; Tindall, 1983; Coetzee, 1995). One view is that cowpea was introduced from Africa to the Indian sub-continent approximately 2000 to 3500 years ago (Allen, 1983). Before 300 BC, cowpeas had reached Europe and possibly North Africa from Asia. In the 17th century AD the Spanish took the crop to West India. The slave trade from West Africa resulted in the crop reaching the southern USA early in the 18th century. Another view was that the Transvaal region of the Republic of South Africa was the centre of speciation of *V. unguiculata*, due to the presence of most primitive wild varieties (Padulosi and Ng, 1997). Presently cowpea is grown throughout the tropic and subtropic areas around the whole world.

Ng (1995) postulated that during the process of evolution of *V. unguiculata*, there was change of growth habit, from perennial to annual breeding and from predominantly outbreeding to inbreeding, while cultivated cowpea (subsp. *unguiculata*) evolved through domestication and selection of the annual wild cowpea (var. *dekindtiana*). During the process of domestication and after the species was brought under cultivation through selection, there was a loss in seed dormancy and pod dehiscence, corresponding with an increase in seed and pod size. The precise location of origin of where cowpea was first domesticated is also still under speculation.

The wide geographical distribution of var. *dekindtiana* throughout sub-Sahara Africa suggests that the species could have been brought under cultivation in any part of the region. However, the centre of maximum diversity of cultivated cowpea is found in West Africa, in an area encompassing the savannah region of Nigeria,

southern Niger, part of Burkina Faso, northern Benin, Togo, and the northwestern part of Cameroon (Ng and Marechal, 1985). Carbon dating of cowpea (or wild cowpea remains from the Kimtampo rock shelter in central Ghana) has been carried out (Flight, 1976), and is the oldest archaeological evidence of cowpea found in Africa. This shows the existence of gathering (if not cultivation) of cowpea by African hunters or food gatherers as early as 1500 BC.

2.1.2 Morphology and biology

Summerfield *et al.* (1974), Kay (1979) and Fox and Young (1982) described cowpea as an annual herb reaching heights of up to 80 cm with a strong taproot and many spreading lateral roots in the surface soil. Growth forms vary and many are erect, trailing, climbing, or bushy, usually indeterminate growers under favourable conditions (Figure 2.1).

Leaves are alternate and trifoliolate. The first pair of leaves is simple and opposite. Leaves exhibit considerable variation in size (6-16 x 4-11 cm) and shape (linear, lanceolate to ovate) and they are usually dark green. The leaf petiole is 5-25 cm long. The stems are striate, smooth or slightly hairy and sometimes tinged with purple.

The flowers are arranged in racemose or intermediate inflorescence at the distal ends of 5-60 cm long peduncles. Flowers are borne in alternate pairs, with usually only two flowers per inflorescence. Flowers are conspicuous, self-pollinating, borne on short pedicels and the corollas may be white, dirty yellow, pink, pale blue or purple in colour. Flowers open in the early day and close at approximately midday. After blooming (opening once) they wilt and collapse.

Figure 2.1: A mature cowpea plant with green pods, dry pods and flower (Source: IITA Research Station Ibadan, 2000)

Fruit are pods that vary in size, shape, colour and texture. They may be erect, crescent-shaped or coiled. They are usually yellow when ripe, but may also be brown or purple in colour.

There are usually 8-20 seeds per pod. Seeds vary considerably in size, shape and colour. They are relatively large (2-12 mm long) and weigh 5-30 g/100 seeds. Seed shape is correlated with that of the pod. Where individual seeds are separate from adjacent ones during development, they become reniform, but as crowding within the pod increases, the seeds become globular. The testa may be smooth or wrinkled, white, green, buff, red, brown, black, speckled, blotched, eyed (hilum white surrounded by a dark ring) or mottled in colour.

2.1.3 Classification

Verdcourt (1970) and Marechal *et al.* (1978) classified cowpea as follow:
ORDER: Fabales
FAMILY: Fabaceae
SUBFAMILY: Faboideae
TRIBE: Phaseoleae
SUBTRIBE: Phaseolinae
GENUS: *Vigna*
SECTION: *Catiang*

Vigna has several species, but the exact number varies according to different authors. All cultivated cowpeas are grouped under *V. unguiculata,* which is subdivided into four semigroups: Unguiculata, Biflora, Sesquipedalis, and Textilis (Westphall, 1974; Marechal *et al.,* 1978; Ng and Marechal, 1985).

2.1.4 Uses

Cowpea has a wide variety of uses namely as a nutritious component in the human diet as well as nutritious livestock feed. Cowpea can be used at all stages of growth as a vegetable crop. The tender green leaves are an important food source in Africa and are prepared as a pot herb, like spinach. Immature snapped pods are used in the same way as snapbeans, often being mixed with other foods. Green cowpea seeds are boiled as a fresh vegetable, or may be canned or frozen. Dry mature seeds are also suitable for cooking and canning. In many areas of the world, cowpea is the only available high quality legume hay for livestock feed. Cowpea may be used green or as dry fodder. It is also used as a green manure crop, a nitrogen-fixing crop or for erosion control (Davis *et al.,* 1991). It is very good for quick growth and establishment and for increasing organic matter and improving soil structure. It has excellent heat tolerance and good drought tolerance (http://www.ii.ctahr.hawaii.edu/sustainag/cowpea.htm). It can also be used for intercropping with the other main crops like pearl millet *(Pennisetum glaucum)* or sorghum (*Sorghum bicolor*).

2.1.4.1 Folk medicine

Cowpeas are sacred to Hausa and Yoruba tribes, and are prescribed for sacrifices to abate evil and to pacify the spirits of sickly children. Hausa and Edo tribes use cowpea medicinally; one or two seeds are ground and mixed with soil or oil to treat stubborn bowels.

2.1.5 Production status

It is rather difficult to obtain reliable statistics on cowpea area and production because most countries do not maintain separate records on cowpea. Probably because of these difficulties, the Food and Agricultural Organisation (FAO) suspended formal publication of cowpea production data several years ago. However, based on information available from FAO and via correspondence with scientists in several countries, cowpea researchers at the International Institute of Tropical Agriculture (IITA) estimated that cowpea is now cultivated on at least 12.5 million hectares, with an annual production of over 3 million tonnes worldwide.

Cowpea is widely distributed throughout the tropics, but central and west Africa account for over 64% of the area (with about 8 million hectares, followed by about 2.4 million hectares in central and southern America, 1.3 million hectares in Asia, and about 0.8 million hectares in eastern and southern Africa). Some cowpea is also cultivated in the Middle East and southern Europe. The important cowpea growing countries are Nigeria, Niger Republic, Mali, Burkina Faso, Senegal, Ghana, Togo, Benin, Cameroon, and Chad in central and west Africa; Sudan, Somalia, Kenya, Malawi, Uganda, Tanzania, Zambia, Zimbabwe, Botswana and Mozambique in east and southern Africa; India, Bangladesh, Nepal, Sri Lanka, Indonesia, China, and Philippines in Asia; and Brazil, Cuba, Haiti, USA, and West Indies in central America. However, a substantial part of cowpea production comes from the drier regions of northern Nigeria (about 4 million ha, with 1.7 million tonnes), southern Niger Republic (about 3 million ha, with 1 million tonnes) and Brazil (about 1.9 million ha, with 0.7 million tonnes) (Singh *et al.*, 1993).

2.1.5.1 Cowpea production systems

Traditionally in west and central Africa, and Asia, cowpeas are grown on small farms often intercropped with cereals such as millet and sorghum by the small scale farmers. Fertilisers and pesticides are generally not used, because they are too expensive or not available for the small farmers. In southern Turkey, Greece, Italy, Bulgaria, and Spain both fodder and grain type varieties are grown mostly as a pure crop.

The commercial production of cowpea is mostly done in the states of Georgia, California, Texas, Mississippi, Arkansas and Tennessee in the USA and most of the cultivation is mechanised (Ferry, 1990).

2.1.5.2 Cowpea production in Cameroon

A joint project between Purdue researchers and the Institute of Agronomic Research of Cameroon focused on developing, testing and extending simple, low cost, and effective technologies that low income farmers can use to abate their losses. The annual production of cowpea in the northern province of Cameroon in the last decade varied from 15 000 to 45 000 MT (Bean/Cowpea CRSP West Africa, 1998). Data on the national production level is uncertain.

2.1.6 Environmental requirements

a. Climate

Cowpea grows primarily under humid conditions. It is tolerant to heat and drought conditions. Cowpea is sensitive to frost. It germinates rapidly at temperatures above 65°F; colder temperatures slow germination. Cowpeas are grown under both irrigated and unirrigated regimes (Davis *et al.*, 1991).

b. Soil

Cowpea is well adapted to a wide range of soils and conditions. It requires well-drained sandy loams or sandy soils where the soil pH is in the range of 5.5 to 6.5 (Davis *et al.*, 1991).

c. Cultural practices
   - seedbed preparation
   - appropriate seeding date
   - the respect of method and rate of seeding
   - the use of selective varieties with high yields
   - weed control

2.1.7 Cowpea production constraints

2.1.7.1 Biotic stress

2.1.7.1.1 Diseases

Cowpea is susceptible to a wide variety of pests and pathogens that attack the crop at all stages of growth (Allen, 1983), for instance cowpea wilt caused by *Fusarium oxysporum*, cowpea root rust caused by a nematode (*Meloidogyne* ssp.*) and* cowpea bacterial blight caused by *Xanthomonas vignicola*. Losses due to pest attacks or diseases can be as high as 90% (IITA, 2000).

2.1.7.1.2 Insects

Some of the major insect enemies of cowpea are cowpea weevil (*Callosobruchus maculatus*), cowpea cuculus (*Chalcodermus sermus*), and the southern cowpea weevil (*Mylabris quadrimaculatus*).

2.1.7.2 Abiotic stress

2.1.7.2.1 Environmental stress (drought stress) in plants

The effects of the environment on plant growth may be divided into enforced damage effects (stress), caused by the environment, and adaptive responses, controlled by the plant (resistance) (Fitter and Hay, 1987). Damage, which may be manifested as death of all or part of the plant, or merely as reduced growth rate due to physiological malfunction, is a common phenomenon and the agents are various: temperature, water availability, soil chemistry, physical properties and others such as air pollution, wind and diseases. However, the most important

environmental agents affecting plant growth in the semi-arid tropical zone is drought.

Linsley *et al.* (1959) defined drought as a sustained period of time without significant rainfall. Katz and Glantz (1977) suggested that there were meteorological and agricultural definitions of drought. A meteorological drought could be defined as that time period when the amount of precipitation is less than some designated percentage of the long term mean. An agricultural drought, on the other hand, could be defined in terms of seasonal vegetation development.

Levitt (1980) reported that drought stress occurs when water uptake from soil cannot balance water loss through transpiration. The subsequent cellular water loss is referred to as dehydration. Drought may start at any time, last indefinitely and attain many degrees of severity. It can occur in any region of the world, with an impact ranging from slight personal inconvenience to endangered nationhood (Hounam *et al.,* 1975).

Agricultural drought occurs when there is not enough moisture available at the right time for the growth and development of crops. As a result, yields and/or absolute production decline (Glantz, 1987). The diagram in Figure 2.2 shows the dimension of drought.

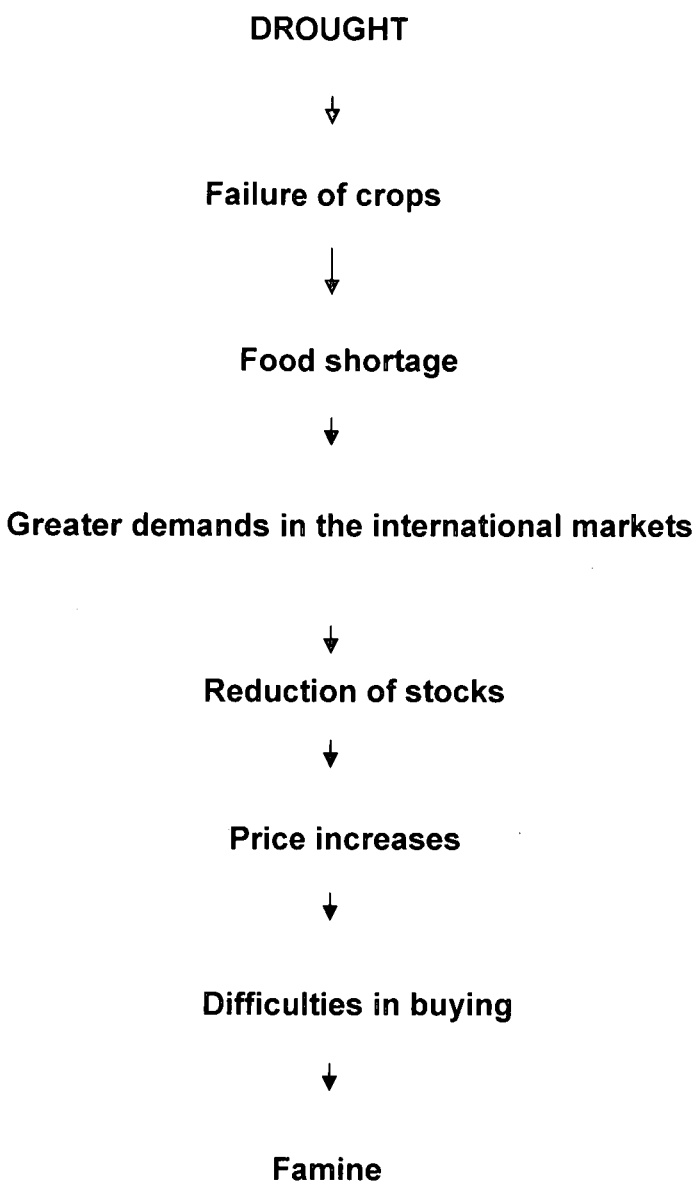

Figure 2.2: The dimensions of drought (Garcia, 1981)

As transpiration occurs as a result of the high temperature common in tropical areas, especially during drought periods, the leaf water potential is reduced. This reduced water potential is then carried down to the roots through the xylem. The soil water potential then decreases because of osmosis into the roots (Raven *et al.,* 1992; Eichhorn, 1992). As a result of a smaller water potential gradient between the root and the soil, less water is absorbed which limits the vegetative growth resulting in low plant yields. Drought does not only affect the yield, but also the quality of the grain and also the appearance of the plant.

Eighty-five percent of the world's cowpea is concentrated in the savannah zone of West Africa between 10° and 20° N latitude (FAO, 1972). Droughts occur frequently in this area, most commonly due to erratic start or early cessation of

the rainfall during the growing season, or occasionally, due to almost no rainfall during the normal growing season for several years in succession (Wien *et al.*, 1976).

Hiler *et al.* (1972) working on drought stress of cowpea found that the flowering stage is the most susceptible to severe imposed stress (-14 to -28 bars leaf water potential). Meanwhile Summerfield *et al.* (1974) found that stress during the vegetative stage irreversibly reduced leaf area and caused significant yield decline.

Water stress is arguably the most important environmental variable affecting plant growth and drought as one of the most important factors threatening the food security of the world (Baker, 1989). The frequency and severity of drought may increase in the future as global warming intensifies.

Furthermore drought stress is highly variable in time (over seasons and years) and space (between and within sites), and is extremely unpredictable. This makes it very difficult to identify a representative drought stress condition (Visser, 1994). The unpredictable and variable forms in which drought stress will manifest itself, makes selection of promising individual plants and breeding for drought tolerance extremely difficult.

Drought tolerance has been shown to be a highly complex trait, influenced by many different genes and should not be regarded as a unique heritable trait, but as a complex of often fully unrelated plant properties (Visser, 1994). Drought can hardly be separated from other important abiotic stresses such as temperature and salinity. Due to these interrelations, no single mechanism exists by which multiple stresses are alleviated. A better understanding of how drought stress affects crop growth and development processes are fundamental. The understanding of the mechanisms of plant adaptations to drought would help breeders to improve drought tolerance of crop plants more effectively. Improved tolerance could sustain productivity and help extend cultivation of certain crops into areas that are currently unsuitable for crop production.

2.1.8 Adaptation to drought stress

Higher plants exposed to water stress, show a variety of morphological and physiological changes at the whole plant level believed to be an adaptation response to stress (Hsiao, 1973). Plants can cope with water stress by avoiding or escaping the periods of drought, in particular during the most sensitive periods of its development. One breeding strategy is to shorten the life cycle of a crop to enable it to mature safely during a rainfall period. For example, in the Sahel, very short season cowpeas developed by researchers at the International Institute of Tropical Agriculture (IITA) avoid drought by maturing in less than 65 days before any substantial stress develops.

Plants can endure or withstand a dry period by maintaining a favourable water balance under drought conditions (Kramer, 1980). Osmotic adjustment, in which the plant increases the concentration of organic molecules in the cell water solution to bind water, is one example. A thicker layer of waxy material at the plant surface and a more extensive and deeper rooting are others. Plants also recover from a dry period by producing new leaves from buds that were able to survive the dry spell.

Many of the drought avoidance and tolerance mechanisms, such as deep root systems, reduced epidermal conductance, increased cuticle thickness, cell membrane stability, proline accumulation, and stomatal closure are due to multigenic expression and involve the whole plant. The common approach in breeding for drought tolerance is to select for drought tolerance components.

2.1.8.1 Cell Membrane Stability (CMS)

The cell membrane can be damaged during stress, causing an increased leakage of electrolytes. The relative rate of this electrolyte leakage is used to estimate the cell membrane stability. Electrolyte leakage is estimated by measuring the electrical conductivity of the medium with which the leaf sample is equilibrated. Cell membrane modification, which results in its perturbed function or total dysfunction, is a major factor in plant environmental stress. The exact structural and functional modification caused by stress is not fully understood. However, the cellular membrane dysfunction due to stress is well expressed in its increased

permeability for ions and electrolytes (Ruter, 1993). Chu-Yung *et al.* (1985) suggested that increased solute leakage is attributed to the loss of membrane integrity through lipid phase transitions and to the effect on membrane bound transport proteins. These proteins play a role in preventing leakage.

The estimation of membrane dysfunction under stress by measuring cellular leakage from affected leaf tissue into an aqueous medium is finding a growing use as a measure of CMS and as a screen for stress tolerance. Tripathy *et al.* (2000) used cell membrane stability to determine drought tolerance of 104 rice genotypes and found the method to be very effective. Ruter (1993) reviewed electrolyte leakage as an effective means of measuring membrane thermostability in leaves and followed sigmodial response curves.

Blum and Ebercon (1981) reported that wheat genotypes grown under conditions of moisture stress, significantly vary in their membrane injury levels. They also noted that injury level ranged from 16.8% to 70% when the genotypes were screened in the laboratory using a 40% PEG solution as a dehydration medium. Mark *et al.* (1991) recommended that cellular rupture due to leaked substances is important for assessing freezing injury in alfalfa.

Using the cell membrane stability test, Blum and Ebercon (1981) found that younger wheat leaf tissues are more tolerant to drought than the older leaf tissues. They also found a variation between bread wheat and durum wheat cultivars on the level of their cell injury percentage under drought stress and concluded that bread wheat cultivars consistently suffered greater injury than durum wheat cultivars. Sullivan *et al.* (1979) used the cell membrane stability test as a selection method for drought and heat tolerance in grain sorghum.

2.1.8.2 The pot evaluation method

This method is performed using plastic pots (both diameter and depth of about 10 to 20 cm, filled with 600 g to 3 kg of soil) in which seeds are sown. This method is usually done under controlled environments, like greenhouses, where drought can be reliably induced to distinguish between tolerant and susceptible genotypes at all stages of growth, particularly at flowering or grain filling stages in seed crops.

Singh *et al.* (1999) found this method more reliable than the field screening method. They reported that germplasm screening for drought tolerance under naturally occurring drought stress does not seem to be reliable. Lack of uniform drought stress in the field will render screening for drought tolerance ineffective and thus limit progress for selection. Selection must occur under controlled environmental conditions. Watanabe *et al.* (1997) used this method as an evaluation method for drought tolerance of cowpea accessions. They found that the degree of wilting and discoloration were fairly uniform among plants of the same accessions. They also found that three replications seemed adequate to correctly evaluate drought tolerance when using this method, provided germination is good and uniform. This method was also reported to be effective in obtaining uniform and healthy seedlings.

2.1.8.3 Stomata

Stomata are small pores in plant leaves through which water vapor and carbon dioxide diffuse mainly during transpiration and photosynthesis (carbon fixation), respectively. It regulates plant carbon, water loss and other physiological functions. Stomata are constantly reacting to environmental stimuli, with responses that can occur in the order of seconds. The primary factors that influence stomatal conductance are light, temperature, humidity, and internal CO_2 concentration.

Stomatal control is often thought to be the first line of defence against water stress. There are two major ideas as to what actually causes stomata to close:

- hydraulic signals (leaf water potential, cell turgor)
- chemical signals (abscisic acid).

The earliest idea for control of stomata in response to soil dryness was that as water supply decreased, leaf water potential and cell turgor declined, and stomatal closure was promoted. However, examples began emerging in which stomatal conductance was reduced, but no reduction in leaf water potential was observed and in these cases, the response of stomata seemed to follow soil water potential more than leaf water potential.

Researchers demonstrated that in some cases, the closing correlated well with abscisic acid (ABA) concentration in leaves. ABA is thought to be produced in plant roots and transported via the xylem stream to stomatal guard cells (Zhang and Davies, 1989; Zhang and Davies, 1990). It is not, however, entirely clear what triggers the production of the ABA within the root, but it may be related to cell turgor.

Elevated levels of atmospheric CO_2 also tend to reduce the area of open stomatal pore space on leaf surfaces thus reducing the amount of water lost to the atmosphere via transpiration. As primary physiological controls on the terrestrial flux of water to the atmosphere, stomata have long been the subject of studies evaluating plant responses to global climate change. The expectation that stomatal conductance will decline with and increase in atmospheric changes has several implications for plant and ecosystem functions.

Barring an increase in leaf area, the most immediate consequence of decreasing stomatal conductance is a reduction in terrestrial evapotranspiration (Field *et al.,* 1995; Jackson *et al.,* 1998). By limiting transpiration, stomatal closure can also improve plant water use efficiency and therefore indirectly influence productivity under water stressed conditions (Polley *et al.,* 1993).

Kanemasu *et al.* (1969) have reported that varieties that offer more resistance to water flow from stomata into the atmosphere have beneficial traits towards drought tolerance. Lal and Moomaw (1977) also confirmed that the rice variety GS6 has higher stomatal diffusive tolerance than IR20, a relatively more drought-sensitive variety. Diallo *et al.* (2002) most recently studied the water status and stomatal behaviour of cowpea plants inoculated with two glomus species at low moisture levels. Tognetti *et al.* (1998) determined that the stomatal conductance of mature oak trees growing near natural CO_2 springs in central Italy were significantly lower than those of similar trees growing further away from the springs during periods of severe summer drought.

2.1.9 Cowpea characterisation

Cowpea yields are low because the environments where they are produced are characterised by various abiotic and biotic stresses. However, even under optimal conditions, the yields are variable and unpredictable, partly due to variability in the growth and development of individual plants. Understanding the extent, distribution and nature of this variation would be useful in the development of cowpea genotypes within both increased yield potential and improved adaptation to environmental stresses.

Phenotypes and genetic diversity can be evaluated using morphological characters, biochemical or molecular markers (DNA markers).

2.1.9.1 Morphological characters

Traditionally, genetic diversity evaluated in crop species are based on differences in morphological characters and qualitative traits (Schut *et al.,* 1997), probably due to the fact that the assay of qualitative traits do not need any sophisticated equipment or complex experiments, they are generally simple, rapid and inexpensive to score. It has been used as a powerful tool in the classification of cultivars and also to study taxonomic status.

Morphological traits continue to be the first step in the studies of genetic relationships in most breeding programmes (Cox and Murphy, 1990; Van Beuningen and Busch, 1997) because, (1) the existing data bases on the germplasm collection or breeding stocks can often be used for genetic analysis; (2) statistical procedures for morphological trait analysis are readily available; (3) morphological information is essential in understanding the ideotype performance relationships; and (4) explanation of heterosis may be enhanced if morphological measures of distances is included as an independent variable.

In cowpea breeding programmes, the major emphasis has been on the collection and conservation of genetic pools. IITA houses over 16000 cultivated and wild accessions of cowpea that cover a wide spectrum of growth habits, environmental responses and varying pest and disease susceptibilities. It is this precious source of material that serves as the essential foundation for the breeding of new

improved varieties. However, the use of morphological traits depends on biochemical traits and most of them are ambiguous descriptors and have limited use for cultivar identification (Stegemann, 1984; Zacarias, 1997). Such characteristics are often controlled by multiple genes and are subject to varying degrees of environmental modification and interaction. Qualitative traits, such as yield performance and quality characters are of major importance in breeding and consequently, these traits are usually focused on during the evaluation of accessions. However, these traits express strong environmental effects, and often also genotype with environment interaction. Liu and Furnier (1993) emphasised the fact that many of the morphological traits are also difficult to analyse because they do not have the simple genetic control assumed by many in genetic models (Tanksley *et al.*, 1989).

Genetic relationship evaluation among germplasm using morphological characteristics are lengthy and costly processes (Cooke, 1984). The genetic control of many morphological characters is assumed to be complex, often including epistatic interactions, and has often not been elucidated (Smith, 1986).

Many morphological markers are recessive and therefore only expressed in the homologous condition. Most elite cultivated and breeding materials do not abound with readily observable morphological markers, a large number of which have deleterious effects on agronomic performance (Smith, 1986). Hence, morphological appearance cannot adequately describe cultivars without extensive replicated trials (Lin and Binns, 1994) and therefore, valid comparisons are only possible for descriptions taken at the same location during the same season (Smith and Smith, 1989).

2.1.9.2 Biochemical markers

2.1.9.2.1 Isozymes

Isozymes are enzymes that share a common substrate but differ in electrophoretic mobility. The procedure to identify their variation is simple. A crude protein extract is made from some tissue sources, usually leaves. The extracts are separated by electrophoresis in a starch gel. The gel is then placed in a solution that contains reagents required for the enzymatic activity of the

enzyme monitoring. In addition, the solution contains a dye that the enzyme can catalyse into a colour reagent that stains the proteins. In this manner, allelic variants of the proteins can be visualised in a gel (Kumar, 1999). Isozymes have long been used because the technique is very robust, technically simple in that the protein extraction and the running of protein molecules on the gel is simple, large numbers of samples can be run in very short time, the bands are expressed co-dominantly.

Direct measures of genetic similarity between individuals have been determined from isozyme markers in many crop plants (Brown, 1979). Isozymes have largely been used in cowpea improvement programmes with emphasis on populations, taxonomy, genetic relationship and diversity studies.

2.1.9.2.2 Isozyme application in cowpea

Ganguly *et al.* (1990) carried out a study on the superoxide dismutase activity in resistant and susceptible cowpea cultivars inoculated with root knot nematode, *Meloidogyne incognita* using isozyme. Superoxide dismutase activity increased in resistant cultivars at all stages of observation. Electrophoretic analyses showed that the isozymes did not vary in number or electrophoretic mobility.

Raman and Dhileepan (1993) studied two oxidereductases and polyphenol oxidase from cowpea infected by *Meloidogyne incognita* race one. Polyacrylamide gel electrophoresis (PAGE) analysis of polyphenol oxidase showed that four new isozymes were produced in roots seven days after inoculation. They were also present after 14 days although their Rb values differed. Phenol concentration also increased with infection.

Vaillancourt and Weeden (1993) showed the lack of isozyme similarity between *Vigna unguiculata* and other species of subgenus *Vigna*. UPGMA cluster analysis was performed and the range of genetic distance among species of subgenus *Vigna* was greater than previously reported in most plant genera. None of the species included in the survey is a close relative of *V. unguiculata,* as shown by the results.

Panella and Gepts (1993) studied the genetic relationships within *Vigna unguiculata* based on isozyme analyses. In general, results of this study concurred with the taxonomic classification within *V. unguiculata* and provided a strong indication that a severe genetic bottleneck occurred during the domestication process of cowpea.

Nevertheless, several drawbacks should be noted with regards to isozymes. Enzyme encoding loci do not constitute a random sample of genes, and they are not randomly dispersed through the genome. Some isozyme variants are not selectively natural and electrophoresis will detect only protein of the actual variability present in amino acid sequence (Bretting and Widrechner, 1995). Furthermore, isozyme expression can be significantly influenced by the environmental factors, management practices and by plant development stage (Bellamy *et al.,* 1996). Therefore, although isozyme analysis is relatively inexpensive and easy to handle, it is not as useful as DNA markers due to the low level of polymorphism and limited number of loci (Bernatzky and Tanksley, 1986).

2.1.9.3 DNA markers

A number of recent publications (Paterson *et al.,* 1991; Weising *et al.,* 1995; Karp *et al.,* 1996; Kumar, 1999) have demonstrated that DNA markers were until now the most promising technique used to differentiate among genotypes at species and subspecies level.

The most closely related cultivars are usually distinguished by DNA fingerprinting methods (Nybom, 1994). Compared to morphological and biochemical characteristics, the DNA genome provides a significantly more powerful source of genetic polymorphism (Beckmann and Soller, 1986). They allow direct comparison of genetic diversity to be made at the DNA level, have the potential to identify a large number of polymorphic loci with an excellent coverage of an entire genome, are phenotypically neutral, allow scoring of plants at any developmental stage and are not modified by environment and management practices (Tanksley *et al.,* 1989; Messmer *et al.,* 1993). They also enable the investigator to detect the exact genetic constitution of an individual plant in a segregating population (Phillip *et al.,* 1994). DNA markers are now widely used in constructing genetic maps, quantitative trait loci (QTL) mapping, diversity analysis and as tool for assisted

selection (MAS) in breeding programmes. In MAS, DNA markers are used to tag desired genes or QTLs to introgressed into elite lines.

DNA markers are considered to be the most suitable means for estimating genetic diversity because of their abundant polymorphism and the fact that they are independent of environment (Gepts, 1993). DNA- based molecular markers such as restriction fragment length polymorphisms (RFLPs) (Federici *et al.,* 1998; Desplanque *et al.,* 1999), randomly amplified polymorphic DNA (RAPD) (Moeller and Schaal, 1999; Rodriquez *et al., 1999*) simple sequence repeats or microsatellites (Dje *et al.,* 1999; Gilbert *et al.,* 1999), sequence tagged sites (Liu 1999; Vanter *et al.,* 1999) and single nucleotide polymorphism (Germano and Klein, 1999) have been used for fingerprinting varieties, cultivars and clones of plants. Amplified Fragment Length Polymorphisms (AFLPs) have emerged as a powerful tool for DNA fingerprinting and genome mapping (Zabeau and Vos, 1993).

2.1.10 Advances in cowpea breeding for drought tolerance

Significant progress has been made at the International Institute of Tropical Agriculture (IITA) in an attempt to develop cowpea drought tolerant genotypes. For example early-maturing cowpea varieties that escape terminal drought has been developed (Singh, 1987).

Different drought tolerant lines have also been identified. Some cease growing as soon as drought stress is imposed, probably to conserve moisture and survive for 2-3 weeks. Others mobilise moisture from lower leaves and remain alive for a longer time. Consequently, these varieties have a better regeneration potential than others do.

A simple technique, using wooden boxes, was developed to screen cowpea germplasm lines at seedling stage, and test their field performance at mature stage under conditions of water deficit. The wooden box technique was found to be more appropriate for breeding programmes in developing countries. Efforts are also being made to combine deep root systems with drought tolerance, to enhance adaptation of cowpeas to low rainfall areas (Watanabe, 1993).

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Chapter 3

MORPHOLOGICAL DIVERSITY ANALYSIS OF COWPEA ACCESSIONS UNDER GLASSHOUSE CONDITIONS

3.1 Introduction

Cowpea (*Vigna unguiculata*) is an important tropical legume with a high protein content of about 25%, a cheap source of protein for the poor in the West African region, where more than 70% of the total production is grown. However, production is constrained by low and variable grain yields, grain quality and susceptibility to diseases and pests, such as aphids (*Oetheca mutabilis)* and the absence of improved cultivars. Although it is reported that yields of 2500 kg/ha are achievable, farmer's yields are consistently low at levels between 350 kg/ha and 700 kg/ha. The genetic improvements are limited by the lack of knowledge of genetic diversity of the indigenous and cultivated germplasm. Moreover, cowpea is a single crop species, but the varietal requirements in terms of plant type, maturity date, seed type (colour preference), and use pattern are extremely diverse from region to region, making breeding programmes for cowpea more complex than for other crops (Barrett, 1987; Paul *et al.*, 1988; Timsina, 1989; Akundabweni *et al.*, 1990; Silva, 1990; Tian and Xu, 1993).

Because no single variety is suitable for all conditions, there is a need to develop varieties with different attributes and resistance to major biotic and abiotic constraints, to suit the specific needs of different regions and cropping systems. Broadening the genetic base by introducing new alleles present in exotic germplasm (Faenza *et al.,* 1982) and a systematic exploitation of heterosis (Masawe, 1994) have been suggested as means to overcome some of the problems. However, identification of parental lines to exploit heterosis and to introduce valuable characters into the cowpea breeding programme will require more reliable information concerning the level of genetic similarity of available gene pools world wide (Mneney *et al.,* 2001). Quantification and classification of diversity in germplasm collections is important for both plant breeders and germplasm curators.

Distance measures between entries can be based on biochemical marker traits, pedigree information, and quantitative or qualitative morphological traits. Although

variation in morphological traits is influenced by environmental factors, their efficiency for selection is still extremely useful. Morphological attributes have traditionally been employed in establishing phylogenetic relationships among genotypes between and within species and for various other purposes including identification of duplicates, studies of genetic variation patterns, and correlation of characteristics of agronomic importance. Marechal *et al.* (1978) used morphological diversity to study taxonomic relationships between genotypes belonging to the genera *Phaseolus* and *Vigna*. Obute (2001) used morphological traits (plant height, number of leaves, leaf length, the number of pods per plant, pod length and number of seeds per pod) to characterize an aneuploid *Vigna unguiculata* from the other cytotypes. Lush (1979) carried out a study on the flower morphology of wild and cultivated cowpea. Pasquet (1993) carried out an intraspecific classification on *Vigna unguiculata* using their morphological traits. Morphological traits of plants can be grouped as either quantitative or qualitative.

The objectives of this study were to:

a) group 20 cowpea accessions from three different African regions into clusters according to their distance as measured by quantitative and qualitative morphological traits,
b) quantify the extent of phenotypic and genetic diversity among these accessions,
c) identify desirable groups that could be utilized in breeding programmes.

3.2 Materials and Methods

3.2.1 Materials

Plant materials

The 20 accessions used in this study were collected from Cameroon, South Africa, and Kenya. The names and country of origin of these accessions are given in Table 3.1. Figure 3.1 shows the morphological differences among the trifoliolate leaves of the accessions studied.

Table 3.1: List of the studied accessions along with their country of origin

No	Accessions	Country of origin
1	MTA22	Cameroon
2	Balen	Cameroon
3	Okhalweni	South Africa
4	AS-94	Cameroon
5	Bafoussam 1	Cameroon
6	Bamougoum	Cameroon
7	Bafoussam 2	Cameroon
8	Bafoussam 3	Cameroon
9	Mpenbeni	South Africa
10	Bafoussam 4	Cameroon
11	Hluhluwa	South Africa
12	Bafoussam 5	Cameroon
13	K.80	Kenya
14	Gacaga	Kenya
15	M.66	Kenya
16	Makueni	Kenya
17	Kamurugu 1	Kenya
18	Ken-Kunde	Kenya
19	Kasuku	Kenya
20	Kamurugu 2	Kenya

MTA22

Balen

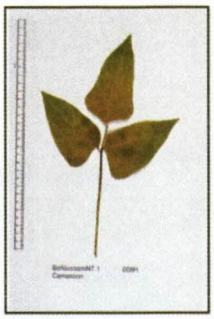
Okhalweni

AS-94

Bafoussam 1

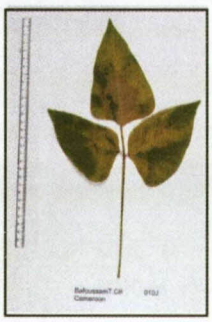
Bamougoum

Bafoussam 2

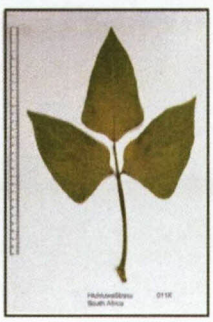


Bafoussam 3

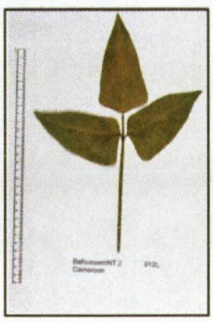
Mpenbeni



Bafoussam 4



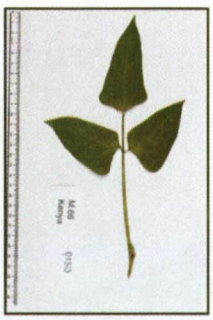
Hluhluwa



Bafoussam 5

K.80

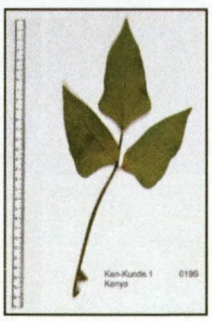
Gacaga



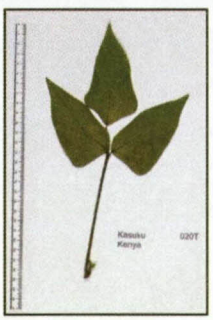
M.66

Makueni

Kamurugu 1



Ken-Kunde



Kasuku

Kamurugu 2

Figure 3.1: Trifoliolate leaves of accessions studied

3.2.2 Methods

3.2.2.1 Experimental environment and methods

The study was conducted in a glasshouse at the University of the Free State (latitude 29.6°, longitude 26.18°, altitude 1351 m above sea level). The maximum temperature was 35°C during the daytime and the minimum night temperature was 15°C. The 20 accessions were evaluated in pots (2.5 liter) that were laid out in a randomized complete block design with five replications. Eight seeds were planted in a red sandy soil at a depth of about 2.5 cm and thinned to four plants per pot a week after germination. The plants were irrigated twice every day from planting date to maturity. Data on the agronomical and morphological characters were collected from 10 randomly selected plants and their means were recorded for all observations. Fifteen qualitative and 12 quantitative traits were measured using the international board for plant genetic resources (IBPGR) cowpea descriptors.

3.2.2.2 Qualitative and quantitative traits evaluation methods

3.2.2.2.1 Qualitative traits

The qualitative traits were evaluated using different scoring scales at maturity and at flowering for flowers.

1- Growth pattern
1= Determinate (apical bud of main stem reproductive)
2= Indeterminate

2- Twining tendency
0 = None
3 = Slight
5 = Intermediate
7 = Pronounced

3- Plant pigmentation (recorded for stem, branches, petioles and peduncles in the 6th week after planting)

0 = None

1 = Very slight

3 = Moderate at the base and tips of petioles

5 = Intermediate

7 = Extensive

9 = Solid

4- Terminal leaflet shape (recorded for the terminal leaflet of a young, mature leaf in the 6th week after planting)

1 = Globose

2 = Sub-globose

3 = Sub-hastate

4 = Hastate

5- Plant hairiness (of stems, leaves and pods)

3 = Glabrescent

5 = Short appressed hairs

7 = Pubescent to hirsute

6-Raceme position (recorded when peduncles have reached full length)

1 = Mostly above canopy

2 = In upper canopy

3 = Throughout canopy

7- Pod attachment to peduncle (recorded when pods were fully-grown)

3 = Pendant

5 = 30 – 90° down from erect

7 = Erect

8- Pod curvature (of mature pods)

0 = Straight

3 = Slightly curved

5 = Curved

7 = Coiled

9- Immature pod pigmentation (pattern of pigment distribution on full grown immature pod)

0 = None
1 = Pigmented tip
2 = Pigmented sutures
3 = Pigmented valves, green sutures
4 = Splashes of pigment
5 = Uniformly pigmented

10- Seed shape
1 = Kidney
2 = Ovoid
3 = Crowder
4 = Globose
5 = Rhomboid

11- Testa texture
1 = Smooth
3 = Smooth to rough
5 = Rough (fine reticulation)
7 = Rough to wrinkled
9 = Wrinkled (coarse folds on the testa)

12- Leaf colour (intensity of green colour)
3 = Pale green
5 = Intermediate green
7 = Dark green

13- Leaf marking (presence/absence of V mark on leaflets)
0 = Absent
1 = Present

14- Splitting of testa
0 = Absent
1 = Present (testa split exposing cotyledons)

15 Flower colour

1=White

2=Violet

3=Mauve-pink

4=Other

3.2.2.2.2 Quantitative traits

1- Seed weight (g)

Weight of 100 seeds with moisture content of 12%.

2- Terminal leaflet length (mm)

Mean length of 10 terminal leaflets from 10 randomly selected plants.

3- Terminal leaflet width (mm)

Mean width of 10 terminal leaflets measured on the broadest part of 10 randomly selected plants.

4- Number of main branches

The branches whose origin is in the leaf axils on the main stem; recorded in the 8^{th} week after planting. Mean of 10 randomly selected plants.

5- Number of nodes on main stem

Recorded between three to four weeks after planting. Mean of 10 randomly selected plants.

6- Number of pods per peduncle

Mean of 10 randomly selected peduncles.

7- Peduncle length (mm)

Recorded when peduncles have reached full length. Mean length of 10 peduncles, one from each of 10 randomly selected plants was measured.

8- Number of pods per plant

Mean number of mature pods from 10 randomly selected plants.

9- Pod length (cm)

Mean of the 10 longest mature pods from 10 randomly selected plants.

10- Plant height (cm)

Mean of 10 randomly selected plants recorded in the 8^{th} week after sowing.

11- Pod weight (g)

Mean weight of the 10 longest mature pods from 10 randomly selected plants.

12- Number of seeds per pod

Mean number of seeds of the 10 longest mature pods from 10 randomly selected plants.

3.2.3 Data analysis

The data were subjected to cluster analysis using the Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998). In the process of hierarchical clustering, the unweighted pair group method of arithmetic average (UPGMA) was employed.

3.3 Results

3.3.1 Qualitative traits

3.3.1.1 Qualitative morphological character analysis

Fifteen qualitative morphological characters of 20 accessions of cowpea are presented in Table 3.2.

1- Growth pattern

MTA22, Okhalweni, AS-94, Mpenbeni, Hlulhuwa, Gacaga, Ken-Kunde, Kasuku, and Kamurugu 2 had a determinate growth pattern. The pattern of Balen, Bafoussam 1, Bamougoum, Bafoussam 2, Bafoussam 3, Bafoussam 4, Bafoussam 5, K.80, M.66, Makueni and Kamurugu 1, was indeterminate.

2- Twining tendency

MTA22, AS-94, K.80, Gacaga, Makueni, Ken-Kunde, Kasuku, and Kamurugu 2 had no twining tendency. Balen, Bafoussam 1, Bamougoum, Bafoussam 2, Bafoussam 4, Bafoussam 5, and Kamurugu 1 had a pronounced twining tendency. The twining tendency of Okhalweni, Mpenbeni, Hluhluwa, and M.66 was slight and that of Bafoussam 3 was intermediate.

Table 3.2: Mean scores of 15 qualitative traits of cowpea

CCESSIONS	GP	TT	PP	TLS	PH	RP	PA	PC	IPP	SS	TT	LC	LM	ST	FC
TA22	1	0	1	4	3	2	3	3	0	5	1	3	0	0	2
len	2	7	3	2	3	1	3	3	0	1	1	5	0	0	2
halweni	1	3	3	2	5	3	3	0	0	5	3	5	0	0	2
-94	1	0	0	4	5	3	5	0	0	5	3	7	0	0	2
foussam 1	2	7	3	2	3	1	3	3	1	1	1	5	0	0	2
mougoum	2	7	1	2	3	1	3	3	0	1	3	5	0	0	2
foussam 2	2	7	1	2	3	1	5	5	1	1	3	5	0	0	2
foussam 3	2	5	5	2	3	1	5	3	4	1	3	5	1	0	2
enbeni	1	3	0	2	5	3	3	0	0	5	5	7	0	0	2
foussam 4	2	7	0	2	3	1	3	5	1	2	3	5	0	0	2
hluwa	1	3	0	2	5	3	3	0	0	4	1	7	0	1	2
foussam 5	2	7	1	2	3	1	3	0	1	1	3	7	0	0	2
0	2	0	0	3	3	2	3	3	5	2	1	5	0	0	2
caga	1	0	0	4	5	2	5	0	0	5	5	7	0	0	2
6	2	3	0	2	5	2	5	0	4	4	1	5	1	0	2
kueni	2	0	0	2	3	2	3	3	0	5	3	5	0	0	2
murugu 1	2	7	0	2	3	1	7	3	0	4	1	7	1	0	2
n-Kunde	1	0	9	2	3	1	5	0	5	3	7	7	0	0	2
suku	1	0	9	3	3	2	5	0	5	3	7	7	0	0	2
murugu 2	1	0	7	3	3	3	5	0	5	3	7	5	1	0	2

GP= growth pattern, TT= twinning tendency, PP= plant pigmentation, TLS= terminal leaflet shape, PH= plant hairiness, RP= raceme position, PA= pod attachment to peduncle, PC= pod curvature IPP= immature pod pigmentation, SS= seed shape, TT= testa texture, LC= leaf colour, LM= leaf marking, ST= splitting of testa, FC= flower colour

3- Plant pigmentation

AS-94, Mpenbeni, Bafoussam 4, Hluhluwa, Gacaga, M.66, Makueni, Kamurugu1, and K.80 had no pigmentation. MTA22, Bamougoum, Bafoussam 2, and Bafoussam 5 had very slight pigmentation on the stem, branches, peduncles, and petioles. At the base and tips of the petiole of Balen, Okhalweni and Bafoussam 1, the pigmentation was moderate. Bafoussam 3 had intermediate pigmentation on its branches and stem and the pigmentation of Kamurugu 2 was extensive. Ken-Kunde and Kasuku had solid pigmentation.

4- Terminal leaflet shape

MTA22, AS-94 and Gacaga terminal leaflet shape was hastate. Balen, Okhalweni, Bafoussam 1, Bafoussam 2, Bamougoum, Bafoussam 3, Mpenbeni, Bafoussam 4, Hluhluwa, Bafoussam 5, M.66, Makueni, Kamurugu 1, and Ken-Kunde had a sub-globose terminal leaflet shape. K.80, Kasuku, and Kamurugu 2 had a sub-hastate terminal leaflet shape. None of the accessions had a globose terminal leaflet shape.

5- Plant hairiness

MTA22, Balen, Bafoussam 1, Bamougoum, Bafoussam 2, Bafoussam 3, Bafoussam 4, Bafoussam 5, K.80, Makueni, Kamurugu 1, Ken-Kunde, Kasuku, and Kamurugu 2 stems, leaves and pods were glabrescent while Okhalweni, AS-94, Mpenbeni, Hluhluwa, Gacaga and M.66 had short appressed hairs on their stems, leaves, and pods.

6- Raceme position

Balen, Bafoussam1, Bamougoum, Bafoussam 2, Bafoussam 3, Bafoussam 4, Bafoussam 5, Kamurugu 1, and Ken-Kunde were mostly above canopy. MTA22, K.80, Gacaga, M.66, Makueni, and Kasuku were in upper canopy while Okhalweni, AS-94, Mpenbeni, Hluhluwa and Kamurugu 2 were throughout the canopy.

7- Pod attachment to peduncle

MTA22, Balen, Okhalweni, Bafoussam 1, Bamougoum, Mpenbeni, Bafoussam 4, Hluhluwa, Bafoussam, K.80 and Makueni full grown pods were pendant. AS-94, Bafoussam 2, Bafoussam 3, Gacaga, M.66, Ken-Kunde, Kasuku, and Kamurugu

2 full grown pods were 30-90° down from erect, while in Kamurugu 1 full grown pods were erect.

8- Pod curvature

MTA22, Balen, Bafoussam 1, Bamougoum, Bafoussam 3, K80, Makueni, and Kamurugu 1 had slightly curved mature pods. Okhalweni, AS-94, Mpenbeni, Hluhluwa, Bafoussam 5, Gacaga, M66, Ken-Kunde, Kasuku, and Kamurugu 2 mature pods were straight. Bafoussam 2 and Bafoussam 4 had curved mature pods.

9- Immature pod pigmentation

MTA22, Balen, Okhalweni, AS-94, Bamougoum, Mpenbeni, Hluhluwa, Gacaga Makueni and Kamurugu 1, had no pattern of pigment distribution on their full grown immature pods. Bafoussam 1, Bafoussam 2, Bafoussam 4, and Bafoussam 5, full grown immature pods had a pigmented tip. Bafoussam 3 and M.66 had splashes of pigment on their full grown immature pods. K80, Ken-Kunde, Kasuku, and Kamurugu 2, full grown immature pods were uniformly pigmented.

10- Seed shape

Balen, Bafoussam 1, Bamougoum, Bafoussam 2, Bafoussam 3, and Bafoussam 5 seeds had a kidney shape. Bafoussam 4, and K.80, seeds had an ovoid shape. Ken-Kunde, Kasuku, and Kamurugu 2 had crowder shape and Hluhluwa, M.66, and Kamurugu 1 a globose shape. MTA22, Okhalweni, AS-94, Mpenbeni, Gacaga, and Makueni had rhomboid shape seeds.

11- Testa texture

The testa texture of MTA22, Balen, Bafoussam 1, Hluhluwa, K.80, M.66, and Kamurugu 1, was smooth while Okhalweni, AS-94, Bamougoum, Bafoussam 2, Bafoussam 3, Bafoussam 4, Bafoussam 5, and Makueni testa texture was smooth to rough. Mpenbeni and Gacaga testa textures were rough and Ken-Kunde, Kasuku, and Kamurugu 2 seed testa textures were rough to wrinkled.

12- Leaf colour

MTA22 leaf colour was pale green. Balen, Okhalweni, Bafoussam 1, Bamougoum, Bafoussam 2, Bafoussam 3, Bafoussam 4, K.80, M.66, Makueni

and Kamurugu 2 leaf colour was intermediate (between pale green and dark green). AS-94, Mpenbeni, Hluhluwa, Bafoussam 5, Gacaga, Kamurugu 1, Ken-Kunde, and Kasuku had dark green colour leaves.

13- Leaf marking

MTA22, Balen, Okhalweni, AS-94, Bafoussam 1, Bamougoum, Bafoussam 2, Mpenbeni, Bafoussam 4, Hluhluwa, Bafoussam 5, K.80, Gacaga, Makueni, Ken-Kunde, and Kasuku had no marking on their leaflets. Bafoussam 3, M.66, Kamurugu 1, and Kamurugu 2 had their leaflets marked with a V.

14- Splitting of testa

Hluhluwa was the only accession with seed testa splitting presence around the hilum and exposing the cotyledons.

15- Flower colour

All 20 accessions had the same violet flower colour.

3.3.1.2 Cluster analysis

The dendrogram constructed on the basis of the data generated from the qualitative traits divided the 20 accessions into two major clusters (A and B), and at a genetic distance of 0.60, each had two subclusters (I and II) (Table 3.3 and Fig 3.2). Cluster A, subcluster I consisted of three accessions and subcluster II of five accessions. Cluster B, subcluster I had two accessions, and subcluster II 10 accessions. Subcluster II had two distinctive groups (a and b), with group a comprising of two accessions and b of eight accessions. In general, most of the accessions in this study were grouped according to their geographic origin. For example, all the accessions in the subcluster I of the major cluster A was from Kenya, and similarly entries in subcluster I in the major cluster B were also all from Kenya. Subcluster II of the major cluster B grouped most of the accessions from Cameroon (8), two accessions from Kenya and no accessions from South Africa. Subcluster II of the major cluster A was the only one where accessions from the three different geographic regions (Cameroon, Kenya and South Africa) were grouped together. However all three accessions from South Africa used in this study were found in this subcluster. Therefore in this study the clustering process reflected the geographic origin of the collections.

Table 3.3: Cluster distribution of the 20 cowpea accessions based on 15 qualitative traits

ter	No. of acc	Name of accessions
ter A		
	3	Kamurugu 2, Kasuku, Ken-Kunde
	5	Hluhluwa, Mpenbeni, Gacaga, AS-94, Okhalweni
ter B		
	2	M.66, K.80
	10	a- (Kamurugu 1, Bafoussam 3) b- (Bamougoum, Balen, Makueni, MTA22, , Bafoussam 1, Bafoussam 5, Bafoussam 2, Bafoussam 4)

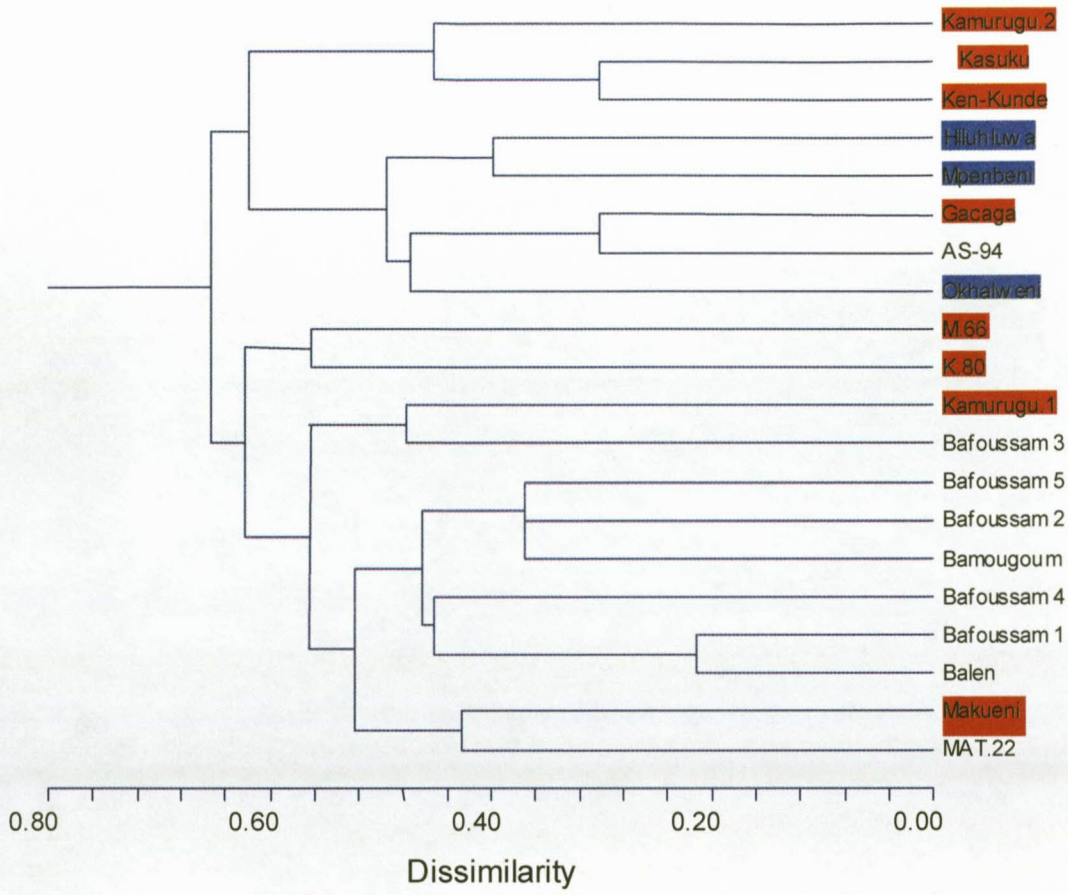


Figure 3.2: Dendrogram of the studied accessions based on 15 qualitative traits

Red: accessions from Kenya

Blue: accessions from South Africa

Normal: accessions from Cameroon

3.3.2 Quantitative traits

3.3.2.1 Quantitative morphological character analysis

Twelve quantitative morphological characters of 20 accessions of cowpea are presented in Table 3.4

Table 3.4: Mean scores of twelve quantitative traits of cowpea

CCESSIONS	SW (g)	PL (mm)	TLL (mm)	TLW (mm)	MB	PP	NMS	PPt	SP	PW (g)	PH (cm)	PL (cm)
TA22	18.08	15.4	51.0	7.20	2	1	6.4	4.0	9.70	2.0	27.0	16.0
alen	18.25	10.0	41.2	22.0	2	1	7.0	4.0	6.60	1.2	18.0	15.0
khalweni	20.21	16.6	84.0	49.0	0	1	9.0	3.2	11.7	3.0	31.0	18.4
S-94	14.40	13.6	60.3	9.20	2	2	8.0	4.0	10.4	2.0	27.0	13.2
afoussam 1	14.02	12.4	37.0	21.2	2	1	6.2	3.4	6.90	1.4	18.2	14.0
amougoum	21.48	14.2	47.0	24.3	2	2	6.1	5.0	7.10	1.2	21.0	15.1
afoussam 2	17.00	13.0	40.0	24.3	2	1	7.1	4.0	7.30	1.4	21.0	13.0
afoussam 3	12.00	17.0	52.4	30.1	1	2	9.0	4.0	8.90	1.4	30.7	15.0
benbeni	22.01	18.0	89.3	59.6	0	1	8.6	3.3	11.8	3.0	31.0	18.4
foussam 4	19.00	13.0	46.0	26.0	2	2	7.1	5.0	8.70	2.0	21.0	15.0
uhluwa	20.00	18.4	80.0	45.4	0	1	9.3	4.0	12.00	3.0	30.6	18.3
foussam 5	20.00	13.8	48.0	28.0	2	1	5.4	4.0	6.50	1.1	18.4	13.3
30	14.25	14.0	49.4	29.4	0	1	8.3	4.0	9.90	2.0	27.4	13.3
caga	11.11	13.1	48.3	8.10	2	1	7.0	5.1	12.1	2.1	27.0	17.0
66	14.00	16.7	48.0	26.3	3	1	8.2	5.0	13.00	2.2	30.8	12.4
kueni	10.06	12.7	76.0	35.3	0	1	8.0	4.0	14.3	2.0	30.5	13.2
murugu 1	11.00	17.6	36.0	20.0	0	2	7.4	5.0	8.60	1.0	33.7	14.0
n-Kunde	13.30	12.4	34.1	20.0	2	2	8.1	6.0	11.6	3.2	20.0	12.0
suku	11.00	9.52	51.0	26.0	2	1	9.3	4.0	11.6	2.0	20.0	12.0
murugu 2	13.00	13.1	62.0	27.0	0	2	9.0	4.0	12.9	2.0	20.0	13.0

SW= seed weight, PL= peduncle length, TLL= terminal leaflet length, TLW= terminal leaflet width, MB= number of main branches, PP= number of pods per peduncle, NMS= number of nodes on main stem, PPt= number of pods per plant, SP= number of seeds per pod, PW= pod weight, PH= plant height, PL= pod length.

1- Seed weight

According to seed weight the accessions could be grouped as follows: Mpenbeni (22.01 g); Bamougoum (21.48 g); Okhalweni, Hluhluwa and Bafoussam 5 (±20.10 g); Bafoussam 4 (19 g); MTA22 and Balen (±18.10 g); Bafoussam 2 (17 g); AS-94, Bafoussam 1, K.80, and M.66 (±14 g); Kamurugu 2 and Ken-Kunde (±13 g);

Bafoussam 3 (12 g); Kamurugu 1, Gacaga, and Kasuku (±11 g); and Makueni (10.06 g). Mpenbeni (22.01 g) had the highest seed weight while Makueni had the lowest (10.06 g).

2- Terminal leaflet length

For the terminal leaflet length the accessions were as follows: Mpenbeni (89.3 mm); Okhalweni (84 mm); Hluhluwa (80 mm); Makueni (76 mm); Kamurugu 2 (62 mm); AS-94 (60.3 mm); Bafoussam 3 (52.4 mm); MTA22 and Kasuku (51 mm); K.80 (49.4 mm); Bafoussam 5, Gacaga, and M.66 (±48 mm); Bamougoum (47 mm); Bafoussam 4 (46 mm); Balen (41.2 mm); Bafoussam 2 (40 mm); Bafoussam 1 (37 mm); Kamurugu 1 (36 mm); and Ken-Kunde (34.1 mm). Mpenbeni had the longest terminal leaflet length (89.3 mm) while Ken-Kunde had the shortest (34.1 mm).

3- Terminal leaflet width

For the terminal leaflet width the accessions were grouped as follows: Mpenbeni (59.64 mm); Okhalweni (49 mm); Hluhluwa (45.4 mm); Makueni (35.3 mm); Bafoussam 3 (30.1 mm); K.80 (29.4 mm); Bafoussam 5 (28 mm); Kamurugu 2 (27 mm); Bafoussam 4, M.66, and Kasuku (26.1 mm); Bamougoum and Bafoussam 2 (24.3 mm); Balen (22 mm); Bafoussam 1 (21.2 mm); Kamurugu 1 and Ken-Kunde (20 mm); AS-94 (9.2 mm); Gacaga (8.1 mm) and MTA22 (7.2 mm). Mpenbeni had the broadest terminal leaflet (59.64 mm) while MTA22 had the narrowest (7.2 mm).

4- Number of main branches

According to the number of main branches counted on each plant the following grouping of the accessions could be made: M.66 (3); MTA22, Bafoussam 1, AS-94, Bafoussam 5, Bafoussam 2, Ken-Kunde, Gacaga, Bamougoum, Bafoussam 4, Balen and Kasuku ,(2); Bafoussam 3 (1); K.80, Makueni, Kamurugu 2, Mpenbeni, Hluhluwa, Kamurugu 1, and Okhalweni (0). M.66 had the highest number of main branches (3) while K.80, Makueni, Kamurugu 2, Mpenbeni, Hluhluwa, Kamurugu 1, and Okhalweni had no main branches.

5- Number of nodes on main stem

For the number of nodes on the main stem, the following grouping of the accessions was made: Okhalweni, Bafoussam 3, Hluhluwa, Mpenbeni, Kamurugu

2 and Kasuku (± 9); AS-94, K.80, M.66, Makueni, and Ken-Kunde (± 8.1); Balen, Bafoussam 2, Kamurugu 1, Gacaga, and Bafoussam 4 (± 7.1); MTA22, Bafoussam 1, and Bamougoum (± 6.1); Bafoussam 5 (5.4). Hluhluwa and Kasuku had the highest number of nodes on their main stem (9.3) while Bafoussam 5 had the lowest (5.4).

6- Number of pods per peduncle

According to the number of pods per peduncle the accessions were grouped as follows: AS-94, Bafoussam 3, Bafoussam 4, Kamurugu 1, Ken-Kunde and Kamurugu 2 (2); MTA22, Bafoussam 1, Bamougoum, Balen, Bafoussam 2, Gacaga, Okhalweni, Hluhluwa, Mpenbeni, Kasuku; K.80, M.66, Bafoussam 5 and Makueni (1). The highest number of pods per peduncle was two and one was the lowest.

7- Peduncle length

According to peduncle length, the following grouping could be made: Mpenbeni, Hluhluwa and Kamurugu 1 (±18 mm); Okhalweni, Bafoussam 3, and M.66 (±16.6 mm); MTA22 (15.4 mm); AS-94, Bamougoum, K.80 and Bafoussam 5 (±13.8 mm); Bafoussam 2, Bafoussam 4, Gacaga, Makueni and Kamurugu 2 (±13.1 mm); Bafoussam 1 and Ken-Kunde (12.4 mm); Balen (10 mm); and Kasuku (9.25 mm). Hluhluwa had the largest peduncle length (18.4 mm) and Kasuku had the smallest (9.25 mm).

8- Number of pods per plant

According to the number of pods per plant the accessions were grouped as follows: Ken-Kunde (6); Kamurugu 1, Bamougoum, Gacaga, Bafoussam 4, and M.66 (±5.2); MTA22, Balen, AS-94, Bafoussam 2, Kamurugu 2, Bafoussam 3, Makueni, K.80, Kasuku, Hluhluwa, and Bafoussam 5; Okhalweni, Bafoussam 1, and Mpenbeni (±3.3); Ken-Kunde had the highest number of pod per plant (6) while Okhalweni had the lowest (3.2).

9- Pod length

For the length of dry mature pods, the following grouping was made: Okhalweni, Mpenbeni, Hluhluwa (±18.3 cm); Gacaga (16.6cm); MTA.22 (15.8 cm); Balen, Bafoussam 3, Bamougoum, and Bafoussam 4 (±14.6 cm); Bafoussam 1 and Kamurugu 1 (14 cm); AS-94, Bafoussam 5, Makueni, Kamurugu 2, Bafoussam2,

and K.80 (13.03 cm); Ken-Kunde, M.66 and Kasuku (±12.20 cm). Mpenbeni had the longest pods (18.46 cm) while Kasuku had the shortest (11.7 cm).

10- Plant height

According to the values of plant height recorded, the accessions were grouped as follows: Kamurugu 1 (33.7 cm); Okhalweni, Mpenbeni, Hluhluwa, M.66, Bafoussam 3, and Makueni (± 30.6 cm); MTA22, AS-94, K.80, and Gacaga (± 27cm); Bamougoum, Bafoussam 2, and Bafoussam 4 (± 20.6 cm); Ken-Kunde, Kamurugu 2, and Kasuku (20 cm); Balen, Bafoussam 1, and Bafoussam 5 (18.2 cm). Kamurugu 1 had the biggest plants (33.7 cm) while Okhalweni and Balen had the smallest (17.9 cm).

11- Pod weight

For the weight of dry mature pods the following grouping was made: Hluhluwa, Mpenbeni and Okhalweni (± 2.7 g); MTA22, AS-94, Bafoussam 4, K.80, Gacaga, M.66, Makueni, Ken-Kunde, Kasuku, and Kamurugu 2 (±1.7 g); Balen, Bamougoum, Bafoussam 2, Bafoussam 1, Bafoussam 5, Bafoussam 3, and Kamurugu 1 (± 1.2 g). Okhalweni had the highest mature dry pod weight (2.8 g) while Kamurugu 1 had the lowest (1.09 g).

12- Number of seeds per pod

For the number of seeds per pod the following grouping of the accessions was made: Makueni (14.3); M.66 and Kamurugu 2 (± 12.9); Okhalweni, Mpenbeni, Hluhluwa, Gacaga, Ken-Kunde, and Kasuku (±11.7); AS-94, MTA22, and K.80 (± 9.9); Kamurugu 1, Bafoussam 4, and Bafoussam 3 (±8.7); Balen, Bafoussam 1, Bamougoum, Bafoussam 2, and Bafoussam 5 (±6.9). Makueni had the highest number of seeds per pod (14.3) while Balen and Bafoussam 5 had the lowest (6.6).

3.3.2.2 Cluster analysis

The dendrogram (Fig 3.3) constructed with the data from the quantitative traits data shows the genetic distance estimates among the accessions. It also divided the 20 accessions into two major clusters (I, II) (Table 3.5), and at a genetic distance of 1.25, the second major cluster had three subclusters (A, B, and C). Cluster I had only one group (A), comprised of three accessions. Subcluster A of

cluster II comprised of only one accession. Subcluster B of the same cluster comprised of five accessions. The subcluster C had two groups (a and b), with group "a" comprising of three accessions and "b" comprising of eight accessions.

The accessions grouped according to their geographic origin or specific collection sites. For example, the major cluster I had three accessions, all from South Africa. Subcluster B of cluster II, grouped five accessions with the majority (4) of the accessions from Kenya, one accession from Cameroon. Subcluster C of the same cluster grouped in total eleven accessions, with three accessions from Kenya and eight accessions from Cameroon. It can therefore be said that in this study, the clustering process did reflect the geographic origin of the accessions.

Table 3.5: Cluster distribution of 20 cowpea accessions based on 12 quantitative traits

Cluster	No. of acc	Name of accessions
Cluster I		
A	3	Hluhluwa, Mpenbeni, Okhalweni
Cluster II		
A	1	Kasuku
B	5	Kamurugu 2, Kamurugu 1, Makueni, K.80, Bafoussam 3.
C (a and b)	11	a- (Ken-Kunde, M.66, Gacaga) b- (Balen, AS-94, Bamougoum, Bafoussam 5, Bafoussam 2), MTA22, Bafoussam 1, Bafoussam 4.

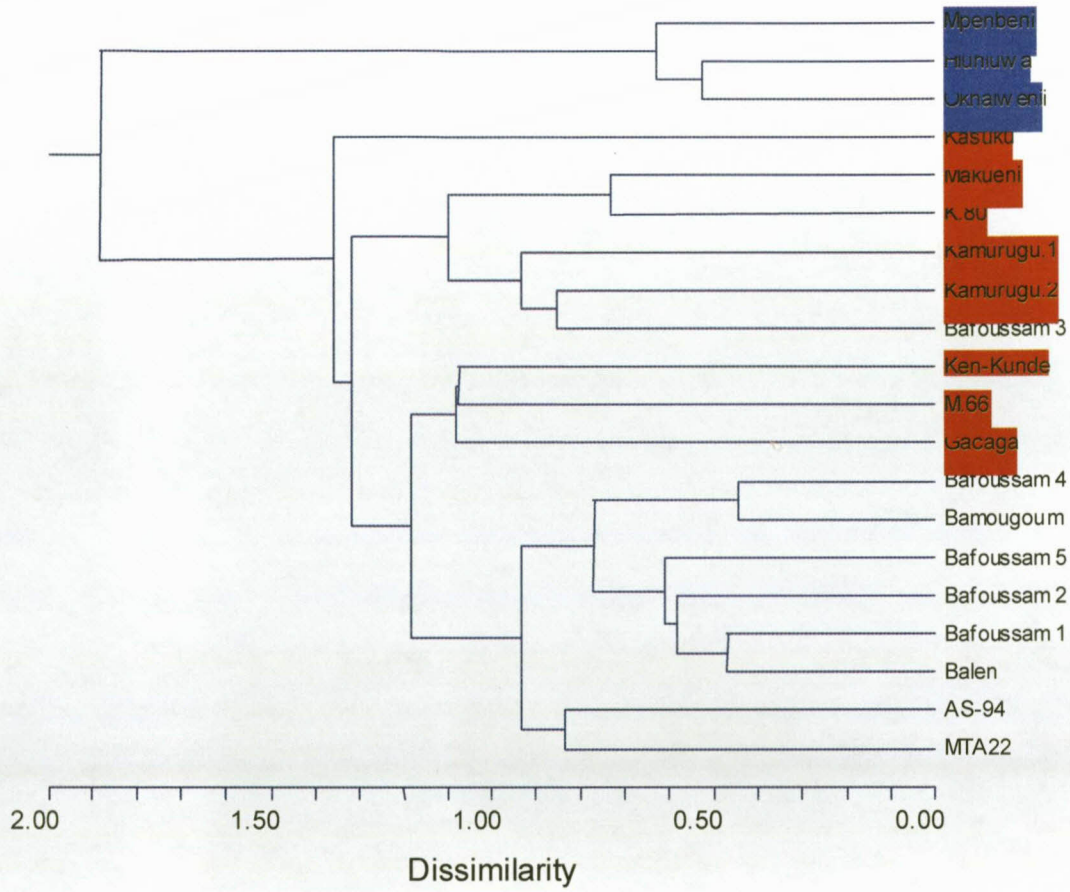


Figure 3.3: Dendrogram of the 20 studied accessions based on 12 quantitative traits

Red: accessions from Kenya
Blue: accessions from South Africa
Normal: accessions from Cameroon

3.4 Discussion

Earlier studies on cowpeas using morphological traits such as plant pigmentation, plant habit, root traits, leaf traits, pod traits, seed traits, grain quality, and yield have been carried out by many researchers. These traits were all found to be of great importance to distinguish genetic variability, and have lead to a better classification of cowpea genotypes. (Apte *et al.*, 1987; Emebiri and Obisesan, 1991; Fery and Dukes, 1994; Karkannavar *et al.*, 1991; Ogunbodede, 1988; Uguru and Uzo, 1991; Brantley and Kuhn, 1983; Fawole, 1988; Drabo *et al.*,

1985; Roquid and Patnaik, 1990). Emebiri (1989) also characterized cowpea cultivars using their flower size and style length, and also reported that both characters were highly heritable.

As in previous studies, this study also found that agro-morphological traits (quantitative and qualitative) are still valuable tools for cowpea genetic diversity studies. For example, some of the morphological traits used in this study, for instance terminal leaflet length, terminal leaflet width, seed weight, twining tendency, plant pigmentation, terminal leaflet shape, immature pod pigmentation, and seed shape had the largest grouping of the accessions and were found to be very efficient in discrimination of the accessions.

Qualitative traits

Cluster analysis substantiated the existence of diversity among the 20 accessions for the morphological traits studied. The clustering pattern shows that accessions from Kenya were genetically more distant among themselves and accessions from Cameroon and South Africa. Furthermore, genetic distance was observed among accessions from the three different geographical regions, presenting a great possibility for the development of suitable varieties for the various agro-ecological zones of Africa with different agro-climatic conditions. That is to say, different cultivars can be developed for specific agro-ecological regions by making use of the available potential of the germplasm.

However, the parallelism observed between the geographical origins (collection areas) and genetic diversity of the accessions studied showed little variability among the accessions from the same areas of collection for most of the characters studied. For example, a high level of relationship was observed among five accessions from the same locality in Cameroon, as well as for the three accessions from South Africa, for most of the traits studied. This close resemblance indicates the possibility that these accessions may have come from the same genetic background. A high level of relationship has also been detected among several cowpea genotypes following the evaluation of the variability in seed proteins among them (D'Urzo *et al.*, 1990). The high level of relationship reported among cowpea varieties may also be due to it being a self-pollinated crop (Padulosi, 1993).

Quantitative traits

The quantitative trait results were very similar to those obtained for the qualitative traits. As for the qualitative traits, the quantitative traits cluster analysis showed the existence of diversity among the 20 accessions for most of the morphological traits studied. The clustering pattern also showed that accessions from Kenya were genetically more distant among themselves as well as from the other accessions from Cameroon and South Africa. There was also very little similarity among accessions from different countries for most of the quantitative traits studied. A high level of similarity was also observed among many accessions from the same region for most of the traits studied.

3.5 Conclusions

A range of observations was made in the current analyses of genetic diversity of cowpea using both their qualitative and quantitative traits. An overall relatively high level of dissimilarity was observed among the accessions for most of the morphological traits analyzed, especially for accessions from different countries. This indicates better possibilities for genetic improvement of the crop through selection and cross breeding. However, a very high level of similarity was revealed between many accessions from the same region for most of the characters studied. The use of material from different geographical origins in any cross breeding programme aiming to develop suitable varieties with specific characters, is therefore strongly recommended. This would avoid the use of material with a similar genetic background, as well as avoiding spending time, money and other resources on materials not having the best chance to produce the best result. For example, the use of Makueni (from Kenya) in a breeding programme aiming to improve Bafoussam 5 (from Cameroon) for the number of seeds per pod would have a better chance of success than the use of Bafoussam 2 from the same region. This study also revealed that some morphological traits (qualitative and quantitative) discriminated more efficiently between the accessions than others. It would therefore be very important to identify beforehand the right agro-morphological characters (those with a high discrimination capacity) before undertaking any genetic diversity studies based on morphological traits.

This study also identified a group of accessions (Makueni, Gacaga, M.66, Mpenbeni, Hluhluwa, MTA22, AS-94 and Okhalweni) that were different from the other accessions for some important characters such as the high number of seeds per pod, pod length, seed weight, number of pods per plant. They can therefore be used for cowpea improvement programmes through cross breeding.

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Chapter 4

POT TEST EVALUATION METHOD FOR DROUGHT TOLERANCE OF COWPEA (*VIGNA UGUICULATA*)

4.1 Introduction

Cowpea (*Vigna unguiculata*) is one of the most important grain legumes grown and consumed by small-scale farmers in the rural areas. The most important production areas are in the less-developed countries of the tropics and subtropics, especially in Sub-Saharan Africa, Asia, Central and South America. Its vegetable utilisation is fully exploited. The crop provides food, cash, and fodder. It has a high potential to increase the income of farmers and traders, thereby contributing to poverty reduction and food security. Another important feature of the cowpea is that it establishes a symbiotic association with *Bradyrhizobium* bacteria enabling it to fix atmospheric nitrogen (Laïty *et al.*, 2003). In doing so the plant does not deplete the natural reserves of soil nitrogen. Findings of many experiments showed that soil nitrogen levels increased following cowpea harvesting. However, owing to scarce and erratic rainfall in these areas, yield of cowpea remains low and unstable (Watanabe *et al.*, 1997). Therefore, cowpea varieties more tolerant to drought are needed, to obtain higher and stable yields. Recent reviews indicated knowledge on different aspects of drought tolerance in crop plants and ways and means to minimise yield losses due to drought (Winter *et al.*, 1988).

Success in breeding for cowpea drought tolerance has not been as pronounced as for the other traits (Singh *et al.*, 1999). This is partly due to the lack of simple, cheap and reliable screening methods to select drought tolerant plants and also because of the complexity of factors involved in drought tolerance. In breeding for enhanced drought tolerance, it is necessary to identify efficient methods to evaluate levels of tolerance in germplasm for crossing and selection of segregated breeding materials.

Several methods have been used to measure the degree of drought tolerance in plants. One of them, for instance, is the morphological characterization of plant traits like the deep rooting and density of roots (Hurd, 1974; Hamblin and

Tennant, 1987; Lorens *et al.*, 1987; Watanabe, 1993), stomatal behaviour (Heichel, 1971; Miskin *et al.*, 1972), leaf rolling (Begg and Turner, 1976; Mathews *et al.*, 1990), osmotic adjustment (Hall and Patel, 1987; Laurie, 1999) and the field screening method. Field screening is difficult due to uncertain rainfall patterns and different temperatures in the dry seasons (Singh *et al.*, 1999).

The pot screening method of varieties at seedling stage is a reliable method to identify drought tolerance. It is practical because of the ease to carry out the study under a controlled environment and the possibility of screening a large number of varieties. Watanabe *et al.* (1997) confirmed the suitability of pot screening techniques for drought tolerance and indicated a variety of differences in plant response to drought stress in cowpea. They also indicated that the phenomenon responsible for drought tolerance in the seedling stage is also manifested at the reproductive stage.

In this study, a pot evaluation method for plants at flowering stage was carried out. The aim of the study was to screen and discriminate among different cowpea accessions for drought tolerance at flowering stage.

4.2 Materials and Methods

4.2.1 Materials

Plant materials used in this study were the same as those used in Chapter 3 for morphological characterization (Table 3.1).

4.2.2 Methods

An adapted method of Watanabe *et al.* (1997) was used to conduct this study. The accessions were screened for drought tolerance at the flowering stage. Seeds were planted in pots filled with 3 kg of sandy sieved soil without fertiliser in a glasshouse at the University of the Free State. An optimum temperature of 15°C (night) and 35°C (day) was kept throughout the study. After germination, plants were thinned to three fairly uniform plants per pot with five replicated pots for each accession. Plants were kept well watered, until they had completed the development stage, after which 500 ml of water was given per day to each pot up to the flowering stage when the watering was terminated. After 21 days, each

plant was scored using the International Board for Plant Genetic Resources (IBPGR) descriptors for cowpea.

They were scored on a 1-7 scale, where 3 = low susceptibility (plant alive with green leaves); 5 = medium susceptibility (plant alive with most of the leaves yellow / or wilting); 7= high susceptibility (plant dead and dry).

The score of three plants in each pot were averaged, after which the scores of the five replicates of each accession were averaged. Using the averages of the evaluation, the tested accessions were then classified into the three categories of susceptibility. (1) With a mean ranging from 5 to 7, the accession was classified as highly susceptible. (2) With a mean ranging from 4 to 4.9, the accession was classified as medium. (3) With a mean ranging from 3 to 3.9, the accession was classified as having a low susceptibility.

4.2.3 Statistical analysis

The data was subjected to analysis of variance using Agrobase (2000) statistical software.

4.3 Results

Table 4.1: Drought susceptibility scores and means after 21 days of drought stress

Accessions	Rep1	Rep2	Rep3	Rep4	Rep5	Mean	Wilted plants (%)
MTA22	7	3	5	5	3	4.60	51.11
Balen	5	3	3	3	5	3.80	42.22
Okhalweni	3	3	3	7	5	4.20	46.66
AS-94	3	5	5	5	5	4.60	51.11
Bafoussam 1	3	3	3	3	3	3.00	33.33
Bamougoum	3	7	7	3	3	4.60	51.11
Bafoussam 2	5	3	3	5	5	4.20	37.77
Bafoussam 3	3	5	3	3	3	3.40	42.22
Mpenbeni	5	3	5	7	3	4.60	51.11
Bafoussam 4	3	3	5	3	5	3.80	42.22
Hluhluwa	3	7	3	3	3	3.80	42.22
Bafoussam 5	3	3	7	5	7	5.00	51.11
K.80	5	3	5	5	3	4.20	46.66
Gacaga	7	5	7	5	7	6.20*	64.44
M.66	3	3	3	3	5	3.40	37.77
Makueni	3	3	3	5	5	3.80	42.22
Kamurugu 1	7	7	5	7	5	6.20*	68.88
Ken-Kunde	7	3	3	7	5	5.00	64.44
Kasuku	3	5	5	3	3	3.80	55.55
Kamurugu 2	7	7	5	5	7	6.20*	68.88

* The highest mean

According to the scores, the accessions were grouped into four groups as follows:

The first group consisted of the accessions that scored 5 and over and comprised of Bafoussam 5 (5.00), Gacaga (6.2), Kamurugu 1 (6.2) and Kamurugu 2 (6.20). The second group had accessions that scored 4.20 to 4.60 and comprised of MTA22, Okhalweni, AS-94, Bamougoum, Bafoussam 2 and Mpenbeni, K.80.

The third group had accessions with a score of 3.8 and comprised of the following accessions: Bafoussam 4, Hluhluwa, Makueni and Kasuku.

The fourth group constituted of accessions scoring less than 3.8 and they were Bafoussam 1, Bafoussam 3 and M.66.

Kamurugu 1, Kamurugu 2 and Gacaca had the highest percentage of wilted or dead plants after 21 days of water deficit stress whereas Bafoussam 1 had the lowest percentage of wilted or dead plants after 21 days without water (33.33%)

A large number of plants died soon after watering ceased. For some accessions it happened after a week and some resisted even longer. Some accessions lost their flowers soon after the watering ceased, some after a week, and others managed to keep their flowers and developed some pods. Wilting of plants was first observed on the lower leaves and progressively the upper leaves became wilted. However, the loss of the lower leaves was common for all the accessions.

Table 4.2 gives the analysis of variance of drought tolerance scores at 21 days of drought stress. It shows that the variation among accessions for drought stress tolerance was highly significant ($p< 0.01$) after 21 days of drought stress.

Table 4.2: Analysis of variance of drought tolerance scores after 21 days of drought stress

Source of variation	Df	F-value
Rep	4	0.940
Accessions	19	4.303*
Residuals	76	1.887

* Significant at p= 0.01

4.4 Discussion

In an attempt to develop a simple and cheap, but still efficient, screening method to select a drought tolerant plant from large numbers of plants, Singh *et al.* (1999) developed a simple technique, using wooden boxes. This technique was used to screen cowpea germplasm for drought tolerance at the seedling stage. The more tolerant lines were later evaluated in the field at a mature stage.

This technique was later expanded by Watanabe *et al.* (1997). In a study carried out to evaluate drought tolerance in cowpea germplasm they used pots instead of wooden boxes as soil containers for the planting of seed. They observed that the use of pots was simple and labour saving. They could evaluate approximately 1000 accessions at a time and found it adequate to correctly evaluate drought tolerance, provided germination was good and uniform. They also found a highly significant correlation between the pot evaluation method results and field evaluation method results. These findings suggested that the pot evaluation technique might be effectively used for screening drought tolerant germplasm or for selecting segregating materials in breeding. Sun *et al.* (1999) also used the pot evaluation method to assess drought tolerance *of Eruca sativa* genotypes from China. They also found the method relatively effective for discriminating drought tolerant genotypes from drought susceptible ones. Stanca (1987) reported in a study in barley genotypes that variability for drought tolerance could be identified at different stages of growth (seed, seedling and adult plants).

In this study, highly significant variation was found between materials tested in their response to drought stress. This shows that the accessions responded

differently. The effect of moisture stress was seen as early as one week after watering was ceased. The different degrees of wilting among accessions were significant and fairly uniform among plants of the same accessions. Watanabe *et al.* (1997) sampled plants showing different degrees of tolerance to obtain a set of criteria for the classification of tolerance. They classified the accessions as highly susceptible (most of the plants of the accession dead and dry); highly tolerant (most of the plants of the accession still growing with green trifoliolate leaves); intermediate tolerance (plants of the accession having different degrees of defoliation, and discoloration of their leaves).

Following the same set of criteria, Kamurugu 1, Kamurugu 2, Gacaga, Ken-Kunde and Kasuku were the most susceptible to drought stress. They showed the highest level of wilted plants soon after watering was ceased, and most of the plants were dead and dry after a week. Consequently they can be classified as highly susceptible.

MTA22, Okhalweni, AS-94, Bamougoum, Mpenbeni, Bafoussam 5, K.80, and Makueni had almost the same reactions to moisture stress. Their plants also started to wilt during the first week but with fewer dead and dry plants than found in the previous accessions. They can be classified as susceptible to drought stress.

Balen, Bafoussam 3, and Hluhluwa showed no sign of wilting after the first week and Bafoussam 4 had only a small number of wilted plants. After two weeks of stress, the number of wilted plants of these four accessions was still low. They were less susceptible than the previous ones. However, after two weeks, the number of wilted plants became more prominent. They can therefore be classified as having intermediate susceptibility.

After 21 days of drought stress, only three accessions were able to withstand the drought stress, M.66, Bafoussam 2, and Bafoussam 1. Most of their plants managed to grow having green leaves and developing new leaves throughout the stressed period. These three accessions also developed two or more pods. Although loss of the lower leaves was common for all the accessions, very few wilted plants were observed for these accessions. They were the most tolerant to drought stress. At the end of the fourth week all the plants were wilted.

From the results of this study, it was clear that almost all the accessions from the humid region of Kenya were highly susceptible, excluding M.66, which was less susceptible to drought. Accessions from the semi arid area of Cameroon and South Africa had fewer numbers of susceptible plants. Two accessions from Cameroon (Bafoussam 1 and Bafoussam 2) were found to be the least susceptible accessions throughout the stress period. It can be that these two accessions have some genetic mechanisms that enable them to withstand drought stress longer.

By inducing drought stress at the flowering stage of each of the accessions, the same maturity stage was used and misevaluation which might be caused by the differences in maturity stage was avoided. However, difficulty was experienced in some accessions to distinguish senescence from the effect of drought. The tolerance scores of three accessions (MTA22, K.80, and Makueni), were not always the same from the five replicated pots. In the same accession, some plants grew better than others, indicating that residual soil moisture sometimes differed. It also indicated that the degree of competition for water between plants was affecting plant growth. It is therefore recommended that the optimum level of soil moisture for discrimination of tolerance must be found and maintained throughout the study. This optimum level of soil moisture for the discrimination of tolerance should also depend on the characteristics of soil used. It is also recommended that after germination, only one plant should be kept in each pot to avoid competition.

4.5 Conclusions

The wide range of drought tolerance observed among the studied accessions suggest the possibility of breeding tolerant cultivars. It also indicates that the method of pot evaluation is quite reliable and would be of benefit for preliminary screening of large numbers of materials for drought tolerance. In the present study two accessions (Bafoussam 1 and Bafoussam 2) were found to be highly drought tolerant and could be recommended to breeders as valuable material for drought tolerance improvement in cowpea. However, further drought tolerance tests, using this method, with plant material at different stages of growth is needed.

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Chapter 5

ASSESSMENT OF DROUGHT TOLERANCE OF COWPEA (*VIGNA UNGUICULATA*) ACCESSIONS FROM CAMEROON, KENYA, AND SOUTH AFRICA BASED ON THEIR STOMATAL BEHAVIOUR (SENSITIVITY AND DENSITY)

5.1 Introduction

Cowpea (*Vigna unguiculata*) is grown as a food crop by small scale farmers in many regions of the world. Its vegetable utilisation is fully exploited. Though native to West Africa, this legume has become a part of the diet about 110 million people (Osondu, 1997). With a protein content of about 25%, this legume is a cheap source of protein for the poor. In the West African semi-arid regions, where more than 70% of the total world production is grown, cowpea has become an integral part of the farming system. Its widespread integration into the farming system is due to its multiple secondary use qualities. The creeping varieties, for example, are applied in weed control; the stalks are an important fodder material, harvested in the dry season when fresh grass and other fodder materials are not available; and cowpea is an atmospheric nitrogen fixing legume which aids soil fertility when intercropped with cereals.

Cowpea in the sahelian (annual rainfall of about 200 to 500 mm) and dry Savannah (annual rainfall of about 500 to 700 mm) zones of West Africa can experience both heat and drought stress (Hall *et al.*, 1997) and several studies have shown how drought stress negatively affects cowpea yield. (Ndunguru and Summerfield, 1975; Dow and Hall, 1986; Mutters *et al.*, 1989; Patel and Hall, 1990; Ntare, 1992; Craufurd *et al.*, 1996).

In the subtropical zones, reduction in cowpea productivity, caused by drought stress, can partially be avoided by choosing a sowing date so that flowering does not coincide with the dry period of the year or by developing species with the ability to escape drought, in particular during the most sensitive periods of its development. One breeding strategy is to shorten the life cycle of the crop to enable it to mature during a rainfall period. For example, in the Sahel, the very short season cowpea avoids drought by maturing (in less than 65 days) before

any stress develops. However, this approach is not always effective in the tropical zones, such as in West Africa, where the occurrence of drought is not predictable (Visser, 1994).

A feasible alternative for tropical and subtropical regions will be to develop cultivars with drought tolerance. The common approach in breeding for drought tolerance is to select for tolerance characteristics. Therefore, breeders need to have a simple, cheap and reliable method to measure those characteristics and select drought tolerant plants from a large number of plants.

Several characteristics have been used to estimate the level of drought tolerance of crop species, including physiological (osmotic adjustment, proline accumulation, timing for plant development, leaf cuticular wax, photosynthesis, translocation, cell membrane stability) and morphological (root architecture, leaf morphology, leaf shedding, leaf angle changes).

Studies showed that stomata constantly react to environmental stimuli and are the first line of defence against water stress (Yordanov *et al.,* 2000). Permanent damage to the plant processes caused by severe moisture stress may be directly or indirectly attributed to stomatal characters (size and number per unit leaf area). These characters showed high heritability in a range of species (Heichel, 1971; Miskin *et al.*, 1972; Walton, 1974; Tan and Dunn, 1976) and breeding for these characters has proved successful. Plant water efficiency can be improved by limiting transpiration by stomatal closure, therefore the plant can survive under conditions of stress (Maherali *et al.*, 2003).

Kanemasu *et al.* (1969) reported that more resistance to water flow from the stomata into the atmosphere is a beneficial drought tolerance trait. Lal and Moomaw (1977) also confirmed that the rice variety Gs6 has higher stomatal diffusive resistance than IR20, a relatively more drought sensitive variety. Although both stomatal conductance and behaviour (sensitivity and frequency) are reasonably heritable, there have been very few studies done on cowpea using these characteristics.

The objective of this study was to characterise cowpea accessions for their drought tolerance ability based on the number of stomata per unit leaf area and

the stomatal pore size after six and 14 days of induced drought in a controlled environment.

5.2 Materials and Methods

5.2.1 Materials

Plant materials used in this study were the same as those used in Chapter 3 for morphological characterization (Table 3.1).

5.2.2 Methods

Growing conditions

The accessions were planted in 2 l pots containing 3 kg of soil, in the glasshouse at the University of the Free State with three replications of each accession for both control and the drought treatment. Five seeds were planted in each pot and were thinned to three after emergence. All the plants in a pot were of the same accession. An optimum temperature of 35°C day and 15°C night was maintained in the glasshouse throughout the study. Plants were kept well watered until they had completed the developmental stage. The watering was terminated at the flowering stage in order to use plant materials of the same stage of maturity.

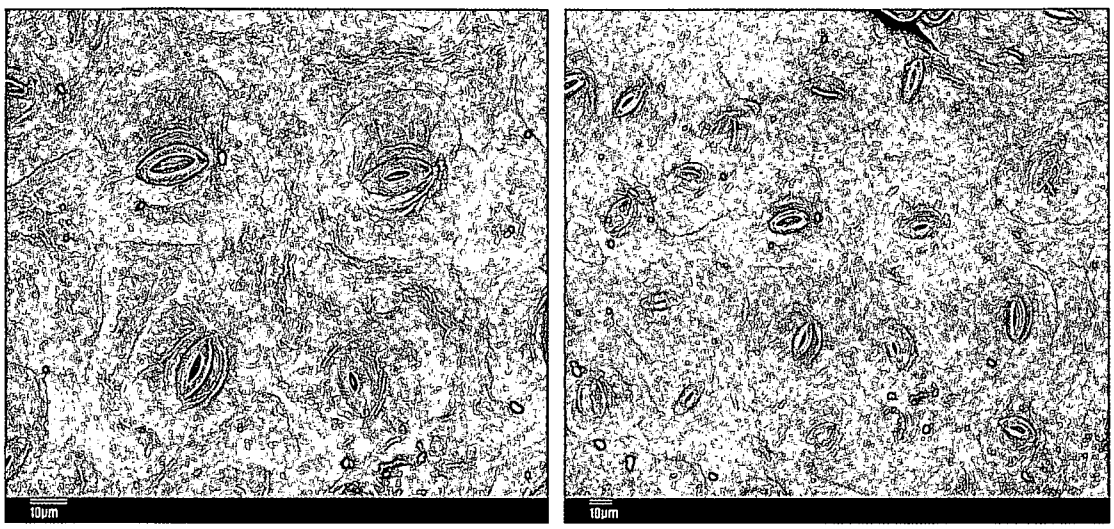
Collection and preparation

Leaflets used in this study were collected on two different dates (after six days of drought and after 14 days of drought) on plants at flowering stage. The middle part of the collected leaflets were sectioned into small pieces and fixed in 3% phosphate-buffered glutaraldehyde.

Scanning electron microscopy (SEM)

The harvested leaves were washed three times in phosphate buffer and then dehydrated in a graded ethanol series (20 min in 30% ethanol; 20 min in 50% ethanol; 20 min in 70% ethanol; 20 min in 90% ethanol; 30 min in 100% ethanol and 30 min again in 100% ethanol). The specimens were critical-point dried with carbon dioxide as the transition fluid. Each dried specimen was mounted on a stub using double-sided tape. All stubs were gold-coated in a Polaron Sputter Coater. Specimens were then examined using a Jeol Winsem 6400 scanning electron microscope operating at 5 kV.

The stomatal pore length and width (Figure 5.1) of 10 randomly selected stomata were measured on the upper side of the leaves at a magnification of x500 using the measurement facility of the microscope. The values were then averaged. The number of stomata per unit area was done by simply counting the number of stomata present at the given area at a magnification of x500. The counting was done on 10 randomly selected areas for each accession and the number of stomata recorded was averaged.



a. **b.**

Figure 5.1: Accession K.80. a. Adaxial (upper) epidermis showing the variation in stomatal pore length and width. b. Upper epidermis showing number of stomata at x500 magnification. Scale bars = 10 µm

5.2.3 Statistical analysis

The data were subjected to variance analysis (ANOVA) using Agrobase (2000) statistical software.

5.3 Results

5.3.1 Stomatal pore length (six and 14 days)

Table 5.1 represents the data of the stomatal pore length collected from stressed and non-stressed plants at six and 14 days of water deficit together with their stomatal pore length reduction percentages.

MTA22 non-stressed plants average stomatal pore length was 13.57 µm. After six days of water stress the average stomatal pore length was 12.61 µm, indicating a 7.08% reduction in pore length. At 14 days of water deficit, the stomatal pore length was 6.68 µm, indicating a 50.78% reduction from day one to day 14 of the water stressed period.

Balen non-stressed plants' average stomatal pore length was 12.01 µm. After six days of water deficit the pore length of stressed plants was 10.43 µm, indicating a reduction of 13.16%. After 14 days of water deficit, the average stomatal pore length was 8.66 µm, indicating a 27.9% reduction. This showed a decrease of stomatal pore length during the water stressed period.

Okhalweni non-stressed plants' average stomatal pore length was 12.13 µm. After six days of water deficit, the pore length was 10.31 µm, indicating a reduction of 15%. At 14 days of water deficit, the stomatal pore length was 10.56 µm, indicating a 12.94% reduction in the stressed plants, 2.06% less compared to the reduction after six days of water stress. This showed that the pore length of the stomata first decreased between day one to day six of the stress then increased between day six to day 14.

AS-94 non-stressed plants average stomatal pore length was 13.97 µm. After six days of water deficit the pore length was 12.31 µm, indicating a reduction of 11.88%. At 14 days of water deficit, the stomatal pore length was 9.25 µm, indicating a 33.78% reduction between day one to day 14 of the water stressed period. This showed a consistent decrease of AS-94 stomatal pore length during the stressed period.

Bafoussam 1 non-stressed plants' average stomatal pore length was 11.95 µm. After six days of water deficit it was 9.77 µm, indicating a reduction of 18.24%. At 14 days of water deficit, the stomatal pore length was 10.01 µm, indicating a 16.23% reduction in the stressed plants, 2.01% less compared to the stomatal pore length reduction after six days of water stress. This showed that the pore length of the stomata first decreased between day one to day six of the stress and then increased between days six to 14.

Bamougoum non-stressed plants' stomatal pore length was 8.72 µm. After six days of water deficit, the stomata pore length was 6.60 µm, indicating a reduction of 24.3% in the stressed plants. At 14 days of water deficit, the stomatal pore length was 11.57 µm, indicating a 32.68% increase in the stomatal pore length, 8.68 % more compared to the reduction at six days of water stress. This showed that the pore length of the stomata first decreased between day one to day six of the stress and then increased sharply between day six to day 14 of stress.

Bafoussam 2 non-stressed plants' average stomatal pore length was 15.94 µm. After six days of water deficit the stomatal pore length of stressed plants was 14.56 µm, indicating a reduction of 8.65%. At 14 days of water deficit, the stomatal pore length was 13.03 µm, indicating a 18.25% reduction of stomatal pore length between day one to day 14 of the water stressed period. This showed a consistent decrease of Bafoussam 2 stomatal pore length during the stressed period.

Bafoussam 3 non-stressed plants average stomatal pore length was 10.40 µm. After six days of water deficit, the pore length was 8.81 µm, indicating a reduction of 15.28%. At 14 days of water deficit, the stomatal pore length was 9.42 µm, indicating a 9.42% reduction in the stressed plants, 5.86 % less compared to the stomatal pore length reduction after six days of water stress. This showed that the pore length of the stomata first decreased between day one to day six of the stress period and then increased between day six to 14.

Mpenbeni non-stressed plants average stomatal pore length was 10.27 µm. After six days of water deficit the pore length was 7.68 µm, indicating a reduction of 25.21%. At 14 days of water deficit, the stomatal pore length was 7.25 µm, indicating a 29.40% reduction between day one to day 14 of the stressed period. This showed a consistent decrease of Mpenbeni stomatal pore length during the stressed period.

Bafoussam 4 non-stressed plants' average stomatal pore length was 11.88 µm. After six days of water deficit the stomatal pore length of stressed plants was 8.80 µm, indicating a reduction of 25.92%. At 14 days of water deficit, the stomatal pore length was 6.88 µm, indicating a 42.08% reduction between day one to day

14 of the water stressed period. This showed a consistent decrease of Bafoussam 4 stomatal pore length during the stressed period.

Hluhluwa non-stressed plants' average stomatal pore length was 11.47 µm. After six days of water deficit the pore length was 8.96 µm, indicating a reduction of 21.88%. At 14 days of water deficit, the stomatal pore length was 7.87 µm, indicating a 31.38% reduction of stomatal pore length between day one to day 14 of the water stressed period. This showed a consistent decrease of Hluhluwa stomatal pore length during stress.

Bafoussam 5 non-stressed plants' average stomatal pore length was 17.22 µm. After six days of water deficit the stomatal pore length was 15.14 µm, indicating a reduction of 12.07%. At 14 days of water deficit, the stomatal pore length was 11.68 µm, indicating a 32.17% reduction of stomatal pore length between day one to day 14 of the water stressed period.

K.80 non-stressed plants' average stomatal pore length was 12.51 µm and after six days of water deficit it was 10.12 µm, indicating a reduction of 19.10%. At 14 days of water deficit, the stomatal pore length was 8.19 µm, indicating a 34.53% reduction between day one to day 14 of the stress period.

Gacaga non-stressed plants' stomatal pore length was 14.01 µm. After six days of water deficit, the stomatal pore length was 11.58 µm, indicating a reduction of 17.34%. At 14 days of water deficit, the length was 12.15 µm, indicating a 13.27% reduction in the stomatal pore length, 4.07% less compared to the length reduction after six days of water stress. This showed that the stomatal pore length first decreased between day one to day six of stress, then increased between days six to 14.

M66 non-stressed plants' stomatal pore length was 14.77 µm and at six days of water deficit, the length was 12.65 µm, indicating a reduction of 14.35%. At 14 days of water deficit, the stomatal pore length was 12.23 µm, indicating a 17.2% reduction between day one to day 14 of the water deficit period. This showed a consistent decrease of stomatal pore length during the stressed period.

Makueni non-stressed plants' stomatal pore length was 11.52 µm. After six days of water deficit, the stomatal pore length was 8.46 µm, indicating a reduction of 26.56%. At 14 days of water deficit, the length was 10.28 µm, indicating a 10.76% reduction, 15.8% less compared to the stomatal pore length reduction after six days of water stress. This showed that the pore length of Makueni stomata first decreased between day one to day six of the stress, then increased considerably between days six to 14.

Kamurugu 1 non-stressed plants' stomatal pore length was 13.30 µm and after six days of water deficit, it was 11.40 µm, indicating a reduction of 14.28%. At 14 days of water deficit, the stomatal pore length was 12.58 µm, indicating a 5.41% reduction in the stressed plants, 8.87% less than the reduction after six days of water stress. This showed that the stomatal pore length of Kamurugu 1 first decreased between day one to day six of the stress, then increased considerably between days six to 14.

Ken-Kunde non-stressed plants' stomatal pore length was 17.01 µm. After six days of water deficit, the pore length was 16.41 µm, indicating a reduction of 3.52% in the stressed plants. At 14 days of water deficit, the stomatal pore length was 15.62 µm, indicating a 8.17% reduction between day one to day 14 of the water deficit period. This showed a consistent decrease of Ken-Kunde stomatal pore length during the stressed period.

Kasuku non-stressed plants' stomatal pore length was 14.38 µm and after six days of water deficit, it was 12.33 µm, indicating a reduction of 14.25%. At 14 days of water deficit, the pore length was 12.29 µm indicating a 14.53% reduction between day one to day 14 of the water deficit period. This showed a decrease of stomatal pore length from day one to day six of the water stressed period which however, remained almost unchanged between days six to day 14 of the stressed period.

Kamurugu 2 non-stressed plants' stomatal pore length was 14.48 µm. After six days of water deficit, the pore length was 12.83 µm, indicating a reduction of 11.39%. At 14 days of water deficit, the stomatal pore length was 12.93 µm, indicating a 10.70% reduction in the stressed plants, 0.69% less than the pore length after six days of water stress. This showed that Kamurugu 2 stomatal pore

length first decreased between day one to day six of the stressed period, then slightly increased between days six to day 14 of the water stressed period.

Table 5.1: Stomatal pore length (μm) of the accessions after six and 14 days of drought stress and control

Accessions	Pore length at normal stage (μm)	Pore length after six days of stress (μm)	Reduction after six days of stress (%)	Pore length after 14 days of stress (μm)	Reduction after 14days of stress (%)
MTA22	13.57	12.61	7.08	6.68	50.78
Balen	12.01	10.43	13.16	8.66	27.90
Okhalweni	12.13	10.31	15.00	10.56	12.94
AS-94	13.97	12.31	11.88	9.25	33.78
Bafoussam 1	11.95	9.77	18.24	10.01	16.23
Bamougoum	8.72	6.60	24.30	11.57	- 32.68
Bafoussam 2	15.94	14.56	8.65	13.03	18.25
Bafoussam 3	10.40	8.81	15.28	9.42	9.42
Mpenbeni	10.27	7.68	25.21	7.25	29.40
Bafoussam 4	11.88	8.80	25.92	6.88	42.08
Hluhluwa	11.47	8.96	21.88	7.87	31.38
Bafoussam 5	17.22	15.14	12.07	11.68	32.17
K.80	12.51	10.12	19.10	8.19	34.53
Gacaga	14.01	11.58	17.34	12.15	13.27
M.66	14.77	12.65	14.35	12.23	17.20
Makueni	11.52	8.46	26.56	10.28	10.76
Kamurugu 1	13.30	11.40	14.28	12.58	5.41
Ken-Kunde	17.01	16.41	3.52	15.62	8.17
Kasuku	14.38	12.33	14.25	12.29	14.53
Kamurugu 2	14.48	12.83	11.39	12.93	10.70

The analysis of variance (ANOVA) of the stomatal pore length after six and 14 days of drought stress presented in Table 5.2 showed highly significant ($p<0.01$) differences between the non-stressed and the stressed plants within each accession and between the accessions.

Table 5.2: Analysis of variance of stomatal pore length after 6 and 14 days of drought stress

Source of variation after six days of stress	**Df**	**F-value**
Rep	9	1.089
Accessions	19	65.878*
Residuals	171	2.152
Source of variation after 14 days of stress		
Rep	9	3.199
Accessions	19	58.519*
Residuals	171	2.469

* $P \leq 0.0\ 1$

Figure 5.2 represents four different types of behaviour produced by the stomatal pore length of the accessions during the water stressed period. In MTA22, Balen, AS-94, Bafoussam 2, Bafoussam 4, Hluhluwa, Bafoussam 5, K.80, M.66, and Ken-Kunde pore length showed a consistent decrease throughout the water deficit period. MTA22 had the highest percentage of stomatal pore length decrease during the water stressed period with 50.78%, followed by Bafoussam 4 (42.08%) and K.80 (34.53%).

Okhalweni, Bafoussam 1, Bafoussam 3, Mpenbeni, Gacaga, Makueni, Kamurugu 1, and Kamurugu 2 first showed a decrease in their stomatal pore length between day one to day six of the water stressed period, and then showed an increase between days six to 14 of the water stressed period. However at day 14, their stomatal pore length was still smaller in size compared to the normal. Makueni had the highest percentage pore length increase from day six to day 14 with 15.80% followed by Kamurugu 1 with 8.87%.

Bamougoum stomatal pore length decreased between day one to day six of the water stressed period and then increased significantly between day six to 14. At 14 days of the water stress the pore length was bigger than the normal.

Kasuku stomatal pore length also decreased between day one to day six, then unlike the others showed no significant variation in stomatal pore length between day six to day 14 of the water stressed period.

5.3.2 Stomatal pore width (six and 14 days)

Table 5.3 represents the stomatal pore width data collected from stressed and non-stressed accessions after six and 14 days of water deficit, together with their width reduction percentages at these days.

MTA22 non-stressed plants' stomatal pore width was 2.63 µm and after six days of water stress it was 2.1 µm, indicating a 20.15% reduction. At 14 days of water deficit, the stomatal pore width was 1.78 µm, indicating a 32.31% reduction from day one to day 14 of the water stress. This showed a consistent decrease of pore width during water stress period.

Balen non-stressed plants' stomatal pore width was 2.54 µm. After six days of water deficit the width was 1.66 µm, indicating a reduction of 34.64%. At 14 days of water deficit, the pore width was 1.58 µm, indicating a 37.79% reduction. This showed a consistent decrease of pore width during water stress.

Okhalweni non-stressed plants' stomatal pore width was 2.07 µm and after six days of water deficit it was 1.12 µm, indicating a reduction of 45.89%. At 14 days of water deficit, the pore width was 1.43 µm, indicating a 30.91% reduction, 14.98% less than after six days of water stress. Therefore the pore width first decreased between day one to day six of the stress, then increased between days six to 14.

AS-94 non-stressed plants' stomatal pore width was 3.00 µm and after six days of water deficit it was 2.83 µm, indicating a reduction of 5.66%. At 14 days of water deficit, the stomatal pore width was 1.97 µm, indicating a 34.33% reduction between day one to day 14 of the water stressed period. This showed a consistent decrease of stomatal pore width during water stress.

Bafoussam 1 non-stressed plants' stomatal pore width was 2.42 µm and after six days of water deficit it was 2.11 µm, indicating a reduction of 12.80%. At 14 days of water deficit, the pore width was 1.64 µm, indicating a 32.23% reduction between days one to 14. This showed a consistent decrease in pore width during water stress.

Bamougoum non-stressed plants' stomatal pore width was 1.87 µm and after six days of water deficit it was 1.16 µm, indicating a reduction of 37.96%. At 14 days of water deficit, the pore width was 1.23 µm, indicating a 34.22% reduction, 3.74% less compared to the pore width reduction after six days of water stress. Therefore the pore width first decreased between day one to day six of the stress, then increased between day six to 14.

Bafoussam 2 non-stressed plants' stomatal pore width was 2.96 µm. After six days of water stress the width was 2.70 µm, indicating a reduction of 8.78%. At 14 days of water deficit, the stomatal pore width was 2.21 µm, indicating a 25.33% reduction between day one and 14 of water stress. This showed a consistent decrease of pore width during stress.

Bafoussam 3 non-stressed plants' stomatal pore width was 2.19 µm and after six days of water deficit it was 1.57 µm, indicating a reduction of 28.31%. At 14 days of water deficit, the pore width was 1.51 µm, indicating 31.05% reduction between days one to 14. This showed a consistent decrease in pore width during the water stressed period.

Mpenbeni non-stressed plants' stomatal pore width was 2.31 µm and after six days of water deficit it was 1.48 µm, indicating a reduction of 35.93%. At 14 days of water deficit, the pore width was 1.09 µm, indicating a 52.812% reduction between day one to days 14 of the water stressed period. This showed a consistent decrease in pore width during stress.

Bafoussam 4 non-stressed plants' stomatal pore width was 2.40 µm. After six days of water deficit the pore width was 1.78 µm, indicating a reduction of 25.83%. At 14 days of water deficit, the pore width was 1.99 µm, indicating a 17.08% reduction between days one to 14 of the water stress, 8.75% less compared to the reduction after six days of stress. The pore width therefore first

decreased between days one to six, and then increased between days six to 14 of the stressed period.

Hluhluwa non-stressed plants' stomatal pore width was 1.85 µm and after six days of water deficit it was 1.29 µm, indicating a reduction of 30.27%. At 14 days of water deficit, the pore width was 1.09 µm, indicating a 41.08% reduction between days one to 14 of the water stressed period. This showed a consistent decrease of stomatal pore width during the stressed period.

Bafoussam 5 non-stressed plants' stomatal pore width was 2.37 µm and after six days of water deficit it was 2.07 µm, indicating a reduction of 12.65%. At 14 days of water deficit, the pore width was 1.93 µm, indicating a 18.56% reduction between days one to 14 of water stress. This showed a consistent decrease of pore width during the stressed period.

K.80 non-stressed plants' stomatal pore width was 2.59 µm. After six days of water deficit the pore width was 2.16 µm, indicating a reduction of 16.60%. At 14 days of water deficit, the pore width was 1.91 µm, indicating a 26.25% reduction between days one to 14 of the water stress. This showed a consistent decrease of pore width during the stressed period.

Gacaga non-stressed plants' stomatal pore width was 2.32 µm and after six days of water deficit, it was 2.20 µm, indicating a reduction of 5.17%. At 14 days of water deficit, the pore width was 2.20 µm, indicating a 5.17% reduction, which shows no difference compared to the reduction after six days of water stress. The pore width therefore decreased between days one to six of the stress, then staid constant between days six to 14.

M66 non-stressed plants' stomatal pore width was 2.38 µm and after six days of water deficit, it was 2.08 µm, indicating a reduction of 12.60%. At 14 days of water deficit, the pore width was 1.91 µm, indicating a 19.74% reduction between days one to 14. This showed a consistent decrease of pore width during stress.

Makueni non-stressed plants' stomatal pore width was 2.43 µm. and after six days of water deficit, it was 1.92 µm, indicating a reduction of 20.98%. At 14 days of water deficit, the pore width was 1.95 µm, indicating a 19.75% reduction in the

stressed plants, 1.23% less than after six days of water stress. The pore width therefore first decreased between days one to six of stress, then increased slightly between days six to 14.

Kamurugu 1 non-stressed plants' stomatal pore width was 2.60 µm and after six days of water deficit, it was 2.65 µm, indicating an increase of 1.92%. At 14 days of water deficit, the pore width was 2.60 µm, indicating a 1.9% reduction from six days of water stress, a 0% reduction compared to the non-stressed plants. The pore width therefore first increased between days one to day six of the stress, then decreased to normal between days six to 14.

Ken-Kunde non-stressed plants stomatal pore width was 3.00 µm and after six days of water deficit, it was 2.72 µm, indicating a reduction of 9.33%. At 14 days of water deficit, the pore width was 2.55 µm, indicating a 15.00% reduction between days one to 14 of the water deficit period. This showed a consistent decrease of pore width during the stressed period.

Kasuku non-stressed plants' stomatal pore width was 2.64 µm and after six days of water deficit, it was 2.34 µm, indicating a reduction of 11.36%. At 14 days of water deficit, the pore width was 2.62 µm indicating a 0.75% reduction between days one to 14, 10.61% less than the reduction after six days of water stress. The pore width therefore first decreased between days one to six of the stress, then increased significantly between days six to 14.

Kamurugu 2 non-stressed plants' stomatal pore width was 2.59 µm and after six days of water deficit, it was 2.13 µm, indicating a reduction of 17.76%. At 14 days of water deficit, the pore width was 2.49 µm, indicating a 3.86% reduction in the stressed plants, 13.9% less than after six days of water stress. The pore width therefore first decreased between days one to six of the stressed period, then increased considerably between days six to 14.

Table 5.3: Stomatal pore width values (μm) of the accessions after six and 14 days of drought stress and control

Accessions	Pore width at normal stage (μm)	Pore width after six days of stress (μm)	Reduction after six days of stress (%)	Pore width after 14 days of stress (μm)	Reduction after 14 days of stress (%)
MTA22	2.63	2.1	20.15	1.78	32.31
Balen	2.54	1.66	34.64	1.58	37.79
Okhalweni	2.07	1.12	45.89	1.43	30.91
KS-94	3.0	2.83	5.66	1.97	34.33
Bafoussam 1	2.42	2.11	12.80	1.64	32.23
Bamougoum	1.87	1.16	37.96	1.23	34.22
Bafoussam 2	2.96	2.7	8.78	2.21	25.33
Bafoussam 3	2.19	1.57	28.31	1.51	31.05
Dipenbeni	2.31	1.48	35.93	1.09	52.81
Bafoussam 4	2.4	1.78	25.83	1.99	17.08
Kuhluwa	1.85	1.29	30.27	1.09	41.08
Bafoussam 5	2.37	2.07	12.65	1.93	18.56
1.80	2.59	2.16	16.60	1.91	26.25
Bacaga	2.32	2.2	5.17	2.20	5.17
1.66	2.38	2.08	12.60	1.91	19.74
Bakueni	2.43	1.92	20.98	1.95	19.75
Bamurugu 1	2.60	2.65	- 1.92	2.60	0.00
Ben-Kunde	3.0	2.72	9.33	2.55	15
Basuku	2.64	2.34	11.36	2.62	0.75
Bamurugu 2	2.59	2.13	17.76	2.49	3.86

The analysis of variance (ANOVA) of the stomatal pore width after six and 14 days of drought stress also showed that there were highly significant ($p<0.01$) differences between the non-stressed and the stressed plants in each accession and between the accessions as well (Table 5.4).

Table 5.4: Analysis of variance of stomatal pore width after six and 14 days of drought stress

Source of variation after 6 days of stress	**Df**	**F-value**
Rep	9	0.227
Accessions	19	2.618*
Residuals	171	0.210
Source of variation after 14 days of stress	**Df**	**F-value**
Rep	9	0.110
Accessions	19	2.264*
Residuals	171	0.215

* $p \leq 0.01$

The behaviour of the stomatal pore width observed in Figure 5.3, also classified the accessions in four different categories: MTA22, Balen, AS-94, Bafoussam 1, Bafoussam 2, Bafoussam 3, Bafoussam 5, Mpenbeni, Hluhluwa, K.80, M.66, and Ken-Kunde showed a constant decrease in their stomatal pore width throughout the water deficit period. Mpenbeni had the most significant percentage stomatal pore width decrease during the water stressed period with 52.81%, followed by Hluhluwa (41.08%) and Balen (37.79%).

Okhalweni, Bamougoum, Bafoussam 4, Kasuku, Makueni, and Kamurugu 2 first showed a decrease in their stomatal pore width between days one to six of the water stressed period, and then showed an increase between days six to 14. Their overall stomatal pore width at 14 days of water stress was still less than the normal. Okhalweni had the most significant pore width increase from days six to 14 of the water deficit period with 14.98% followed by Kamurugu 2 with 13.9%.

Kamurugu 1 showed an increase in its stomatal pore width between days one to six of water stress, then decreased and at 14 days of water stress, its stomatal pore width was the same size as for the non-stressed plants.

Gacaga showed a decrease in its stomatal pore width between days one to six of the water stressed period, then showed no significant variation between days six to 14 of the water stressed period.

5.3.3 Stomata density

Table 5.5 presents number of stomata per mm^2 for each accession. According to the number of stomata recorded the accessions can be grouped as follows: Kamurugu 1 (477.5/mm^2); Bafoussam 2, K.80, Kamurugu 2, Kasuku, MTA.22, Ken-Kunde, and Bafoussam 5 (±367.5/mm^2); AS-94, Makueni, Balen, Bafoussam 3, Gacaga, Bamougoum, and Bafoussam 4 (±292.5/mm^2); M.66, Bafoussam 1, Okhalweni, Hluhluwa, and Mpenbeni (±250/mm^2).

Table 5.5: Stomata density (mm^2) of the accessions after 14 days of drought stress

Accessions	Number of stomata (mm^2)
MTA22	337.5
Balen	287.5
Okhalweni	247.5
AS-94	305.0
Bafoussam 1	250.0
Bamougoum	280.0
Bafoussam 2	387.5
Bafoussam 3	287.5
Mpenbeni	230.0
Bafoussam 4	277.5
Hluhluwa	245.0
Bafoussam 5	330.0
K.80	367.5
Gacaga	282.5
M.66	257.5
Makueni	292.5
Kamurugu 1	477.5
Ken-Kunde	332.5
Kasuku	352.5
Kamurugu 2	367.5

There was a significant ($p<0.001$) variation among the accessions studied for their stomata density (Table 5.6).

Table 5.6: Analysis of variance of stomata density scores after 14 days of drought stress

Source of variation	DF	F-value
Replications	9	2.131
Accessions	19	58.178*
Error	171	1.608

*$p \leq 0.001$

Figure 5.3 shows the histogram representing stomata density levels at 14 days of water stress. Kamurugu 1 had the highest stomata density (477.5/mm^2) followed by Bafoussam 2 (387.5/mm^2), whereas Mpenbeni had the lowest stoma density (230/mm^2) followed by Hluhluwa (245/mm^2).

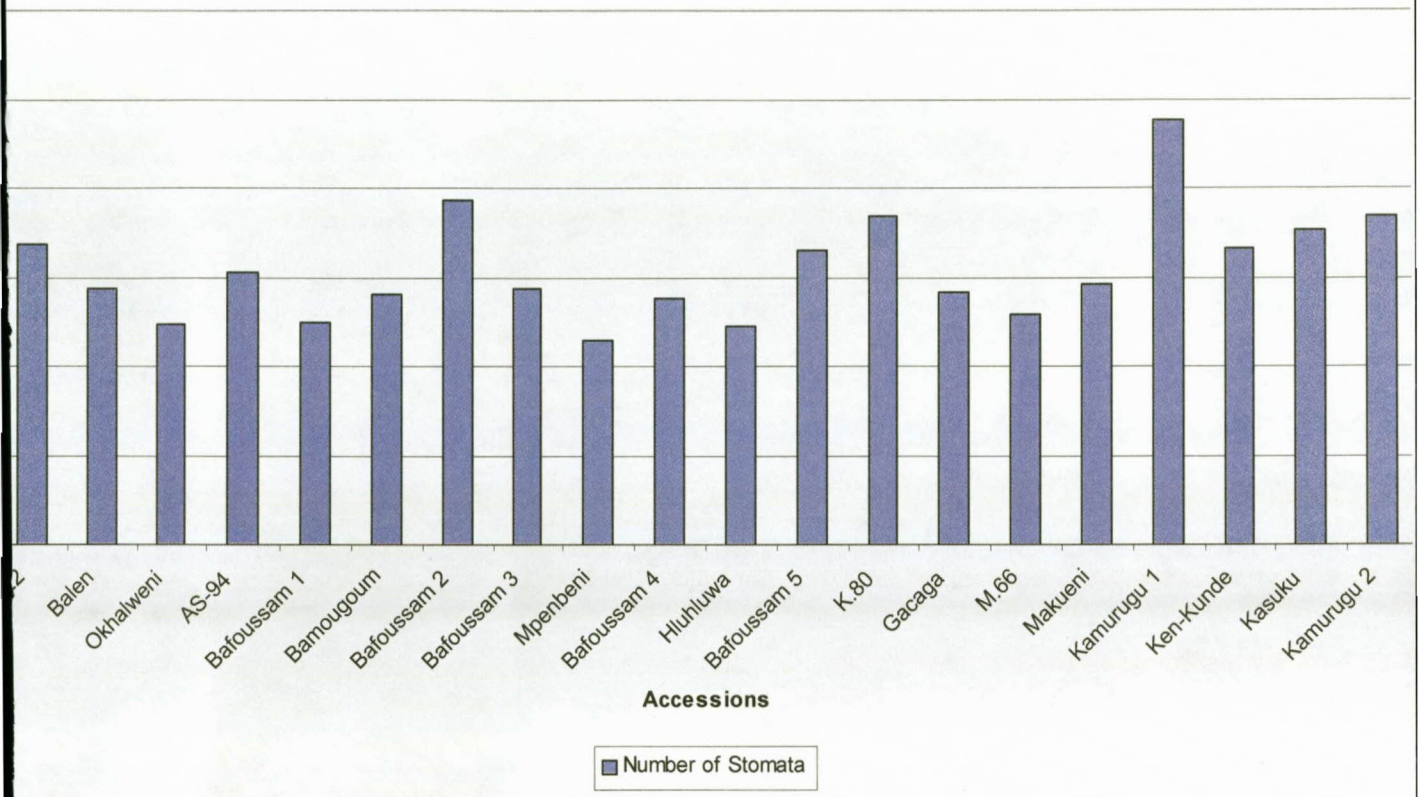


Figure 5.2: Histogram representing the stomata density levels at 14 days of drought stress

5.4 Discussion

Stomatal behaviour (sensitivity and frequency) has been suggested as a potentially useful trait in developing crop plants with improved water use efficiency (De Michele and Sharpe, 1974; Shawcroft *et al.*, 1974). Levitt (1972) showed how stomatal behaviour could affect plant water use efficiency (WUE) under semi-arid conditions. He associated high WUE with plants that avoid drought. Muchow and Sinclair (1989) reported that the ability of a sorghum plant to survive severe water deficits, depends on its ability to restrict water loss through the leaf epidermis, by stomata attaining minimum aperture size. At such stage, the rate of water loss is regulated by the epidermal conductance. Low epidermal conductance would therefore be a useful selection criterion to identify genotypes with enhanced survival capability.

Omara and Hussain (1988) observed genotypic differences in the stomatal frequency per unit area for both surfaces of barley flag leaf. Stomatal frequency increased with increasing water stress. Walton (1974) found that the general combining ability for stomatal length and frequency in *Bromus inermis* was highly significant. The partial segregation coefficients showed that stomatal frequency was related to yield. These results reflected the importance of stomatal size and frequency in a drought tolerant breeding programme. He also found that in barley, stomatal frequency, stomatal resistance, and transpiration varied among genotypes within species and was under genetic control.

The results of this study confirm the findings of the above studies that there is a reduction of the stoma opening under moisture stress and a positive correlation between stomatal frequency and drought tolerance. Different levels of stomatal closure were observed among the accessions studied from day one to day 14 of the water stressed period. The general trend was that the stomatal pore length and width of the stressed plants after six and 14 days of water stress was lower ($p< 0.01$) than those of the normal plants in almost all the accessions studied because most of the accessions reduced both their stomatal pore length and width between day one to 14 of the water deficit period. This showed that under moisture stressed conditions most of the accessions had the ability to regulate their stomatal opening. For example MTA22, Balen, AS-94, Bafoussam 2, Mpenbeni, Bafoussam 4, Hluhluwa, Bafoussam 5, K.80, M66, Ken-Kunde and Kasuku significantly reduced the degree of their stomatal aperture (length and width) between days one to 14 of the water stressed period. Reitz (1975) in a study of stomatal behaviour in upland cotton found that the lines which had the ability to effectively regulate their stomata during the water stressed period were more tolerant to drought than the ones which could not. Therefore under drought stress, MTA22, Balen, AS-94, Bafoussam 2, Mpenbeni, Bafoussam 4, Hluhluwa, Bafoussam 5, K.80, M66, Ken-Kunde and Kasuku may be able to effectively reduce water loss by decreasing their stomatal opening, by which the plants become more tolerant to drought. Meanwhile, Okhalweni, Bafoussam 1, Bamougoum, Bafoussam 3, Gacaga, Makueni, Kamurugu 1, and Kamurugu 2 were more tolerant to water stress, since no significant difference was observed in their degree of stomatal opening between six to 14 days of water stress. These accessions could not effectively close their stomata and this shows that they

could not effectively reduce transpiration under drought conditions, making them less tolerant to drought. MTA22 had the highest decrease in its stomatal aperture between six to 14 days, indicating a greater ability than all the other accessions in regulating stomata opening. Gay (1994) demonstrated that stomatal closure will have great effect on the water balance at field scale and will prevent excessive desiccation during the later stages of drought, when a small volume of water could make the difference between life and death. He also recommended the use of accessions with greater ability in regulating their stomatal opening in drought tolerance breeding programmes.

According to the number of stomata found in each accession, Balen, Okhalweni, AS-94, Bafoussam1, Bamougoum, Bafoussam 3, Mpenbeni, Bafoussam 4, Hluhluwa, Gacaga, M66, and Makueni had a relatively low stomata density compared to the others. Ray *et al.* (1975) found in a genetic modification of cotton plants for more efficient water use study, that cotton lines that had low stomata density, transpired less water than did lines with more stomata. The study associated the lines with low stomata density with greater drought tolerance.

5.5 Conclusions

The present study indicates that cowpea (*Vigna unguiculata*) in Kenya, South Africa and Cameroon have a number of drought tolerant accessions. Accessions with a significant drought tolerance include Bafoussam 4, MT22, AS-94, Balen, Hluhluwa, Mpenbeni, Bafoussam 5 and K.80. These accessions can be recommended as good accessions for drought tolerance breeding programmes.

On one hand the general trend showed that accessions from the humid areas of Kenya and Cameroon had a relative high stomata density and also had the poorest ability to regulate the degree of their stomatal opening under drought stress. On the other hand accessions from the arid and semi-arid areas of South Africa, Cameroon, and Kenya did show a relatively high ability to regulate the degree of their stomata opening under the imposed drought stress and had a relatively low stomata density. This suggests that cowpea has adapted to water deficit environments over a long history of cultivation and natural selection and contains valuable genetic resources for drought tolerance breeding programmes.

However, a re-evaluation of the drought tolerance found in the accessions in this study, using other related characteristics under conditions of drought stress is desirable to further confirm the results found in this study.

Bafoussam 4 was the only accession clearly found to have both a low stomata density and a good ability to regulate the degree of its stomata opening under the imposed drought stress. Therefore it was not possible to confirm any correlation between the ability to regulate the degree of stomata opening and stomata density among the accessions studied.

The results presented in the tables and figures show that the use of laboratory test systems, like the scanning electron microscope, can reveal characteristics of plant tolerance to drought.

Stomatal behaviour (sensitivity) and density measurements have the advantage of being rapid, requiring little space, and allowing precise control of environmental conditions and can be recommended to cowpea breeders as a valuable method for drought tolerance screening.

5.6 References

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Chapter 6

EVALUATION OF CELL MEMBRANE STABILITY OF 20 COWPEA (*VIGNA UNGUICULATA*) ACCESSIONS IN RESPONSE TO OSMOTIC STRESS WITH PEG 6000

6.1 Introduction

Cowpea (*Vigna unguiculata*) is of major importance in the livelihoods of millions of relatively poor people in less developed countries. From the production of this crop, rural families derive food, animal feed and cash. Because of its high protein content (20-25%), cowpea has been referred to as "poor man's meat" (Nielsen *et al.*, 1997). However, cowpea is extensively grown in the Sub-Saharan region of Africa and in other semi-arid and arid regions of the world where drought stress is often a major problem in cowpea production, resulting in reduced cowpea quality and yield loss.

Current germplasm screening for drought tolerance relies on field and whole plant techniques, which are often inefficient and inaccurate due to environmental interactions and the particularly low heritability of yield in such conditions. A rapid, accurate procedure allowing simultaneous screening of large numbers of genotypes is needed.

It is known that under stress conditions the plasmalemma is the primary object of injury (Levitt, 1980). Membranes are considered as one of the primary sites of lethal damage in cells. A consequence of the altered membrane is the increase of cell permeability, which is accompanied by electrolyte leakage from the cell and desiccation of the plant (Crowe and Clegg, 1978; Blum and Ebercon, 1981; Leopold, 1986).

An important strategy in the development of drought tolerance in plants is the maintenance of cell membrane integrity during imposition of water stress (Vasquez-Tello, 1990). Sullivan (1972) developed a rapid, efficient method for determining cell membrane stability (CMS) in grain sorghum by measuring the amount of electrolyte leakage from leaf segments exposed to heat shock. This method was used in many studies and was found to be rapid, inexpensive and

required little space, enabling screening of many genotypes in a short period of time. Electrolyte leakage has been used effectively to measure CMS in a number of plants, including sorghum (Sullivan and Ross, 1979), soybeans (Martineau *et al.*, 1979), wheat (Saadalla *et al.*, 1990), potato and tomato (Chen *et al.*, 1982), and turfgrass (Wallner *et al.*, 1982; Venkateswarlu and Ramesh, 1993; Van Rensburg, 1993; Gavuzzi, 1997; Bandurska, 2000). In all these studies, large differences in CMS were found among genotypes and between individual representatives of genotypes after being exposed to stress like drought or heat. CMS has been used to characterise the drought tolerance ability of different plants and helped in designing effective breeding programmes to develop drought tolerant plants. However, crops like cowpea, known to be an important food crop in the semi-arid and arid regions of the world, and an extremely resilient crop, cultivated under some of the most extreme agricultural conditions in the world has received very little attention.

The aim of this study was to use electrolyte leakage to measure CMS in cowpea accessions from three different African countries (Kenya, Cameroon and South Africa) and to characterise its drought tolerance ability using the CMS results.

6.2 Materials and Methods

6.2.1 Materials

Plant materials used in this study were the same as those used in chapter 3 for morphological characterization (Table 3.1).

6.2.2 Methods

Growing conditions

The accessions were planted in pots containing 3 kg of soil in a glasshouse with three replications each in a complete randomised design. Five seeds were planted per pot and were thinned to three after emergence. An optimum temperature was maintained, in the glasshouse, throughout the study period. After six weeks leaf samples were taken from fully expanded young leaves.

Sampling

Leaf samples of about 10 mm in diameter were taken from fully expanded young leaves. Five samples were taken from two or three leaves per accession. Samples were kept in airtight test tubes, wetted with a drop of water and transferred to the laboratory within an hour.

Drought tolerance test

The test-system for detecting cell membrane stability (CMS) developed by Sullivan (1972) was followed. Samples were washed with three changes of distilled water to remove surface adhered electrolytes. Five leaf samples were placed in a test tube with 10 ml solution of 20% polyethylene glycol 6000 (PEG). For the control treatment five samples were placed in 10 ml of distilled water. All the samples were incubated at 10°C for 24 hours and then equilibrated in a water bath at 25°C. Conductivity of the incubated medium was read using a conductivity meter. After the first readings, the samples were autoclaved for 15 min to release electrolytes from the leaf samples and a second conductivity reading was taken after the sample temperature reached room temperature (25°C).

Calculation of percentage injury due to desiccation was done as follows:

Percentage Injury = 1-[1-(T1/T2)/1-[C1/C2)] X100 where T and C refer to the mean of the treatment and control reading respectively, and the numbers 1 and 2 refer to initial and final conductivities.

6.2.3 Statistical analysis

The data were subjected to the analysis of variance (ANOVA) using Agrobase 2000.

6.3 Results

Table 6.1 represents the conductivity values of the treated and control leaf samples before and after they were autoclaved. Table 6.2 represents all the repetitions and mean percentages of injury due to desiccation.

Table 6.1: Conductivity values of the treated and control leaf samples before and after they were autoclaved

Accessions	T1	T1	T1	T1	C1	C1	C1	C1	T2	T2	T2	T2	C2	C2	C2	C2
MTA22	86.7	88.2	96.0	82.4	18.9	36.2	40.1	25.0	318	328	408	299	539	582	405	589
Balen	83.2	87.5	79.8	78.0	42.5	45.0	24.6	25.8	336	366	345	353	1135	553	597	614
Okhalweni	87.3	97.8	87.2	80	94.3	40.6	70.3	48.1	390	417	408	364	881	534	674	651
AS-94	80.6	94.7	91.4	97.2	21.3	29.0	25.4	24.2	325	437	337	97.2	383	526	668	558
Bafoussam 1	89.9	84.7	85.6	90.4	26.7	19.1	21.5	19.4	384	404	419	364	688	544	628	665
Bamougoum	93.3	79.4	82.1	96.3	23.6	27.3	41.3	56.6	437	354	322	379	613	926	773	663
Bafoussam 2	99.3	87.2	81.2	99.0	60.6	94.3	43.0	60.0	491	390	324	489	853	881	430	851
Bafoussam 3	86.0	104.8	95.4	98.1	44.0	36.6	37.2	27.0	381	414	401	366	381	512	331	514
Mpenbeni	81.2	83.8	91.4	86.5	94.3	40.6	70.3	48.1	390	417	408	364	881	534	674	651
Bafoussam 4	86.2	79.0	81.9	70.2	42.2	74.3	42.1	50.3	372	309	424	410	661	832	760	886
Hluhluwa	81.5	88.4	89.6	88.1	32.0	32.5	20.2	31.0	351	319	342	297	504	635	435	746
Bafoussam 5	85.0	84.5	76.7	77.4	25.5	23.4	20.3	21.1	387	381	316	323	544	456	272	449
K.80	116	146.3	105.7	95.4	28.4	33.0	25.1	21.5	329	343	317	384	504	288	549	498
Gacaga	87.0	86.0	96.3	94.1	28.4	32.1	56.6	54.9	396	366	372	379	629	676	663	873
M.66	87.2	86.2	87.1	88.1	35.2	30.9	34.4	46.6	254	289	317	405	484	500	495	671
Makueni	126.9	99.3	85.4	97.2	38.7	60.6	37.5	45.5	461	491	329	97.2	595	853	551	609
Kamurugu 1	75.5	76.7	75.8	73.6	15.3	32.9	26.4	15.1	355	358	276	355	475	553	661	409
Ken-Kunde	81.7	152.6	80.3	87.5	24.9	34.3	31.9	28.7	212	372	315	424	652	738	738	425
Kasuku	88.6	85.1	80.0	82.6	37.1	34.9	34.9	34.6	287	314	266	178	465	481	537	485
Kamurugu 2	91.4	80.0	88.6	87.1	25.1	34.9	37.1	30.0	324	266	287	297	504	537	465	745

T1: Conductivity values of the treated samples before they were autoclaved
T2: Conductivity values of the treated samples after they were autoclaved
C1: Conductivity values of the control samples before they were autoclaved
C2: Conductivity values of the control samples after they were autoclaved

The results of the conductivity measurements showed that except for three accessions (Okhalweni, Bafoussam 2, and Mpenbeni) where one control treatment reading (C1, Table 6.1) was higher than the stress treated samples (T1, Table 6.1), all the other control treatment readings were lower than the readings of the stress treatment. After the samples were autoclaved, AS-94, Bafoussam 2, Bafoussam 3, and Bafoussam 5 had one control treatment reading that was lower than the stress treatment. All the other control treatment readings were higher than the stress treatment readings.

According to the results recorded from the percentage injury (Table 6.2), the accessions could be divided into five groups. The first group had accessions with an injury level ranging from 25% to 31% and comprised of the following accessions: Kamurugu 2, Ken-Kunde, Kasuku, and K.80. The second group contained the accessions where the injury level ranged from 22.0% to 24.9% and comprised of MTA.22, Hluhluwa, and M.66. The third group had accessions with injury levels that ranged from 19% to 21.9% and comprised of Bamougoum, Balen, Kamurugu 1, Gacaga, Bafoussam 1, Makueni, AS-94, Mpenbeni. The fourth group had injury levels between 17% and 18.9% and comprised of Bafoussam 3 and Bafoussam 5. The fifth group of accessions had injury levels from 14% to 16.9% and comprised of Bafoussam 2, Okhalweni, and Bafoussam 4. The percentage injury of plants within the same accessions was fairly uniform except for Kasuku, Ken-Kunde, M.66, Makueni, Kamurugu 1, and Bafoussam 3 (Table 6.2).

Table 6.2: Injury percentages and mean of the percentage of injury due to desiccation

Accessions	Rep1 injury %	Rep2 injury %	Rep3 injury %	Rep4 injury %	Mean injury %
MTA22	23.8	21.5	19.7	22.9	22.0
Balen	21.9	17.2	19.6	18.7	19.4
Okhalweni	13.0	17.1	12.2	15.6	14.5
AS-94	20.3	17.2	22.7	21.3	20.4
Bafoussam 1	20.3	18.9	17.6	22.6	19.9
Bamougoum	18.3	20.1	18.3	19.7	19.1
Bafoussam 2	14.0	16.7	13.6	12.8	14.3
Bafoussam 3	12.5	19.6	14.2	22.7	17.3
Mpenbeni	16.8	21.3	24.0	19.7	20.5
Bafoussam 4	17.9	18.3	14.6	11.9	15.7
Hluhluwa	18.0	23.8	22.6	26.6	22.8
Bafoussam 5	18.2	18.0	18.2	20.3	18.7
K.80	31.5	35.3	30.1	24.1	30.3
Gacaga	18.3	19.7	19.0	22.0	19.8
M.66	29.1	25.2	22.1	16.0	23.1
Makueni	22.5	14.1	20.6	22.5	19.9
Kamurugu 1	25.0	18.0	16.0	19.0	19.5
Ken-Kunde	36.1	38.2	21.7	14.3	27.7
Kasuku	29.0	21.4	26.0	42.3	29.7
Kamurugu 2	27.6	24.0	26.0	29.0	26.7

The analysis of variance showed a highly significant ($p< 0.001$) variation in percentage injury among the accessions (Table 6.3). Figure 6.1 provides the three levels (low, medium and high) of injury for each accession. The injury levels of Bafoussam 2, Okhalweni, Bafoussam 4 and Bafooussam 3 were low whereas Mpenbeni, AS-94, Makueni, Bafoussam 1, Gacaga, Kamurugu 1, Balen, Bamougoum, Bafoussam 5, had a medium level and MTA22, Hluhluwa, M.66, Kamurugu 2, Ken-Kunde, Kasuku and K.80 a high level of injury.

Table 6.3: Analysis of variance of percentage injury due to desiccation of the cowpea accessions studied

Source of variance	DF	F-value
Replications	3	0.60
Accessions	19	4.30*
Error	57	

*Significant $p < 0.001$

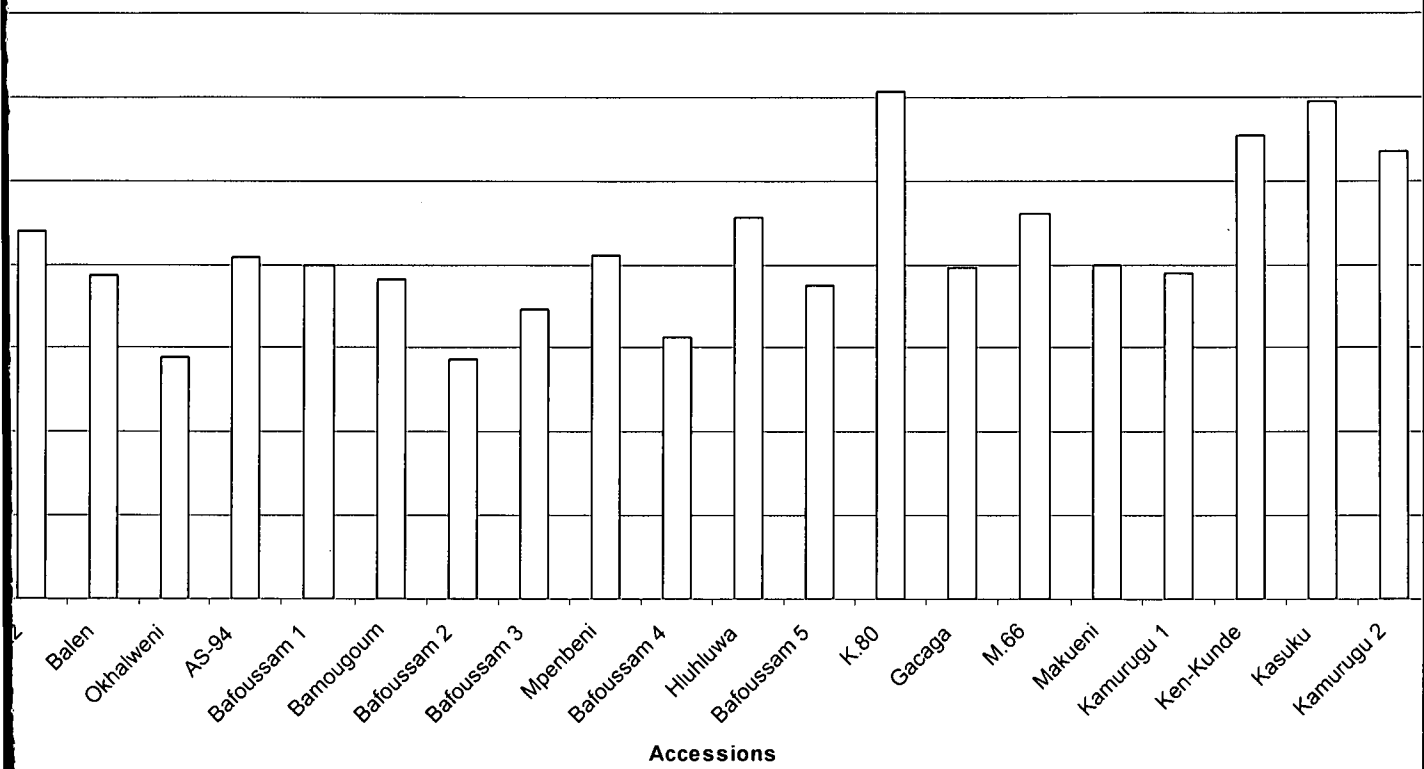

Figure 6.1: Histogram showing the levels of injury due to desiccation of the accessions studied

6.4 Discussion

The study of drought tolerance in food crops using the cell membrane stability test and its significant contribution as a screening method was reported in different crops like wheat (Bewley, 1979; Blum and Ebercon, 1981; Mark *et al.,* 1991), barley (Stanca, 1987), sorghum (Sullivan and Ross, 1979), soybeans (Martineau *et al.*, 1979), potato and tomato (Chen *et al.*, 1982), and turfgrass (Wallner *et al.*, 1982; Venkateswarlu and Ramesh, 1993; Van Rensburg, 1993; Gavuzzi, 1997; Bandurska, 2000). These studies showed large ranges of

electrolyte leakage in plants subjected to water deficit which indicate that under the same water deficit pressure, the damage to the cell membrane is different. Some plants resist better than others. The authors concluded in their reports that those genotypes or accessions, which exhibit a low percentage of cell membrane injury during water stress, could be considered as drought tolerant and those with a high cell membrane injury could be considered as susceptible to drought.

In this study, a large variation was seen among the accessions studied for CMS under water deficit stress (Table 6.1). The percentage of injury to the membranes estimated from the electrolyte leakage data ranged from 14.3% to 30.3% (Table 6.2). Three accessions Bafoussam 2, Bafoussam 3 and Bafoussam 4 showed a low level of injury (Figure 6.1) and can be considered as tolerant to drought stress. In a similar study Blum and Ebercon (1981) classified such type of adaptation as osmotic adjustment by the cell membrane. It is the phenomena of cell membrane adjustment to drought stress. The degree of injury caused to cell membranes by controlled dehydration was also found to decrease in plants subjected to previous dehydration. K.80, Kasuku, Ken-Kunde and Kamurugu 2 with a high level of injury can be considered as susceptible accessions.

The highest percentage of cell membrane injury level obtained in this study was 30%, which is low compared to up to the 70% injury level reported by Blum and Ebercon (1981) in wheat. The reason is probably the low polyethylene glycol (PEG) concentration (20%) used in this study compared to 40% PEG concentration used in most of the other studies.

The results of this study also showed that accessions from the humid regions of Kenya and Cameroon had the highest level of injury, and were therefore the most susceptible to drought. Accessions from the arid and semi-arid regions of Cameroon, Kenya and South Africa were the accessions with the lowest and intermediate level of injury. They were therefore more tolerant to drought stress than those from the humid areas. This is probably due to some kind of adaptation mechanism developed with time in the arid and semi-arid regions.

6.5 Conclusions

This study showed that CMS measurements following drought stress can predict drought tolerance among cowpea accessions. Currently, cowpea breeders usually screen for drought tolerance in the field, which is complicated by a number of factors. For example it is known that environmental factors, such as unpredictable rainfall patterns, different temperatures in the dry seasons (Singh *et al.*, 1999), and relative air humidity (Perdomo *et al.*, 1996) interact with drought stress. Variability in year to year weather patterns further complicate field drought tolerance determinations. Because of the many interacting factors, comparing the drought tolerance results from separate screening tests may not be valid unless environmental conditions (including pre-test conditions) are identical (Martineau *et al.*, 1979).

The results from this study showed that the use of a laboratory test system such as PEG, simulating drought by the application of agents, can reveal drought tolerance characteristics in crop plants. Cell membrane stability measurements have the advantage of being rapid, requiring little space, and allowing precise control of environmental conditions. It can be recommended to cowpea breeders as a valuable method for drought tolerance screening. However, the optimum level of polyethylene glycol (PEG) concentration capable to induce drought stress that will show differences in tolerance, need to be established before the studies are carried out. Although the results seem to be promising in discriminating between the accessions, further field screening is required to confirm the results obtained in this study.

6.6 References

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Chapter 7

SUMMARY

Cowpea (*Vigna unguiculata*) is a staple food crop of significant economic importance worldwide. Cowpea is valued for the high vitamin and mineral content present in young leaves, pods and seed. The crop is unique in that it provides food, cash and fodder. However, cowpea production is limited by numerous biotic (insects, microbial and fungal diseases, and other pests) and abiotic (high temperature, drought) factors. Because of its widespread use, numerous initiatives have been undertaken to improve various agronomic and nutritional traits of cowpea. Analysis of genetic relationships among and within crop species is a prerequisite for any genetic improvement and central to successful breeding programmes. This can be achieved through characterisation of germplasm either using morphological, biochemical or DNA markers. Fifteen qualitative morphological traits and 12 quantitative morphological traits were used in this study to group 20 cowpea accessions from three different African regions into clusters according to their genetic distance, in order to quantify the extent of phenotypic and genetic diversity among these accessions and identify desirable groups that could be utilised in breeding programmes.

The results showed a relatively high level of dissimilarity among the accessions for most of the morphological traits analyzed, especially for accessions from different countries. This indicates better possibilities for genetic improvement of the crop through selection and cross breeding. However, a very high level of similarity was revealed between many accessions from the same region for most of the characters studied. Makueni, Gacaga, M.66, Kamurugu 1, Bafoussam 1, MTA22, AS-94 and Okhalweni were the group of accessions different from the other accessions for most of the traits studied and can therefore be used for cowpea improvement programmes through cross breeding.

Drought is a serious environmental stress affecting cowpea production throughout the world. Twenty cowpea accessions were characterised for drought tolerance using three different screening methods (pot screening method, cell membrane stability and stomatal behaviour).

For the pot screening method, seeds were planted in pots in the glasshouse at the University of the Free State. After germination plants were kept well watered, until they had completed the early development stage, after which 500 ml of water was given per day per pot up to the flowering stage when the watering was terminated. After 21 days, each plant was scored using the International Board for Plant Genetic Resources (IBPGR) descriptors for cowpea. The results showed a wide range of drought tolerance among the studied accessions and this suggests the possibility of breeding cowpea drought tolerant cultivars. Bafoussam 1 and Bafoussam 2 were found to be highly drought tolerant and could be valuable materials for drought tolerance improvement in cowpea.

Drought tolerance potential of the accessions was also characterised based on their ability to close their stomata, and the number of stomata per unit leaf area under water deficit conditions. The results showed that cowpea (*Vigna unguiculata*) in Kenya, South Africa and Cameroon were rich in drought tolerant accessions. The general trend showed that accessions from the humid areas of Kenya and Cameroon had a relative high stomata density and also had the lowest ability to regulate the degree of their stomatal opening under drought stress. Meanwhile accessions from the arid and semi-arid areas of South Africa, Cameroon, and Kenya did show a relatively good ability to regulate the degree of their stomatal opening under the imposed drought stress and had a relatively low stomatal density. Materials with good drought tolerance included Bafoussam 4, MT22, AS-94, Balen, Hluhluwa, Mpenbeni, Bafoussam 5 and K.80. However, Bafoussam 4 was the only accession found to have both a low stomatal density and a good ability to regulate the degree of its stomatal opening under the imposed drought stress. Therefore it was not possible to confirm any correlation between the ability to regulate the degree of stomatal opening and stomatal density among the accessions studied.

The results of the cell membrane stability test also showed that there was significant variation among the accessions for electrolyte leakage. Kamurugu 2, Ken-Kunde, Kasuku, and K.80 had the highest percentage level of injury and were found to be the most susceptible to drought stress, when the accessions were exposed to simulated osmotic stress. Bafoussam 2, Okhalweni, and Bafoussam 4 had the lowest percentage level of injury and were found to be the least susceptible to drought stress.

The results of the three techniques seemed to be promising in discriminating the accessions tested for their drought tolerance ability as presented in Appendix. However, extensive field screening is required to confirm the results obtained in this study.

Appendix

Table 7.1: Summary of drought tolerance of cowpea accessions tested

Accessions	Pot test	Stomatal behaviour test	Cell membrane stability test
MTA22	Susceptible	Tolerant	Susceptible
Balen	Intermediate	Tolerant	Intermediate
Okhalweni	Susceptible	Susceptible	Tolerant
AS-94	Susceptible	Intermediate	Intermediate
Bafoussam 1	Tolerant	Susceptible	Intermediate
Bamougoum	Susceptible	Highly Susceptible	Intermediate
Bafoussam 2	Tolerant	Intermediate	Tolerant
Bafoussam 3	Intermediate	Susceptible	Intermediate
Mpenbeni	Susceptible	Tolerant	Intermediate
Bafoussam 4	Intermediate	Tolerant	Tolerant
Hluhluwa	Intermediate	Tolerant	Susceptible
Bafoussam 5	Susceptible	Intermediate	Intermediate
K.80	Susceptible	Tolerant	Highly Susceptible
Gacaga	Highly susceptible	Susceptible	Intermediate
M.66	Tolerant	Intermediate	Susceptible
Makueni	Susceptible	Susceptible	Intermediate
Kamurugu 1	Highly susceptible	Susceptible	Intermediate
Ken-Kunde	Highly susceptible	Intermediate	Highly Susceptible
Kasuku	Highly susceptible	Intermediate	Highly Susceptible
Kamurugu 2	Highly susceptible	Susceptible	Highly Susceptible

OPSOMMING

Akkerbone (*Vigna unguiculata*) is 'n stapelvoedselgewas van betekenisvolle ekonomiese waarde. Akkerbone is belangrik vir die hoë vitamien en minerale inhoud van jong blare, peule en sade. Die gewas is uniek deurdat dit voedsel, kontant en voer verskaf. Die produksie van akkerbone word deur verskeie biotiese (insekte, mikrobiese en fungi siektes, en ander peste) en abiotiese (hoë temperature, droogte) faktore beperk. Weens die wydverspreide gebruik van akkerbone, is verskeie inisiatiewe reeds onderneem om die agronomiese en voedingswaarde van akkerbone te verbeter. Analise van die genetiese verwantskappe onder gewasspesies is 'n voorvereiste vir enige verbetering en sentraal tot suksesvolle teelprogramme. Dit kan bereik word deur die karakterisering van kiemplasma deur morfologiese, biochemiese of DNA-merkers te gebruik. Vyftien kwalitatiewe en 12 kwantitatiewe morfologiese kenmerke is in hierdie studie gebruik om 20 akkerboon genotipes van drie Afrika-lande volgens hul genetiese afstand te groepeer om die omvang van die fenotipiese en genetiese diversiteit tussen die genotipes te kwantiviseer en groepe wat vir teelprogramme gebruik kan word, te identifiseer.

Die resultate toon 'n relatiewe hoë graad van morfologiese verskil tussen die genotipes van die drie lande. Dit toon dat daar moontlikhede is vir genetiese verbetering deur seleksie en kruisteling. 'n Hoë graad van ooreenkoms kom voor tussen genotipes van dieselfde gebied. Makueni, Gacaga, M.66, Kamurugu 1, Bafoussam 1, MTA22, AS-94 en Okhalweni is die genotipes wat van die ander verskil en dus in teelprogramme gebruik kan word om akkerbone te verbeter.

Droogte is 'n ernstige probleem wat die produksie van akkerbone wêrelwyd beïnvloed. Twintig akkerboon genotipes is met behulp van drie verskillende metodes (pottoetsmetode, selmembraanstabiliteittoets en stomatagedrag) vir droogteweerstandbiedendheid getoets.

Die pottoetsmetode is uitgevoer deur sade in potte, in 'n glashuis, aan die Universiteit van die Vrystaat te plant. Tot en met die blomstadium is die plante elke dag natgemaak waarna geen water meer toegedien is nie. Na 21 dae is die plante volgens die Internasionale Raad van Plant Genetiese Bronne beoordeel. Die resultate het 'n wye reeks van droogtebestandheid in die bestudeerde

genotipes getoon wat aandui dat daar 'n moontlikheid is om droogtebestande akkerbone te teel. Die resultate het getoon dat Bafoussam 1 en Bafoussam 2 hoogs droogteweerstandbiedend is en waardevolle materiaal kan wees om droogteweerstandbiedendheid in akkerbone te verhoog.

Droogteweerstandbiedendheid van die genotipes is ook bepaal volgens hul vermoë om stomata onder toestande van watertekort te sluit en die aantal stomata per blaar per eenheidsoppervlakte. Die resultate het getoon dat akkerbone (*Vigna unguiculata*) in Kenia, Suid-Afrika en Kameroen ryk is aan droogteweerstandbiedende genotipes. Die algemene neiging was dat genotipes van die vogtige gebiede van Kenia en Kameroen baie stomata per eenheidsoppervlakte gehad het en ook die laagste vermoë om hul stomata-opening onder droogtetoestande te reguleer. Genotipes van die droë en half-droë gebiede van Suid-Afrika, Kameroen en Kenia het relatief goeie vermoë getoon om stomata-opening onder droogtetoestande te reguleer en het relatief min stomata per eenheidsoppervlakte gehad. Genotipes wat goeie droogteweerstandbiedendheid getoon het, is Bafoussam 4, MTA22, AS-94, Balen, Hluhluwa, Mpenbeni, Bafoussam 5 en K.80. Die enigste genotipe met goeie regulering van stomata-opening en min stomata per eenheidsoppervlakte was Bafoussam 4. Dit was dus nie moontlik om enige korrelasie tussen stomata-opening regulering en stomata digtheid in die bestudeerde genotipes te bevestig nie.

Die resultate van die selmembraanstabiliteittoets het ook getoon dat daar beduidende variasie tussen die genotipes t.o.v. elektrolietdeurlating bestaan. Kamurugu 2, Ken-Kunde, Kasuku en K.80 het die hoogste persentasie besering getoon en is dus die mees vatbare genotipes teen droogte. Bafoussam 2, Okhalweni en Bafoussam 4 het die laagste persentasie besering getoon en is dus die meeste bestand teen droogte.

Die resultate van die drie tegnieke blyk belowend te wees vir die toetsing van weerstandbiedendheid in akkerboon genotipes (Aanhangsel). Baie veldtoetse is egter nodig om die resultate van hierdie studie te bevestig.

Aanhangsel

Tabel 7.1: Opsomming van droogteweerstandbiedendheid van akkerboon genotipes

Genotipes	Pottoets	Stomatagedragtoets	Selmembraanstabiliteittoets
MTA22	Vatbaar	Verdraagsaam	Vatbaar
Balen	Intermediêr	Verdraagsaam	Intermediêr
Okhalweni	Vatbaar	Vatbaar	Verdraagsaam
AS-94	Vatbaar	Intermediêr	Intermediêr
Bafoussam 1	Verdraagsaam	Vatbaar	Intermediêr
Bamougoum	Vatbaar	Hoogs vatbaar	Intermediêr
Bafoussam 2	Verdraagsaam	Intermediêr	Verdraagsaam
Bafoussam 3	Intermediêr	Vatbaar	Intermediêr
Mpenbeni	Vatbaar	Verdraagsaam	Intermediêr
Bafoussam 4	Intermediêr	Verdraagsaam	Verdraagsaam
Hluhluwa	Intermediêr	Verdraagsaam	Vatbaar
Bafoussam 5	Vatbaar	Intermediêr	Intermediêr
K.80	Vatbaar	Verdraagsaam	Hoogs vatbaar
Gacaga	Hoogs vatbaar	Vatbaar	Intermediêr
M.66	Verdraagsaam	Intermediêr	Vatbaar
Makueni	Vatbaar	Vatbaar	Intermediêr
Kamurugu 1	Hoogs vatbaar	Vatbaar	Intermediêr
Ken-Kunde	Hoogs vatbaar	Intermediêr	Hoogs vatbaar
Kasuku	Hoogs vatbaar	Intermediêr	Hoogs vatbaar
Kamurugu 2	Hoogs vatbaar	Vatbaar	Hoogs vatbaar