RESPONSE OF DUAL-PURPOSE COWPEA LANDRACES TO WATER STRESS

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Dr. Alfred Odindo (Supervisor)

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Prof. Hussein Shimelis (Co-supervisor)

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DEDICATION

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GENERAL ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp) is an important protein-rich grain legume of major economic importance. It is widely grown by small-scale farmers in the arid and semi-arid regions of the world where it is cultivated for its leaves, fresh immature pods and dry grains. However, it is also an underutilized grain legume. In sub-Saharan Africa where most of the cowpea is produced, drought stress is one of the major factors limiting its productivity. Despite the inherent capacity to survive drought stress, several cowpea varieties are affected by mid and late season drought. Therefore, varieties with a higher tolerance to drought stress are required to obtain higher and more stable yields. The objectives of this study were: (i) to determine morphological responses of four dual-purpose cowpea landraces to water deficits during vegetative and reproductive stages (ii) to determine physiological responses of four dual-purpose cowpea landraces to water deficits and recovery during the reproductive stage (iii) to determine yield performance of cowpea landraces after recovery from water stress and how this relates to (ii) above.

Four cowpea landraces namely; Lebudu, Lehlodi, Sejwaleng and Morathathane collected from Kgohloane and Ga-Mphela villages, Limpopo Province, South Africa were used in the study. Pot experiments were conducted under glasshouse conditions at the Controlled Environment Facility (CEF), University of KwaZulu-Natal. The first pot experiment evaluated the morphological responses of four cowpea landraces to water stress and recovery. The study was conducted as a single factor experiment laid out in randomized complete block design (RCBD). The treatments (four cowpea landraces) were each planted in 40 pots giving a total of 160 experimental units (drained polyethylene pots with a 5 litre capacity). Each plant in each pot was treated as a replicate. Plants were well-watered until the formation of six fully expanded trifoliates, then irrigation was withheld for 28 days to simulate drought stress during the vegetative growth. The imposition of drought stress was terminated by re-watering all plants after 28 days. The cowpea plants were re-watered sufficiently and allowed to grow until the four landraces reached 50% flowering stage. Watering was withheld again at 50% flowering for a two-week period for all the four landraces to simulate drought stress during the reproductive growth.

The second experiment was conducted to investigate physiological responses of the four cowpea landraces to water stress during the reproductive stage. The experiment was laid out as a 4 x 2 factorial treatment structure in randomized complete design (CRD) with the following three factors: cowpea landraces – 4 levels (Lebudu, Lehlodi, Sejwaleng and Morathathane), water regimes – 2 levels (stressed and well-watered) treatment combinations each replicated 20 times (20 pots each containing one plant) giving a total of 160 experimental units (drained polyethylene pots with a 5 litre capacity).

Data on morphological responses were collected and included: number of green vs. senesced leaves, visual assessment of leaf greenness, stem, branch greenness and survival percentage. Physiological responses to water stress were determined during the reproductive stage and included: leaf water potential, relative water content, stomatal conductance, proline content, chlorophyll content, carotenoid content, chlorophyll a content, phenolics (free and membrane-bound), total antioxidant capacity and chlorophyll fluorescence parameters (*Fv/Fm*). Genstat 14th edition (VSN International, UK) was used to perform analyses of variance (ANOVA) and differences between means were determined by the Least Significant Differences (LSD) at the 5% probability level.

Landraces showed different morphological responses during both vegetative and reproductive growth stages. Lebudu, Lehlodi and Sejwaleng displayed a strong ability to maintain stem greenness longer as compared to Morathathane during vegetative growth. Lebudu delayed leaf senescence more than other landraces; no differences in survival were observed. All landraces survived for 28 days without water and resumed growth after re-watering. During the reproductive stage, Lebudu displayed a strong ability to maintain leaf, branches and stem greenness longer and showed relatively higher tolerance to drought stress compared to other three landraces. Water stress caused a decline in leaf water potential, relative water content, carotenoid content, chlorophyll content, stomatal conductance and increased proline content, phenolics, chlorophyll a content, total antioxidant capacity and while chlorophyll fluorescence parameter, *Fv/Fm*, was not affected. All landraces maintained higher relative water content above a critical threshold with Sejwaleng maintaining a significantly higher RWC (69%) than Lehlodi, Lebudu and Morathathane. Morathathane developed a more negative leaf water

potential at maximum stress than Lebudu, Lehlodi and Sejwaleng. Stomatal closure was observed in all cowpea landraces during water stress, but re-opened after re-watering. Chlorophyll content was considerably reduced in Morathathane as compared to Lebudu, Lehlodi and Sejwaleng. No significant differences were observed between the cowpea landraces with respect to carotenoid content at maximum stress. Chlorophyll *a* content increased significantly for Morathathane as compared to Lebudu, Lehlodi and Sejwaleng. High accumulation of proline was observed for Lebudu, Lehlodi and Morathathane as compared to Sejwaleng, which showed a very slow accumulation of proline. Lebudu, Lehlodi and Sejwaleng showed an increase in phenolic compounds while a decline was observed for Morathathane. Total antioxidant capacity (TAOC) was high in all cowpea landraces during water stress. Also, all chlorophyll fluorescence parameters showed that cowpea landraces had efficient photo-protection mechanisms during drought stress. After re-watering, relative water content, leaf water potential, stomatal conductance, chlorophyll content, carotenoids, chlorophyll *a*, proline content and TAOC recovered and reached the same level as that of well-watered plants.

All four landraces were re-watered after the imposition of stress and above ground biomass, pod mass and number and seed yield determined. Although there was a reduction in the total above-ground biomass, pod mass and number in all four landraces under water stress compared to the well–watered treatment; this was not statistically significant ($P > 0.05$). Furthermore, no significant differences ($P > 0.05$) were observed between the four landraces with respect to seed yield under stressed and well-watered conditions. This study established that cowpea landraces vary with respect to the various morphological and physiological adaptive mechanisms in response to water deficits. Such adaptive mechanisms probably ensure their survival under severe water stress conditions until the next rainfall and therefore allowing them to produce reasonably relatively higher leaf and seed yield. Detailed knowledge of these mechanisms in the landraces could be useful in the genetic enhancement and breeding for drought tolerance in the existing cowpea germplasm.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. GENERAL INTRODUTION

Cowpea (*Vigna unguiculata* [L]. Walp) is an important protein-rich grain legume of major economic importance amongst small-scale farmers in semi-arid regions of the world. Cowpea is cultivated for its leaves, fresh immature pods and dry grains. It is estimated that over 12.5 million tons of cowpea grains are produced worldwide, with Africa's arid Sahel region accounting for 64% of total cowpea seed production (Fery, 2002). In addition to its high nutritional value (22-23% protein content) (Bressani, 1985) and ability to fix nitrogen in relatively poor soil, cowpea has greater drought tolerance than most other crop plants (Ehlers and Hall, 1997; Hall, 2004). The crop is often grown under water limited field conditions. For example, cowpea is widely cultivated by smallholder/subsistence farmers in South Africa who mainly live in dry marginal areas. South Africa with an annual long-term rainfall average of about 500mm per year can be considered as a drought risk country. The frequency and intensity of droughts have increased over the last 30 years in many parts of the world (Hall *et al.*, 2003) and these trends will likely continue in the future in many regions including , South Africa. There is therefore a need to develop cultivars that are more drought tolerant and can adapt to these changing environmental conditions. In addition, such cultivars should be able to use less water more efficiently for improved productivity. The availability and adoption of such cowpea cultivars with high levels of drought tolerance and increased water-use efficiency particularly by resource poor famers living in marginal areas would contribute to ensuring food security (Fatokun *et al.*, 2012).

Phenological variation with respect to flowering and maturation times have been previously investigated to understand the mechanisms related to drought escape (Gwathmey and Hall, 1992). Breeding cultivars for tolerance against mid and late-season drought has been achieved through development of early and late-maturing cowpea cultivars (Cisse *et al.*, 1978; Cisse *et al.*, 1995). Early maturing genotypes may be able to complete their reproductive cycle in time to escape late-season drought (Grantz and Hall, 1982; Ehlers and Hall, 1997). These genotypes, however, have been shown to be sensitive to mid-season drought (Thiaw *et al.*, 1993). Indeterminate and late-maturing cultivars may be suitable for environments characterized by intermittent drought stress (Hall and Patel, 1985), however, they are damaged by late season drought. Therefore, it seems that efforts made to develop drought tolerant cowpea cultivars have met with partial success. It would therefore be important to identify physiological and morphological traits that could improve the tolerance of cowpea to mid and late-season drought. In South Africa, most poor resource farmers grow unimproved cowpea landraces (Asiwe, 2009; Shiringani and Shimelis, 2011). Personal communication with local farmers, Mrs Mmapitsi Kobe and Mrs Mahlong who have grown these landraces for many years, revealed that these farmers have been in possession of these landraces for about 60 years. According to the farmers, the cowpea landraces provide insurance against hunger because during drier years when maize and other crops fail, they would be able to harvest substantial quantities of cowpea. These landraces may have adapted to local conditions which are often water limited and may possess genes that could be used in developing improved cowpea varieties with high levels of drought tolerance and higher yields. Although cowpea is relatively more drought tolerant than other crops, the level of drought tolerance could still be improved through genetic improvement (Fatokun *et al.*, 2012). However, there is little information documented about the responses of cowpea landraces grown by subsistence farmers in the Limpopo province to water deficits. As a first step to enhancing drought tolerance in the existing local cowpea landraces, it is important to evaluate the ability of these landraces to tolerate drought stress. From a crop improvement perspective, it is important to understand their variability with regards to morphological, physiological and genetic traits contributing to their adaptability to water-deficits. Such information may be useful to plant breeders to identify traits in these landraces that could be used to generate segregating populations from where selection could be carried for the development of cowpea cultivars with better adaptation to drought and improved water use efficiency.

Aim of the study

The aim of the study was to examine whether there is any variation with respect to morphological and physiological traits among local cowpea landraces grown by smallholder/subsistence farmers in Limpopo province, and determine the extent to which this may relate to yield performance under water limited conditions.

Specific objectives of the study

1. To examine whether there are differences in leaf greenness, stem greenness, branch greenness, leaf number and senescence amongst four cowpea landraces subjected to water deficits during vegetative and reproductive growth stages

2. To examine whether there are differences among the landraces with respect to changes in plant water status osmolyte accumulation , pigment content , total antioxidant capacity and photosynthetic capacity when subjected to water deficits during the reproductive growth stage

3. To determine yield performance as indicated by pod number, pod mass, seed yield, biomass and harvest index and correlate this with objective 2 above.

Dissertation structure

Chapter 1 is the general introduction and describes the problem statement and specific objectives of the study.

Chapter 2 reviews the literature on plant responses to water deficits. The review considers the overall strategies and the mechanisms involved in the drought tolerance of plants in general and focus specifically on cowpea.

Chapter 3 describes morphological responses of four dual-purpose cowpea landraces to water deficits during the vegetative and reproductive growth stages. Variation in morphological responses such as leaf senescence, maintenance of green leaf area, plant survival, stem greenness and branches greenness and their implications as indicators of drought tolerance in cowpea landraces are discussed.

Chapter 4 describes physiological responses of four dual-purpose cowpea landraces when subjected to drought stress and recovery during the reproductive growth stage. Changes in plant water status (e.g. leaf water potential and relative water content), pigment content (e.g. chlorophyll content, carotenoid content, chlorophyll , proline content, phenolics and chlorophyll fluorescence (*Fv/Fm*) and total antioxidant capacity (TAOC) are discussed and related to drought tolerance and recovery after drought relief. Furthermore, yield performance with respect to biomass, pod number and seed yield are discussed and related to physiological responses and recovery after stress relief.

Chapter 5 discusses insights on the understanding of morphological and physiological responses in relation to the future improvement of cowpea landraces with regards to better adaptation to drought stress and yield performance within the context of climate variability (uncertain and erratic rainfall and prolonged dry spells) typical of smallholder/subsistence farming in Africa and more specifically in South Africa. This chapter also highlights the major limitations to the study and points out directions for further research.

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2. LITERATURE REVIEW

2.1 Cowpea: origin, genetic diversity and distribution

Cowpea (*Vigna unguiculata* (L) Walp., Fabaceae, 2n = 2x = 22) is a major grain legume, fodder for livestock, vegetable and cover crop cultivated around the world. Cowpea is often referred to as crowder pea, black-eyed and southern pea and is known internationally with other various names including lubia, niebe, coupe, or frijole (Ng and Marachel, 1985). Cowpea is thought to have originated from West Africa, Central Africa and South Africa (Ng and Padulosi, 1988).

The cultivated cowpea has been reported to have a narrow genetic base indicating the crop might have gone through a 'genetic bottleneck' during domestication. Four cultigroups of cowpea are recognized (Baudoin and Martchal, 1985) and they are: (1) *unguiculata*, which is the common form; (2) *biflora* or catjang, which is characterized by small erect pods and found mostly in Asia; (3) *sesquipedalis*, or yard-long bean, also mostly found in Asia and is characterized by its very long pods and (4) *textilis*, found in West Africa and which are used for fiber obtained from its long peduncles (Ehlers and Hall, 1997). The primitive and wild relatives of *V. unguiculata* are reportedly found in Southern Africa including Namibia, Botswana, Zambia, Zimbabwe, Mozambique and the Republic of South Africa. The Limpopo province in the Republic of South Africa is the centre of diversity due to the presence of most primitive wild botanical varieties including *rhomboidea*, *prottracta*, *tennis* and *stenophylla* (Ng and Marachel, 1985). 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 North-Western part of Cameroon (Ng and Marechal, 1985).

2.2 Description and classification

Cowpea is an annual herb reaching a height of up to 80 cm with a strong taproot system and many spreading lateral roots in the surface soil (Kay, 1979). The crop exhibits a wide range of growth habits which are generally described by their appearance at maturity as being either erect, semi-erect, semi-spreading, or spreading (prostrate), and as either extra-early, early, medium, or late (Timko *et al.*, 2008). There is also variability in the growth habit which includes indeterminate and determinate cowpea types (Patel and Hall, 1990; van Rensburg *et al.*, 2007).

Leaves are alternate and trifoliate exhibiting considerable variation in size and shape (e.g. linear, lanceolate to ovate) and they are usually shiny and dark green. The stems are striate, smooth or slightly hairy and sometimes purple in colour (Kay, 1979).

Flowers are born in multiple racemes on 8 to 20 cm flower stalks (peduncles) that arise from the leaf axis (Hadley *et al*., 1983). Flowers are large and conspicuous, self-pollinating and typically white, yellow, pink, pale blue or purple in colour (Ehlers and Hall, 1997). Two or three pods per peduncle are common and often four or more pods are carried on a single peduncle (Adcock and Lawes, 1976). Fruits are pods that vary in size, shape, colour and texture. As the seeds dry, pod color of the green and yellow types becomes tan or brown. There are usually 8-20 seeds per pod. Seed shape is a major characteristic correlated with seed development in the pod. Seed develop into a kidney shape if not restricted within the pod and globular when restricted by the pod. Cowpea seed coat can be smooth or wrinkled (Saunders, 1960) are characterized by a wide variation in seed coat colour ranging from white to dark red and black and the seed is often mottled or shows a black “eye” at the hilum (van Rensburg *et al.*, 2007; Timko and Singh, 2008).

2.3 Cowpea utilization and production

Cowpea is an important crop for the nutrition and livelihoods of millions of people in less developed countries (Singh *et al.*, 2003; Timko and Singh, 2008). The crop may be consumed at various stages of its development including green leaves, green pod, green peas, and dry grains (Ibrahim *et al.*, 2010). The leaves are prepared like spinach, lettuce, cabbage or amaranthus and eaten along with maize meal in Southern Africa (Enyi, 1975; Sebetha *et al.*, 2010). Fresh seeds and immature pods are frozen or canned and consumed in the United States of America (Rahman *et al.*, 2008). Cowpea leaves and seeds are low in fat, high in carbohydrate, moderate in protein, and low in anti-nutritive factors. The dried seeds contain 22 -23 % protein content (Bressani, 1985) while cowpea leaf protein is about 9.3 to 12.4% (Sebetha *et al.*, 2010). Leaves also are a good source of minerals, specifically Fe, Ca, K, and Zn (Imungi and Potter, 1983). Raw leaves are high in vitamin C, carotene, and folacin, although 80% of these vitamins can be lost during cooking. Cooked cowpea leaves are suitable for further processing (Imungi and Potter, 1983), such as compacting and drying to create a rehydratable product with shelf life. In South Africa, the leaves are usually harvested and used fresh or cooked and formed into cakes and sundried.

The end product is called “Morogo” (in the local Sepedi language) is stored for winter use when leafy vegetables are scarce. The crop fixes nitrogen (Sanginga *et al.*, 2000) and is used as a soil cover and green manure for maintaining the productivity of soils (Blade *et al.*, 1997). In addition to human consumption, cowpea stems and leaves are also a very important nutritious fodder for livestock (Singh and Tarawali, 1997). Cowpea fodder plays a particularly critical role in feeding animals during the dry season in many parts of West Africa (Singh and Tarawali 1997).

Dry grain production is the only commodity of cowpea for which production estimates are generated on a worldwide basis (Timko and Singh, 2008). Available data on area under cowpea cultivation and production shows that 11.3 million hectares (ha) is under cowpea cultivation and 3.6 million tons (t) is produced (Singh *et al.*, 2002). Africa’s arid Sahel region accounts for 70% of total cowpea seed production produced annually (Timko and Singh, 2008). The estimated area and production in over 50 other countries in Asia, Africa, and Central and South America that grow cowpea would make a world total of over 14 million ha and 4.5 million t (Singh *et al.*, 2002). Nigeria is the largest producer and consumer of cowpea with about 5 million ha and over 2 million tons produced annually followed by Niger and Brazil (Singh *et al.*, 2003).

2.4 Cowpea production in South Africa

Cowpea is one of the important leafy vegetables in South Africa (van Rensburg *et al.*, 2007). It is also the third important grain legume after groundnut (*Arachis hypogea* L.) and dry beans (*Phaseolus vulgaris* L.) (Asiwe, 2009a). In South Africa, cowpea production is largely confined to the Limpopo, Mpumalanga, North West and KwaZulu-Natal provinces (Department of Agriculture Forestry and Fisheries, 2011). In these Provinces, it is solely grown as a sole crop and as a mixed crop with maize, bambara groundnut, soybeans and dry beans. Most of the cowpea production is by poor resource farmers and production is very low. The land area planted by local farmers ranges between 0.25 and 2.0 ha per farmer with grain yield ranging between 0.25 and 1.0 ton/ha with an average of 0.5 tons/ha per farmer (Asiwe, 2009b). These is much lower than cowpea’s grain yield potential of 3.0 tons/ha (Singh *et al.*, 1997). This may be due to the fact that research and production of cowpea has been neglected in South Africa in the last 30 years due to lack of funding and interest of researchers to work on the improvement of the crop. The consequences of these are that cultivated varieties are unimproved and the lack of knowledge of good agronomic practices worsens the limitations to cowpea production (Asiwe 2009b).

2.5 Biotic factors limiting cowpea production

2.5.1 Diseases

Diseases affecting cowpea are numerous and generally grouped into three main classes: viral, bacterial and fungal diseases (Asiwe, 2009b). The mainly seed borne viruses are blackeye cowpea mosaic potyvirus (BICMV), cowpea aphid-borne mosaic potyvirus (CABMV), cucumber mosaic cucumovirus (CMV), cowpea mosaic (CPMV) and cowpea severe mosaic (CPSMV) comoviruses, southern bean mosaic sobemovirus (SBMV), and cowpea mottle carmovirus (CPMoV) (Hampton *et al.*, 1997). Major fungal diseases include: Anthracnose (*Colletotrichum lindemuthianum*), Ascochyta blight, Black leaf spot or leaf smut, Brown blotch, Brown rust, Cercospora leaf spots (*Cercospora canescens* Ellis and G. Martin), Powdery mildew, Pythium soft stem rot, Septoria leaf spot, Sphaceloma scab, and Web blight fungus (*Rhizoctonia solani* Kuhn). Cowpea bacterial blight (CoBB) and bacterial pustule caused by *Xanthomonas axonopodis* pv. *Vignicola* (*Xav*) are major bacterial diseases of cowpea (Ehlers and Hall, 1997; Bouker and Fatokun, 2007).

2.5.2 Insect pests

The major insect pests of cowpea are aphids (*Aphis craccivora*), thrips (*Megalurothrips sjostedti*), Maruca pod borer (*Maruca vitrata*), a complex of pod sucking bugs (*Clavigralla* spp., *Acanthomia* spp., *Riptortus* spp.), and the storage weevil *Callosobruchus maculatus* (Ehlers and Hall, 1997; Bouker and Fatokun, 2007; Asiwe, 2009a).

2.5.3 Parasitic weeds

The important parasitic weeds attacking cowpea in Africa are *Alectra vogelii* [Benth.] and *Striga gesnerioides* [Wild.] Vatke. Both are flowering plants that parasitize cowpea plants in the field. These weeds have been reported to cause considerable cowpea yield losses (Ehlers and Hall, 1997; Bouker and Fatokun, 2007; Asiwe, 2009a).

2.6 Abiotic factors limiting cowpea production

In the semi-arid cowpea growing regions, drought and heat stress are major production constraints (Hall and Patel, 1985; Bouker and Fatokun, 2007). However, the most environmental factor affecting plant growth is drought stress (Agbicodo *et al.*, 2009). Drought is currently the most important abiotic stress limiting cowpea production worldwide (Singh *et al.*, 1999; Hall, 2004).

2.7 Definition of drought and water stress

From a meteorological point of view, drought is defined as the absence of rainfall for a long period of time to cause moisture depletion in the soil and a decrease of water potential in plant tissue (Kramer, 1980). Turk and Hall (1980b) and Hall and Schulze (1980) defined drought as the occurrence of substantial water loss in the soil, atmosphere and plant. Agriculturally, drought is defined as the inadequacy of water that is available to the plants, including precipitation and soil moisture storage capacity, in quantity and distribution during the life cycle of the crop plant, which restricts the expression of full genetic potential of the plant from reaching the genetically determined theoretical maximum yield (Begg and Turner, 1967). Generally water stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation (Jaleel *et al.*, 2009). Water stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and a decrease in cell enlargement and growth. Severe water stress may inhibit photosynthesis, impair metabolism processes, and finally lead to dehydration and plant death (Kamara *et al.*, 2003). However, the reaction of plants to drought differs significantly depending upon intensity and duration of water stress as well as plant species and the stage of development (Chaves *et al.*, 2003).

2.8 Types of drought stress

Drought can be classified in various ways: according to the time of occurrence into intermittent and terminal, its intensity into mild, moderate and severe and its duration into short and long duration. Intermittent drought is the one that occurs at any time during the crop's vegetative growth stage. Terminal drought is the one that occurs at the end of the crop growth stage, affecting mostly the reproductive stage such as flowering and seed development (Ludlow and

Muchow, 1990). Intermittent drought results from variable amount and distribution of rainfall during the crop growth stage while terminal drought results from cessation of rainfall. Both intermittent and terminal drought affect crop production but the impacts may vary (Turk and Hall, 1980a; Turk *et al.*, 1980; Abayomi and Abidoye, 2009). Therefore, a clear understanding of drought stress occurring in a particular environment is important.

2.9 Drought tolerance mechanisms in plants

Several mechanisms operate independently or jointly to enable plants to cope with drought stress (Agbicodo *et al.*, 2009). Drought tolerance is therefore manifested as a complex trait (Mitra, 2001). Mitra (2001) defined drought tolerance as the ability of a crop plant to produce its economic product with minimum loss in water-deficit environment relative to the water-constraint-free environment. Strategies of drought-avoidance or drought-tolerance involving plant mechanisms that provide the plants with the ability to respond and survive drought have been reported (Levitt, 1980). According to Mitra (2001), the mechanisms that plants use to cope with drought stress can be grouped into three categories namely: drought escape, drought avoidance and drought tolerance.

Drought escape is the ability of a plant to complete its life cycle before serious soil and plant water deficits occur. This mechanism involves rapid phenological development (e.g. early flowering and early maturity), developmental plasticity (variation in duration of growth depending on the extent of water deficit) and remobilization of photo-assimilates (Chaves *et al.*, 2003). Flowering time is an important trait related to drought adaptation, where a short life cycle can lead to drought escape (Araus *et al.*, 2002). Drought escape occurs when phenological development is successfully matched with periods of soil moisture availability, where the growing season is shorter and terminal drought stress is prevalent (Araus *et al.*, 2002). The time to flowering is a major trait of a crop adaptation to the environment, particularly when the growing season is restricted by terminal drought and high temperatures. Developing short-cycle varieties has been an effective strategy for minimizing yield loss from terminal drought, as early maturity helps the crop to avoid the period of stress (Farooq *et al.*, 2009).

Drought avoidance is the ability of plants to maintain relatively high tissue water potential despite a shortage of soil moisture (Hall and Schulze, 1980). Mechanisms for improving water uptake, storage in plant cell and reduction in water loss confer drought avoidance while responses of plants to tissue deficit determine drought tolerance (Mitra, 2001). Plants develop strategies for maintaining turgor by increasing root depth or developing an efficient root system to maximize water uptake, and by reducing water loss through reduced stomatal conductance, reduced absorption of radiation, by leaf rolling or folding and reduced leaf area (Turner, 1986; Mitra, 2001). The root characters such as biomass, length, density and depth are the main drought avoidance traits that contribute to final yield under terminal drought environments (Subbarao *et al.*, 1995).

Drought tolerance is the ability of plants to withstand water-deficit with low tissue water potential (Chalves *et al.*, 2003; Mitra, 2001). Plants can also tolerate drought stress conditions by avoiding dehydration of tissues and maintaining high tissue water potential or tolerating low tissue water potential. These is achieved by minimizing water loss and maximizing water uptake (Chaves *et al.*, 2003). Plants that use tolerance mechanism maintain turgor through osmotic adjustment through an accumulation of compatible solutes in the cell, increase cell elasticity, decreased cell volume and resistance to desiccation (Turner, 1986; Agbicodo *et al.*, 2009). However, plants may use more than one mechanism at a time to cope with drought (Agbicodo *et al.*, 2009). However, these adaptation mechanisms to cope with drought have some disadvantages with respect to yield potential. Mechanisms that confer drought avoidance by reducing water loss (such as stomatal closure and reduced leaf area) decrease carbon assimilation due to reduction in physical transfer of carbon dioxide and increase leaf temperature thus reducing biochemical processes, which negatively affects yield. Therefore, the development of drought tolerant cowpea cultivars must reflect a balance among escape, avoidance and tolerance while maintaining adequate productivity (Agbicodo *et al.*, 2009). Drought escape, avoidance, and tolerance mechanisms have been described in cowpea.

2.10 Mechanisms of drought escape, avoidance and tolerance in cowpea

2.10.1 Drought escape in cowpea

Early maturity is an important phenological trait in avoiding late-season drought stress such cultivars are able to complete their life cycle before the onset of severe drought conditions (Grantz and Hall, 1982; Ehlers and Hall, 1997). Early maturity in cowpea is desirable and has proven to be useful in dry environments because of their ability to escape drought. Early maturing genotypes depend on drought escape mechanisms which enable them to complete their reproductive cycle in time to escape late-season drought (Ehlers and Hall, 1997). Evidence of early flowering in cowpea has been reported. Suliman and Ahmed (2010) reported that cowpea varieties grown under water stressed conditions flowered 1-15 days earlier than those grown under well watered conditions. Water stress had a similar effect even on days to maturity, whereby water stressed cowpea matured faster than the control treatment. Early flowering cowpea genotypes can produce a crop of dry grain in 60 days, while longer season genotypes may require more than 150 days to mature, depending on photoperiod (Timko and Singh, 2008). However, these genotypes have been reported to perform poorly when exposed to mid-season drought (Thiaw *et al.*, 1993; Mai-Kodomi *et al.*, 1999a; Ogbonnaya *et al.*, 2003). Therefore, it is important to develop early maturing cultivars that are more tolerant to a mid-season drought. Muchero *et al.* (2009) suggested that combining early maturity with delayed drought-induced senescence or "stay-green" trait should be efficient in managing both early and late season drought stress in cowpea. The authors observed that several of the recombinant inbred lines expressed the combined traits of early maturity with delayed drought-induced senescence.

2.10.2 Drought avoidance mechanisms in cowpea

Drought avoidance in cowpea appears to be mainly due to several mechanisms of water loss minimization (Turk *et al.*, 1980). Avoidance of water loss through reduced leaf area and increased leaf abscission (Turk and Hall, 1980b; Turk *et al.*, 1980; Anyia and Herzog, 2004b) and stomatal closure (Bates and Hall, 1981; Cruz de Carvalho *et al.*, 1998; Anyia and Herzog, 2004a; Souza *et al.*, 2004; Hamidou *et al.*, 2007b) are drought avoidance mechanisms in cowpea. Several other mechanisms may explain the extreme dehydration avoidance of cowpea. Cowpea is able to maintain high leaf water potential or high leaf relative water content during water stress

(Bates and Hall, 1981; Hall, 2004), thus avoiding tissue dehydration (Hall, 2004). In California, cowpea cultivar 'CB5' was reported to survive a vegetative stage drought that would have killed most other crop species and showed high recovery rate when re-watered and produced grain yields of about 4000 kg/ha that were similar to a weekly irrigated control treatment (Turk *et al.*, 1980). Cowpea also changes the position of leaflets under drought stress. The leaves become oriented in a way that light interception is reduced on both sides of the leaflet (Shackel and Hall, 1979), thus reducing transpiration. The leaves are generally cooler when they take this position (Shackel and Hall, 1979).

2.10.3 Drought tolerance mechanisms in cowpea

Two types of drought tolerance mechanisms have been described in cowpea as type 1 and type 2 (Mai-Kodomi *et al.*, 1999b). Under water stress "type 1" drought tolerant lines (e.g. TVu 11986 and TVu 11979) stopped growth after the onset of drought stress probably to conserve moisture and survived for 2-3 weeks. The "type 2" drought tolerant lines (e.g. Danila and Kanannado) continued slow growth of the leaves. However, with continued water stress the unifoliates of these lines showed early senescence and dropped off but the growing tips remained turgid and alive for a longer time suggesting that moisture was mobilized from the unifoliates to the growing tips. The type 2 mechanism of drought tolerance is more effective in keeping the plants alive for a longer time and ensures better chances of recovery than type 1 after stress release (Mai-Kodomi *et al.*, 1999b). Stomata closure to reduce water loss through transpiration and cessation of growth (for Type 1 drought avoidance) and osmotic adjustment and continued slow growth (drought tolerance in Type 2) have been suggested as the possible mechanisms for drought tolerance in cowpea (Lawan, 1983). The two types of drought tolerance responses by cowpea under drought stress indicate that cowpea genotypes have evolved different mechanisms to cope with drought stress (Mai-Kodomi *et al.*, 1999b; Muchero *et al.*, 2008).

2.11 Effect of water stress on crop growth and development

2.11.1 Crop establishment and growth

Well-distributed rainfall is vital for normal plant growth and development (Jaleel and Llorente, 2009). The first and foremost effect of drought is impaired germination and poor stand establishment (Harris *et al.*, 2002). Growth is one of the most drought sensitive physiological process due to a reduction in turgor pressure (Shao *et al.*, 2009). Water stress affects cell division and meristematic tissue enlargement (Jaleel *et al.*, 2009) and limits plant growth and establishment (Jaleel and Llorente, 2009; Jaleel *et al.*, 2009; Shao *et al.*, 2009). Cell division is the principal determinant of meristematic activity and determines plant growth rate (Bartels and Ramanjulu, 2005) and it affects elongation and expansion (Shao *et al.*, 2009). Water stress caused leaf and plant growth reduction in cowpea (Lobato *et al.*, 2008). Stem length in soybean was decreased under water stress conditions (Specht *et al.*, 2001). Water stress reduced plant height in cowpea (Hiler *et al.*, 1972; Abayomi and Abidoye, 2009). Growth arrest can be considered as a possibility to preserve carbohydrates to sustain metabolism, prolonged energy supply and better recovery after stress relief (Bartels and Ramanjulu, 2005). Leaf area, number of leaflets and leaf area index reduced in cowpea in response to water stress in cowpea (Hiler *et al.*, 1972; Anyia and Herzog, 2004b; Suliman and Ahmed, 2010). Reduction in leaf area is considered to be a plant's first line of defense against drought (Taiz and Zeiger, 1998).

2.11.2 Effect of water stress on photosynthesis

Water stress decreases the rate of net photosynthesis (Turner and Begg, 1981). It has been reported that stomata play a role in controlling the decline of net carbon dioxide (CO_2) uptake, thus leading to a decrease in leaf internal CO_2 concentrations (Cornic, 2000). Stomatal closure allows plants to limit transpiration, however, it may also limits CO_2 absorption, which leads to a decreased photosynthetic activity (Yang *et al.*, 2006). It was found in cowpea that photosynthesis was correlated with stomatal conductance under water stress conditions indicating that photosynthesis was limited by stomatal closure which restricts CO_2 diffusion (Anyia and Herzog, 2004a; Souza *et al.*, 2004; Hamidou *et al.*, 2007b; Singh and Raja Reddy, 2011). In common bean, stomatal conductance and net photosynthetic rate were significantly reduced by water deficits (Santos *et al.*, 2009). A combination of stomatal and non-stomatal effects on photosynthesis has also been reported. Tezara *et al.* (1999) concluded that water stress inhibits photosynthesis through diminished ribulose-1,5-bisphosphate (RuBP) supply caused by low ATP synthesis. Water stress can also increase the oxygenase activity of the RuBP carboxylase/oxygenase (Rubisco), reducing carboxylation efficiency. Therefore, decreases in the rate of photosynthesis in drought-stressed plants can be caused by stomatal closure (*i.e.* reduction of CO_2 availability) and/or impairments in photochemical (*i.e.* decrease in NADPH and ATP supply) and/or biochemical (*i.e.* reduced RuBP regeneration and carboxylation efficiency) reactions (Santos *et al.*, 2009). Other non-stomatal limitations may include changes in chlorophyll synthesis, functional and structural changes in chloroplast, and interruption in processes of accumulation, transport, and distribution of assimilates (Farooq *et al.*, 2008). Photosynthetic pigments including chlorophylls and carotenoids are normally reduced during drought stress (Yordanov *et al.*, 2000), primarily with the production of reactive oxygen species (ROS) in the thylakoids (Reddy *et al.*, 2004). Carotenoids levels were reduced by drought stress in wheat (Chandrasekar *et al.*, 2000). Total chlorophylls content and carotenoids were reduced during drought stress in cowpea (Singh and Raja Reddy, 2011). Also, Manivannan *et al.* (2007) and Saeedipour (2011) reported that chlorophylls a and b, and total chlorophyll content in sunflower and wheat plants declined in response to water stress. Similar results were reported by Efeoglu *et al.* (2009) who also reported a reduction in chlorophyll content (a, b, $a + b$) in maize. According to Anjum *et al.* (2011) loss of chlorophyll content under water stress is considered as the main cause of inactivation of photosynthesis.

2.11.3 Effect of water stress on root development

Drought-tolerance mechanisms in legume crops are closely related to the root system or rooting pattern (Pandey *et al.*, 1984). Root growth is very sensitive to water stress since they are the main transport of water in the plant (Kage and Ehlers, 1996). According to Matsui and Singh (2003) root length density, rooting depth and root dry matter are parameters of the root system that could be used for screening drought tolerance in cowpea. Moisture stress resulted in low root volume in cowpea genotypes indicating that the development of roots was inhibited or stopped during water stress (Watanabe and Terao, 1998b; Hamidou *et al.*, 2007b). On the contrary, water stress increased root length (Alyemeny, 1997) and root biomass in cowpea (Turk *et al.*, 1980). Increased root biomass may be due to the ability of cowpea to divert assimilates to enhance root growth so as to exploit deeper soil profile to absorb water. The development of an extensive root system under drought stress enhancing the ability of the plant to absorb water in the subsoil efficiently is a fundamental adaptation mechanism against water stress (Ludlow and Muchow, 1990; Bartels and Ramanjulu, 2005; de Ronde and Spreeth, 2007). Therefore, developing cowpea cultivars with a well-developed root system is vital for increasing productivity under water limited environments.

2.11.4 Effect of water stress on dry matter production and partitioning

A common adverse effect of water stress on crop species is the reduction of fresh and dry biomass production. Crop productivity under water stress conditions relies greatly on dry matter partitioning to root and shoot (Kage *et al.*, 2004). Leaf area development is controlled by the amount of assimilates allocated to the leaves and determines light interception and dry matter production (Jones, 1992). Anyia and Herzog (2004a) observed a reduction in the total dry matter when cowpea plants were subjected to water stress. A reduction in shoot weight reduced under moderate and severe water stress (Hiler *et al.*, 1972). Drought stress decreased plant total dry mass in vegetable Amaranth (*Amaranthus spp.*) (Liu and Stutzel, 2004). Total plant biomass production depends on the amount of water used for growth (Anyia and Herzog 2004b). Water stress can also affect dry matter allocation to different parts (Alyemeny, 1997). Drought stress reduces both shoot and root growth (Liu and Stutzel, 2004). Root: shoot ratio for dry matter increased for different vegetable amaranth genotypes subjected to water stress (Liu and Stutzel, 2004). Water stress increased root: shoot ratio in cowpea (Alyemeny, 1997; Suliman and Ahmed, 2010). Increase in root: shoot ratios under drought has been attributed to the fact that shoot growth is more sensitive to increasing soil water stress than root growth. Inhibition of shoot and root growths are well known effects and therefore, an increase in root to shoot dry mass ratio has been considered as one of the mechanisms involved in the adaptation of plants to drought stress (Alyemeny, 1997; Turner, 1997).

2.11.5 Effect of water stress on grain yield

Grain yield, which is positively correlated with leaf area and dry matter partitioning, may also be reduced by leaf area reductions induced by water stress (Summerfield *et al.*, 1976; Anyia and Herzog, 2004a; Suliman and Ahmed, 2010). Cowpea is sensitive to water stress at different stages of growth (Kumaga *et al.*, 2003). The effect of water stress occurring during vegetative growth has been shown to have little effect compared with water stress occurring during the reproductive growth stage (Hiler *et al.*, 1972; Turk *et al.*, 1980; Ziska *et al.*, 1983; Hamidou *et al.*, 2007a; Hamidou *et al.*, 2007b; Ahmed and Suliman, 2010). Turk *et al.* (1980) reported that cowpea can survive water stress and high evaporative demands encountered during the vegetative stage and produce high seed yield. The reproductive stage of development is the most sensitive to water deficit in cowpea, causing a reduction in water-use efficiencies and seed yields

(Hiler *et al.*, 1972; Turk *et al.*, 1980; Ahmed and Suliman, 2010). During the flowering period, the reproductive phase in cowpea is shortened and formation of new floral nodes and flowers are delayed or aborted leading to low grain yield (Turk and Hall, 1980a). In cowpea, grain yield in cowpea is determined by three components: mean number of pods per plant that reach maturity, average number of seeds per pod and average weight of seed (Aryeetey and Laing, 1973). The most important yield component is the number of pods that reach maturity (Doku, 1970). Reduced number of pods per plant contributes low yield under drought stress (Summerfield *et al.*, 1976; Turk *et al.*, 1980; Hamidou *et al.*, 2007b; Abayomi and Abidoye, 2009). In water stressed soybean, seed yield was lower when compared to well-watered plants (Specht *et al.*, 2001). Number of seeds per pod and seed weight also contribute to low yield (Ahmed and Suliman, 2010; Suliman and Ahmed, 2010). Other researchers have pointed out that reduced leaf area after flowering (Summerfield *et al.*, 1976; Turk and Hall, 1980a) and reduced carbon dioxide (CO_2) (Turk and Hall, 1980a; Mitra, 2001; Anyia and Herzog, 2004a) assimilation may also be responsible for reduced grain yield.

2.12 Physiological responses to water stress

Water stress leads to a decrease in plant water content, turgor reduction and consequently causing a decrease in cellular expansion and alteration of various vital physiological and biochemical processes (Costa *et al.*, 2008; Lobato *et al.*, 2008). During stressful conditions, various solutes that play important roles in stabilizing enzyme complexes, protecting membranes, and ensuring the osmotic adjustment required for maintenance of turgor, are synthesized in response to water stress (Mitra, 2001; Shao *et al.*, 2009). These include the accumulation of osmotically active solutes (e.g. proline, carbohydrates, organic acids and amino acids) as well as enzymatic and non-enzymatic antioxidants (Morgan, 1984; Wang *et al.*, 2000; Blokhina *et al.*, 2002). These solutes are low-molecular-weight, highly soluble compounds that are usually nontoxic even at high cytosolic concentrations (Farooq *et al.*, 2009). Under water deficit, solute accumulation lowers the osmotic potential of the cell, which attracts water into the cell and helps with the maintenance of turgor (Ludlow and Muchow, 1990). Osmotic adjustment is thought to enable the maintenance of turgor which might help in sustaining physiological processes such as stomatal opening, photosynthesis, cell enlargement and plant growth under drought stress conditions (Morgan, 1984; Munns, 1988; Ludlow and Muchow, 1990; Kusaka *et* *al.*, 2005). Osmo-protection mechanisms may probably be not functional until severe dehydration occurs and therefore, osmotic adjustment may be critical for survival rather than to increase plant growth and yield under drought conditions (Munns, 1988; Ludlow and Muchow, 1990; Serraj and Sinclair, 2002; Shao *et al.*, 2009).

The accumulation of the amino acid proline is a widespread plant adaptation to water stress (Hare *et al.*, 1999). In plants, proline is synthesized in the cytosol and mitochondria from glutamate via Δ^1-pyrroline-5-carbohydrate (P5C) by two successive reductions catalysed by P5C synthetase (PC5S) and P5C reductase (PC5R), respectively (Hare *et al.*, 1999). Several studies in higher plants have demonstrated that proline accumulates when plants are exposed to environmental stresses (e.g. heat, drought and cold stress). Increased levels of proline have been reported in water stressed cowpea plants (Campos *et al.*, 1999; Hamidou *et al.*, 2007b; Lobato *et al.*, 2008). Proline content increased in wheat and mulberry plants in response to water deficit and has been considered an index for water stress tolerance (Ramanjulu and Sudhakar, 2000; Rampino *et al.*, 2006). It has been reported that proline accumulation under water stress conditions contributes to osmotic potential (Ingram and Bartels, 1996; Souza *et al.*, 2004). Accumulation of proline has also been associated with the prevention of protein denaturation and preservation of enzyme structure and activity (Rajendrakumar *et al.*, 1994) and protein of membranes from damage by reactive oxygen species (ROS) produced under water stress conditions (Hamilton and Heckathorn, 2001). However, the role of proline in osmotic adjustment has been questioned. Proline accumulation has been argued not to play a role in osmotic adjustment and that its accumulation is a potential sign of injury (Irigoyen *et al.*, 1992; Campos *et al.*, 1999; Souza *et al.*, 2004). Despite contradicting views about the role of proline accumulation in plants, proline has emerged as a suitable index in drought tolerance screening studies.

2.13 Oxidative stress and antioxidants

Water stress induces reactive oxygen species (ROS) in plants (Brou *et al.*, 2007). ROS can also be induced by salinity, low and high temperatures (Foyer and Noctor, 2000; Lee *et al.*, 2007b). These include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO^-) and the singlet oxygen (${}^1O_2$) (Foyer and Noctor, 2000; Chaves *et al.*, 2003; Cavalcanti *et al.*, 2004a; Brou *et al.*, 2007). High levels of ROS can damage lipids, cell structure and macromolecules, DNA and carbohydrates, proteins, while causing photo-inhibition of photosynthetic apparatus which might lead to rapid leaf senescence (Prochazkova *et al.*, 2001) resulting in oxidative stress (Alscher *et al.*, 2002; Lee *et al.*, 2007a; Ahmad *et al.*, 2010). Oxidative stress is a general term used to describe a state of damage caused by reactive oxygen species (Chaves *et al.*, 2003). Therefore, efficient removal of ROS from the chloroplast is important for normal functioning in plants (Badawi *et al.*, 2004). Oxidative damage caused by ROS is alleviated by a combined action of both enzymatic and low molecular mass antioxidants (Asada, 1999). Low molecular antioxidants include β-carotene, ascorbic acid, phenols, proline, vitamin E and enzymatic antioxidants include superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT) and other enzymes of the ascorbate glutathione cycle (Cavalcanti *et al.*, 2004b; Lee *et al.*, 2007a; Lee *et al.*, 2007b). SOD is considered the first line of defense converts superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and molecular oxygen (Alscher *et al.*, 2002; Cavalcanti *et al.*, 2004a; Cavalcanti *et al.*, 2007; Lu *et al.*, 2010). POD and APX reduces H_2O_2 to water while CAT dismutase H_2O_2 into water and oxygen (Foyer and Noctor, 2000; Cavalcanti *et al.*, 2007; Lu *et al.*, 2010). Acclimation to water stress in plants is generally associated with higher antioxidant capacity which keeps the ROS concentration at lower levels (Smirnoff, 1998). In wheat, the activities of peroxidase (POX), glutathione reductase (GR), APX, phenols and proline increased upon imposition of drought stress and has been used as an index for drought tolerance (Chakraborty and Pradhan, 2012). Furthermore, the comparatively less decrease in antioxidants, higher activities of POX, GR, CAT and higher phenolic contents in tolerant tea were found in comparison to susceptible ones (Upadhyaya *et al.*, 2008). Therefore, drought tolerant cultivars must have an efficient antioxidant defense mechanism against ROS to survive severe drought stress and adapt to drought conditions.

2.14 Morphological indicators of drought tolerance in cowpea

2.14.1 Stay-green/delayed leaf senescence trait

An important morphological trait that may contribute to drought tolerance is the "stay-green" also referred to as delayed leaf senescence trait in cowpea (Gwathmey *et al.*, 1992; Hall, 2004). Stay-green can be defined as extended foliar greenness during grain-filling under post-anthesis drought or resistance against drought-induced post-flowering senescence (Muchero *et al.*, 2008; Muchero *et al.*, 2009). The "stay-green" trait has been reported in sorghum and maize and describes the phenotype of plants that retain their leaves green longer and produce increased grain yield (Borell *et al.*, 2000b; Borras *et al.*, 2003; Burke *et al.*, 2010). In cowpea, this trait enhances plant survival after a mid-season drought has damaged the first flush of pods, which enables a substantial production of second flush of pods (Gwathmey and Hall, 1992). In Senegal, DLS cowpea cultivars began flowering about 35 days, produced about 2000 kg ha^{-1} of grain in 60 days followed by second flush of pods with potential to produce additional 1000 kg ha^{-1} by 100 days from sowing (Hall *et al.*, 2003). Cowpea varieties with the stay-green trait also have enhanced leaf production because their leaves remain green and attached to the plant until harvest (Hall *et al.*, 1997a; Fatokun *et al.*, 2012).

In general, the stay-green trait is a complex phenomenon, which can be classified in five types, three of which are functional (i.e. are associated with prolonged photosynthesis), while the other two being 'cosmetic' rather than functional (Thomas and Howarth, 2000). In practice, the stay-green trait often results from a combination of different types. The expression of stay-green seems to be similar in many crops species, but the genetics and physiology of stay-green are diverse (Thomas and Howarth, 2000). For example, in cowpea the DLS trait seems to be controlled a single gene (Gwathmey and Hall, 1992; Ismail *et al.*, 2000). The DLS trait in cowpea has simple inheritance and can be selected effectively with advanced lines under field conditions (Ismail *et al.*, 2000). In soybean (*Glycine max* (L.) Merr.), a single major gene, assumed to be Dt_1,dt_1, was involved in the expression of the DLS trait (Pierce *et al.*, 1984). In sorghum, the ability to delay leaf senescence is also genetically controlled (Rosenow *et al.*, 1983; Van Oosterom *et al.*, 1996). Furthermore, expression of the DLS trait is strongly influences by environmental factors (e.g. drought) (Pierce *et al.*, 1984). The trait expresses best in environments in which the crop is dependent upon stored soil moisture, but where it is sufficient to meet only a portion of the transpiration demand (Mahalakshmi and Bidinger, 2002).

2.14.2 The physiological basis of stay green/delayed leaf senescence trait

Expression of delayed leaf senescence trait in cowpea is thought to be promoted by accumulation of non-structural carbohydrates (e.g. starch and sucrose) in the base of the stems and probably roots (Gwathmey *et al.*, 1992). In sorghum, stay-green genotypes contains more cytokinins (McBee, 1984) and basal stem sugars than senescent genotypes. Increased accumulation of sugars is associated with greater functional leaf area during seed filling, thereby reducing their reliance of stored assimilates from stem to fill the grains (McBee *et al.*, 1983). Sorghum stay-green hybrids have also been shown to have higher leaf-nitrogen concentrations at flowering and maintain these during grain filling which is associated with higher transpiration efficiency (Borrell and Hammer, 2000). There appears to be limited understanding regarding the physiological mechanisms underlying delayed leaf senescence in cowpea during drought stress. Understanding the mechanisms underlying the trait may be useful for improving drought tolerance of cowpea.

2.14.3 Stem greenness

Another morphological trait that may contribute to drought tolerance is stem greenness (Muchero *et al.*, 2008). These researchers observed differences amongst cowpea genotypes for their ability to preserve stem greenness under drought stress. The significant correlations between stem greenness with survival and recovery dry weights further suggest that maintaining green stems is a manifestation of stem viability rather than cosmetic pigmentation.

2.14.4 Indeterminacy in growth habit

Another trait that has also been associated with drought tolerance in cowpea is indeterminacy in growth habit (Anyia and Herzog, 2004a). According to Ehlers and Hall (1997), an indeterminate growth habit makes possible a longer reproductive period that contributes to drought adaptation because during drought seasons, water stress do not occur during the entire reproductive period, and such cultivars can resume vegetative and reproductive growth more quickly once moisture stress is alleviated. Early flowering, delayed leaf senescence (DLS) trait, and indeterminate growth habit are characteristics which are being combined to improve drought adaptation in cowpea breeding programs. Cowpea cultivars that will combine the ability to withstand mid-season and terminal droughts consisting of early flowering and an indeterminate growth habit with the DLS trait should exhibit drought adaptation and yield stability in many environments. Early flowering is useful in years when the rainy season is short, while the DLS trait allows the crop to stay alive through mid-season drought and recover when rainfall resumes. In Senegal, it was indicated that a plant of this type could begin to flower in about 35 days and produce 2 tons/ha grain in 60 days followed by a second flush of pods with the potential to produce an additional 1 ton/ha in 100 days after planting. Also, an early flowering cultivar with the DLS trait would produce more biomass and may fix more atmospheric nitrogen than an early flowering cultivar without the DLS trait (Hall *et al.*, 2003).

2.15 Screening approaches for drought tolerance

Drought tolerance has been shown to be a highly complex trait, expression of which depends on action and interaction of different morphological, physiological and biochemical characters that are controlled by products expressed by different genes (Mitra, 2001). Furthermore, it is difficult to study isolated single gene and to understand its role of drought tolerance in crop plants (Mitra, 2001). Efforts made in the past to develop drought tolerant cowpea varieties have met with little success. This may have to do with the complexity of the factors that are associated with the trait. Plant breeders need traits that can be readily used to identify resistant and susceptible plants in order for selection to be effective. Two approaches have been proposed for screening and breeding for drought tolerance. The first is the empirical or performance approach that utilizes grain yield and its components as the main criteria, since yield is the integrated expression of the entire array of traits related to productivity under water stress. The second approach involves analysis of physiological or morphological mechanisms that will contribute significantly to growth and yield under water stress conditions (Agbicodo *et al.*, 2009). A method which focus on some specific physiological, biochemical and morphological traits, an integrated approach which combines cellular water relations, rooting characteristics, leaf area and biochemical and morphological changes to screen cowpea for drought tolerance has been proposed by Slabbert *et al.*(2004). The different screening techniques that were tested included: the antioxidative response in the form of superoxide reductase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), proline accumulation, 2, 3, 5 - triphenyltetrazolium chloride (TTC) assays, early drought screening at the seedling stage, cell membrane stability (CMS), relative water content (RWC), leaf water potential (LWP), leaf area, chlorophyll a and b and carotenoid content and chlorophyll fluorescence. Many studies have successfully used a combination of these parameters to screen for drought tolerance in many crops species including maize, tea, beans, cowpea and wheat (Chiulele and Agenbag, 2004; Rampino *et al.*, 2006; Efeoglu *et al.*, 2009;

Santos *et al.*, 2009; Chakraborty and Pradhan, 2012). Since no single method is completely successful in screening for drought tolerance, the combination of different methods to screen for drought tolerance may likely produce better results (Timko and Singh, 2008). A combination of both approaches has been proposed and might facilitate greater progress in the development of drought tolerant cowpea varieties (Fussell *et al.*, 1991).

2.16 Drought tolerance genes in cowpea

Plants respond to water deficit and adapt to drought conditions by various physiological changes including transition in gene expression during water deficit. The mechanisms of drought response have been investigated most extensively in a model plant, *Arabidopsis thaliana* (Shinozaki and Yamaguchi-Shinozaki, 1997). In Arabidopsis, the drought signal is mediated through abscisic acid (ABA)-dependent and -independent pathways to regulate expression of genes that are involved in drought tolerance. For example, these gene products are thought to function in the accumulation of osmoprotectants, such as sugars, proteins, stress-signaling pathways, transcriptional regulation, and so on (Shinozaki and Yamaguchi-Shinozaki, 1997). Cowpea is relatively drought-tolerant as compared to other crops (Singh *et al.*, 1999) and is an excellent crop for investigating the genetic basis of drought tolerance (Barrera-Figueroa *et al.*, 2011). Efforts have been made to identify genetic elements that are involved in drought stress response in cowpea and several gene transcripts have been isolated. These includes cDNAs homologous to 12-oxo-phytodienoic acid reductase, NADPH-dependent aldehyde reductase, alcohol dehydrogenase, dehydrin (Iuchi *et al.*, 1996a), lipoxygenase (Iuchi *et al.*, 1996b), multicystanin (Diop *et al.*, 2004), 9-cisepoxycarotenoid dioxygenase (VuNCED1) (Iuchi *et al.*, 2000), phospholipase D (El Maarouf *et al.*, 1999), galactolipid acyl hydrolase (Matos *et al.*, 2001), phosphatidylinositol-specific phospholipase C (El-Maarouf *et al.*, 2001), ascorbate peroxidase (D'arcy-Lameta *et al.*, 2006) and glutathione reductase (Contour-Ansel *et al.*, 2006) have been described. The functions of these candidate genes are presented in Table 2-1.

Table 2-1. Different genes identified and their role in drought tolerance in cowpea (Agbicodo *et al.*, 2009).

Gene name	**Gene function**	**Authors**
VuNCED1	9-Cis-epoxycarotenoid dioxygenase catalyses the key step involved in ABA biosynthesis	Iuchi *et al*. (2000)
CPRD86	9-Cis-epoxycarotenoid dioxygenase catalyses the key step involved in ABA biosynthesis	Iuchi *et al*. (2000)
VuABA1	Zeanthin epoxidase, an enzyme involved in in early step of ABA biosynthesis	Iuchi *et al*. (2000)
CPRD12	Cowpea response to dehydration stress	Iuchi *et al*. (1996b)
CPRD46	Water stress-inducible gene for neoxanthin cleavage enzyme involved in ABA biosynthesis	Iuchi *et al*. (1996b)
CPRD8	Cowpea response to dehydration stress	Iuchi *et al*. (1996a)
CPRD14	Cowpea response to dehydration stress	Iuchi *et al*. (1996a)
CPRD22	Cowpea response to dehydration stress	Iuchi *et al*. (1996a)
dtGR	Dual-targeted glutathione reductace enzyme involved in detoxification of reactive oxygen species	Contour-Ansel *et al*. (2006)
cGR	Cytosolic gluthathione reductase key enzyme involved in detoxification of reactive oxygen species	Contour-Ansel *et al*. (2006)
VucAPX	Cytosolic ascorbate peroxidase key enzyme involved in detoxification of reactive oxygen species	D'Arcy-Lamenta *et al*. (2006)
VupAPX	Peroximal ascorbate peroxidase key enzyme involved in detoxification of reactive oxygen species	D'Arcy-Lamenta *et al*. (2006)
VutAPX	Thylakoid ascorbate peroxidase key enzyme involved in detoxification of reactive oxygen species	D'Arcy-Lamenta *et al*. (2006)
VusAPX	Stromatic ascorbate peroxidase key enzyme involved in detoxification of reactive oxygen species	D'Arcy-Lamenta *et al*. (2006)
VuPLD1	Putative phospholipase D a major lipid-degrading enzyme in plants	El-Maarouf *et al*. (1999)
VuPAP-α	PAP important for enzymatic cascade leading to membrane lipid degradation during environmental stresses or senescence	Marcel *et al*. (2000)
VuPAP-β	PAP important for enzymatic cascade leading to membrane lipid degradation during environmental stresses or senescence	Marcel *et al*. (2000)
VuC1	Protein inhibitors of cysteine proteinases belonging to the papain family	Diop *et al*. (2004)
VuPAT1	Galactolipid acyl hydrolase involved in membrane degradation induced by drought stress	Matos *et al*. (2001)

In addition, ten drought tolerance quantitative trait loci (QTL) associated with tolerance in seedlings have been mapped in cowpea (Muchero *et al.*, 2009). The development of molecular markers or identification of genes associated with seedling drought tolerance for use in marker-assisted selection, and breeding for drought tolerance in cowpea was suggested by these authors. Studies show that the application of genetic engineering can lead to improved drought tolerance and increased yield under drought. For example, transgenic rice overexpressing the *SNAC1* gene had 22–34 % higher seed setting in the field than the negative control under severe drought stress conditions at the reproductive stage. *SNAC1* transgenic rice plants are drought tolerant not only due to the increased expression of genes encoding proteins functioning in the production of osmolytes, detoxification and redox homeostasis, and in protection of macromolecules, but also because of the increased stomatal closure in transgenic leaves which prevents water loss from the plant (Hu *et al.*, 2006). Transgenic soybean plants overexpressing the *Arabidopsis* Δ 1-pyrroline-5- carboxylate synthase gene, *P5CR* , showed greater tolerance to drought stress due to an increased free proline level and RWC and reduced levels of reactive oxygen species, particularly hydrogen peroxide (de Ronde *et al.*, 2004; Kocsy *et al.*, 2005). Nelson *et al.* (2007) also reported that under water limited conditions, transgenic maize plants with increased expression of the ZmNFYB2 gene showed tolerance to drought based on the responses of a number of stress-related parameters, including chlorophyll content, stomatal conductance, leaf temperature, reduced wilting, and maintenance of photosynthesis. These stress adaptations contribute to a grain yield advantage to maize under water-limited environments. In cowpea, though many genes conferring drought resistance have been discovered, no success stories have been reported in creating drought tolerance by genetic engineering of functional genes. There is need for isolating drought stress-related genes in cowpea for the development of genetically engineered cowpea with improved drought resistance and increased yield.

2.17 Conclusion

To summarize, cowpea has evolved various mechanisms to avoid, escape and tolerate drought stress. Also, changes at morphological, physiological, biochemical and molecular levels occur in response to drought stress. These changes are useful indicators in the selection and breeding of drought tolerant cowpea cultivars.

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3. MORPHOLOGICAL RESPONSE OF FOUR DUAL-PURPOSE COWPEA LANDRACES TO DROUGHT STRESS AT VEGETATIVE AND REPRODUCTIVE STAGES

ABSTRACT

Dual-purpose cowpea (*Vigna unguiculata* L) landraces are grown for their seed and consumed as leafy vegetables mainly by rural small-scale/subsistence farmers in South Africa. These landraces are adapted to harsh environments which are often water limited. However, there is little information in the literature on morphological responses of landraces to drought stress and how this may relate to drought tolerance. The objective of this study was to investigate whether variation is present in leaf greenness, stem greenness, branch greenness, leaf number and senescence in four cowpea landraces subjected to water deficits during vegetative and reproductive growth stages. Controlled experiment was conducted under glasshouse conditions with four cowpea landraces namely: Lebudu, Lehlodi, Sejwaleng and Morathathane. The experiment was laid out using the randomised complete block design (RCBD) with cowpea landrace as the treatment or factor. There were four treatments (landraces) each planted in 10 pots which were replicated 20 times (single plants). Plants were well watered until the formation of six fully expanded trifoliates then irrigation was withheld for 28 days to simulate drought stress during vegetative growth. The imposition of drought stress was terminated by re-watering all plants after 28 days. The cowpea plants were then given sufficient moisture and allowed to grow until 50% flowering stage. Watering was withheld at 50% flowering for a two-week period for all the four landraces to simulate drought stress during reproductive growth. Data collected included number of green leaves, senesced leaves, plants survival percentages, stem greenness, leaf greenness and branches greenness. Significant differences were observed for leaf greenness, stem greenness, number of senesced leaves, number of green leaves and plant survival. Lebudu had lower senesced leaves than Lehlodi, Sejwaleng and Morathathane during the vegetative and reproductive growth stages. Lebudu also had greener leaves than Lehlodi, Sejwaleng and Morathathane during the vegetative stage whereas all landraces had yellowish leaves during the reproductive stage. Lebudu, Lehlodi and Sejwaleng had greener stems than Morathathane during the vegetative stage whereas only Lebudu had a greener stem during the reproductive stage. Lebudu maintained greener branches than other landraces during the reproductive stage. All landraces had 100% plant survival during the vegetative stage while during the reproductive stage, 100% plant survival was recorded for Lebudu, 60% for Morathathane, 40% for Sejwaleng and 20% for Lehlodi. After drought-relief, recovery in all landraces was from apical meristems. Thus it can be concluded that variability in morphological responses in local cowpea landraces differ during drought encountered during the vegetative and reproductive growth stages. These results suggest that based on morphological responses to drought stress, Lebudu may tolerate drought better during both vegetative and reproductive growth phases; whereas Lehlodi, Sejwaleng and Morathathane may tolerate drought during the vegetative growth stage. It is possible that the observed differences in the ability of the landraces to tolerate drought and the association with morphological traits may be further linked to differences in the underlying physiological mechanisms in response to drought stress.

Keywords: Cowpea, drought-stress, drought-tolerance, reproductive stage, vegetative stage

3.1 INTRODUCTION

Cowpea (*Vigna unguiculata*) is a drought-tolerant legume of major economic importance in the tropical and subtropical regions of sub-Saharan Africa. The crop is grown for its fresh leaves, fresh and dry grains. Most of the leaves are harvested during the vegetative stage while fresh immature pods and dry grains are also consumed. Drought is one of the most important abiotic factors limiting cowpea productivity (Agbicodo *et al.*, 2009). Improving the drought tolerance of cowpea is one of the most important objectives of plant breeders working on this crop, to minimize the yield losses resulting from moisture stress, which is a regular feature of most cowpea growing environments (Muchero *et al.*, 2008; Muchero *et al.*, 2009). Significant differences among cowpea cultivars to tolerate drought stress have been reported by several authors (Anyia and Herzog, 2004a; Abayomi and Abidoye, 2009; Belko *et al.*, 2012). The differences among cowpea cultivars with respect to drought tolerance have also been associated with various morphological traits (Gwathmey *et al.*, 1992; Mai-Kodomi *et al.*, 1999b; Muchero *et al.*, 2008; Belko *et al.*, 2012). Such traits may include delayed leaf-senescence (DLS) or the stay green trait and stem greenness which is thought to confer drought tolerance in cowpea cultivars possessing these traits (Gwathmey *et al.*, 1992; Mai-Kodomi *et al.*, 1999b; Muchero *et al.*, 2008).

The 'stay-green' is an important morphological trait that has been associated with drought tolerance under drought stress conditions. Plants exhibiting the 'stay-green' phenotype are characterized by maintenance of a significant green leaf area during drought stress. It is believed that the maintenance of green leaf area contributes to continued carbohydrate formation during drought and faster recovery following drought-relief (Borell *et al.*, 2000b). In sorghum, the stay-green phenotype manifests itself at the post-anthesis growth stage by retention of green-leaf area. Gwathmey *et al*. (1992) demonstrated a link between delayed leaf senescence (DLS), a trait similar to 'stay-green', and grain yield in cowpea. This trait is thought to be genetically controlled (Mai-Kodomi *et al.*, 1999a) and can be linked to the timing of senescence initiation and also in the subsequent rate of leaf senescence during drought stress which may affect survival and reproductive ability after drought-relief in cowpea. Gwathmey *et al*. (1992) reported 53-98% survival of stay-green cowpea genotypes after production of the first flush of pods compared with 15-18% survival of two non-DLS lines Gwathmey *et al*. (1992). Therefore, delayed leaf senescence is effective in keeping plants alive longer and ensuring better chances of recovery after drought relief (Mai-Kodomi *et al.*, 1999b). Moreover, delayed leaf senescence can be easily measured by visual scoring as used by Maikodomi *et al*. (1999), Belko *et al*. (2012) and Muchero *et al*. (2008) to discriminate cowpea genotypes that exhibit significant genetic variation for drought tolerance.

Stem greenness is another important morphological trait that has been associated with drought tolerance in cowpea (Muchero *et al.*, 2008). Muchero *et al*. (2008) reported that stem greenness under glasshouse conditions and field drought-induced senescence under field conditions is a reliable and cost-effective approach in screening and selecting for vegetative drought tolerance in cowpea. Furthermore, the authors reported that stem greenness was a reliable indicator of survival and recovery. Stem greenness can also be easily measured by visual scoring (Muchero *et al.*, 2008).

The ability of cowpea branches to maintain greenness has not been exploited as a morphological trait that can be used to screen for drought tolerance. Cowpea branches especially of creeping, indeterminate varieties are the site of many mature pods and ability to retain branches greenness under drought stress conditions may be an indicator of drought tolerance. Maintenance of branches greenness may allow water and nutrient translocation to developing pods and seeds.

In South Africa, most resource-poor farmers still grow unimproved cowpea landraces (Shiringani and Shimelis, 2011) under drought-prone dry-land conditions characterized by insufficient moisture. In fact, Limpopo Province in the Republic of South Africa is suggested to be the centre of diversity due to the presence of most primitive wild botanical varieties including *rhomboidea*, *prottracta*, *tennis* and *stenophylla* (Ng and Marachel, 1985). Many of these landraces may exhibit significant variations with respect to genetic, physiological and phenotypic differences which could probably be associated with their ability to survive drought conditions. Although research work on drought tolerance has been conducted elsewhere, limited information exists on drought tolerance in locally adapted cowpea germplasm from South Africa. It is hypothesized that differences in the responses of these landraces to drought stress during vegetative and reproductive growth phases could be associated with the ability of a particular landrace to delay the initiation of leaf senescence during drought and also to maintain stem and branches greenness. The ability to maintain leaf, stem and branches greenness would allow the cultivar to recover when drought stress is alleviated and resume normal metabolic plant function which is critical for biomass production and yield performance. The objective of this study was to investigate variation with respect to leaf number, stem greenness, plant survival, leaf greenness and branches greenness of four cowpea landraces and whether these can be associated with differences in their response to drought during vegetative and reproductive growth.

3.2 MATERIALS AND METHODS

3.2.1 Experimental procedure

3.2.1.1 Plant material

Four cowpea landraces collected from Moletjie Ga-Mphela in the Limpopo Province, were used for the study. The landraces are Lehlodi (A), Sejwaleng (B), Morathathane (C) and Lebudu (D) (Figure 3-1).

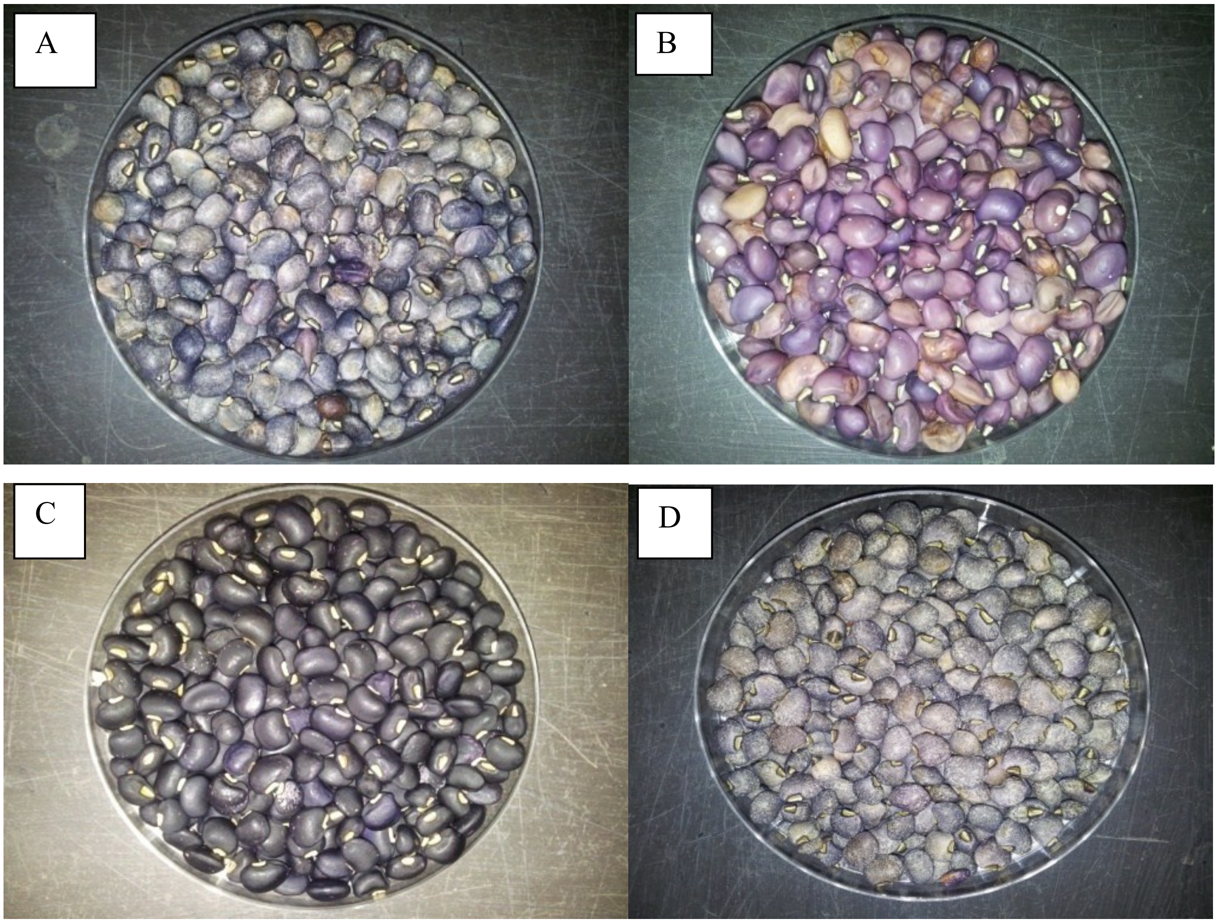

Figure 3-1. Differences in seed coat colour of four cowpea landraces used in the study.

The landraces are grown by resource-poor farmers who engage in dual-purpose cowpea production, i.e., for their dry grain and vegetative parts e.g. leaves and fresh immature pods for many years (Mmapitsi Kobe, personal communication). A detailed description of morphological traits of these landraces is presented in Table 3-1. These landraces don't show much variation for plant growth pattern and growth habit. All have indeterminate growth pattern and their growth habit is indeterminate spreading and climbing. All landraces have a dark green leaf colour except for Morathathane which has a light-green leaf colour. Flower colour and leaf shape is purple and sub-hastate for all landraces respectively. All landraces can reach 50% flowering in approximately 100 days while Lebudu matures earlier than Morathathane, Lehlodi and Sejwaleng (Table 3-1).

Table 3-1. Morphological description of four cowpea landraces used in the study (Shiringani and Shimelis, 2011).

	Landrace			
	Lebudu	Morathathane	Lehlodi	Sejwaleng
Traits				
GP	2	2	2	2
GH	4	4	4	4
ACB	3	3	3	3
TL	1	1	1	1
LGC	3	2	3	3
PC	5	3	3	3
SCC	1	2	3	4
FC	1	1	1	1
ST	R	S	R	S
LS	SH	SH	SH	SH
IPP	0	0	0	5
TD	5	5	5	5
DF	101	103	103	107
DM	118	134	135	138

GP = Growth pattern: 1 = determinate, 2 = indeterminate; GH = growth habit: 1 = determinate, 2 = indeterminate bush, 3=indeterminate spreading not climbing, 4= indeterminate spreading and climbing; ACB = anthcynanin colouration of branches: 1 = absent, 3 = light, 5= medium, 7 = dark; TL = tendrils: 1 = absent, 3 = few, 5 = medium, 7 = many; LGC = leaf green colour: 1 = light, 2 = medium, 3 = dark; PC = pod curvature of mature pods: 0 = straight, 3 = slightly curved, 5 = curved, 7 = coiled; SCC = seed coat colour: 1 = grey, 2 = black, 3 = blue, 4= purple; FC= flower colour: 1= purple, 2= white, 3=red, 4= yellow; ST = seed texture of surface of testa: s = smooth; r= rough; TLS= terminal leaf shape: H=hastate, SH=sub-hastate, G = globose, SG=sub-globose; IPP= immature pod pigmentation: 0= none, 3= splashes of pigments, 5= uniformly pigmented: TD=Twinning tendency: 0 =none, 3=slight, 5=intermediate, 7= pronounced: DF = days to 50% flowering; DM = days to maturity.

3.2.1.2 Experimental setup and design

A pot experiment was conducted under simulated drought conditions in a glasshouse at the Controlled Environment Facility (CEF), University of KwaZulu-Natal, Pietermaritzburg Campus (29° 35' S, 30° 25' E). Temperature, photosynthetic ally active radiation and relative humidity were monitored electronically using a Hobo Data logger (Onset Computer Corporation, Bourne, USA). The experiment was laid out using randomised complete block design (RCBD) with cowpea landrace as the treatment factor. The treatments (four cowpea landraces) were each planted in 40 pots giving a total of 160 experimental units (drained polyethylene pots with a 5 litre capacity). Each plant in each pot for each landrace was treated as a replicate

3.2.1.3 Soil preparation, potting and planting

Soil of known physical properties (Odindo, 2007) (Appendix 1) was collected from the University of KwaZulu-Natal Ukulinga Research Farm (29°39'48.82"S; 30°24'19.89"E) and screened through a 1cm mesh to remove clods and stones. Each pot was carefully filled with 5kg soil and the mass of the pot and soil determined and recorded. The pots were filled with water until full saturation and allowed to drain for 24h until field capacity then weighed. A compound fertilizer 2:3:2 (22) was added to the soil at a rate of 150 kg/ha according to soil analysis results (Appendix 2); and two seeds were planted in each pot at a 20 mm depth. Plants were well watered until the formation of six fully expanded trifoliates then irrigation was withheld for 28 days to simulate drought stress during vegetative growth. The imposition of drought stress was terminated by re-watering all plants after 28 days. The cowpea plants were then well-watered and allowed to grow until four landraces reached 50% flowering stage. Watering was withheld at 50% flowering for a two-week period for all the four landraces to simulate drought stress during reproductive growth. Drought stress was then terminated after 12 days by re-watering all plants.

3.2.2 Data collection

Number of senesced leaves was determined as the number of completely senesced trifoliates per replicate after 2 and 4 weeks during vegetative growth and 1 and 2 weeks during reproductive growth phase, respectively. Number of green leaves was determined by counting the number of green leaves per replicate after 2 and 4 weeks during vegetative growth phase and 1 and 2 weeks during reproductive growth phase, respectively.

3.2.2.1 Stem greenness

Stem greenness was scored on a scale of 0 to 5, according to Muchero *et al*. (2008) with slight modification where 0= completely yellow stem, 1 = yellowish-grey stem, 2 =green with severe yellowing, 3=green with moderate yellowing, 4 =green with slight yellowing, 5 completely green stem. Forty plants were scored for stem greenness during vegetative growth whereas twenty plants were scored during the reproductive growth stage. Stem greenness was scored on drought-stressed plants at the end of the drought period.

3.2.2.2 Leaf greenness

Leaf greenness was scored on a scale of 1 to 5 according to Maikodomi *et al*. (1999b) with 1 = normal green turgid leaves, 2 = green and slightly wilted, 3 = green-yellow and wilted, 4 = yellow and light brown leaves with severe wilting, and 5 = completely dried leaves. Leaf greenness was scored only on drought-stressed plants at the end of the drought period. Fourty plants were scored for stem greenness during vegetative growth whereas twenty plants were scored during the reproductive growth stage.

3.2.2.3 Branch greenness

Branch greenness was scored only during the reproductive stage on a scale of 1 to 5 where with 1 = normal green branches, 2 = green and slightly wilted, 3 = green-yellow and wilted, 4 = yellow and light brown branches with severe wilting, and 5 = completely dried branches. Branch greenness was scored on twenty drought-stressed plants at the end of the drought period.

3.2.2.4 Plant survival

Plant survival was scored after re-watering on a scale of 0 to 1 according to Muchero *et al*. (2008) with 1 = recovery and 0 = no recovery was observed. Number of plants that survived was counted and percentage plant survival was determined after re-watering.

3.2.2.5 Data analysis

Genstat 14th edition (VSN International, UK) was used to perform analyses of variance (ANOVA) and the differences between means were compared using Least Significant Differences (LSD) at α=95% ($P \leq 0.05$).

3.3 RESULTS

Drought stress period during the vegetative phase was characterized by high light intensities and temperature (Figure 3-2). Average temperature during the day and night was 26°C and 18°C respectively (Figure 3-2A). Whereas maximum and minimum temperature recorded were 36°C and 20°C respectively. On average, relative humidity during the day was 58% while during the RH at night was 82% (Figure 3-2B). Maximum and minimum relative humidity during the day where recorded at 75% and 35% respectively. Maximum and minimum light intensity during the day were 2000 and 350 μMol photons m^{-2} s^{-1} respectively. On average, light intensity during the day was 1141 μMol photons m^{-2} s^{-1} (Figure 3-2C).

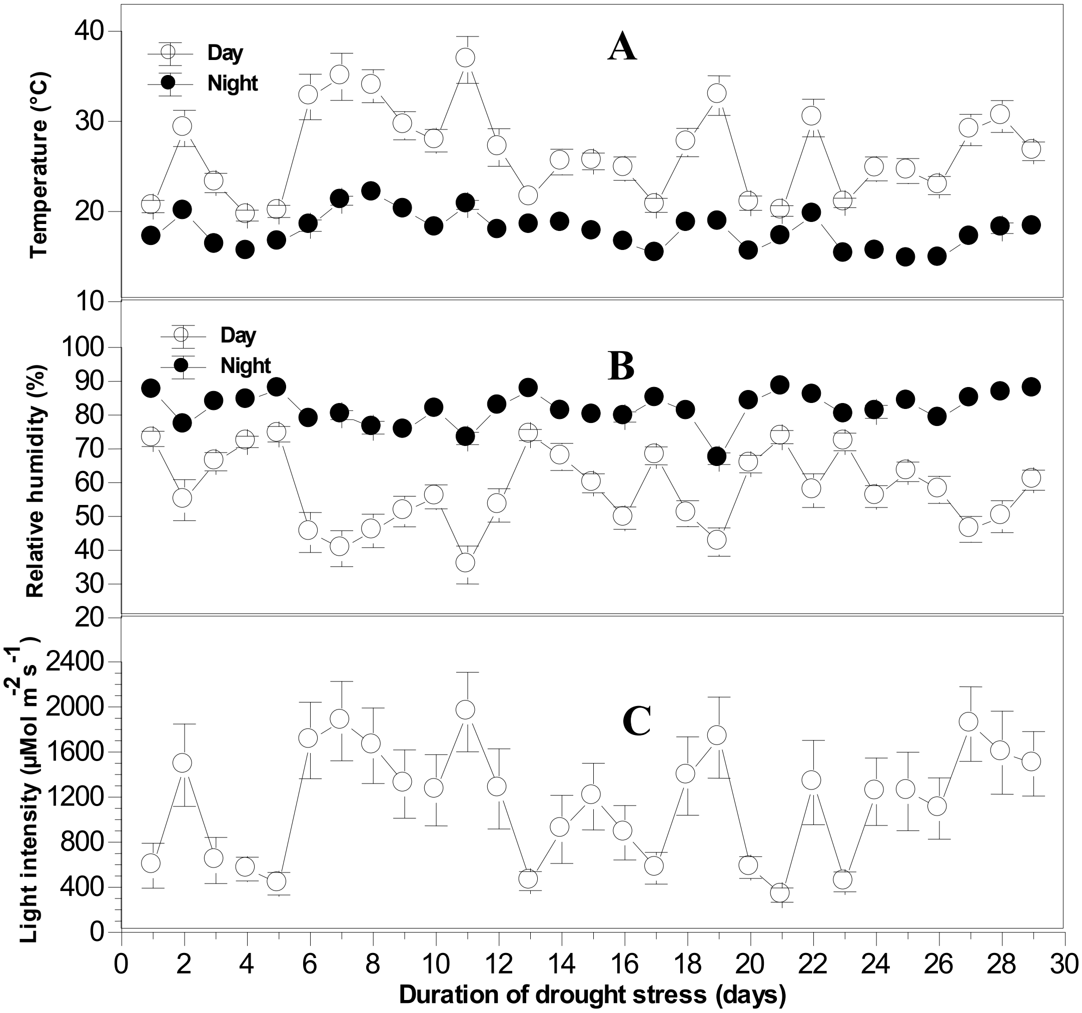

Figure 3-2. Changes in temperature (A), relative humidity (B) and light intensity (C) in the glasshouse during drought stress imposed during the vegetative stage. Bars indicate standard error.

Drought stress period during the reproductive phase was also characterized by high light intensities and temperature (Figure 3-3). Average air temperature during the day and night was 36.2°C and 22.5°C respectively (Figure 3-3A). Whereas maximum and minimum temperature recorded were 50°C and 21°C respectively. Generally, temperature increased from early in the morning and decreased slowly in the afternoon and during the night. As expected, relative humidity drops throughout the day as the temperature increases (Figure 3-3B). On average, relative humidity during the day was 47% while during the night RH was 74%. Maximum and minimum relative humidity during the day was recorded at 72% and 25% respectively. Maximum and minimum light intensity during the day were 2100 and 600 µMol photons m^{-2} s^{-1} respectively. On average, light intensity during the day was 1521 µMol photons m^{-2} s^{-1} (Figure 3-3C).

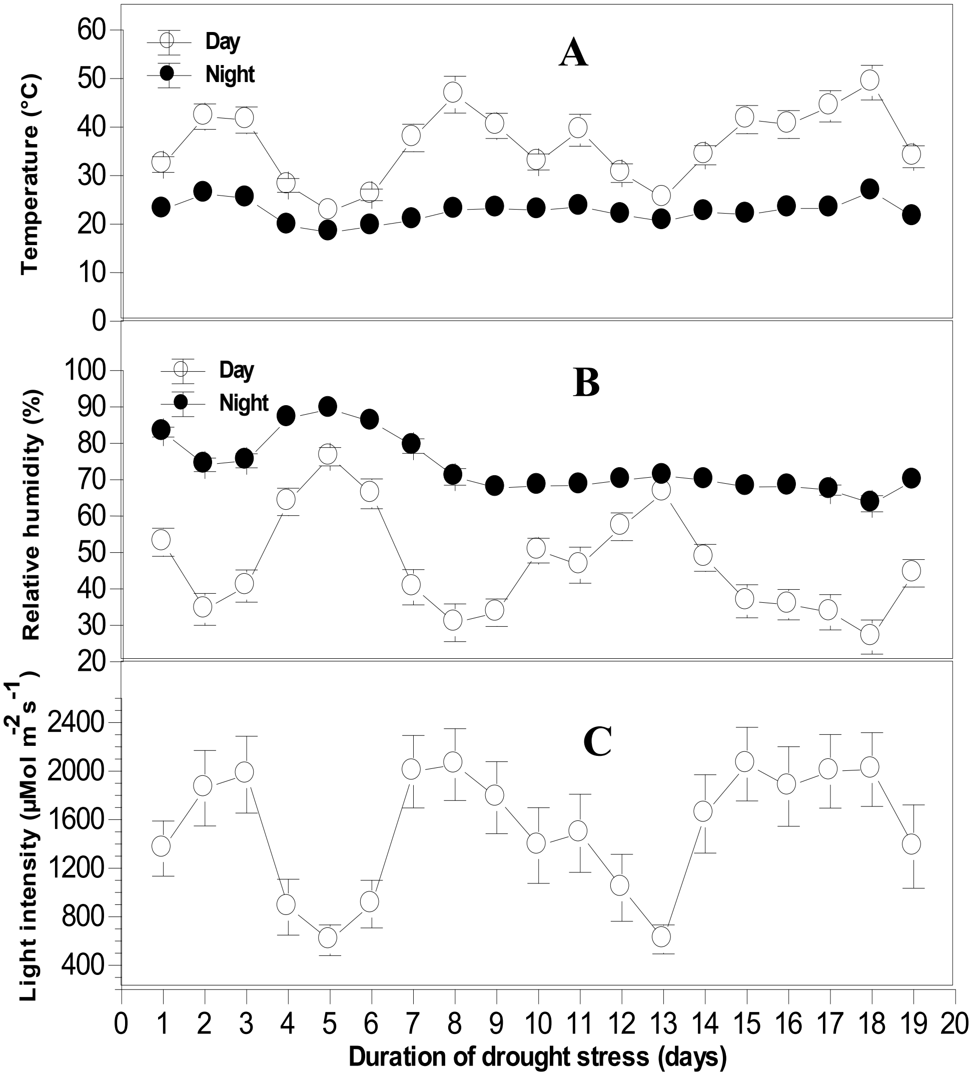

Figure 3-3. Changes in relative humidity (A), temperature (B) and light intensity (C) in the glasshouse during drought stress imposed during the reproductive stage. Bars indicate standard error.

After exposure to drought stress, significant differences ($P < 0.05$) between the cowpea landraces with respect to the average number of senesced leaves after 2 and 4 weeks of drought stress during vegetative growth (Figure 3-4A) and 1 and 2 weeks during reproductive growth (Figure 3-4B) were observed. Lebudu had significantly lower number of senesced leaves than

Morathathane, Sejwaleng and Lehlodi after a 2 week drought period during vegetative growth (Figure 3-4A & 3-5) and one week of drought during the reproductive growth (Figure 3-4B). Although the number of senesced leaves increased after a 4-week drought stress during vegetative growth and a 1-week drought during reproductive growth, Lebudu maintained a significantly lower number of senesced leaves than the other three landraces during both growth phases. Of the three landraces, Morathathane and Lehlodi had the highest number of senesced leaves compared to Sejwaleng; however, these were not significantly different. Also under well-watered conditions and during pod filling and at maturity, Lebudu had more green leaves than the other landraces (Figure 3-6) indicating that Lebudu may possess the stay-green trait.

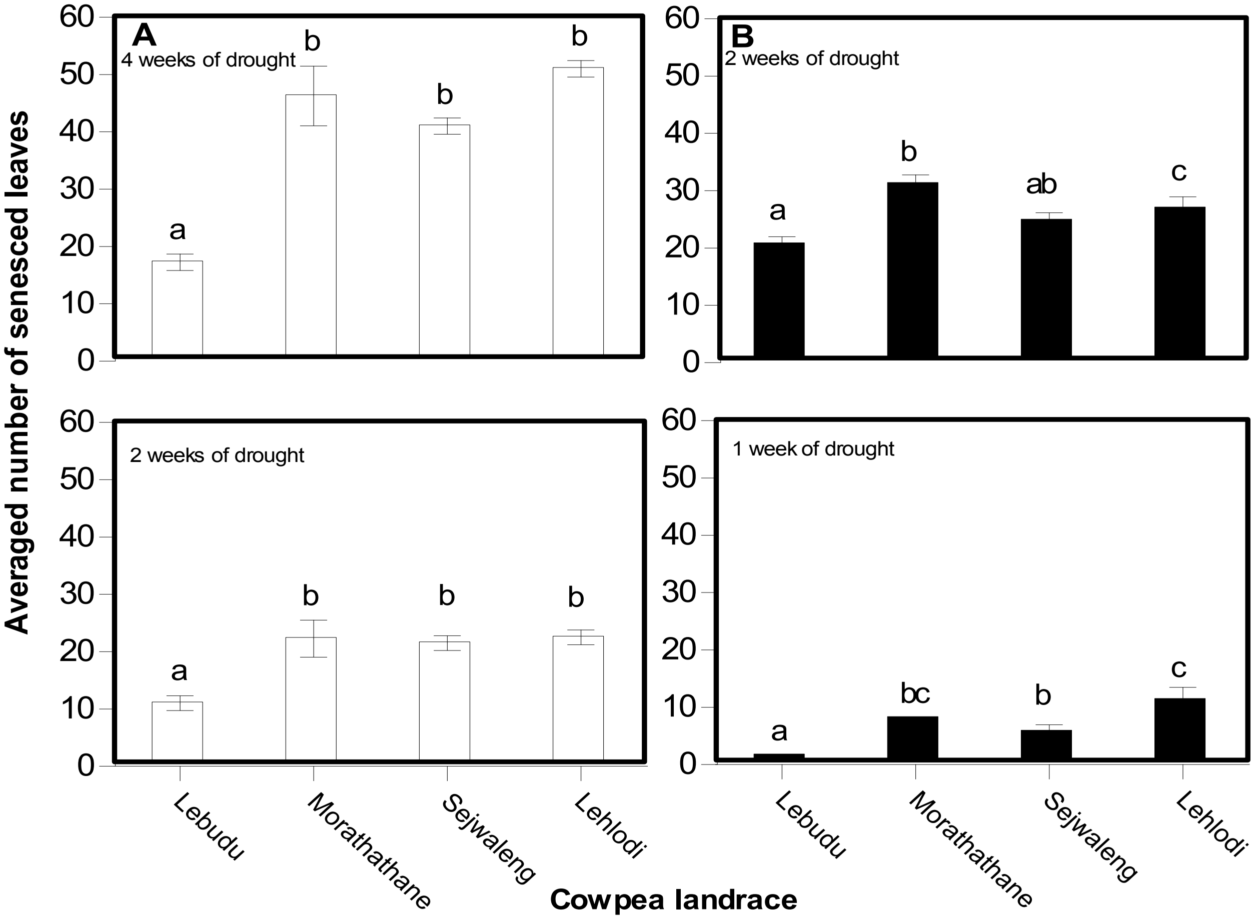

Figure 3-4. Average number of senesced leaf trifoliates of cowpea landraces after 2 and 4 weeks of drought stress at vegetative phase (A) and after 1 and 2 weeks of drought at reproductive phase (B). Bars indicate standard errors. Different letters indicates significant differences, $P \leq 0.05$.

Figure 3-5. Differences in leaf senescence after 1 week of drought stress during the vegetative growth stage. Grey=Lebudu; Black =Morathathane; Purple =Sejwaleng; Blue =Lehlodi.

Figure 3-6. Differences in ability to delay leaf senescence between cowpea landraces at physiological pod maturity under well-watered conditions. A = Lebudu, B = Morathathane, C = Sejwaleng, D = Lehlodi.

There was generally a lack of clear differences with respect to the number of green leaves among the four landraces after 2 weeks of drought during the vegetative phase (Figure 7A). The average number of green leaves was higher in Sejwaleng than Lebudu and Morathathane but these were not significantly different. The landrace Lehlodi had a significantly higher number of green leaves than Lebudu and Morathathane but was not significantly different from Sejwaleng after 2 weeks of drought stress during the vegetative growth phase. Drought caused a considerable loss of green leaves after 4 weeks, however; no significant differences were observed between the cowpea landraces.

Significant differences ($P < 0.05$) with respect to the number of green leaves were observed between the cowpea landraces when drought was imposed for a period of 1-week during reproductive growth (Figure 3-7). Drought stress caused a considerable loss of green leaves after 1 week during the reproductive growth stage (Figure 3-7B). Lebudu had significantly higher number of green leaves after 1 week of drought compared to other landraces. Morathathane and Lehlodi also had the highest number of green leaves but these were not significantly different. Sejwaleng had the lowest number of green leaves after 1 week of drought stress. However the number of green leaves were considerably reduced for all landraces when drought was imposed for 2 weeks during vegetative growth; Lebudu maintained the highest number of green leaves than Sejwaleng whereas Morathathane and Lehlodi had the lowest number of green leaves (Figure 3-7A).

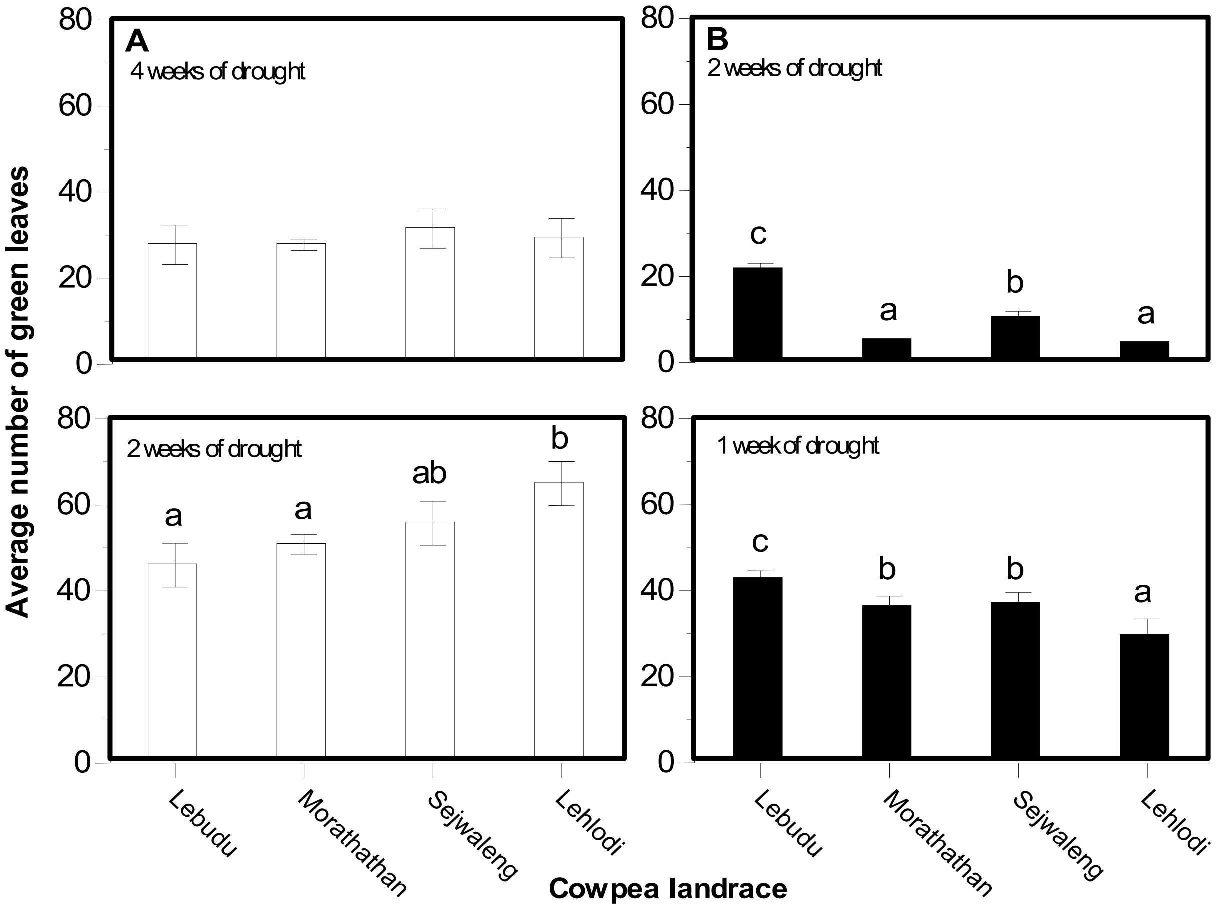

Figure 3-7. Average number of green leaves of cowpea landraces after 2 and 4 weeks of drought stress at vegetative phase (A) and after 1 and 2 weeks of drought at reproductive phase (B). Bars indicate standard errors. Different letters indicates significant differences, $P \leq 0.05$.

Leaf greenness scores also differed among cowpea landraces during both vegetative and reproductive growth stages (3-8A). Lebudu had the lowest score (2) than Lehlodi, Sejwaleng and Morathathane (3, 3.1 and 3, respectively) during vegetative growth. During reproductive growth, all landraces had the highest scores (5 for Morathathane and 4 for Lebudu, Lehlodi and Sejwaleng) as compared to drought stress during the vegetative growth stage (Figure 3-8A).

Differences in stem greenness between cowpea landraces were observed after the imposition of drought stress conditions for 4 weeks during the vegetative phase and two weeks during the reproductive phase (Figure 3-8B, 3-9 and 3-10). Lebudu and Morathathane had high scores (5 and 5 respectively) then, Sejwaleng and Lehlodi (3.9 and 4.5, respectively) during the vegetative stage. During the reproductive growth stage, Lebudu had the highest score whereas (4.5); Morathathane, Lehlodi and Sejwaleng had the lowest scores (3, 2.8, and 2.9, respectively).

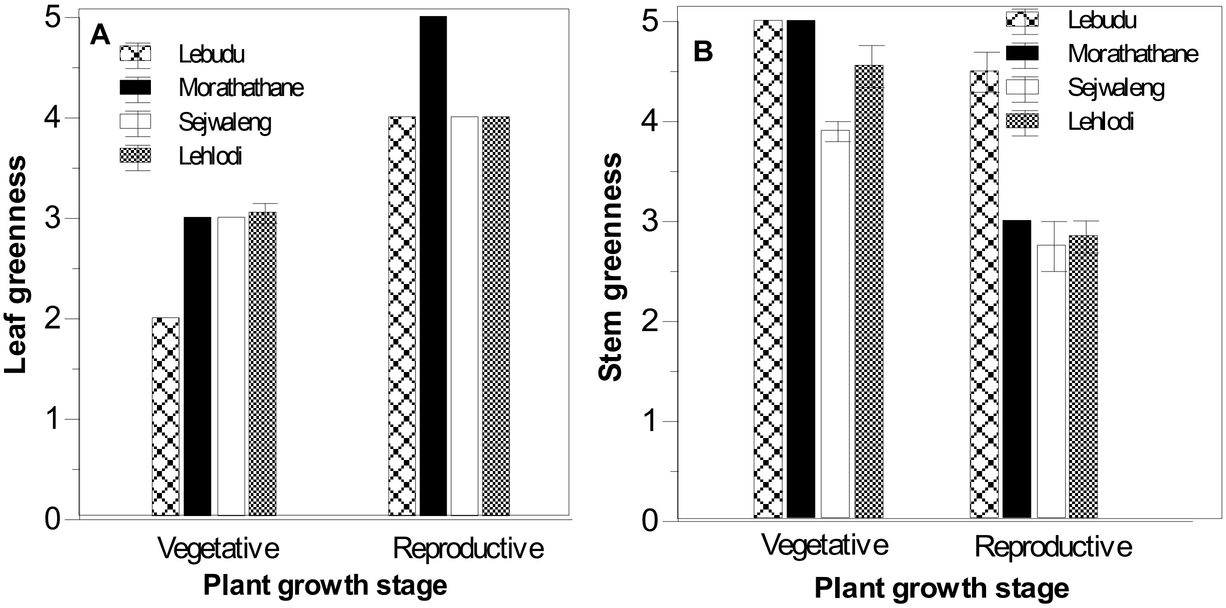

Figure 3-8. Scores of leaf greenness and stem greenness of cowpea landraces during drought stress at the vegetative and reproductive growth stages. Values are means ± S.E. Standard error are not shown where the values are smaller.

Figure 3-9. Stem greenness of cowpea landraces after 4 weeks of drought stress during the vegetative phase. A=Lebudu, B=Morathathane, C=Sejwaleng, D=Lehlodi.

Figure 3-10. Differences in stem greenness of cowpea landraces after 2 weeks of drought stress during the reproductive phase.

Branches greenness was only scored during the reproductive growth stage only, and differences between cowpea landraces were observed. Lebudu had the highest score (3-11A) whereas Morathathane, Lehlodi and Sejwaleng had the lowest scores (3, 3 and 3, respectively). After re-watering, growth resumed from the apical meristems during both the vegetative and reproductive growth stages (Figure 3-11B). All landraces had 100% plant survival during vegetative phase. However, during the reproductive growth stage, Lebudu had 100% survival whereas Morathathane, Sejwaleng and Lehlodi had 60%, 40% and 20% plant survival respectively.

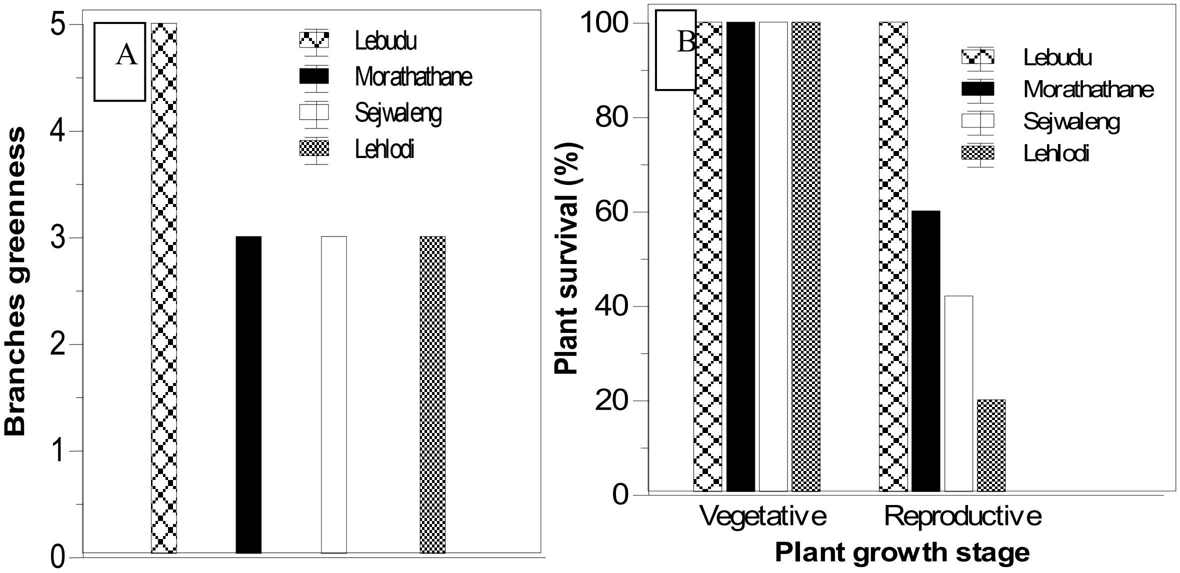

Figure 3-11. Scores of branches greenness (A) and percentage plant survival (B) of four cowpea landraces subjected to drought stress.

3.4 DISCUSSION

This study investigated whether variation in morphological traits (delay in the initiation of leaf senescence stem and branch greenness) of four cowpea landraces (Lebudu Lehlodi, Sejwaleng and Morathathane) can be associated with differences in their response to drought during vegetative and reproductive growth. The results showed considerable variability with respect to morphological traits among the four cowpea landraces when subjected to drought conditions. During both vegetative and reproductive growth stages, Lebudu had less number of senesced leaves whereas Sejwaleng, Lehlodi and Morathathane had most number of senesced leaves. It has been shown that drought stress can affect many aspects of plant growth including reduced leaf expansion (Hsiao, 1973); a reduction in leaf number as a result of accelerated leaf senescence and abscission (Bala Subramanian and Maheswari, 1992; Suliman and Ahmed, 2010). Cowpea has also been documented to have a loss of leaf area as a result of drought (Turk and Hall, 1980a; Gwathmey and Hall, 1992). The shedding of leaves is commonly observed under drought conditions as a method for decreasing the leaf area from which water evaporates (Ludlow and Muchow, 1990). This can consequently lead to decreased leaf area and affect the source capacity (leaf canopy) and result in reduced photosynthetic capacity (Gwathmey and Hall, 1992). The drought-induced senescence of leaves for these cowpea landraces might be an adaptation to escape extreme drought conditions and indicates an avoidance strategy Bala Subramanian and Maheswari, 1992).

The results also showed significant variability between landraces with respect to the number of green leaves during both vegetative and reproductive stages. Despite, the loss of leaves, all landraces maintained more green leaves during the vegetative stage at the end of the drought period (4 weeks). However, Lebudu maintained more green leaves as compared to Lehlodi, Sejwaleng and Morathathane during the reproductive stage. Clear differences were further observed with respect to leaf greenness during drought stress between the four cowpea landraces. Lebudu maintained greener leaves as evidenced from the lower score, than Sejwaleng, Lehlodi and Morathathane which showed higher scores during vegetative growth stage. Similar findings were reported by Belko *et al.* (2012) who noted that several cowpea genotypes preserved leaf greenness more than others during drought stress. Drought tolerant cowpea genotypes Mouride, Suvita 2 and IT97K-499-39 maintained greener leaves than the drought-sensitive Bambey 21, IT82E-18, IT97K-556-6, and UC-CB46. Maikodomi *et al*. (1999a) also reported that drought-tolerant cowpea varieties such as Dan Ila and Kanannado remained green for longer under drought stress conditions. Anyia and Herzog (2004a) also reported that cowpea varieties UCR 386 and RCXAC were less susceptible to drought stress by delaying leaf senescence which in the literature is sometimes referred to as the "stay-green" trait. The stay-green colour observed for Lebudu could be probably attributed to delayed leaf senescence (DLS) trait. According to Gwathmey and Hall (1992), delayed leaf senescence or the "stay-green" trait may contribute to drought adaptation during drought stress encountered during the reproductive stage by enhancing plant survival. The DLS trait enables cowpea to recover after drought and produce a second flush of pods that compensate for the low yield by the first flush of pods (Gwathmey and Hall, 1992). The "stay-green" trait has also been reported in sorghum (*Sorghum bicolor* L. Moench) (Borell *et al.*, 2000a; Subudhi *et al.*, 2000). Stay-green is a drought tolerance mechanism exhibited in some sorghum genotypes subjected to post-flowering drought stress. The trait allows tolerant genotypes to maintain green leaves which are photosynthetically efficient during the grain-filling stage, thereby allowing more productivity (Borell *et al.*, 2000b). In sorghum, retention of green leaf area at physiological maturity has been shown to be an excellent indicator of the stay-green trait, and has been successfully used to select for drought tolerance in sorghum (*Sorghum bilocor* L.) (Rosenow *et al.*, 1983). Lebudu also retained more green leaves during pod filling and at maturity confirming its ability to stay green longer as compared to the other landraces. Results in this study suggest that all cowpea landraces may tolerate drought during the vegetative stage while Lebudu may tolerate drought during the reproductive stage better than the other landraces.

Stem greenness between the four cowpea landraces also differed during drought stress at the vegetative and reproductive growth stages. Lebudu, Lehlodi and Sejwaleng had greener stems while Morathathane showed a light-green stem during drought stress at the vegetative growth stage. Plants were re-watered after imposing drought during the vegetative phase and the intensity of stem greenness recovered. The landrace Lebudu had a greener stem compared to other three landraces (Lehlodi, Sejwaleng and Moranthathane). Furthermore, Lebudu had the highest plant survival compared to other three landraces. These results are in agreement with those of Muchero *et al.* (2008) who found that some genotypes preserved stem greenness much more than others, and stem greenness was a reliable predictor of survival. In their study, cowpea genotypes IT93K503-1 and CB46 exhibited significant differences in tolerance to seedling-stage drought stress. IT93K503-1 displayed strong ability to maintain a viable green stem and survive greenhouse drought stress up to 4 weeks after last watering. IT93K503-1 also exhibited minor drought-induced senescence under field drought conditions with only slight tip burning observed on the leaves. Compared to IT93K503-1, CB46 exhibited reduced ability to maintain a green stem and survive in response to greenhouse drought stress and displayed increased drought-induced leaf senescence under field conditions (Muchero *et al.*, 2008).

The variability in morphological traits that may be associated with physiological responses during drought stress were further evident from the observed differences with respect to branch greenness. Branch greenness differed among the four cowpea landraces during drought stress. Lebudu had more green branches compared to other landraces. Green branches might be an indication of viability, and could be a morphological trait that can be used to select for drought-tolerance. Intermediate genotypes could be distinguished from highly tolerant genotypes by progressive yellowing of branches during drought stress.

CONCLUSION

Considerable variation in morphological responses (delay in the initiation of leaf senescence, stem and branch greenness) of four cowpea landraces (Lebudu Lehlodi, Sejwaleng and Morathathane) subjected to drought stress were observed. All the landraces maintained stem greenness and had high plant survival when drought was imposed during the vegetative phase; but only Lebudu showed delayed leaf senescence; (stay-green), retained stem and branches greenness and had highest plant survival than other landraces when drought was imposed during the reproductive stage. Thus it can be concluded that variability in morphological traits in local cowpea landraces can be associated with differences in their response to drought during vegetative and reproductive growth. These results suggest that based on morphological responses to drought stress, Lebudu may tolerate drought better during both vegetative and reproductive growth phases; whereas Lehlodi, Sejwaleng and Morathathane may tolerate drought during the vegetative growth stage. It is possible that the observed differences in the ability of the landraces to tolerate drought and the association with morphological traits may be further linked to differences in the underlying physiological mechanisms in response to drought stress.

Furthermore, drought-tolerance associated morphological traits in these cowpea landraces provide a platform for the identification of genetic factors underlying their morphological responses.

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4. PHYSIOLOGICAL RESPONSES OF DUAL-PURPOSE COWPEA LANDRACES TO TERMINAL DROUGHT STRESS AND RECOVERY

ABSTRACT

Dual-purpose cowpea (*Vigna unguiculata* L) landraces are grown for immature fresh pods and dry grains mainly by rural small-scale/subsistence farmers in South Africa. These landraces are adapted to local conditions often characterized by water limiting conditions such as drought but are able to produce fresh biomass (leaves) and seed. This may be attributed to several factors including variation in their physiological responses to water deficits. There is little information about the variation with regards to physiological responses to water deficits occurring during reproductive growth and how this may relate to recovery from drought and yield performance of local cowpea landraces. The objective of this study was to determine firstly, whether there are changes in chlorophyll and carotenoid content, proline, phenolic compounds and total anti-oxidant capacity in response to drought stress occurring during the reproductive stage of four local dual-purpose landraces. Secondly it was to determine whether these changes can be associated with recovery from drought and yield performance. Controlled environment study was conducted under glasshouse conditions as a 4 x 2 factorial treatment structure with the following two factors: Dual-purpose landraces – (Lebudu, Lehlodi, Sejwaleng and Morathathane) and two levels of water stress (stressed and well-watered). Drought stress was imposed by withholding irrigation at 50% flowering for 12 days followed by re-watering after 15 days. Data were collected on pre-dawn leaf water potential and relative water content to quantify the level of stress, and stomatal conductance, chlorophyll content, chlorophyll *a*, carotenoids, proline content, phenolic compounds, total antioxidant capacity and chlorophyll fluorescence (*Fv/Fm*). Total above-ground biomass production, pod number, pod mass and seed yield upon relief from water stress were determined at maturity. The landrace Morathathane showed the highest decline in leaf water potential, total phenolics and chlorophyll content compared to Lebudu, Lehlodi and Morathathane. Sejwaleng maintained high relative water content compared to the other landraces but showed the lowest proline accumulation while Lebudu, Lehlodi and Morathathane showed high proline accumulation. Carotenoids declined while chlorophyll a increased in all landraces. There was no significant effect of water deficit on chlorophyll fluorescence. No significant differences in membrane-bound phenols were observed between landraces, however, Sejwaleng and Lehlodi showed the highest accumulation of free phenols. Total above-ground biomass production was reduced for all cowpea landraces. However, Lebudu showed moderate loss in biomass production after relief from water stress compared to Lehlodi, Morathathane and Sejwaleng. Pod mass and number were reduced by water stress despite the availability of sufficient moisture after relief from stress for Lebudu and Lehlodi compared to Sejwaleng and Morathathane. Seed yield was reduced greatly for Lebudu compared to Lehlodi, Morathathane and Sejwaleng. The major findings of this study suggest that significant variation exist among cowpea landraces with respect to their physiological responses to water stress. However the variation in the responses may not be associated with the ability of a particular land race to recover from drought stress and its yield performance.

Keywords: Cowpea, drought stress, physiology, recovery,

4.1 INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a protein-rich grain legume crop widely grown by resource-poor farmers in arid and semi-arid regions of the world (Muchero *et al.*, 2009). Most farmers grow cowpea under dryland conditions with no irrigation facilities and drought is the most important abiotic factor limiting cowpea production in semi-arid regions due to unpredictable and intermittent rainfall patterns (Bala Subramanian and Maheswari, 1992). The damaging effect of drought stress is most critical when it occurs during the post-flowering and pod filing stage of crop growth and is often referred to as “terminal drought” (Hiler *et al.*, 1972; Turk *et al.*, 1980; Ahmed and Suliman, 2010). Cowpea is commonly exposed to terminal drought stress due to reduced rainfall or plant available water and high temperatures, especially during floral development (Dadson *et al.*, 2005). Many studies have shown that drought stress at the reproductive stage has a negative impact on seed yield. Turk *et al.* (1980) and Shouse *et al.* (1981) reported that drought stress during the flowering and pod-filling stages reduced grain yield of cowpea. Yield losses in cowpea have been minimized by breeding for early maturity (Cisse *et al.*, 1995). Early maturity in cowpea is a desirable trait and has proven to be useful in dry environments because it enhances the ability to escape drought. Early maturing genotypes depend on drought escape mechanisms which enable them to complete their reproductive cycle in time to escape late-season drought (Ehlers and Hall, 1997). Early maturing cowpea cultivars can produce up to 2000 kg/ ha in 60 to 70 days in many cowpea growing regions (Ehlers and Hall, 1997). Hall (2004) indicated that this is achieved by selecting plants that began flowering early and had erect plant habit and synchronous flower production (1st type) or more sequential rather than synchronous flowering, medium cycle from sowing to maturity and more spreading plant habit (2nd type). The early erect and more synchronous flowering enables the plants to escape terminal drought while the spreading growth habit and sequential flowering enables them to escape mid-season drought (Hall, 2004). Unfortunately, early erect and more synchronous flowering cultivars are damaged by mid-season drought (Thiaw *et al*., 1993) due to detrimental effects of drought on pod set and pod filling of erect synchronous flowering cultivars (Turk *et al*., 1980). Cowpea exhibits drought tolerance under water stress conditions by a combination of dehydration avoidance and tolerance mechanisms that enable plant survival until stress relief (Hall and Schulze, 1980; Turk and Hall, 1980b; Lawn, 1982). The effectiveness of these drought response mechanisms influences both the capacity to survive water deficit and productivity after relief from stress (Likoswe and Lawn, 2008). Cowpea landraces grown by resource poor farmers in Limpopo Province are spreading types, late maturing and indeterminate. This could be a disadvantage when drought is terminal and there is no chance for recovery. However, farmers have highlighted that in dryer years when other crops including maize fail, they would harvest relatively reasonably high cowpea leaf and seed yield (Mmapitsi Kobe, personal communication). The ability to survive both intermittent and terminal drought and produce high leaf and seed yield under these conditions is not well understood and could probably be related to a combination of genetic, physiological and morphological traits in these land races.

Water stress tolerance in cowpea has been associated with various physiological responses. This includes water use efficiency (WUE), leaf gas exchange, relative water content, leaf water potential, and membrane stability, accumulation of osmolytes like proline, soluble sugars, proteins and resistance of photosynthetic apparatus (Anyia and Herzog, 2004a, 2004b; Hamidou *et al.*, 2007a; Labuschagne *et al.*, 2008). Physiological responses to water stress in cowpea landraces can be determined by observing changes in leaf water potential. For example, leaf water potential decreases with increasing plant water deficit and is therefore used to measure drought tolerance (Anyia and Herzog, 2004a). It has been reported that drought tolerant cowpea cultivars have the ability to maintain higher leaf water potential with decreasing soil moisture content (Anyia and Herzog, 2004a; Chiulele and Agenbag, 2004; Slabbert *et al.*, 2004). Cowpea is also regarded as a drought-avoiding crop by maintaining high leaf relative water content (RWC) in response to drought stress (Turk *et al.*, 1980; Petrie and Hall, 1992; Anyia and Herzog, 2004b). Generally, studies have shown that cultivars maintain high RWC are drought tolerant (Chakraborty *et al.*, 2002; Kumar *et al.*, 2008; Upadhyaya *et al.*, 2008). Relative water content and leaf water potential have been widely used to quantify the effects of water deficits in leaf tissues. Leaf water content is a useful indicator of plant water balance, since it expresses the relative amount of water present on the plant tissues. On the other hand, water potential measures the energy status of water inside the leaf cells (Slatyer and Taylor, 1960). Low leaf water potential in cowpea landraces may be suggestive of an increase in osmotic potential and this could be significant with regards to the ability of these land races to extract water from extremely dry soils.

Osmotic adjustment is defined as the accumulation of solutes within the plant tissue (either in roots or shoot) in response to a lowering of soil water potential leading to the lowering of the water potential, which provides the driving force for water extraction at low water potential (Morgan, 1984; Flower and Ludlow, 1986; Ramanjulu and Sudhakar, 2000). This means that the presence of the osmoregulation in leaf cells subjected to dehydration can be an indication of the adaptation to drought conditions (Chandrasekar *et al*., 2000). Among known compatible solutes proline is probably the most widely distributed osmolyte, and its accumulation seems to be involved in the process of adaptation to osmotic stress. Genotypic differences in proline accumulation during drought stress have been reported and a positive correlation between magnitude of free proline accumulation and drought tolerance has been suggested as an index for determining drought tolerance potential of cultivars (Ramanjulu and Sudhakar, 2000; Chiulele and Agenbag, 2004; Chakraborty and Pradhan, 2012) though there are reports of negative correlation between proline accumulation and drought tolerance (Singh and Raja Reddy, 2011).

Cowpea landraces exposed to drought stress may also respond by adjusting the stomatal conductance. Stomatal conductance is a measure of the rate of passage of carbon dioxide (CO_2) entering, or water vapor exiting through the stomata of a leaf. Stomatal closure is a common drought-avoidance response mechanism of plants to drought stress which may increase plant water-use efficiency (Parry *et al.*, 2005; Santos *et al.*, 2009). Several studies have shown that cowpea reduced its stomatal conductance when subjected to water stress (Cruz de Carvalho *et al.*, 1998; Anyia and Herzog, 2004a, 2004b; Hamidou *et al.*, 2007a). Dehydration avoidance in cowpea by stomatal closure in order to minimize water loss is one of the drought adaptation mechanism to survive severe water stress (Turk *et al.*, 1980; Souza *et al.*, 2004). Stomatal closure results in an increase in water-use efficiency (Hall *et al.*, 1997b). An increase in plant water-use efficiency could have positive impacts on yield and improve the performance of landraces with this response. There is very little information on comparisons between local cowpea landraces with respect to stomatal conductance in response to water deficits and how this may relate to yield performance.

Drought adaptation in crop plants is often associated with the induction of defense mechanisms necessary for protection of the photosynthetic apparatus. Photosynthetically active radiation (PAR) in plants is absorbed by chlorophyll and accessory pigments of chlorophyll-protein complexes, and it migrates to the reaction centers of photosystem I (PS I) and photosystem II (PS II), where the conversion of the quantum photosynthetic process takes place (Horton *et al.*, 1996). Chlorophyll stability after subjecting leaves to heat stress is a rapid method for the determination of drought adaptation and forms one of the indices for estimating resistance to dehydration. The chlorophyll breakdown or destruction commences rapidly at critical temperature and this property of chlorophyll stability has been used to evaluate genotypic tolerance potentials and found to correlate well with drought tolerance (Ramanjulu and Sudhakar, 2000). On the contrary, loss of chlorophyll is a drought avoidance mechanism associated with minimization of light absorption by chloroplasts (Pastenes *et al.*, 2005; Manivannan *et al.*, 2007) by reducing the possibility of damage to the photosynthetic apparatus (Epron *et al.*, 1992).

Chlorophyll fluorescence of dark adapted leaves is a direct indicator of the photosynthetic activity (Lichtenthaler and Babani, 2000) which gives an indication of status of photosynthetic apparatus (Maxwell and Johnson, 2000). The measurement of this parameter allows estimation of the degree of injuries and their place in photosystem II and to study the protection mechanisms involved in the removal of the excess of excitation energy through the emission of heat/fluorescence emitted from the photosynthetic apparatus (Araus *et al.*, 1998; Lu and Zhang, 1999). The analysis chlorophyll fluorescence parameters such as *Fo* (initial fluorescence), *Fv/Fo* (maximal primary yield PS II photochemistry), and *Fv/Fm* (maximum quantum yield of PS II photochemistry) are considered as an important approach for evaluating for drought tolerance (Li *et al.*, 2006). The lack of significant of reduction of these parameters tolerance provides a rapid and accurate technique of detecting and quantifying plants tolerance to drought stress (Li *et al.*, 2006). It would be interesting to determine whether differences could exist with respect to photosynthetic efficiency among cowpea landraces grown by farmers in the Limpopo and whether such differences can be associated with yield performance under water limiting conditions.

It has been well established that reactive oxygen species accumulate in plants in response to a number of stress factors including water deficits. Crop plants are able to adapt and survive by producing anti-oxidants to scavenge these free radicals. The antioxidant levels of a plant are also a good indicator of the redox state, which is vital for the stress tolerance development (Sanchez-Rodrı´guez *et al.*, 2010). Both enzymatic and non-enzymatic antioxidants provide protection against oxidative damage. Several studies have reported that increased enzymatic antioxidants including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalyse (CAT) and polyphenol oxidase (PPO) correlated with drought tolerance in different crops (Badawi *et al.*, 2004; Ahmad *et al.*, 2010; Lu *et al.*, 2010). There is very little information on comparisons between local cowpea landraces with respect to changes in anti-oxidant capacity in response to water deficits and how this may relate to yield performance.

Phenolics and carotenoids are also well known for their antioxidant activity within the chloroplasts, scavenging singlet oxygen (Havaux, 1998; Munne-Bosch and Alegre, 2000; Bilger et al., 2001). Accumulation of phenolic compounds in leaf tissues can also reduce an excess of absorbed light during drought (Hura *et al.*, 2009b), therefore, limiting the chlorophyll excitation during conditions unfavourable for the photosynthetic apparatus (Burchard *et al.*, 2000). The variation in the accumulation of phenolic compounds in local cowpea landraces in response to water deficits has not been established and whether this may relate to yield performance is not clear.

There is little information in the literature on the physiological responses of dual-purpose landraces to drought stress occurring during reproductive growth and recovery and how this may relate to yield. This knowledge could be used in future for the improvement of local cowpea landraces which are adapted to water limited conditions. The aim of this study was to determine the physiological responses of four dual-purpose cowpea landraces to drought stress at the reproductive stages (terminal drought). The underlying hypothesis was that genotypic variability to terminal drought tolerance and recovery exists in local cowpea landraces. The specific objectives were:

(i) To examine whether there are differences among the landraces with respect to changes in plant water status osmolyte accumulation, pigment content, total antioxidant capacity and photosynthetic capacity when subjected to water deficit during the reproductive growth stage, and

(ii) To determine yield performance as indicated by pod number, pod mass, seed yield, biomass and harvest index and correlate this with objective 2 above.

4.2 MATERIALS AND METHODS

4.2.1 Experimental procedure

4.2.1.1 Plant material

Four dual-purpose cowpea landraces (Lebudu, Lehlodi, Morathathane and Sejwaleng) were used in the study. The details of the landraces are presented in section 3.2.1.1.

4.2.1.2 Experimental procedure and design

Controlled experiment was conducted under glasshouse conditions at the Controlled Environment Facility (CEF), University of KwaZulu-Natal. Temperature, solar radiation (PAR) and relative humidity (RH) were monitored electronically using a Hobo Data logger (Onset Computer Corporation, Bourne, USA) (Figure 4-1).

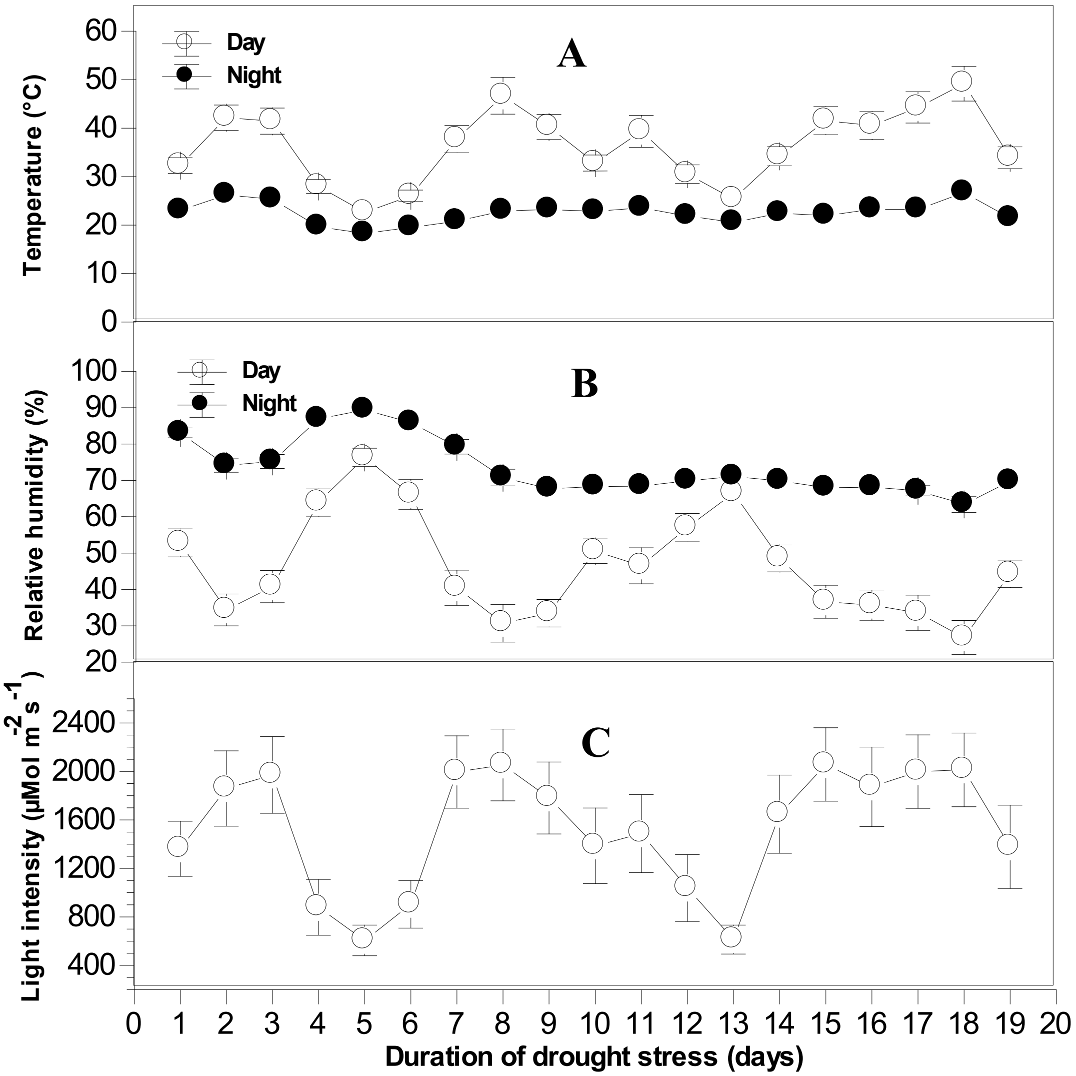

Figure 4-1. Changes in relative humidity (A), temperature (B) and light intensity (C) in the glasshouse during drought stress imposed during the reproductive stage. Bars indicate standard error.

The experiment was laid out as a 4 x 2 factorial treatment structure in randomized complete blocking design (RCBD) with the following two factors: cowpea landraces – 4 levels (Lebudu, Lehlodi, Sejwaleng and Morathathane) and water regimes – 2 levels (stressed and well-watered) giving a total of 8 treatment combinations each replicated 20 times (20 pots each pot containing one plant) giving a total of 160 experimental units (drained polyethylene pots with a 5 litre capacity).

4.2.1.3 Soil preparation, potting and planting

Soil of known physical properties (Appendix 1) was collected from the University of KwaZulu-Natal Ukulinga Research Farm (29°39'48.82"S; 30°24'19.89"E) and sieved through a 1cm mesh to remove clods and stones. Each pot was carefully filled with 5kg soil and the mass of the pot and soil determined and recorded. The pots were filled with water until full saturation and allowed to drain for 24h until field capacity then weighed. Two seeds were planted in each pot by placing a depth of 2 cm and later thinned to one. A compound fertilizer 2:3:2 (22) was applied during planting at the rate of 150 kg/ha based on soil analysis results (Appendix 2). Plants were well-watered (field capacity) up to 50% flowering and irrigation was withheld for 12 days to impose water stress. After 12 days, plants were re-watered to relief the stress and recovery measurements taken after 15 days.

4.2.2 Data collection

4.2.2.1 Soil moisture content monitoring

Changes in soil water content (SWC) of the upper 6-10 cm of soil on a percentage by volume was monitored after every 3 days using a handheld Theta-Probe ML 2x soil water sensor (Delta devices, Cambridge, England). Eight (8) pots/plants were selected randomly for monitoring changes in soil water content.

4.2.2.2 Determination of leaf water potential

Pre-dawn leaf water potential measurements were made using a Scholander pressure chamber (Scholander *et al.*, 1965). A fresh leaf selected from a pair of fully developed second lateral leaflets was selected and excised from the plant using a sharp razor blade. The leaf was wrapped in a cling film and quickly sealed in a chamber. Pressure was increased slowly at approximately the rate of 100 kPa using nitrogen gas from a cylinder until sap droplets appeared at the position of the xylem vessels at the cut surface. The droplets were examined using a hand lens. The endpoint was checked by decreasing the pressure and then slightly increasing it again. Leaf water potential is the negative of the applied pressure (– MPa).

4.2.2.3 Determination of leaf relative water content

Relative water content (RWC) measurements were made at the same time as leaf water potential measurement using the entire second lateral leaflet of the trifoliate leaf (Shackel and Hall, 1983). Four pairs of leaflets were excised and weighed immediately and recorded as fresh weight (FW). After that they were placed in plastic bags containing water and kept on ice for 4 hours. The turgid weights (TW) were measured as well as dry weight (DW) after drying the leaves in the oven for 24 hours at 80ºC. Relative water content was determined according to Shackel and Hall (1983) using the formula:

$$RWC = (FW–DW)/ (TW–DW) \times 100\%$$

4.2.2.4 Determination of chlorophyll content

Chlorophyll content index (CCI) in leaves was measured non-destructively with a hand-held chlorophyll content meter (CCM- 200 plus, Biosciences). Twelve leaves were randomly sampled for determining chlorophyll content.

4.2.2.5 Determination of stomatal conductance

Stomatal conductance was measured non-destructively on twelve second fully expanded leaves for each landrace selected randomly using a steady diffusion leaf porometer (model SC–1, Decagon devices). The sensor head was placed on the leaf and abaxial stomatal conductance measurements (mmol m^{-2} s^{-2}) were recorded. Stomatal conductance measurements were only done on sunny days from around 10h00 to 14h00.

4.2.2.6 Determination of total anti-oxidant capacity

Total anti-oxidant capacity (TAOC) was determined according to Benzie and Strain (1996) with slight modifications. These authors developed the FRAP assay which is based on the reduction of the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) complex by a reductant, therefore determining the combined anti-oxidant capacity of anti-oxidant molecules present in the tissue under investigation. Aliquots of 0.2 g freeze-dried plant material (leaves) were extracted with 1 N perchloric acid, vortexed for 10 seconds and centrifuged at 10, 000 rpm for 10 min at 4°C. A fresh FRAP reagent solution (300 mM sodium acetate buffer pH 3.6, 10 mM Fe(II)-TPTZ prepared in 40 mM HCl, 20 mM $FeCl_3$ x 6H2O (10:1:1)) was prepared prior to measurement. Subsequently an aliquot of the samples (30 µl) was mixed with 900 µl FRAP reagent solution and the absorbance was measured at 593 nm after 10 min using a spectrophotometer. The total anti-oxidant capacity was expressed as mg $FeSO_4 \times 7H_2O \times g\ DW^{-1}$ equivalent.

4.2.2.7 Determination of phenolics

Phenols were determined according to Bohm *et al.* (2006). Briefly, freeze-dried tissue (leaves) (0.1 g) was mixed with 10 ml 99.8% (v/v) methanol and homogenized for 30 s using the Ultra-Turrax homogenizer. The mixture was then manually shaken using IKA® (ks 130, Staufen, Germany) and left overnight at room temperature to extract the free phenols. Subsequently the mixture was centrifuged, the supernatant filtered through Whatman® no. 4 filter paper and the pellet repeatedly rinsed with 10 ml solvent until colour was no longer released. Membrane-bound phenols were released from the remaining plant residue by alkaline hydrolysis. A 10 ml portion of alkaline solution (2 M NaOH) in 75% (v/v) aqueous methanol was added to each sample, which was then incubated at 90°C for 60 min. Samples were allowed to cool before the supernatant was filtered and analyzed. 5 mL ultrapure water, 1mL sample, and 1 mL Folin-Ciocalteu reagent (Sigma®) were placed in a test-tube. The mixture was mixed and allowed to stand for 5-8 minutes at room temperature. 10 mL of 7% sodium carbonate was added followed by addition of ultrapure water to bring the volume to 25 mL. The solution was mixed and allowed to stand at room temperature for 2 hours. Free and membrane-bound phenols concentrations were determined spectrophotometrically at 750 nm (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan) and expressing the results in 'gallic acid equivalents' (GAE).

4.2.2.8 Determination of chlorophyll a and total carotenoid content

Aliquots of 0.2 g freeze-dried plant material (leaves) were extracted with 10 mL of methanol (99.9%, v/v), vortexed for 10 seconds and centrifuged at 10, 000 rpm for 10 min at 4°C. Chlorophyll a and total carotenoids were determined according to Lichtenthaler *et al.* (1987) by computing the absorbance values of the plant tissue extracts at wavelengths of 470, 646.8, 652, 663.2 and 665.2 nm using a spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan). The formula's to determine chlorophyll a and total carotenoids content are presented below:

$$\text{Chlorophyll a } (C_a) = 11.75\ A_{662} - 2.35\ A_{645}$$

$$\text{Chlorophyll b } (C_b) = 18.61\ A_{645} - 3.96\ A_{662}$$

$$\text{Total carotenoids content} = 1000A_{470} - 2.270 \text{ Chl a} - 81.4 \text{ Chl b}/227$$

4.2.2.9 Determination of proline content

Proline content was determined using the method of Bates *et al.* (1973) with some modifications. Samples of 0.2 g freeze-dried leaf material were homogenized in 10 mL of 3% sulfosalycic acid (w/v). The homogenate was sieved through a Whatman filter paper) no 4. Two millilitres (mL) of the supernatant was reacted with 2 mL acid-ninhydrin and 2 mL of glacial acetic acid in a test tube for 1 hour at 100°C, and the reaction was terminated in an ice bath. The reaction mixture was extracted with 4 mL toluene, and stirred with an iron rod for 15–20 sec. The chromophere containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve and calculated on a dry weight basis as follows:

[(µg proline/ mL x mL toluene)/ (115µg/µmole)]/ [(g sample)/5] = µmoles proline/g of dry weight material. This was converted to mg/g dry weight.

4.2.2.10 Determination of chlorophyll fluorescence

Chlorophyll fluorescence was measured using the Plant Efficiency Analyzer (PEA) (Hansatech Instruments Ltd, Norfolk, England). Six second fully expanded leaves selected randomly from and used for measurements. The leaves were dark adapted with a lightweight plastic leaf clip for 30 min before the measurement. During measurement, the PEA sensor unit was held over the clip and the shutter opened. A single button-press activates the high intensity of the LED array of the sensor head which provides a maximum light intensity of 3000 µmol/m^2/s. Maximal PS II photochemical efficiency Fv/Fm, the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm), was calculated automatically.

4.2.2.11 Biomass and seed yield determination

Above ground biomass and seed yield were determined at maturity. All pods from each treatment were picked and pod mass, pod length and number of seeds per pod were determined.

The pods were then shelled and the grain was removed and its mass determined. Biomass was determined by drying plant parts except pods at 60 ºC for 3 days. All observations were replicated ten times (1 plant = 1 replicate).

4.2.2.12 Data analysis

Genstat 14th edition (VSN International, UK) was used to perform analyses of variance (ANOVA) and the differences between means was determined by Least Significant Differences (LSD) at α=95% ($P \leq 0.05$).

4.3 RESULTS

Highly significant ($P < 0.001$) were observed between moisture content of soil that was well-watered compared to soil that was allowed to dry-out. Soil water content of stressed plants declined after irrigation was withheld. Soil water content of well-watered treatments was high (above 30%) during the entire drought period There was also a highly significant ($P < 0.001$) water stress x time interaction signifying that stress intensity intensified overt time (Figure 4-2). However, it should be noted that the time of treatment was long enough to find some leaf age related modifications (Figure 4-3). Stressed plants lost most the leaves compared to well-watered plants which showed no symptoms of water stress (Figure 4-3).

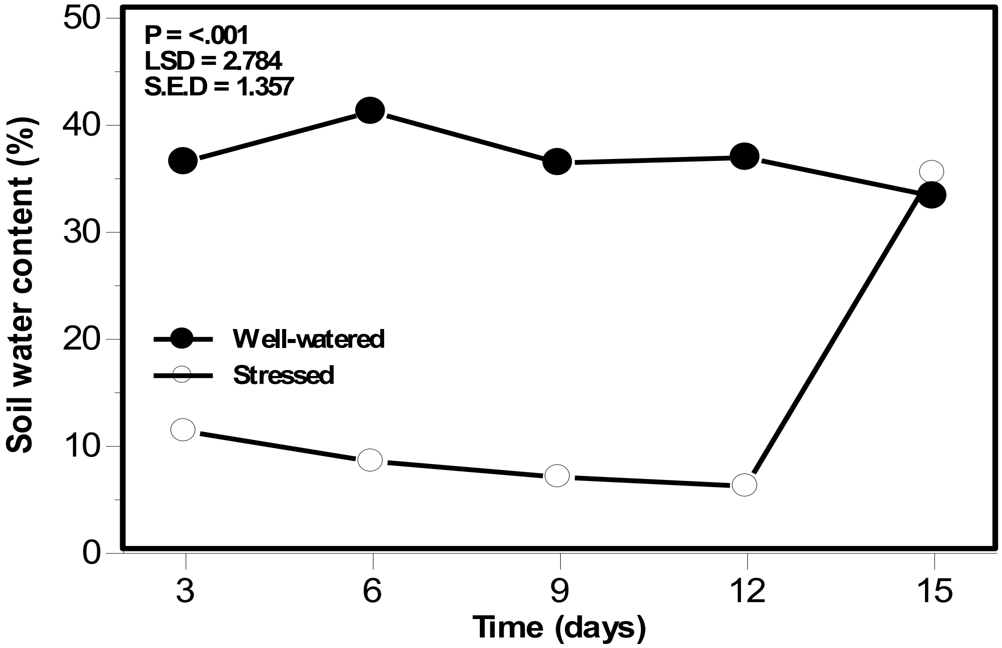

Figure 4-2. Effect of withholding irrigation on changes in soil water content.

Figure 4-3. Cowpea plants under well-watered (left) and stressed (right) conditions.

Highly significant differences ($P < .001$) between stressed and well-watered plants were observed with regards to leaf water potential. Leaf water potential under well-watered conditions was generally below – 0.5 MPa while that of stressed plants became more negative for all cowpea landraces. A significant ($P < 0.05$) landrace x stress interaction was observed and this could suggest that the response to water deficit was influenced by the land race type (differences between the land races). Also, a significant ($P < 0.05$) landrace x stress x time interaction was observed signifying that cowpea landraces differed with regards to a decline in leaf water potential over time during water stress (Figure 4-4). At maximum stress (12 days), Morathathane showed a more negative value (– 2.2 MPa) as compared to Lebudu, Lehlodi and Sejwaleng (– 1.85, 1.8 and 1.75 MPa, respectively). After re-watering, Lebudu showed rapid recovery of leaf water potential (– 0.23 MPa) as compared to Sejwaleng, Lehlodi and Morathathane (– 0.48, 0.41, 0.4 MPa, respectively) (Figure 4-4).

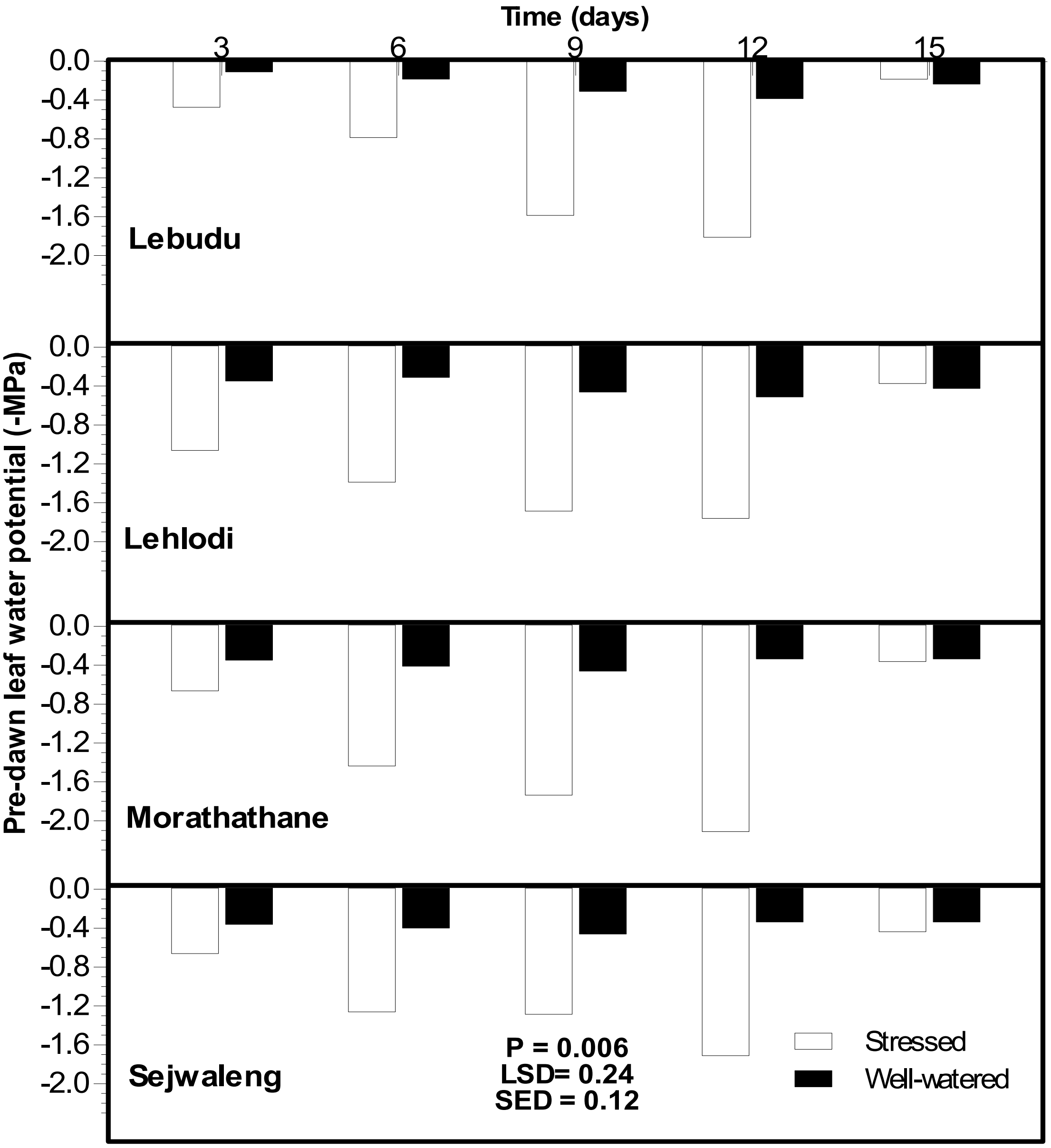

Figure 4-4. Changes in the pre-dawn leaf water potential of four cowpea landraces subjected to well-watered (control) and water stress conditions during reproductive growth for 12 days.

Highly significant differences ($P < 0.001$) were observed between stressed and well-watered plants with respect to relative water content (RWC). Relative water content of well-watered plants remained high (~ 90%) while water stress decreased RWC of stressed cowpea plants. A significant variety x stress interaction ($P < 0.05$) was observed, signifying that RWC between cowpea landraces differed in response water stress. Also, a highly significant interaction ($P < 0.001$) landrace x water stress x time interaction observed indicates that the four landraces responded differently to water stress over time with regards to RWC (Figure 4-5). At maximum stress (12 days without water) Sejwaleng showed a lesser decline in RWC (69%) than Lebudu, Lehlodi and Morathathane (58.2%; 56% and 55%, respectively). The recovery from water stress (after 15 days) was evidenced by increase in RWC after re-watering, but, no significant differences were observed between landraces (Figure 4-5).

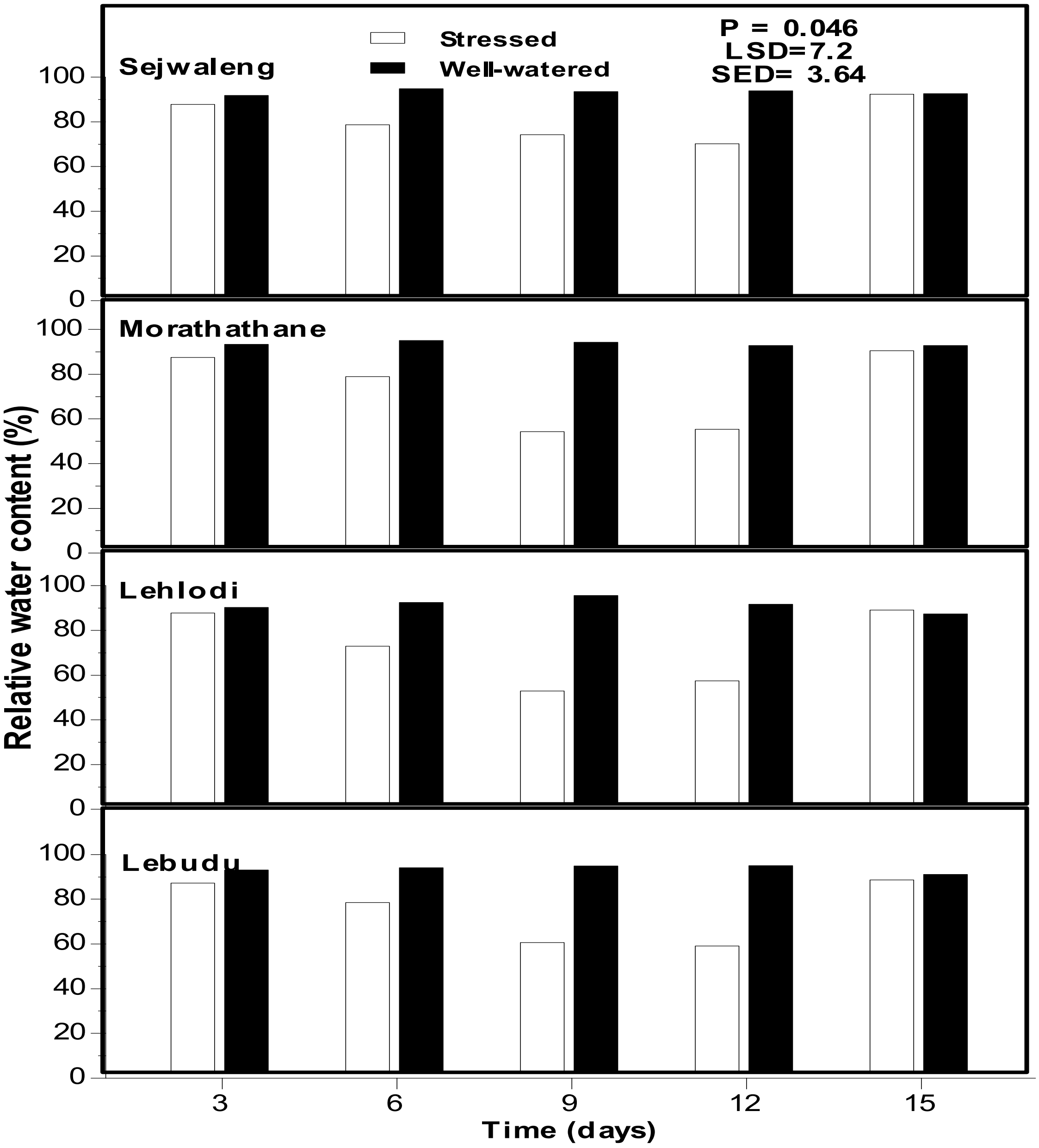

Figure 4-5. Changes in relative water content of four cowpea landraces subjected to well-watered (control) and water stress conditions during reproductive growth for 12 days.

Highly significant ($P < 0.001$) differences were observed for well-watered and stressed plants with regards to stomatal conductance. No stomatal conductance was observed for stressed plants from 3 to 12 days without water for all cowpea landraces. A highly significant landrace x stress interaction shows that different landraces responded differently to water stress. Also, a highly significant ($P < 0.001$) landrace x stress x time interaction was observed. This could suggest that different landraces responded differently to water stress over time (Figure 4-6). Stomatal activity did not differ between landraces after withholding water but differed after re-watering (15 days). Lebudu and Lehlodi showed lower stomatal conductance (149 and 116 mmol m^{-2} s^{-2}, respectively) as compared to Morathathane and Sejwaleng (234 and 284 mmol m^{-2} s^{-2}, respectively).

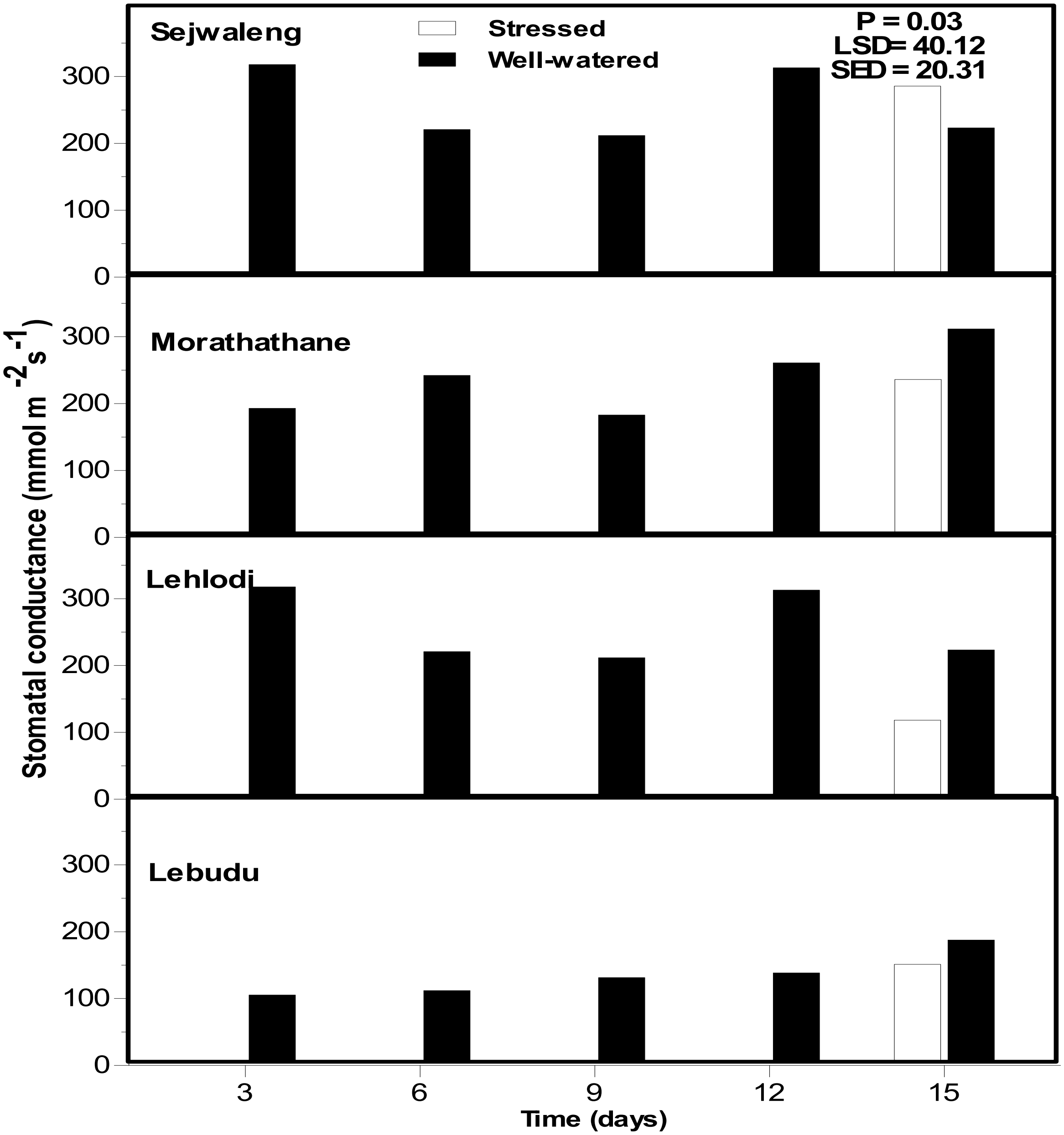

Figure 4-6. Changes in stomatal conductance of four cowpea landraces subjected to well-watered (control) and water stress conditions during reproductive growth for 12 days. NB: Values for stressed plants from 3 to 12 days do not appear on the graph because their values were zero.

Water stress caused a reduction in chlorophyll content of cowpea all landraces. Chlorophyll content of well-watered plants was generally higher while that of stressed plants declined with increasing stress intensity. A highly significant landrace x stress interaction (*P* < 0.001) was observed, this suggests that the response of the four cowpea landraces to water deficits may be influenced by differences between the landraces. Also, a significant interaction (*P* < 0.05) landrace x water stress x time interaction observed indicates that the four landraces responded differently to water stress over time with regards to chlorophyll content (Figure 4-7). After 12 days without water, Morathathane and Lehlodi showed significantly lower chlorophyll content (12.9 and 16.94, respectively) than Lebudu and Sejwaleng (20.53 and 21.5, respectively). After re-watering, (15 days) Lebudu and Lehlodi showed significantly higher chlorophyll content (48.95 and 45.44, respectively) as compared to Morathathane and Sejwaleng (36.44 and 37.49, respectively).

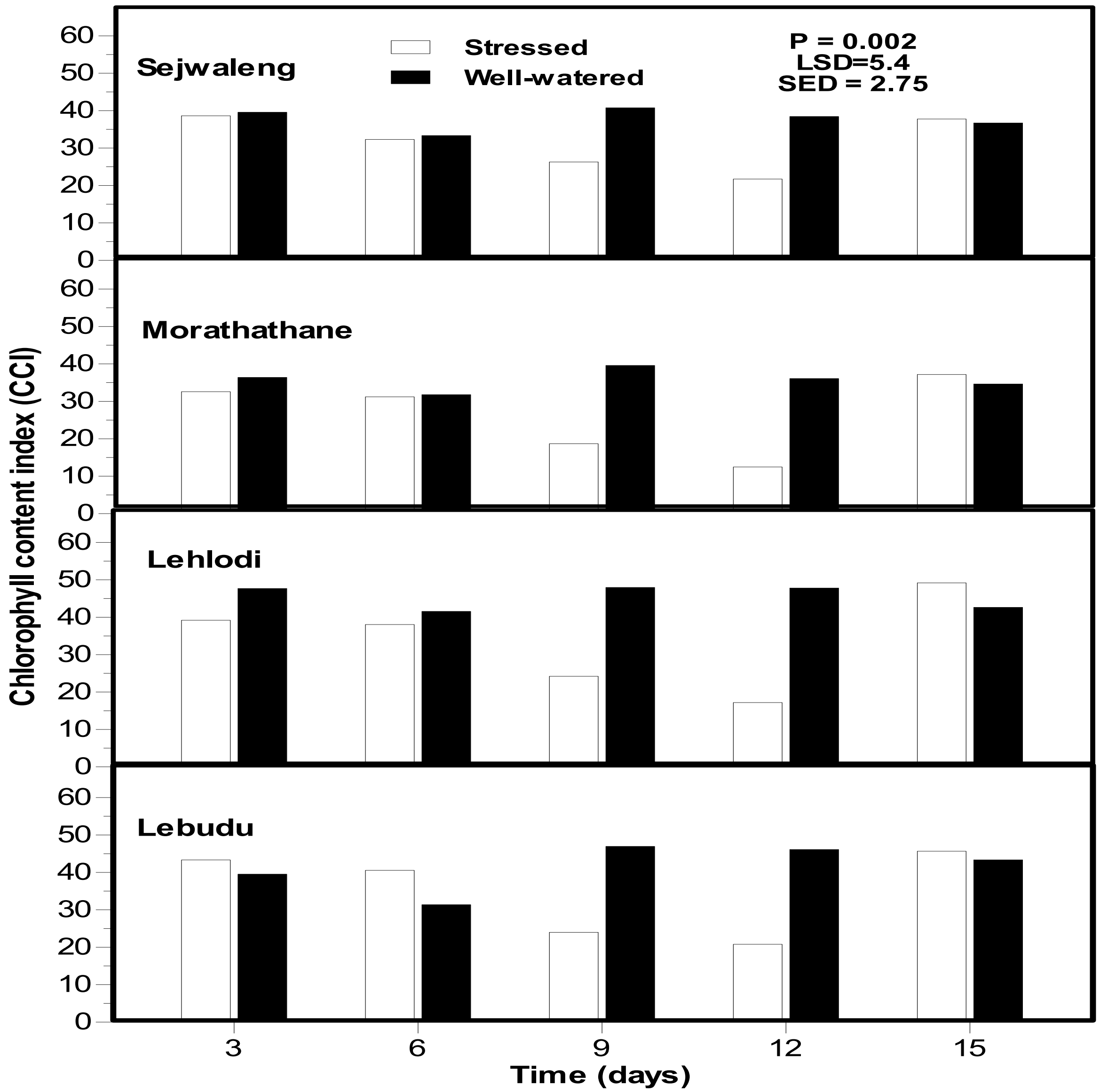

Figure 4-7. Changes in chlorophyll content index of four cowpea landraces subjected to well-watered (control) and water stress conditions during reproductive growth for 12 days.

Highly significant differences ($P < 0.001$) were observed between stressed and well-watered plants with respect chlorophyll a content. Stressed plants showed an increase in chlorophyll a content as compared to well-watered plants. A significant landrace x stress interaction ($P < 0.05$) was observed and it implies that the four landraces responded differently to water stress. Also, a significant landrace x stress x time ($P < 0.05$) interaction shows that different landraces differed under stress conditions at various time intervals with respect to chlorophyll a content (Figure 4-8). There were no significant different between landraces observed after 3 and 6 days without water while significant differences were observed after 9 and 12 days without water. After 9 days without water, Morathathane and Lehlodi showed the highest increase in chlorophyll content (1.38 and 1.36 µg/g dry weight, respectively) as compared to Lebudu and Sejwaleng (1.265 and 1.271 µg/g dry weight, respectively). After 12 days without water, Morathathane showed the highest increase in chlorophyll a content (1.546 µg/g dry weight, respectively) than Sejwaleng, Lebudu and Lehlodi (1.346, 1.443 and 1.475 µg/g dry weight, respectively). After, re-watering (15 days) chlorophyll a content of stressed plants increased in and was similar to those of the well watered treatments (control) however, no significant differences between landraces were observed.

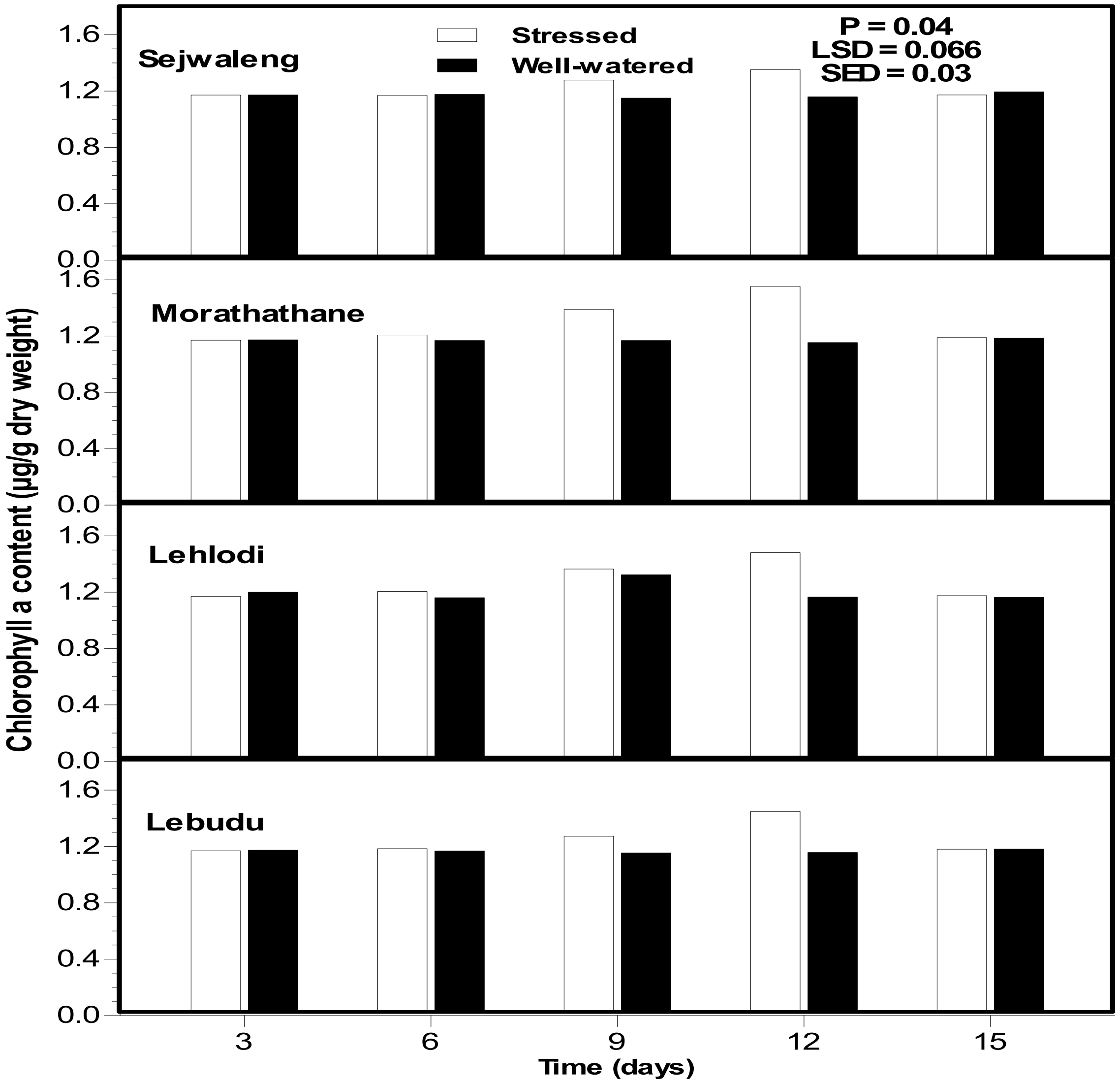

Figure 4-8.Chlorophyll a content of four cowpea landraces in response to water stress during the reproductive growth stage compared to well watered plants (control).

Significant differences (*P* < 0.05) were found between well-watered and stressed plants with regards to total antioxidant capacity (TAOC). TAOC of stressed plants increased only after 9 and 12 days while that of well-watered plants was generally low between 9 and 12 days without water. A significant landrace x water stress interaction was observed on TAOC and indicates that the four landraces responded differently to water stress. Also, a highly significant interaction (*P* < 0.001) landrace x water stress x time interaction indicates that the four landraces responded differently to water stress over time (Table 4-1). Lehlodi and Sejwaleng showed higher TAOC (0.73 and 0.59 mg/g dry weight) as compared to Lebudu and Sejwaleng (0.43 and 0.41 mg/g dry weight) while no differences between landraces were observed after 12 days without water and after re-watering. After re-watering and recovery period, total antioxidant capacity of stressed plants returned to levels similar to control plants.

Table 4-1. Changes in total antioxidant capacity (mg/g DW) of four cowpea landraces subjected to water stress conditions by withholding water for 12 days compared and re-watering at 15 days.

	Landrace							
	Lebudu		**Lehlodi**		**Morathathane**		**Sejwaleng**	
Time	**Well-watered**	**Stressed**	**Well-watered**	**Stressed**	**Well-watered**	**Stressed**	**Well-watered**	**Stressed**
3	0.42^{ghijk}	0.4^{fghi}	0.41^{ghijk}	0.39^{efgh}	0.39^{efghi}	0.42^{ghijk}	0.41^{fghij}	0.39^{defgh}
6	0.39^{efghi}	0.36^{cdefg}	0.36^{cdef}	0.38^{defg}	0.36^{cdef}	0.39^{fghi}	0.37^{defg}	0.41^{ghijk}
9	0.32^{bc}	0.43^{hijkl}	0.41^{ghijk}	0.7^{p}	0.34^{bcd}	0.41^{ghijk}	0.34^{bcde}	0.59^{o}
12	0.29^{ab}	0.48^{lmn}	0.26^{a}	0.49^{mn}	0.25^{a}	0.46^{klmn}	0.24^{a}	0.48^{lmn}
15	0.4^{lmn}	0.47^{lmn}	0.51^{n}	0.46^{jklmn}	0.46^{klmn}	0.49^{mn}	0.5^{n}	0.44^{ijklm}
***P*=<.001**								
LSD=0.05								
SED=0.03								

Means followed by the same letter (s) are not significantly different

No significant differences ($P > 0.05$) were observed between well-watered and stressed plants with regards to total phenolics. However, total phenolics of well-watered and stressed plants increased for all cowpea landraces. The non-significant landrace x stress interaction implies that landraces did not differ with regards to total phenolics. A highly significant ($P < 0.001$) landrace x stress x time interaction signifies that different landraces responded differently to water stress over time (Table 4-2). Morathathane showed a general decline in total phenols (303 µg/g dry weight) as compared Lebudu, Lehlodi and Sejwaleng which showed an increase after 12 days without water (314, 311 and 350 µg/g dry weight, respectively). Increased levels of total phenols were observed for all cowpea landraces after re-watering, however, no significant differences were observed between landraces.

Table 4-2. Changes in total phenols (µg/g DW) of four cowpea landraces subjected to water stress conditions by withholding water for 12 days compared to well-watered plants (control).

	Landrace							
	Lebudu		**Lehlodi**		**Morathathane**		**Sejwaleng**	
Time	**Well-Watered**	**Stressed**	**Well-watered**	**Stressed**	**Well-watered**	**Stressed**	**Well-watered**	**Stressed**
3	271 ^{ab}	282 ^{abc}	321 ^{abcdef}	289 ^{abcd}	291 ^{abcd}	319 ^{bcdef}	264 ^{a}	312 ^{bcdef}
6	293 ^{abcde}	422 ^{l}	297 ^{abcde}	338 ^{defghi}	308 ^{abcdef}	342 ^{efghi}	323 ^{cdefg}	340 ^{efghi}
9	280 ^{abc}	362 ^{cdefgh}	299 ^{abcde}	312 ^{abcdef}	291 ^{abcd}	306 ^{abcdef}	352 ^{fghij}	323 ^{cdefg}
12	391 ^{jkl}	314 ^{bcdef}	371 ^{ghijk}	311 ^{abcdef}	395 ^{jkl}	304 ^{abcdef}	408 ^{kl}	351 ^{fghij}
15	340 ^{efghi}	373 ^{hijkl}	395 ^{jkl}	377 ^{ijkl}	348 ^{fghij}	332 ^{defghi}	319 ^{bcdef}	408 ^{kl}

***P*=0.015**
LSD=48.8
SED=24.5

Means followed by the same letter (s) are not significantly different

Highly significant differences (*P* < 0.001) were observed between well-watered and stressed plants with regards to free phenolics. Free phenolics of well-watered plants were generally higher than that of stressed plants. There was a non-significant landrace x stress interaction which implies that landraces did not differ with regards to total phenolics under both stressed and well-watered conditions. However, a highly significant landrace x time (*P* < 0.001) interaction shows that landraces differed over time with respect to free phenols (Table 4-3). Free phenolics of well-watered and stressed plants increased for all cowpea landraces after 12 days withholding water. Sejwaleng showed higher accumulation of free phenols (144.7 µg/g dry weight) as compared to Lebudu, Lehlodi and Morathathane (113, 117 and 116 µg/g dry weight, respectively). A non-significant (*P* > 0.05) landrace x stress x time interaction signifies that different landraces did not differ in response to water stress over time. Increased levels of total phenols were observed for all cowpea landraces after re-watering (day 15).

Table 4-3. Changes in free phenols (µg/g dry weight) of four cowpea landraces subjected to water stress conditions by withholding water for 12 days compared to well-watered plants (control).

	Landrace							
	Lebudu		**Lehlodi**		**Morathathane**		**Sejwaleng**	
Time	**Well-Watered**	**Stressed**	**Well-Watered**	**Stressed**	**Well-Watered**	**Stressed**	**Well-Watered**	**Stressed**
3	115.9±2.27	118.1 ±6.83	105.3±5.7	114.6±1.66	111±3.02	121.8±3.67	122.2±3.49	131.14±2.9
6	91.76±2.13	85.3.9±4.46	104.3±3.09	98.1±4.25	96.1±2.99	87.14±4.39	112.4±3.59	96.1±4.37
9	123.6±2.68	119±2.68	117.7±9.7	115.1±5.63	120.9±1.05	108.8±1.2	134.4±6.44	126.93±2.74
12	143.2±2.68	113.3±4.8	145.2±4.74	117.1±1.01	145.9±4.46	116.7±0.33	155.93±3.6	144.8±2.6
15	167.1±7.22	146±3.1	147.9±9.1	140.4±9.47	151.2±3.59	150.3±2.82	186.9±3.8	165±3.02
LSD	13.2							

Highly significant differences ($P < 0.001$) were observed between well-watered and stressed plants with regards to membrane-bound phenolics. Withholding water caused an increase in the content of membrane-bound phenolics. Also, a significant landrace x stress x time ($P < 0.05$) interaction shows that landraces differed under stress conditions at various time intervals with respect to membrane-bound phenolics (Table 4-4). There were no significant different between landraces observed after 3, 9 and 12 days without water while significant differences were observed after 6 days without water and after re-watering (15 days). Lebudu showed the highest increase of membrane-bound phenols after 6 days (337 µg/g dry weight) as compared to Lehlodi, Sejwaleng and Morathathane (240, 244 and 255 337 µg/g dry weight, respectively). After, re-watering (15 days) Lehlodi showed the highest increase in membrane-bound phenols (236 337 µg/g dry weight) as compared to Lebudu, Sejwaleng and Morathathane (226, 242 and 181 337 µg/g dry weight, respectively).

Table 4-4. Changes in membrane-bound phenols (µg/g dry weight) of four cowpea landraces subjected to water stress conditions by withholding water for 12 days compared to well-watered plants (control).

	Landrace							
	Lebudu		**Lehlodi**		**Morathathane**		**Sejwaleng**	
Time	**Well-watered**	**Stressed**	**Well-watered**	**Stressed**	**Well-watered**	**Stressed**	**Well-watered**	**Stressed**
3	155.5±11.83	163.9±7.04	197.6±10.05	174.8±5.36	180.2±8.08	197.1±22.97	141.8±9.8	184.3±8.79
6	201.6±23.58	336.7±43.05	195.2±7.29	240±10.92	211.9±16.27	255±26.6	210.1±2.91	244.2±12.01
9	157.2±20.82	207.1±6.1	181±16.11	196.9±7.02	169.8±8.19	196.7±2.55	217.3±10.91	196±4.73
12	247.9±8.62	200.7±5.96	225.5±6.59	194.7±8.85	248.9±9.42	187±17.86	252.4±10.89	205.8±5.34
15	173±5.49	226.6±13.3	247±8.13	236.4±12.86	196.9±30.3	181.2±17.0	132.2±31.16	242.8±17.59
LSD	**43.75**							

Highly significant differences ($P < 0.001$) were observed between stressed and well-watered plants with respect carotenoid content. Stressed plants showed a decline in carotenoid content as compared to well-watered plants. A significant landrace x stress interaction ($P < 0.05$) was observed and it implies that the four landraces responded differently to water stress. Also, a significant landrace x stress x time ($P < 0.05$) interaction shows that different landraces differed under stress conditions at various time intervals with respect to carotenoid content (Figure 4-9). There were no significant different between landraces observed after 3, 6, 12 and days without water while significant differences were observed after 9 days without water. At 9 days without water Sejwaleng showed the highest decline in carotenoid content (158.7 µg/g dry weight) as compared to Lebudu, Morathathane and Lehlodi (184.2, 184.2 and 186 µg/g dry weight). After, re-watering (15 days) carotenoid content of stressed plants increased in all landraces after rewatering and was similar to those of the well watered treatments (control); however, no significant differences between landraces were observed.

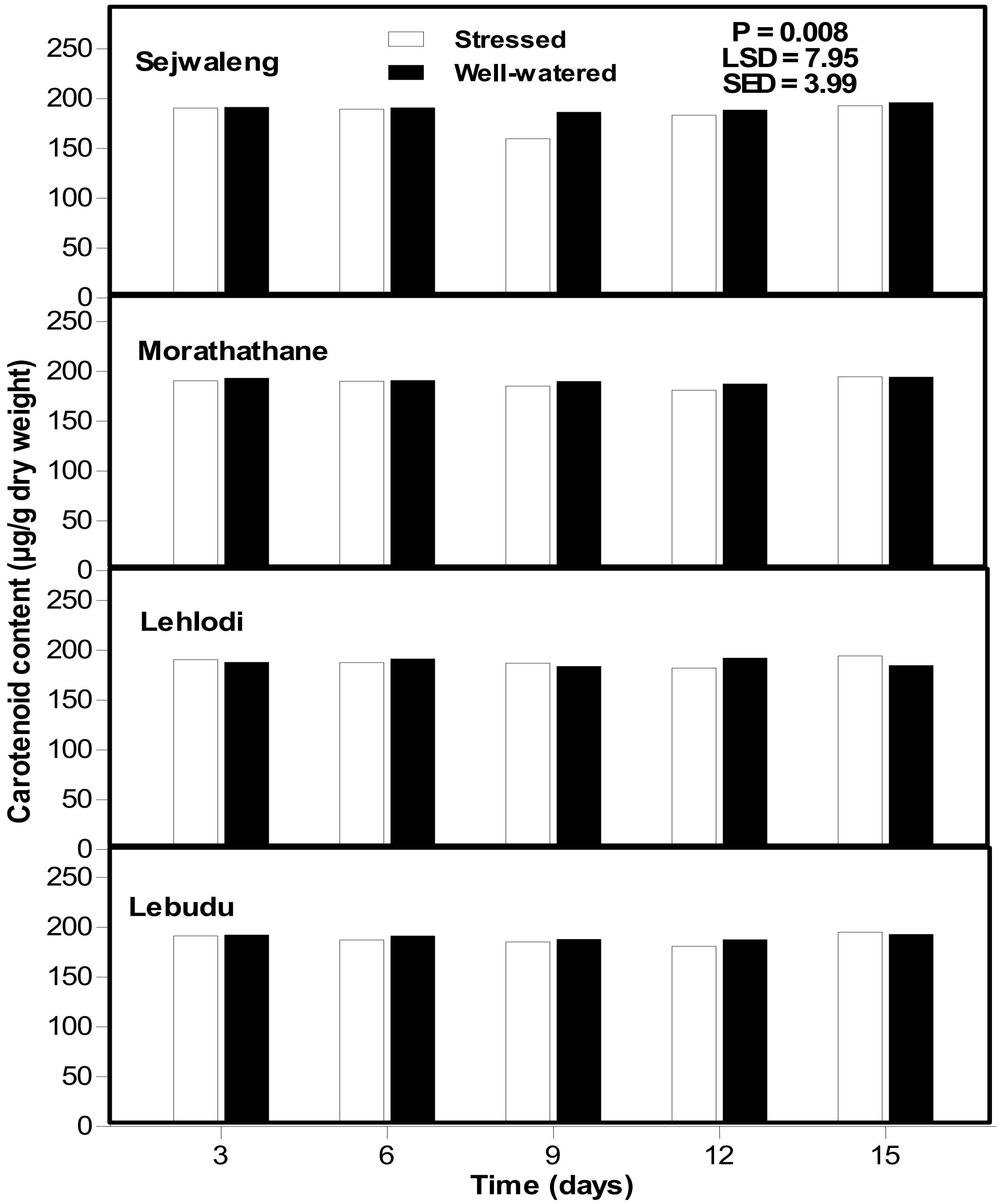

Figure 4-9. Changes in carotenoid content of four cowpea landraces subjected to water stress conditions by withholding water for 12 days compared to well-watered plants (control).

Highly significant differences (*P* < 0.001) were observed between stressed and well-watered plants with respect to proline content. Stressed plants showed an increase in proline content as compared to well-watered plants which maintained low proline content. A highly significant landrace x stress interaction (*P* < 0.001) was observed and it implies that the four landraces responded differently to water stress with respect to proline content. Lebudu, Lehlodi and Morathathane showed the highest accumulation of proline as compared to Sejwaleng. Also a highly significant landrace x stress x time ($P < 0.05$) interaction was observed and shows that different landraces differed under stress conditions at various time intervals with respect to proline content (Figure 4-10). Landraces Lebudu, Lehlodi and Morathathane showed rapid increase in proline content with increasing water deficits as compared to Sejwaleng which showed a slow increase in proline. At maximum stress (12 days without water), Sejwaleng showed less proline accumulation (116.5 µmol/g dry weight) as compared to Lebudu, Lehlodi and Morathathane which accumulated high proline (190.2, 199.6 and 189.7 µmol/g dry weight, respectively). After, re-watering (15 days) proline content of stressed plants decreased and was similar to those of the well watered treatments (control) however, no significant differences between landraces were observed.

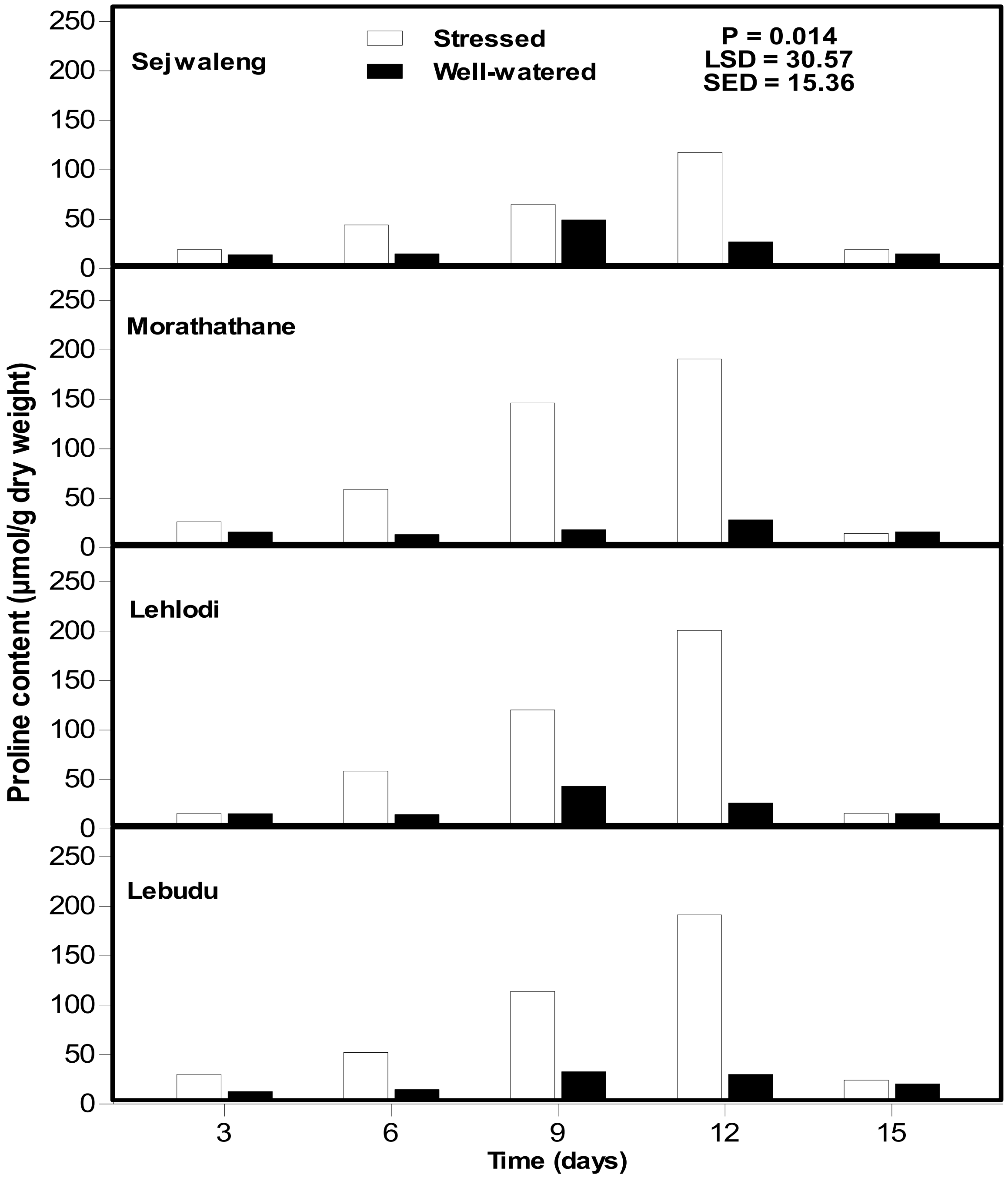

Figure 4-10. Changes in proline content of four cowpea landraces subjected to water stress conditions by withholding water for 12 days compared to well-watered plants (control).

No significant differences ($P > 0.05$) were observed between well-watered and stressed plants with regards to photochemical efficiency of PS II (*Fv/Fm*). Both stressed and well-watered plants had *Fv/Fm* value of 0.7 respectively. A significant variety x time interaction ($P < 0.05$) was observed signifying that *Fv/Fm* values of different cowpea landraces differed over time, however, no significant differences ($P > 0.05$) were observed between landraces. There was also non-significant landrace x stress interaction ($P > 0.05$) implying that landraces did not differ with regards to chlorophyll fluorescence after withholding water. Also, a non-significant ($P > 0.05$) landrace x stress x time interaction was observed and signifies that different landraces did not respond differently to water stress over time with respect to *Fv/Fm* (Figure 4-11).

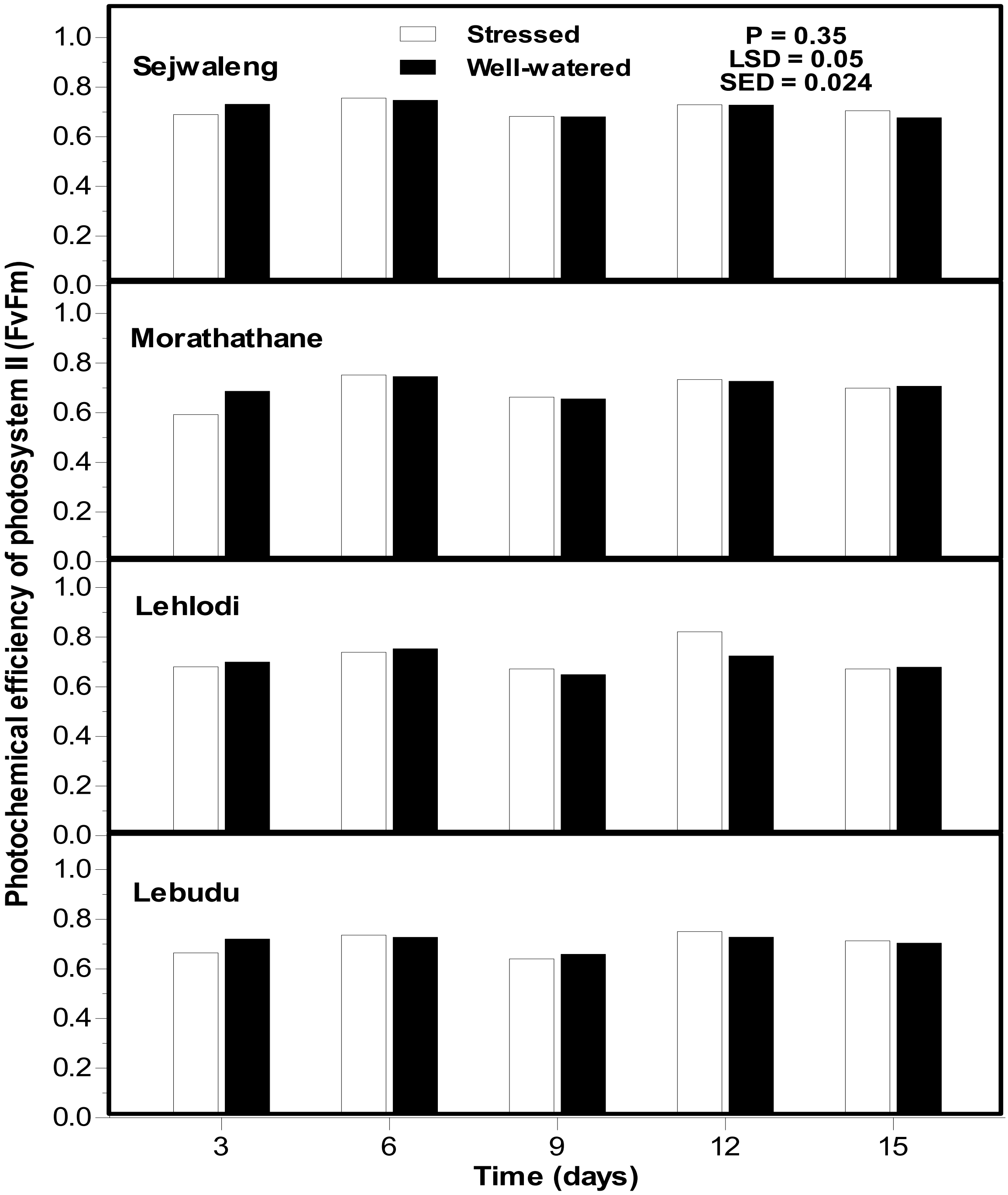

Figure 4-11. Changes in *Fv/Fm* of four cowpea landraces subjected to water stress conditions by withholding water for 12 days compared to well-watered plants (control).

Effect of water stress and recovery on total biomass, pod number, pod mass and seed yield are presented in Figure 4-12. Highly significant differences (*P* < 0.01) were observed between cowpea landraces with respect to total biomass. Lebudu had higher biomass after relief from stress (39.7g/plant) compared to Lehlodi, Morathathane and Sejwaleng (18.8, 24.4, 21.4 g/plant, respectively). Highly significant differences (*P* < 0.01) were observed between stressed + recovery treatment and well-watered treatment on total biomass at maturity. This suggests that stress treatment reduced total biomass despite the availability of adequate moisture after relief from stress. The stressed + recovery treatment had lower total biomass (20.7 g/plant) in relation to well-watered plants (31.5 g/plant). There was non-significant water stress x landrace interaction (*P* > 0.05) on total biomass; however, stress + recovery treatment resulted in lower biomass for all cowpea landraces compared to well-watered plants (Figure 4-12A).

Significant differences (*P* < 0.05) were observed between cowpea landraces with respect to number of pods/plant. Morathathane and Sejwaleng had higher number of pods/plant (19.8 and 15.8 respectively) compared to Lebudu and Lehlodi (8.7 and 11.1 respectively). No significant differences (*P* > 0.05) were observed between stressed + recovery treatment and well-watered treatment on number of pods/plant. There was a non-significant water stress x landrace interaction (*P* > 0.05) on number of pods/plant (Figure 4-12B).

Significant differences (*P* < 0.05) were observed between cowpea landraces with respect to number of pod mass. Lehlodi and Sejwaleng had higher pod mass/plant after relief from stress (37.5 and 40.8 g/plant respectively) compared to Lebudu and Morathathane (24.2 and 28.3 g/plant, respectively). No significant differences (*P* > 0.05) were observed between stressed + recovery treatment and well-watered treatment on pod mass. There was a non-significant water stress x landrace interaction (*P* > 0.05) on pod mass (Figure 4-12C).

Significant differences (*P* < 0.05) were observed between cowpea landraces with respect to seed yield. Morathathane and Sejwaleng had higher seed yield (39.9 and 32.9 g/plant respectively) compared to Lebudu and Lehlodi (20 and 28.1 g/plant, respectively). No significant differences (*P* > 0.05) were observed between stressed + recovery treatment and well-watered treatment on seed yield. There was a non-significant water stress x landrace interaction (*P* > 0.05) on seed yield (Figure 4-12D).

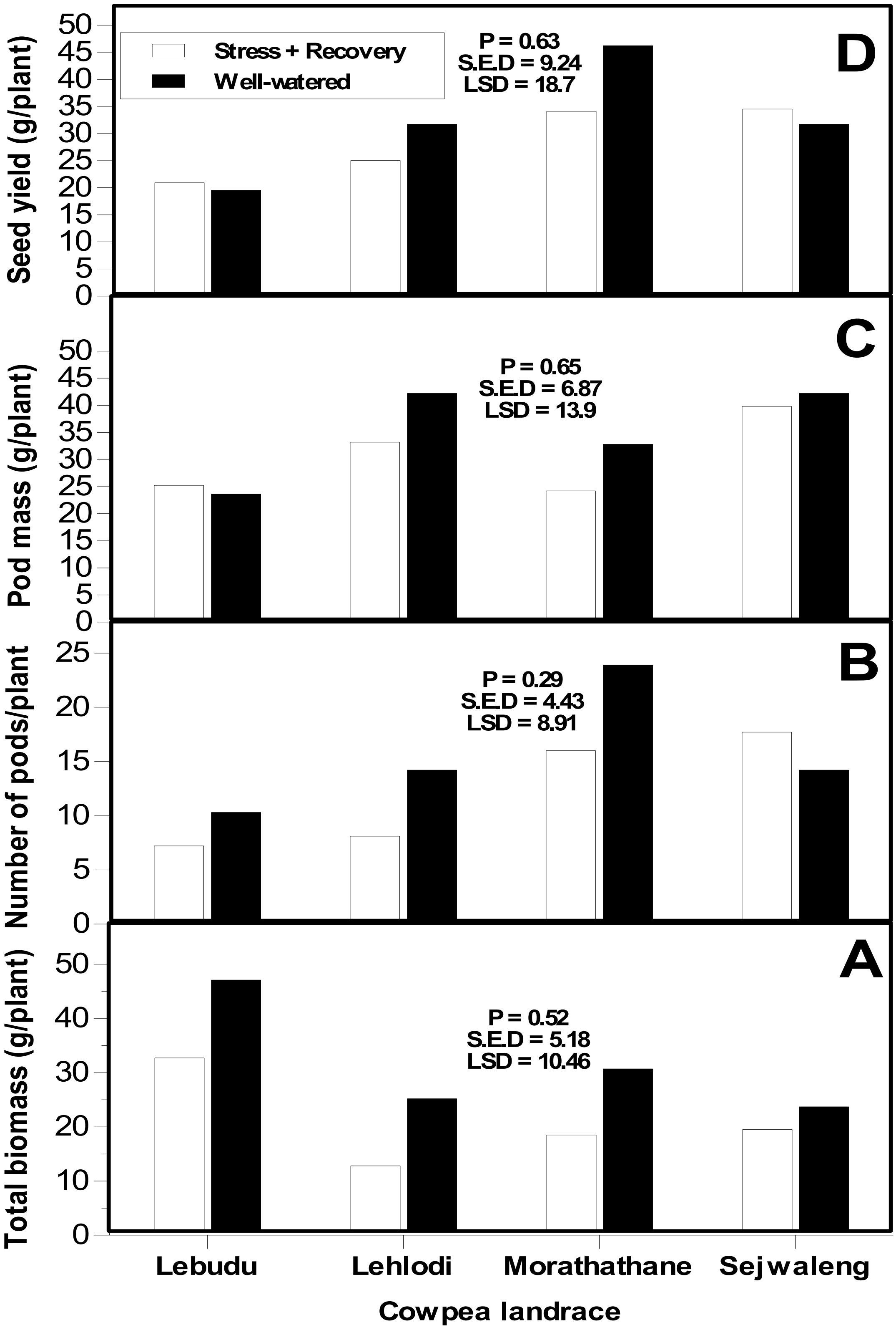

Figure 4-12. Effect of water stress at early flowering and recovery on total biomass (A), pod number (B), pod mass (C) and seed yield (D) four dual-purpose cowpea landraces.

4.4 DISCUSSION

This study investigated the physiological responses to water deficit and recovery during reproductive growth of four dual-purpose cowpea landraces. Furthermore, a secondary objective was also to examine whether physiological responses to water deficit and their recovery can be associated with yield performance. The results showed that all cowpea landraces were affected by water deficits, but their response and adaptive mechanisms were different. Leaf water potential of stressed plants declined but stressed plants of Lebudu, Lehlodi and Sejwaleng showed less negative values at maximum stress (– 1.8 MPa) as compared to a more negative leaf water potential (– 2.2 MPa) for Morathathane. These observations are in agreement with those observed by Turk and Hall (1980a) and Hiler *et al.* (1972) who reported that cowpea plants subjected to severe stress conditions maintained predawn leaf water potential values above – 1.8 and – 2.8 MPa, respectively. Hamidou *et al.* (2007a) also reported that cowpea genotypes that maintained less negative leaf water potential values between – 1.7 and – 2.4 MPa showed better drought tolerance than genotypes which maintained more negative leaf water potentials values between – 2.6 to – 3.6 MPa. Rapid recovery of leaf water potential was observed after re-watering. This suggests that cowpea landraces avoided irreversible cell damage by maintaining leaf water potential above a critical threshold which recovered rapidly after re-watering.

Relative water content declined in all cowpea landraces evaluated, but the responses were different. Sejwaleng had a slower decline in RWC and maintained high relative water content of 69% at maximum stress (12 days) as compared to Lebudu, Lehlodi and Morathathane which showed rapid decline in RWC and maintained values of 58.2, 57 and 55% respectively at maximum stress. These findings suggest that Sejwaleng responded to water deficit by maintaining higher tissue water content than other landraces. Generally, a minimum of 28-30% of water is required in plant cells for the maintenance and functioning of membrane structures (Blum, 1988). Despite differences between landraces, their maintenance of RWC above the critical level under stress is indicative of the ability of these landraces to sustain metabolic processes for their survival. Results in this study are in agreement with those by other workers that cowpea is able to maintain high RWC (Anyia and Herzog, 2004b; Anantharaju and Muthiah, 2008). Therefore, a water regulative function by maintaining leaf relative water content above a certain threshold is assumed for the cowpea landraces, as this has been described as a characteristic of cowpea under drought conditions (Likoswe and Lawn, 2008).

Further evidence of drought-avoidance mechanisms by cowpea landraces in this study was shown by stomatal closure during drought stress. The combination of water stress, high temperatures (average day T = 36.2°C) and low air relative humidity (average RH during the day = 47%) probably led to stomatal closure in all cowpea landraces (Figure 4-2). Dehydration avoidance by closing stomata is one of the most important mechanisms used by cowpea to survive severe drought stress (Bala Subramanian and Maheswari, 1992; Anyia and Herzog, 2004a, 2004b; Souza *et al.*, 2004). Stomata of all cowpea landraces were closed for the entire stress duration and recovered after re-watering. Results in this study are in agreement with Singh and Raja Reddy (2011) and Hamidou *et al.* (2007a) who reported a decrease in stomatal conductance to zero under severe water stress conditions. Stomatal closure probably reduced transpiration rate (Souza *et al.*, 2004; Galle *et al.*, 2007; Hamidou *et al.*, 2007a) which could have resulted in maintenance of leaf water potential and relative water content of cowpea landraces above a critical threshold (Bala Subramanian and Maheswari, 1992; Souza *et al.*, 2004). Upon re-watering, stomatal conductance of plant subjected to water deficits recovered and reached values close to or higher than those of well-watered plants (Figure 4-8). Increased stomatal conductance after relief from stress indicates increased carbon gain by cowpea landraces (Flexas *et al.*, 2006). These further suggest an increase in the rate of photosynthesis as carbon balance of a plant during water stress and recovery may depend as much on the velocity and degree of photosynthetic recovery, as it depends on the degree and velocity of photosynthesis decline during water depletion (Bala Subramanian and Maheswari, 1992; Flexas *et al.*, 2006). The results in this study therefore suggest that cowpea landraces tolerate water deficits by stomatal closure to probably reduce transpiration rates and maintain high tissue water content.

The reduction in chlorophyll content observed among the landraces in this study agrees with those by Singh and Raja Reddy (2011) who made similar observations in cowpea. Morathathane and Lehlodi showed the highest reduction in chlorophyll content as compared to Lebudu and Sejwaleng. Reduction in chlorophyll content in this study might be regarded as a drought response mechanism associated with minimization of light absorption by chloroplasts (Pastenes *et al.*, 2005; Manivannan *et al.*, 2007) thus, enhancing photo-protection under severe water stress, as has been proposed for stressed beech trees and rosemary plants (Munne-Bosch and Alegre, 2000; Gallé and Feller, 2007). On the contrary, chlorophyll maintenance has been shown to be essential for photosynthesis under drought stress (Chandrasekar *et al.*, 2000). Although there are contradicting arguments about whether higher chlorophyll content (i.e., stay green trait) contributes to yield under drought conditions or not (Thomas and Smart, 1993; Thomas and Howarth, 2000), many studies indicated that stay-green is associated with improved yield and transpiration efficiency under water-limited conditions in sorghum and maize (Borell *et al.*, 2000b; Borras *et al.*, 2003). Maintenance of higher chlorophyll content by landraces Lebudu and Sejwaleng and slow decline suggest that they are more drought tolerant as compared to Lehlodi and Morathathane. These observations agree with Chandrasekar *et al.* (2000) who reported that drought-tolerant wheat cultivar showed lower reduction in chlorophyll content than susceptible one. Also, findings by Li *et al.* (2006) showed that drought tolerant barley cultivars showed the least decline in chlorophyll content as compared to drought-sensitive genotypes which showed the highest reduction in chlorophyll content. Therefore, maintaining of higher chlorophyll content for a longer period of time is one of the strategies for increasing crop production, particularly under water-limited conditions (Guo *et al.*, 2008). These results further suggest that Lebudu and Sejwaleng which showed a lesser decline in chlorophyll content may perform better with respect to yield (biomass and seed yield) under drought conditions than Morathathane and Lehlodi (Figure 4-6).

Chlorophyll *a* increased for all landraces during drought stress. Morathathane showed the highest increase in chlorophyll a than other landraces. An increase in chlorophyll a has been reported in drought-tolerant maize and triticale respectively (Efeoglu *et al.*, 2009; Hura *et al.*, 2009a). An increase in chlorophyll *a* suggests that water stress did not cause a loss of photosynthetic reaction centres (PS I and PS II) of cowpea landraces (Efeoglu *et al.*, 2009).

Plant contains substantial amounts of carotenoids that serve as non-enzymatic antioxidants and scavenge reactive oxygen species (Jung *et al.*, 2000). Carotenoids are responsible for scavenging singlet oxygen; hence their high content has been suggested to be a measure of drought tolerance (Chandrasekar *et al.*, 2000). In this study carotene content was reduced among the four landraces. However, there were no significant differences in reduction between stressed and well-watered plants (4 μg/g dry weight). The lack of significant differences in carotenoid content among the four cowpea landraces is inconsistent with reports by Singh, Raja and Reddy (year) who observed large reductions carotenoid content in cowpea and suggested that this could be due to damage by oxidative stress. A low concentration of carotenoids could be indicative of a high level of drought tolerance in the four cowpea landraces used in this study. Similar observations have been alluded to in wheat (Chandrasekar *et al.*, 2000). Contrastingly, Hura *et al.* (2009a) reported an increase in carotenoid content in drought-tolerant triticale. The association between the production of anti-oxidants such as carotenoid during stress in crop plants and the link to drought tolerance is not clear as evidenced by the reports in the literature and need further investigation.

Acclimation to water stress is generally associated with higher antioxidant capacity, which keeps reactive oxygen species at lower levels (Smirnoff, 1998). Total antioxidant capacity increased with increasing drought stress intensity for all cowpea landraces. It has been reported that drought-tolerant varieties have a better capacity to protect themselves from drought-induced oxidative stress by maintaining high antioxidant molecules under stress conditions (Turkan *et al.*, 2005; D'arcy-Lameta *et al.*, 2006; Brou *et al.*, 2007; Nair *et al.*, 2008; Lu *et al.*, 2010). This suggests that Lebudu, Lehlodi, Morathathane and Sejwaleng may possess an efficient antioxidant system to tolerate drought stress. The increase in TOAC correlates to a certain extant to high levels of carotenoids, phenols and proline for cowpea landraces.

Maximum quantum efficiency of PS II (*Fv/Fm*) is related to photosynthetic efficiency and results in this study showed that photosynthetic apparatus were not affected during drought stress. Generally, a decline in *Fv/Fm* is a good indicator of photoinhibitory damage when plants are subjected to water and heat stress (Havaux, 1992; Angelopoulous *et al.*, 1996). Tolerance to water stress associated with high light and temperature, and the absence of permanent photoinhibition is an indication of tolerance to water stress (Epron *et al.,* 1992). The stability of *Fv/Fm* confirms the high PS II resistance to dehydration (Havaux, 1992) and suggests cowpea landraces protected their photosynthetic apparatus during water stress. These results agrees with Santos *et al.* (2009) who showed that water stress caused no photoinhibition, as both water-stressed and well watered plants had *Fv/Fm* value of 0.725 at the maximum water deficit. It has been indicated that the photosynthetic apparatus of PS II are relatively tolerant of desiccation and that damage occurs at very low leaf relative water content of 40% or less (Blum, 1996). Lethal relative water content or leaf water potential at which leaves die is an expression of the extent plants are able to withstand dessication without dying (Ludlow and Muchow, 1990). The lethal values can be determined only when 50% of the leaves of the plant are dead, or when 50% of the surface area of a leaf is dead, or when there is only one leaf on a plant subjected to slow drying cycle (Ludlow and Muchow, 1990). In this study the stress treatment was very severe and reduced the number of leaves to about 95% after 12 days without water (Figure 4-5), with no impairment of photosystem II. The maintenance of RWC above 50% was probably due to stomatal closure and leaf shedding which could have reduced transpiration rates (Bala Subramanian and Maheswari, 1992), thus protecting the photosynthetic apparatus. The resistance of photosynthetic apparatus under drought for cowpea landraces in this study could have been an important drought tolerance mechanism for photoinhibition protection under drought stress as reported by several workers (Souza *et al.*, 2004; Hamidou *et al.*, 2007b; Santos *et al.*, 2009; Singh and Raja Reddy, 2011).

In this study, Lebudu, Lehlodi and Morathathane showed higher proline accumulation during water deficits as compared to Sejwaleng which showed slow proline accumulation. The slow accumulation of proline for Sejwaleng is attributed to its high relative water content (~ 69%) than Lebudu, Lehlodi and Morathathane which showed high proline accumulation at low relative water content. Proline accumulation in this study correlated with changes in relative water content ($r^2 = 0.82$) and leaf water potential ($r^2 = 0.83$) confirming that proline accumulation was a consequence of declining relative water content and leaf water potential. These observations are in agreement with work by other researchers who reported that proline accumulation under water stress conditions correlated to changes in leaf relative water content and water potential (Chiulele and Agenbag, 2004; Reddy *et al.*, 2004; Rampino *et al.*, 2006). High accumulation of proline suggests a possible high stress tolerance mechanism for Lebudu, Lehlodi and Morathathane as compared to Sejwaleng. Results in this study agrees with work by other researchers who reported high proline content in drought tolerant cowpea cultivars (Chiulele and Agenbag, 2004; Anantharaju and Muthiah, 2008). The synthesis of proline under drought stress probably helped sustain cell and tissue activity during drought stress (Serraj and Sinclair, 2002; Souza *et al.*, 2004; Efeoglu *et al.*, 2009). Proline accumulation in cowpea landraces could have also reduced the photo damage in the thylakoid membranes by scavenging and/or reducing the production of $^1O_2$ (Reddy *et al.*, 2004). However, despite its known role in osmotic adjustment, several authors have argued that increased proline accumulation in cowpea does not contribute to osmotic adjustment but its accumulation is a symptom of "injury" rather, and may not necessarily indicate drought tolerance (Campos *et al.*, 1999; Slabbert *et al.*, 2004; Souza *et al.*, 2004; Singh and Raja Reddy, 2011). It is difficult to agree with the symptom of "injury" theory because accumulation of osmolytes including proline results in a decrease of the cell osmotic potential which then lower water potentials and allow additional water to be extracted from dry soils under water stress conditions (Ludlow and Muchow, 1990; Ramanjulu and Sudhakar, 2000). Also, genetic engineering for enhanced synthesis of osmoprotectants including proline is reported to contribute to drought tolerance. For example, transgenic soybean plants overexpressing the *Arabidopsis* Δ 1-pyrroline-5- carboxylate synthase gene (P5CR), one of the key enzyme involved in proline synthesis (Ramanjulu and Sudhakar, 2000), showed greater tolerance to drought stress due to increased free proline levels, RWC and a reduced level of reactive oxygen species, particularly hydrogen peroxide (de Ronde *et al.*, 2004; Kocsy *et al.*, 2005).

Increase of phenolic compounds during drought stress has been proposed as one of the protective mechanisms in response to water deficit (Hura *et al.*, 2009b). In this study, no significant differences between stressed and well-watered plants with regards to phenolic compounds were observed. Generally, both stressed and well-watered plants showed an increase in phenolic compounds. The increase in phenols in well-watered plants could have been due to high temperatures and light intensity while that of stressed plants could have been due to a combination of water deficits, high light intensity and temperature in the glasshouse during the study (Figure 3-3). Average temperature and light intensity in the tunnel were 36.2°C and 1521 µMol photons m^{-2} s^{-1} respectively. Tesfay and Modi (2013) reported an increase in phenolic compounds under high temperature stress in Moringa seedlings. The authors observed the highest accumulation of total phenolics at 35/18°C followed by 30/15°C and 25/12°C. A thermoprotection mechanism under high temperature by increased phenolic compounds was suggested. Studies in tomato also showed that heat stress (25 and 35°C) caused an accumulation of total phenols (Rivero *et al.*, 2001). It has been shown that phenolic compounds present in leaf tissues can protect the deeper situated mesophyll, by absorbing light reaching the leaf and transforming it into a blue fluorescence which is less destructive to the cellular structures of the leaf, including the photosynthetic apparatus (Bilger *et al.*, 2001). Phenolic compounds can, in this way, function as photoprotectors reducing damage of photosynthetic apparatus during conditions of water deficit in leaves (Hura *et al.*, 2008; Hura *et al.*, 2012). Therefore, this function makes it possible for them to play the role of protective filters, so preventing possible injuries to the photosynthetic apparatus which is more sensitive to radiation, including UV, during the water deficit (Hura *et al.*, 2007; Hura *et al.*, 2008). Therefore, increased phenolic compounds could have played a photoprotective and thermoprotective role in cowpea landraces under both stressed and well-watered conditions.

However, despite the lack of significant differences, increased levels of total phenols were observed for Lebudu, Lehlodi and Sejwaleng while a decline was observed for Morathathane. Morathathane and Lebudu showed a decline in free phenols whereas Sejwaleng and Lehlodi showed an increase. Landraces, Lebudu, Lehlodi and Lebudu showed an increase in membrane-bound phenols while Morathathane showed a decline. The decline in total, free and membrane-bound phenols for Morathathane suggest that phenols-based drought tolerant mechanism is not present in this landrace while an increase of either total, free and membrane-bound phenols for Lebudu, Lehlodi and Sejwaleng suggests drought-tolerance. Results in this study agree with findings by Chakraborty *et al.* (2002) and Chakraborty and Pradhan (2012) who reported drought stress-induced accumulation of phenols in tolerant cultivars of tea. Results also agree with findings by Hura *et al.* (2009b) who reported an increase in total and membrane-bound phenolics in triticale. In their study, an increase in the content of membrane-bound and total phenolics was shown in the variety that was drought resistant.

Reduced total biomass in all cowpea landraces despite adequate moisture after relief from water stress at the reproductive phase disagrees with findings by Anyia and Herzog (2004a) who observed an increase in dry matter among stressed genotypes, with stressed plants showing higher gain than well-watered plants after re-watering. This was associated with increased availability of assimilates due to enhanced green leaf area duration after stress relief. Lehlodi, Morathathane and Sejwaleng showed the highest reduction in biomass while Lebudu showed the highest gain after stress relief. Probably Lebudu had the highest compensatory growth after relief from water stress compared to Lehlodi, Morathathane and Sejwaleng as indicated by the highest biomass production at maturity. Results in this study are in agreement with Anyia and Herzog (2004a) who observed that cowpea cultivars UCR

1340, UCR 328 and Lagreen showed the highest growth rates after drought relief and produced the highest biomass at maturity than other cultivars with relatively low compensatory growth. The reduction in biomass production by cowpea landraces in this study was probably due to stomatal closure. Stomatal closure has been shown to decrease CO_2 fixation which further caused a decline in photosynthesis (Hamidou *et al.*, 2007a) which further reduces yield (Mitra, 2001). Furthermore, an increase in leaf senescence and abscission of older leaves coupled with reduced growth and expansion of new leaves observed in this study could have also contributed to loss in biomass after relief from stress as also observed by Anyia and Herzog (2004b, 2004a). This further shows that the shedding of leaves, reduced growth and decreased gas exchange and recovery of gas exchange and plant water status (e.g. leaf water potential and relative water content) after relief from drought stress which led to resumption of vegetative and reproductive growth were successful survival strategies to cope with terminal drought by these indeterminate cowpea landraces (Anyia and Herzog, 2004a). These mechanisms explains why Limpopo farmers are able to harvest relatively reasonably higher leaf and grain yield from these landraces even when planted under field conditions where severe drought conditions are prevalent.

Pod mass was greatly reduced by water stress despite the availability of sufficient moisture after relief from stress for Lebudu and Lehlodi compared to Sejwaleng and Morathathane. Reduction in pod mass in the stress + recovery treatment indicated assimilate limitation after relief from water stress as observed by Bala Subramanian and Maheswari (1992) in cowpea. Pod number and seed yield were reduced by water stress in all cowpea landraces, though, this was not statistically significant. Pod number is the most sensitive yield component to water stress (Hamidou *et al.*, 2007b; Abayomi and Abidoye, 2009). Lehlodi and Lebudu showed the most reduction in pod number compared to Sejwaleng and Morathathane, though; this was not statistically significant. This may be attributed to new growth and flushes of flowers which formed after relief from stress. It has been reported that cowpea has the ability to produce new flushes of flowers when stress is relieved late in the life cycle (Lawn, 1982). Also, the indeterminate growth habit of this landraces could have also extended their reproductive period as observed by compensatory growth after relief from stress. According to Ehlers and Hall (1997), an indeterminate growth habit makes possible a longer reproductive period that contributes to drought adaptation because during drought seasons, water stress do not occur during the entire reproductive period, and such cultivars can resume vegetative and reproductive growth more quickly once moisture stress is alleviated. Probably compensatory growth after growth counteracted the adverse effects of water stress at flowering on final growth and productivity although stressed plants showed reduced biomass production (Bala Subramanian and Maheswari, 1992).

Sinclair *et al*. (1987) reported that the high yield tendency of cowpea under water limited conditions is closely related to the ontogenetic flexibility of the crop than to any physiological responses. The present study showed that physiological responses during water deficits may not necessarily be related to a particular landrace to recover from drought stress. However, compensatory growth after relief from stress due to indeterminacy in growth habit by these cowpea landraces may be related to their ability to recover from stress and produce leaf and seed yield. The current results are in agreement with Bala Subramanian and Maheswari (1992) who reported that whole plant physiological responses under adequate moisture after a period of water deficits such as increase in leaf area, extended green leaf area duration, shift in dry matter partitioning and increase in the number of pods are related to cowpea productivity. Results in this study also are in agreement with Anyia and Herzog (2004a) who showed that growth before and after a terminal drought appears to be more important in determining the final biomass yield than plant responses during drought. These partially compensating growth responses probably reduce adverse effects of water deficits on growth and ensure reasonable productivity of these cowpea landraces rather than physiological responses during water deficits (Bala Subramanian and Maheswari, 1992; Anyia and Herzog, 2004a).

CONCLUSION

Significant variation exists among cowpea landraces with respect to their physiological responses to water stress and recovery with respect to plant water status, osmolyte accumulation, pigment content, total antioxidant capacity and photosynthetic capacity; however the variation in the responses could not be associated with the ability of a particular land race to recover from drought stress. Furthermore the variation in physiological responses of the cowpea land races in this study could not be associated with yield performance.

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5. GENERAL DISCUSSION, CONCLUSIONS AND FUTURE RESEARCH

5.1 Introduction

The development of more drought-tolerant cultivars is a future goal necessary to alleviate food security threats. However, this requires comprehensive exploration of the many potential genetic resources and in-depth understanding of the mechanisms and responses to drought stress (Rampino *et al.*, 2006). Drought stress is currently the most important abiotic factor responsible for low yields in cowpea. In Africa, cowpea is commonly grown in semi-arid regions, where drought stress occurs due to intermittent rainfall. Cowpea makes a valuable contribution to the diet of many people around the world. Its fresh leaves, immature pods and dry grains are consumed (Ehlers and Hall, 1997; Rahman *et al.*, 2008). Therefore, drought stress occurring at any time during growth and development may hamper the crop's productivity. Identification of drought-tolerant cowpea cultivars able to survive early, mid or late season drought is needed in order to obtain higher and more stable yields. The aim of the study was to evaluate whether there is any variation with respect to morphological and physiological traits among local cowpea landraces, and how this may relate to performance under water limited conditions. In order to achieve this, several objectives were set out: Firstly, to examine whether there are differences in leaf greenness, stem greenness, branch greenness, leaf number and senescence amongst the four cowpea landraces when subjected to water deficits during vegetative and reproductive growth stages; secondly to examine whether there are differences among the landraces with respect to changes in plant water status (i.e. leaf water potential and relative water content), osmolyte accumulation, pigment content, total antioxidant capacity and chlorophyll fluorescence when subjected to water deficits during the reproductive growth stage; and thirdly whether the differences if any in objective 2 relate to yield performance after stress relief.

Chapter 3 investigated the morphological responses of four cowpea landraces to drought stress during two phenological stages: vegetative and reproductive. The results showed that all cowpea landraces had the ability to tolerate drought stress during the vegetative growth stage. However, different responses to drought stress were observed. Lebudu, Lehlodi and Sejwaleng maintained a greener stem than Morathathane. Lebudu delayed leaf senescence, that is, maintained green leaf area more than other landraces during the vegetative stage. During the reproductive growth stage, all landraces demonstrated an ability to tolerate drought stress, however, significant differences in ability to tolerate terminal drought stress were observed. The use of drought related phenotypes associated with drought tolerance including ability of leaves and stem to stay green have been reported in cowpea (Muchero *et al.*, 2008; Agbicodo, 2009; Muchero *et al.*, 2009; Muchero *et al.*, 2010). Delayed leaf senescence and stem greenness enhances plant survival and recovery during drought stress (Muchero *et al.*, 2008). It has been suggested that cowpea cultivars that will combine the ability to withstand mid-season and terminal droughts consisting of early flowering and an indeterminate growth habit with the stay-green trait should exhibit drought adaptation and yield stability in many environments (Ehlers and Hall, 1997). As observed for Lebudu in this study, we also propose that a cultivar with a spreading and indeterminate growth habit with the ability to delay leaf senescence, maintain stem and branches greenness may also have a strong ability to tolerate mid and late season drought stress or long-term drought stress encountered in semi-arid regions.

Breeding for drought tolerance requires knowledge on the physiological mechanisms involved in drought tolerance (Subbarao *et al.*, 1995). Understanding the variability in the physiological responses to drought among different cowpea genotypes may help identify underlying regulatory processes at the genetic level and how these impact yields and this could be important for the identification of genes that may determine responses to drought. In chapter 4, variability in physiological responses of cowpea landraces to terminal drought and recovery and how this may relate to yield performance was investigated. Adaptive mechanisms to limit physiological damage and restore metabolic processes and maintain optimal plant function under conditions of water stress included the maintenance of higher tissue water content, stomatal closure, reduction in chlorophyll content and increase in chlorophyll a, increase in total antioxidant capacity, proline content and phenolics. The ability to resume normal physiological function and recover after drought relief is critical. Rapid recovery of various physiological mechanisms was observed in all cowpea landraces after re-watering. This was also evident by resumption of growth from apical meristems. According to Slabbert *et al.* (2004) recovery from severe drought stress is a physiological advantage in areas of unpredictable rainfall patterns. The results from this study show that these cowpea landraces have different responses after relief from stress. After relief from drought, all cowpea landraces compensated for reduced biomass during drought and rapidly formed new leaves and flowers and produced sufficient yield at maturity. Lebudu accumulated more biomass than Lehlodi, Sejwaleng and Morathathane while compromising seed yield whereas the latter three landraces produced more seed yield and compromised biomass production. These suggest that Lebudu allocated resources towards biomass production while Lehlodi, Sejwaleng and Morathathane allocated resources towards seed development. Though these cowpea landraces are often grown for dual-purposes both for grain and leaf biomass, the present study suggest that under field conditions where these landraces are often grown, Lebudu may be more suited for leaf biomass while Lehlodi, Sejwaleng and Morathathane may be more suited for grain production.

In many drought stress studies, pot experiments have been widely used to screen cultivar response to water deficits (Anyia and Herzog, 2004a; Slabbert *et al.*, 2004; Abayomi and Abidoye, 2009). Similarly in this study, only pot experiments were conducted to study the morphological and physiological responses of cowpea land races to water deficits and the effect on yield performance. However, there is need for further research to validate findings from this study under field conditions.

5.2 Recommendations for future research

The current work has shown that cowpea landraces can potentially be used to enhance drought tolerance in existing cultivars. However, there are some major gaps which still need to be researched. Studies on genetic control of physiological mechanisms involved in drought tolerance of these cowpea landraces should be carried out in order to provide insights into regulatory processes that control their adaptation of these cowpea landraces to water deficits. More concerted efforts are required to identify, isolate and combine such genes for higher levels of drought tolerance.

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APPENDICES

Appendix 1. Physical properties of the soil used in the study (Odindo, 2007).

Parameters measured	Value
Bulk density (kg m^{-3})	1562
% gravimetric water content at near saturation (-1KPa)	23.73
% gravimetric water content at field capacity (-33KPa)	17.47
% gravimetric water content at permanent wilting point (-1500KPa)	12.26
Clay (%)	31.86
Silt (%)	48.94
Coarse sand (%)	10.35
Medium sand (%)	2.2
Fine sand (%)	2.5
Very fine (%)	4.15
Total porosity (%)	41.06
Plant available water (mm m^{-1})	114

Appendix 2. Chemical physical properties of the soil used in the study.

Parameters measured	Value
Sample density g/mL	1
P (mg/L)	20
K (mg/L)	227
Ca (mg/L)	1613
Mg (mg/L)	443
Exchangeable acidity (cmol/L)	0.08
Total cations (cmol/L)	12.36
Acid saturation %	1
pH (KCL)	5.16
Zn (mg/L)	7.2
Mn (mg/L)	19
Cu (mg/L)	8.1
Organic carbon %	1.7
Nitrogen %	0.23