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MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES  
OF COWPEA (*Vigna unguiculata* (L) Walp.) CULTIVARS TO  
INDUCED WATER STRESS AND PHOSPHORUS  
NUTRITION

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by  
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## DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any University for a degree

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## ABSTRACT

Cowpeas are produced under low and irregular rainfall in most of arid and semi-arid areas of sub-Saharan Africa. Growth and yield are therefore reduced due to the occurrence of water stress during the growing season. Knowledge of the responses and adaptive mechanisms of cowpeas to water stress may help to improve the management practices for these areas. Therefore, three glasshouse experiments were conducted at Welgevallen Experimental Farm of the University of Stellenbosch to test the responses of two cowpea cultivars to water stress. In the first experiment, physiological responses were used to identify those physiological parameters, which can be used to distinguish between drought tolerant and susceptible cowpea cultivars. In the second experiment, some of the identified physiological parameters together with some morphological growth responses, yield and grain protein content of the same two cowpea cultivars were used to identify which is the more tolerant cultivar. In the third experiment, the hypothesis that increased phosphorus supply may improve the tolerance of cowpea plants to water stress and their ability of recover from the stress was tested. The results showed that water stress affected water relations, morphological growth parameters, yield and grain protein content, but increasing P supply reduced the effect of water stress and promoted more rapid recovery after re-watering. Water relations were affected by water stress because it reduced relative water content, which resulted in reduced water potential and increased leaf diffusive resistance and proline accumulation. Morphological growth responses and yields were affected because water stress reduced the leaf area, which resulted in reduced biomass production and seed yield. Lower leaf area under water stress was the result of the reduced number of leaves and leaf expansion rate, but the number of leaves was the most important parameter. Reduced seed yield was due to reduced number of pods. The responses of the two cultivars tested were different. AB Wit, which performed better under well-watered conditions was more affected by water stress due to its larger leaf area that resulted in excessive water loss by transpiration. ACH14 was more drought tolerant than AB Wit due to a combination of a more rapid stomatal closure and proline accumulation, which induced osmotic adjustment, and which in turn helped to maintain higher water potentials. The increased P supply reduced the effect of the water stress. High-P level plants showed higher root growth, which resulted in more water uptake and larger leaf area during the water stress period, and after re-watering these plants recovered

more rapidly. The more rapid recovery from stress was the result of enhanced root growth and leaf expansion rate and most probably due to increased water uptake. High-P level plants also showed more rapid leaf appearance and plant growth at earlier stages compared to the low-P level plants.

## UITTREKSEL

Akkerbone word onder toestande van lae en wisselvallige reënval in baie ariede en semi-ariëde gebiede van Afrika verbou. In hierdie gebiede word groei en produksie dikwels beperk deur water tekorte gedurende die groei seisoen. Kennis van reaksies en aanpassingsmeganismes van akkerbone teenoor water tekorte mag dus help om produksietegnieke in bogenoemde gebiede te verbeter. Om hierdie rede is drie glashuiseksperimente onder gekontroleerde toestande op die Welgevallen Proefplaas van die Universiteit van Stellenbosch uitgevoer. In die eerste eksperiment is fisiologiese reaksies van twee cultivars gebruik om eienskappe te identifiseer wat gebruik kan word om tussen droogteweerstandbiedende en droogte gevoelige cultivars te onderskei. In die tweede eksperiment is sommige van die geïdentifiseerde eienskappe asook morfologiese groei, opbrengs en kwaliteitsreaksies van dieselfde twee cultivars gebruik om die meer droogte weerstandbiedende cultivar te identifiseer. In die derde eksperiment is die hipotese dat P-bemesting die droogteweerstandbiedendheid teen en herstelvermoë na droogte kan verbeter, getoets. Die resultate toon dat water tekorte beide plant-waterverhoudings, morfologiese eienskappe asook opbrengs en proteïëinhoud beïnvloed, maar dat hoë P-peile die invloed van water tekorte verminder en herstelvermoë na die droogte verbeter. Plant-waterverhoudings is beïnvloed omdat water tekorte relatiewe waterinhoud van plante verlaag wat aanleiding gee tot verlaagde plantwaterpotensiale, verhoogde huidmondjie weerstand en 'n toename in prolien inhoud.

Morfologiese eienskappe en opbrengs is benadeel weens 'n verlaging in blaaroppervlakte wat fotosintetiese vermoë en gevolglik ook biomassa-produksie en saad opbrengs benadeel. Verlaagde blaaroppervlakte tydens water tekorte was hoofsaaklik die gevolg van 'n vermindering in aantal blare, terwyl verlaagde saadopbrengs grootliks die resultaat van 'n vermindering in aantal peule was.

Die cultivar AB Wit wat die hoogste opbrengs onder gunstige groeitoestande gelewer het, is die meeste beïnvloed deur water tekorte omdat die welige blaargroei van hierdie cultivar, laukse waterverbruik en groter transpirasie verliese veroorsaak het. Die cultivar ACH14 daarteenoor het waterverliese beperk deurdat die huidmondjies vinniger gesluit het en verhoogde prolien-inhoude, osmotiese aanpassings veroorsaak het. Dit het gehelp

om waterpotensiale instand te hou. Hierdie cultivar was gevolglik meer droogte weerstandbiedend as AB Wit.

Hoë vlakke van P-bemesting het die effek van water tekorte verminder weens verbeterde wortelgroei. Dit het wateropname gedurende en na die periode van water stremming verbeter sodat plante vinniger herstel het na die droë periode. Plante wat by hoë P-peile gegroei is het ook 'n verhoogde blaarverskyningstempo en 'n toename in groei tydens die vroeë ontwikkelingsstadiums getoon.

## ABSTRACT

O feijão nhemba é produzido em regime de sequeiro na maior parte das regiões áridas e semi-áridas da África sub-Sahariana. Portanto, o seu crescimento e rendimento são reduzidos devido à ocorrência do stress hídrico. O conhecimento das respostas e dos mecanismos de adaptação da cultura às condições de limitada disponibilidade de água pode contribuir para melhorar algumas práticas culturais em áreas sujeitas à seca. Por isso, três experiências foram conduzidas em Welgevallen, estação experimental da Universidade de Stellenbosch, com vista a testar a resposta de dois cultivares do feijão nhemba ao stress hídrico. A primeira experiência foi conduzida com o objectivo de avaliar as respostas fisiológicas (relação de água da planta e mudanças osmóticas) de dois cultivares do feijão nhemba ao stress hídrico e usá-las para identificar dentre os parâmetros fisiológicos (potencial de água da planta, resistência estomática, conteúdo relativo de água, conteúdo da prolina e estabilidade da membrana da célula) aqueles que podem ser usados para distinguir o cultivar mais tolerante ao stress hídrico. Na segunda experiência, os parâmetros fisiológicos identificados juntamente com alguns parâmetros morfológicos, o rendimento e o conteúdo proteico do grão, foram usados para identificar o cultivar mais tolerante ao stress hídrico. Na terceira experiência, a hipótese de que a adição de elevados níveis de fósforo pode ajudar a melhorar a tolerância do feijão nhemba ao stress hídrico foi testada. Os resultados mostraram claramente que o stress hídrico afectou tanto a relação de água da planta e mudanças osmóticas como o crescimento, o rendimento e o conteúdo proteico do grão, mas a adição de fósforo reduziu o efeito do stress hídrico e contribuiu para a rápida recuperação do stress. A relação de água da planta e mudanças osmóticas foram afectadas pelo stress hídrico porque este reduziu o conteúdo relativo de água que resultou na redução do potencial de água e no aumento da resistência estomática e do conteúdo da prolina. O crescimento e o rendimento foram afectados porque o stress hídrico reduziu a área foliar, que resultou na pouca produção de biomassa e do rendimento do grão. A reduzida área foliar em condições de stress hídrico foi devido ao reduzido número de folhas e a reduzida taxa de expansão foliar, mas o número de folhas foi o factor que mais contribuiu para reduzir a área foliar. O baixo rendimento de grão foi resultado dum menor número de vagens por planta. Os dois cultivares testados responderam diferentemente ao stress hídrico. O cultivar Akkerbone Bechuana Wit (AB Wit), que mostrou um bom performance em condições de boa disponibilidade de água, foi o mais

susceptível ao stress hídrico devido à sua maior área foliar que contribuiu para uma excessiva perda de água por transpiração. Akkerbone CH 14 (ACH14), foi o mais tolerante ao stress hídrico do que o AB Wit, devido ao rápido fecho dos estomas e à acumulação da prolina, que induziu ao ajustamento osmótico e contribuiu para manter alto o potencial da água. A adição de elevados níveis de fósforo reduziu o efeito do stress hídrico. Plantas que cresceram em elevados níveis de fósforo tiveram um elevado crescimento de raízes, o que resultou em maior absorção de água e maior área foliar durante o período do stress hídrico. Depois do restabelecimento de água, plantas que cresceram em elevados níveis de fósforo, mostraram uma rápida recuperação do stress. A rápida recuperação do stress foi o resultado dum maior crescimento de raízes e maior taxa de expansão foliar e muito provavelmente devido à maior absorção de água. As plantas que cresceram em elevados níveis de fósforo, mostraram também nos primeiros estágios de crescimento, um rápido crescimento e aparecimento de folhas do que as plantas que cresceram em baixos níveis de fósforo.



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Dedico este trabalho aos meus pais Marcos Chiulele e Virgínia Jalane. Igualmente dedico  
`a Isabel.

A todos “Kanimambo”.

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## CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

### 1.1 The cowpea crop

Cowpea (*Vigna unguiculata* (L.) Walp.) is the most important food legume in the arid and semi-arid regions of the tropics and sub-tropics where water stress is its major production constraint due to low and erratic rainfall (Mai-kodomi *et al.*, 1999a). It is mainly grown for seed production, but it can also be used to obtain fresh pods and or leaves for human consumption. Cowpeas are also used to feed livestock (Nell, 1992). The cowpea grain as well as the leaves are high in protein content (Steele, Allen & Summerfield, 1985), therefore it may be regarded as a multi-purpose crop.

In Mozambique, cowpeas are one of the most important food crops, which guarantee the food security and supplement the diet with protein in rural areas. Cowpeas are produced under rainfed conditions by small-scale farmers, mostly in sandy soils, which are regularly subjected to moisture deficits. Most farmers in this country grow late maturing and photoperiod sensitive cultivars, usually intercropped with maize or cassava. The average yields of cowpeas are generally low (less than 500 kg/ha). Many factors may contribute to the low yields, namely pests, diseases and weeds, poor agricultural practices, low soil fertility such as phosphorus deficiencies, lack of good seed quality, the use of unproved landraces (traditional varieties) and the occurrence of water stress.

Water stress has been reported as a major constraint for cowpea production due to its unpredictability (Turk, Hall & Asbell, 1980; Akyeampong, 1986; Mai-kodomi *et al.*, 1999a; Mai-kodomi *et al.*, 1999b; Singh, Mai-kodomi & Terao, 1999a; Singh, Mai-kodomi & Terao, 1999b). Water stress affects seedling growth as well as the growth of reproductive and maturing stages due to changes in metabolic processes. As a result the production of biomass and seed yield are reduced (Singh *et al.*, 1999a).

In recent times the Sub-Saharan Africa region has experienced cyclic periods of drought, which affected crop production. Therefore, the development of cowpea cultivars, which are resistant to drought, may be an important factor to ensure food security in this region.

Drought resistance is a combination of adaptive mechanisms that enable the crop to grow, survive and yield satisfactorily in areas subjected to periodic water deficits (Turner, 1979). These mechanisms include drought escape, dehydration postponement (avoidance) and dehydration tolerance (Begg & Turner, 1976; Turner, 1986). Drought escape consists of shortening the growth cycle (earliness) or the development of phenological plasticity, which enables the plants to mature before soil water becomes limiting or by extending the period of flowering and reproductive phases to escape water deficits (Turner, 1986). Dehydration postponement may be obtained via the maintenance of cell turgor during periods of water stress. Maintenance of turgor is obtained by maintaining water uptake, reducing water loss or osmotic adjustment while the maintenance of cell volume under water stress is obtained by decreasing the cell elasticity (Turner, 1986). Dehydration tolerance depends on the ability of the cells to withstand mechanical injury, the ability of membranes to withstand degradation and the ability of the membranes and cytoplasm to prevent denaturation of the proteins and maintain enzyme activity during water stress conditions.

Studies on cowpeas have shown that they are drought resistant during vegetative growth stages (Singh *et al.*, 1999b), but not during the reproductive stages (Turk *et al.*, 1980). Drought resistance in cowpeas was found to be associated with drought avoidance due to drought-induced regulation of water loss (Turk & Hall, 1980a; Turk & Hall, 1980b; Mai-kodomi *et al.*, 1999b) and improved water use efficiency under drought conditions (Turk & Hall, 1980c).

These strategies may be the result of the combination of a large variety of plant morphological and physiological responses, which enable plants to avoid or tolerate water stress.

## 1.2 Morphological responses

### 1.2.1 Effect of water stress on growth and development of plants

Water stress generally affects plant growth, development, yield and quality (Gardner, Pearce & Mitchell, 1985; Hale & Orcutt, 1987). The response of crop plants to water stress is dependent on the type of crop (determinate or indeterminate), and the timing and the intensity of the water stress. There is evidence that most determinate crops are more sensitive to water stress from the time of flower initiation, during flowering, and to a lesser extent, during fruit and seed development (Begg & Turner, 1976). In indeterminate crops where these stages overlap, the situation is less clear.

Several authors have investigated the responses of crop plants to water stress at different growth stages (Wien, Littleton & Ayanaba, 1979; Turk *et al.*, 1980; Turk & Hall, 1980a; Turk & Hall, 1980b; Winkel, Renno & Payne, 1997). Most of the studies showed that water stress has a severe effect during flower initiation, flowering, fruit and seed development, but less during the vegetative growth stages. Water stress during the vegetative growth stage was reported to reduce leaf area (Turk & Hall, 1980b; Hale & Orcutt, 1987; Maiti *et al.*, 1996), stem elongation (Wien *et al.*, 1979) and above-ground biomass production (Turk & Hall, 1980b; Gardner *et al.*, 1985; Winkel *et al.*, 1997). Leaf area reduction by water stress was found to be the result of the reduction in the leaf expansion rate due to the sensitivity of cell enlargement to water stress (Hsiao, 1973; Akyeampong, 1986) or enhanced leaf senescence (Gardner *et al.*, 1985). The reduction in leaf area reduces the radiation interception (Mollier & Pellerin, 1999) and thus biomass production (Akyeampong, 1986). Because leaf area is positively correlated with seed yield (Turk & Hall, 1980b), its reduction due to water stress reduces seed yield (Akyeampong, 1986). For example, Summerfield, Huxley & Dart (1976) found that seed yield of cowpeas was reduced because of leaf area reduction by water stress during the vegetative growth stage. Water stress prior to flowering and at the beginning of flowering time was reported to delay or totally inhibited the flowering of tillers in pearl millet (Winkel *et al.*, 1997), delayed tassel initiation and pollination in maize and flowering in other crops (Gardner *et al.*, 1985; Upendra *et al.*, 2000). Similarly, stress during ripening was reported to cause early senescence and maturity (Upendra *et al.*, 2000).

The effect of stress intensity on growth and development of crop plants is also well documented (Turk & Hall, 1980b). Turk & Hall (1980b) reported that mild water stress increased earliness while severe water stress delayed the development of cowpeas. Severe water stress was also reported to cause early senescence and abscission of leaves and fruits due to accumulation of abscissic acid (ABA) (Levitt, 1980; Sanaullah & Bano, 1999; Chandrasekar, Sairam & Srivastava, 2000; Kulkarni, Prasad & Sashidhar, 2000; Asch *et al.*, 2001; Yang *et al.*, 2001).

Plant organs and physiological processes responded differently to a particular degree of water stress (Malik, Dhankar & Turner, 1979). For example, cell enlargement was reported to be more sensitive than cell division (Begg & Turner, 1976), while root growth was less affected by water stress than shoot growth, as indicated by the increase in root to shoot ratio (Wu & Cosgrove, 2000). However, there are some controversies with regard to root growth during water stress conditions. Malik *et al.* (1979) and Meyer & Ritchie (1980) reported that taproot growth of cotton was not affected by water stress while Turk & Hall (1980c); Zou *et al.* (2000) and Huang & Huang (2001) reported that root growth of both cowpeas and fescue were reduced by water stress. Younis, *et al.* (2000) reported that root elongation was less affected by water stress than stem elongation and above-ground dry mass. In general, it is believed that increased root growth under conditions of water deficiency is an important factor contributing to drought tolerance (Malik *et al.*, 1979; Sullivan & Ross, 1979; Bajji, Littus & Kinet, 2000; 2001), because plants exhibiting high rates of root growth may exploit larger volumes of the soil profile for water and nutrient uptake.

### **1.2.2 Effect of water stress on yield and yield components**

The effect of water stress on yield and yield components depend on its timing and intensity (Gardner *et al.*, 1985) as well as the duration of the stress period (Begg & Turner, 1976). Although above-ground biomass is often found to be more sensitive than the reproductive part, water stress during critical stages may have a significant effect on economic yield without any effect (or having only a small effect) on total above-ground dry mass (Begg & Turner, 1976).

The timing of water stress is important with regard to the effect on seed yield. Most studies show that water stress during flower initiation, flowering and pod filling affects the



seed yield more than the vegetative growth (Wien *et al.*, 1979; Gardner *et al.*, 1985; Turk *et al.*, 1980; Winkel *et al.*, 1997). Water stress during flower initiation and flowering time, for example, reduced the seed yield of cowpeas (Turk *et al.*, 1980; Turk & Hall, 1980a) and pearl millet (Winkel *et al.*, 1997), while a severe, but short period of water stress had no effect when occurring during the vegetative growth stage (Gardner *et al.*, 1985).

The intensity and duration of water stress are also important in determining the seed yield, because these factors may affect the length of the reproductive growth phase and the development of indeterminate crops. Hearn (1975a) showed that both watering frequency and the date of final watering, in a tropical environment with a definite dry season, influenced the length of flowering and boll set in cotton. Less frequent and earlier termination of watering reduced both the period of flowering, boll development and yield. Increases in the frequency of watering on the other hand resulted in increased seed yield of cotton (Hearn, 1975b).

The effect of water stress on yield components depends largely on the timing and intensity of the stress in relation to the development of that portion of the plant used for economic yield. It has been reported that water stress prior to ear emergence in determinate cereals, reduces the number of grains set per spikelet (Winkel *et al.*, 1997). On cowpeas water stress during flowering and pod filling reduce the number of pods per plant due to flower abscission and smaller seed dry mass (Turk *et al.*, 1980). Reduced seed dry mass, in case of high intensity water stress during seed filling, may be the result of reduced translocation of the carbohydrates to the seed.

### **1.3 Plant quality responses**

The effects of water stress on quality of yield may either be beneficial or detrimental depending on the crop. For example, water stress increases the percentage of protein of the grains such as wheat (Agenbag & De Villiers, 1995) and cowpeas (Wien *et al.*, 1979). The increase in protein content will result in an improved feeding quality of cowpea and bread baking quality of wheat. Mild stress conditions shortly before harvesting can increase the accumulation of soluble carbohydrates, a feature that increases the quality of some fruits (Begg & Turner, 1976).

## 1.4 Physiological responses

### 1.4.1 Plant water relations and osmotic changes during water stress

Water stress may be induced by withholding water or by applying a solute such as polyethylene glycol as an osmoticum. The inducement of water stress by withholding water has the advantage that the water stress that develops is similar to the water stress that develops under field conditions, but has the disadvantages that the soil water potential is not controlled and it changes as the soil water deficits develops (Kramer, 1969). In contrast the use of polyethylene glycol (PEG) as osmoticum allows easier control of the water potential, since the osmotic potential of a certain molecular weight may be determined easily, but toxicity effects attributed to the use of PEG and associated to the presence of some impurities have been reported (Lesham, 1966). Lawlor (1970) found that although toxicity effects were not due to the presence of impurities, PEG caused the leaves to die. High molecular weights of PEG may have damaged the roots and caused blockages of water movement, reduced water absorption and caused desiccation of the leaves (Lawlor, 1970). Other properties of PEG which make it less suitable for use as osmotic solute include the lowering of surface tension and increasing the viscosity of the solution (Lawlor, 1970).

Water stress develops as the result of excessive water loss, which is not replaced by root uptake (Begg & Turner, 1976; Ramanjulu & Sudhakar, 2000). This causes a decrease in water potential (Begg & Turner, 1976; Al-karaki, Clark & Sullivan, 1996; Szegletes *et al.*, 2000), relative water content (Hale & Orcutt, 1987; Naidu, Raju & Narayanan, 2001) and cell turgor. Osmotic potential decreases due to the accumulation of solutes in the root and leaf cells to adjust to the osmotic stress (Levitt, 1980; Hale & Orcutt, 1987; Al-karaki *et al.*, 1996; Szegletes *et al.*, 2000).

Water stress may be measured by the determination of the plant water content, leaf relative water content (RWC) (Noggle & Fritz, 1976; Turner, 1981; Matin, Brown & Ferguson, 1989), total leaf water potential, leaf rolling, leaf temperature, leaf thickness, stomatal resistance, photosynthetic rate, leaf permeability (Turner, 1981), proline content (Bates, Waldren & Teare, 1973; Van Heerden & De Villiers, 1996) and cell membrane stability (Sullivan & Ross, 1979).

Water content involves the measurement of the fresh weight at sampling and dry mass after oven drying. Noggle & Fritz (1976) and Turner (1986) indicated that water content is not a good indicator of water stress, since it can vary with species and previous growth conditions. The use of relative water content (RWC) as tool to measure water stress has been reported by many authors (Noggle & Fritz, 1976; Turner, 1981; Matin *et al.*, 1989). RWC expresses the water retention capability of the leaf tissue. RWC of the leaves decreases under water stress and provides an important indication of water stress. RWC has been used to differentiate between drought resistant and susceptible cultivars, since drought-resistant cultivars show higher RWC compared to drought susceptible cultivars (Schonfeld *et al.*, 1988; Matin *et al.*, 1989).

Water potential decreases with decrease in the osmotic potential of the roots as the result of water stress (Begg & Turner, 1976). Levitt (1972) and Blum (1974) have suggested that total water potential of plant tissue may be used to differentiate between drought resistant and susceptible cultivars. These studies concluded that higher total leaf water potentials in specific cultivars within populations indicated increased drought resistance.

Stomatal response, a major factor controlling plant water loss, has long been considered to be a valuable method to screen for drought tolerance. It can be easily evaluated by measuring leaf diffusive resistance (LDR). Differences in sensitivity of diffusive resistance of different crop species have been reported (Blum, 1974; Jones, 1974; Jones, 1979). Blum (1974) suggested that insensitive stomata might lead to drought avoidance under semiarid conditions whereas Jones (1974) suggested that rapid stomatal response to water stress might be a drought resistance mechanism to conserve soil water for later use and maintaining higher leaf water potential.

Proline has been reported to accumulate in a range of plant species and cultivars as response to water stress (Bates *et al.*, 1973; Van Heerden & De Villiers, 1996; Ain-Lhout *et al.*, 2001; Naidu *et al.*, 2001) due to reduction in water potential (Handa *et al.*, 1986; Ober & Sharp, 1994; Bussis & Heineke, 1998) or relative water content (Rajagopal, Balasubramanian & Sinha, 1977; Argandona & Pahlich, 1991). Proline accumulation seems to aid in drought tolerance providing energy and nitrogen (Gardner *et al.*, 1985) after stress and stabilizing membranes, reducing enzymes denaturation (Gardner *et al.*,

1985; Ain-Lhout *et al.*, 2001) and acting as a neutral osmoticum. Proline accumulation has been suggested as a criterion for selecting drought tolerant crops (Singh, Aspinall & Paleg, 1972), since proline accumulation was found to be positively correlated with drought tolerance in wheat (Van Heerden & De Villiers, 1996) and mulberry (Ramanjulu & Sudhakar, 2000). However, Lawlor (1979) reported that proline accumulates only with severe stress. This indicates that proline accumulation may be an ineffective indicator during the initial stages of stress development.

Cell membrane stability, measured by electrical leakage, has been reported as an important contributor to drought tolerance in plants (Franca *et al.*, 2000; Nyachiro & Briggs, 2000). Levitt, (1980) suggested that tolerance to all stresses, including dehydration, depends on the maintenance of the membranes in an undenatured and unregated form or at least on the repair of lipid structure to its bilayer state after the initial damage has occurred. Premanchendra *et al.*, (1992) reported that cell membrane stability increased with exposure to water stress, indicating that the plants acclimate to water stress.

### **1.5 Effect of nutrient supply on growth, development and yield of plants grown under water deficit conditions**

The uptake of several elements has been shown to decrease under water deficit conditions (Greenway & Klepper, 1969). Greenway, Hughes & Klepper (1969) showed that the uptake of phosphorus was reduced slightly when the potential of the root medium was reduced to -2 bars and decreased linearly as the potential of the root medium was reduced further. At root water potentials of -10 bars, phosphorus uptake was shown to be negligible. These results clearly suggest that reduced growth observed as a result of moderate water deficits may, in part, arise from the disruption in mineral nutrition as well as the direct effects of water deficits.

The soil nutrient status can markedly influence the water use by crops, and hence the time of onset of water stress where soil water is limited, or the strategy for irrigation where water supply is unrestricted. In situations of limited water supply, high nitrogen fertilizer application rates or wheat after a legume crop resulted in vigorous vegetative growth, which depleted the plant available soil water very rapidly and led to a lower yield than with low fertilizer application rates (Fisher & Kohn, 1966a, 1966b, 1966c; Bond,

Power & Willis, 1971). Fisher & Kohn (1966a,b) showed that the application of nitrogen increased the leaf area and evapo-transpiration in the vegetative phase and reduced the available soil water in the root zone at ear emergence. This resulted in a reduction of leaf RWC during grain filling. In indeterminate cotton, Hearn (1975b) found that in a dry tropical environment there was little advantage of nitrogen fertilization to obtain high lint yield unless irrigation was prolonged, because high rates of nitrogen fertilization prolonged flowering and increased the period of boll maturation.

Phosphorus is an important nutrient element for plant growth and development. It has been reported that phosphorus deficiency reduces leaf initiation (Chiera, Thomas & Rufty, 2002), leaf appearance (Elliot, Reuter & Abbot, 1997; Gutierrez-Bloem & Thomas, 1998), relative growth rate (RGR), root length, plant dry mass and relative leaf area expansion rate (RLAER) (Lynch, Lauchli & Epstein, 1991; Colomb, Kiniry & Debaeke, 2000), as well as stem and leaf dry mass accumulation, particularly in early stages of growth in wheat (Rodriguez & Gourdiaan, 1995). Relative root elongation is also shown to be reduced (Ma *et al.*, 2003). The sensitivity of leaf area expansion rate to P deficiency causes a reduction in carbon availability to the leaves and biomass partitioning to the heterotrophic tissues (Lynch *et al.*, 1991). Hormonal changes, as result of P deficiencies, are known to reduce cytokinin supply to shoots (Horgan & Wareing, 1980). In some legume species P deficiency has been demonstrated to induce rhizosphere acidification (Elliot *et al.*, 1997) and increases mycorrhizal colonization (Lynch *et al.*, 1991). The association between mycorrhiza colonization and P deficiency were also reported by Mosse (1973) who found that increased P supply reduces the survival of mycorrhizas.

Root growth is less sensitive to P deficiency than shoot growth (Lynch *et al.*, 1991). Root tolerance to P deficiencies have been suggested to be the result of reduced transport of P from roots to shoots, higher export rate of photosynthates to the roots (Mollier & Pellerin, 1999; Nielsen, Eshel & Lynch, 2001) and reduced shoot water availability (Radin & Eidenbock, 1984). However, if the P deficiency continues, the root growth may be reduced due to a reduced leaf area and consequent reduced capacity of light interception (Mollier & Pellerin, 1999).

Root to shoot ratio increase under P deficiency. Marshner (1995) reported that the increase in root: shoot ratio in P deficient plants was correlated with an increase in the

partitioning of carbohydrates towards roots, as indicated by a sharp increase in the sucrose content in the roots (Khamis, Chaillou & Lamaze, 1990). Despite the adaptive response to an increased P acquisition by the roots, flower initiation was delayed in subterranean clover (Rossiter, 1978) and seed formation was restricted in maize (Barry & Miller, 1989), mainly due to premature senescence of leaves. While mild P deficiency stimulated root growth relative to shoot growth, acute P deficiency in wheat caused visible symptoms such as stunted and spindly growth (Elliot *et al.*, 1997), necrosis and dead tips of the oldest blades (Grundon, 1987) and reddening of veins of mature leaves (Atkinson, 1973).

It seems that high-P nutrition may improve the tolerance of crop plants to water stress. This tolerance to water stress was found to be associated to a higher rate of water extraction and maintenance of a higher water potential in white clover (Singh, Sale & McKenzie, 1997), increased water and P uptake in white clover (Singh & Sale, 1998), increased P uptake in wheat (Gutierrez-Bloem & Thomas, 1998), reduced resistance to water flow and increased rate of water uptake in white clover (Singh & Sale, 2000). The ability of plants grown with high levels of P to tolerate water stress was found to be the result of increased root growth and their ability to absorb larger amounts of water due to increased hydraulic conductance (Radin & Eidenbock, 1984; Singh & Sale, 2000), and increased xylem diameter (Singh & Sale, 2000). The tolerance to water stress by plants grown with high levels of P may be due to improved water relations as a result of higher proline accumulation as reported by Al-Karaki *et al.* (1996) and Singh *et al.* (2000).

Thus, nitrogen and phosphorus both influence water use in different ways, i.e. by increasing leaf area, prolonging the development period, increasing root growth and root water uptake. However, these effects may be detrimental to the growth and yield when the soil moisture deficit is prolonged, because the larger root volume increases water uptake and higher leaf areas result in more rapid water depletion, which may reduce the ability of the plants to cope with the water stress for a long period of drought.

## 1.6 Plant recovery from water stress.

The recovery after water re-establishment in water stressed plants is an important characteristic of drought tolerance. For example, BassiriRad & Cadwell (1992) and Brady *et al.* (1995) suggested that recovery of root growth was important for rapid exploitation of water and nutrients following rainfall or irrigation events. Huang, Duncan & Carrow (1997a) found that drought resistant turfgrass plants recovered completely after re-watering due to enhanced root growth, rapid root water uptake, maintenance of root viability in dry soil and rapid root regeneration. This was confirmed by Bajji *et al.* (2000), but Eissenstat *et al.* (1999) reported that root regeneration was delayed after stressed citrus plants were re-watered, so that water and P uptake occurred from existing roots.

Loveys & Kriedemann (1973) reported that re-watering results in a rapid increase in leaf water potential and recovery of turgor, but the opening of stomata and the recovery of photosynthesis are often delayed. The delay of stomatal opening in tobacco and bean leaf discs placed into water to recover from stress was shown to be dependent on the intensity and duration of the stress treatment (Fisher, Hsiao & Hagan, 1970). Although a small number of guard cells remained permanently closed and the intercellular CO<sub>2</sub> concentration had a small carry-over effect (Fisher *et al.*, 1970), the major after-effect of stress clearly resided in the guard cells and was subsequently shown to be due to the persistence of abscissic acid, which has a direct effect on guard cells (Turner, 1986). However, photosynthesis does not recover from stress as quickly as leaf stomatal conductance and abscissic acid levels. Angelopoulos, Dichio & Xiloyannis (1996) showed that severely stressed olive trees recovered only partially in 4 to 5 days with regard to the photosynthetic activity and chlorophyll fluorescence indices after re-watering.

A frequently observed effect on recovery from stress is a more rapid rate of growth and development of stressed plants compared to well-watered plants. For example, re-watering of previously stressed plants resulted in higher root growth compared to that of well-watered plants in tussock grasses (BassiriRad & Cadwell, 1992), increased shoot dry mass in both cassava and turfgrass (Huang, Duncan & Carrow, 1997b; Alves & Setter, 2000) and increased rates of leaf enlargement in both sunflower and maize (Boyer, 1970; Hsiao, Acevedo & Henderson, 1970; Acevedo, Hsiao & Henderson, 1971). If the stress period was short, full recovery was possible (Acevedo *et al.*, 1971), but with long or

severe water stress, full recovery did not occur (Boyer, 1970; Acevedo *et al.*, 1971). The rapid increase in growth rate shortly after re-watering is most probably due to the expansion of previously existing cells rather than the production of new cells.

Recent studies have shown that recovery from water stress can be enhanced by phosphorus nutrition. The rapid recovery after re-watering was found associated with higher water and P uptake (Singh *et al.*, 1997), higher accumulation of proline, and an increased leaf expansion rate (Singh *et al.*, 2000).

Although literature studies provide valuable information with regard to the response and adaptive mechanisms of different cowpea cultivars to water stress, more studies combining morphological and physiological aspects are needed to understand the responses and adaptive mechanisms of cowpeas to water stress. Knowledge about these responses and mechanisms may contribute to improving management practices and to develop drought resistant cultivars for semi-arid areas of the Sub-Saharan Africa.



## 1.7 The objectives of the study

This study was conducted to examine the growth responses, yield and grain protein content as well as water relations, cell membrane stability and proline accumulation in the leaves of two cowpea cultivars grown under water stress conditions and under water stress conditions combined with different levels of phosphorus nutrition.

- The first objective is to evaluate the responses of physiological parameters (water potential, leaf diffusive resistance, relative water content, proline accumulation and cell membrane stability) of two cowpea cultivars to water stress and to identify those parameters, which can be used to distinguish between drought tolerant and drought susceptible cowpea cultivars.
- The second objective is to evaluate the morphological responses (shoot growth, yield, and grain protein content) of the two cowpea cultivars to induced water stress and therefore to identify the drought tolerance of the tested cultivars.
- The third objective is to test the hypothesis that phosphorus nutrition may induce drought tolerance in cowpea plants. Drought tolerance was evaluated by examining plant growth responses (shoot and root growth) and water relations (leaf diffusive resistance and proline accumulation in the leaves) during water stress and after re-watering, in plants grown at high and low P nutritional levels.

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**CHAPTER 2**

**PLANT WATER RELATIONS AND PROLINE  
ACCUMULATION AS A RESPONSE TO WATER STRESS**

**Abstract**

Cowpea crops produced under rainfed conditions of arid and semiarid areas of Sub-Saharan Africa often experience periods of water stress. It is therefore important to identify drought tolerant cultivars of the cowpea and the mechanisms involved in their ability to withstand these conditions. The aim of this study is to evaluate the responses of physiological parameters (leaf water potential, leaf diffusive resistance, relative water content, leaf proline content and cell membrane stability) to water stress and to identify those parameters, which can be used to distinguish between drought tolerant and drought susceptible cowpea cultivars, Akkerbone Bechuana Wit (AB Wit) and Akkerbone CH14. Water stress was applied from 16 to 30 days after emergence (DAE) by withholding water to the stressed plants. Well-watered plants were watered regularly to keep the soil moisture content at field water capacity. Water stress increased leaf diffusive resistance (LDR) and proline accumulation and decreased relative water content (RWC) in both cultivars and water potential only in AB Wit. Increases in proline content seemed to correlate more closely to changes in relative water content than changes in water potential. Water potential, relative water content and proline accumulation, therefore, seemed to be useful parameters to distinguish between drought tolerant and drought susceptible cowpea cultivars.

**Keywords:** Cell membrane stability, cowpea, leaf diffusive resistance, proline, relative water content, water potential, water stress.

## 2.1 Introduction

Water stress is the most important constraint for crop production in semiarid areas without irrigation. Water stress may be determined by physiological parameters such as leaf water potential, leaf osmotic potential, osmotic adjustment, leaf diffusive resistance (LDR), relative water content (RWC), leaf temperature, leaf thickness, leaf rolling (Turner, 1981), proline content (Bates, Waldren & Teare, 1973) and cell membrane stability (Sullivan & Ross, 1979; Blum & Ebercon, 1981).

Leaf water potentials are used to measure water stress since it decreases with increasing plant water deficit. It has been suggested that leaf water potential may be used to differentiate between cultivars with regard to their drought resistance. Levitt (1972) suggested that drought resistant cultivars might have the ability to maintain higher total water potentials during dry periods.

Leaf diffusive resistance (LDR) measure stomata openings, which respond directly to the changes in leaf water potential and leaf turgor pressure (Turner, 1986). But the primary factor determining the stomata opening is the accumulation of abscissic acid (ABA) (Leminechez *et al.*, 2001). Blum (1974) suggested that insensitive stomata might lead to drought avoidance under semiarid conditions whereas Jones (1974) and Maroco, Pereira & Chaves (1997) suggested that rapid stomatal response to water stress might be a drought resistance mechanism to conserve soil water for later use and maintain higher leaf water potentials. For these reasons, LDR are considered to be a criterion to select drought tolerant cultivars.

Relative water content (RWC) has been successfully used to monitor water content and water status in peanuts (Bennet, Boot & Hammond, 1984). Sinclair & Ludlow (1985) are of the opinion that RWC is a more useful parameter for plant water balance than total water potential and it should therefore provide universal relationships between physiological parameters and the level of water stress. Matin, Brown & Ferguson (1989) however, reported that RWC is a more reliable method to separate cultivars regarding their drought tolerance than LDR, because changes in LDR are very inconsistent.

Proline content has been reported to increase in response to a decrease in water potential (Handa *et al.*, 1986; Ober & Sharp, 1994; Bussis & Heineke, 1998) or RWC

(Rajagopal, Balasubramanian & Sinha, 1977; Argandona & Pahlich, 1991). Proline accumulation affects osmotic potential (Bussis & Heineke, 1998) and is therefore regarded as an important criterion for selecting drought tolerant crops (Singh, Aspinall & Paleg, 1972). For instance, Van Heerden & De Villiers (1996) showed that proline accumulation is positively correlated with drought tolerance in wheat.

Cell membrane stability may also be used as a criterion of stress tolerance (Sullivan, 1972). The polyethylene glycol (PEG) test measures cell membrane stability (CMS) and has been shown as an efficient method to determine drought resistance in many crops such as sorghum (Sullivan & Ross, 1979; Premachandra *et al.*, 1992) and wheat (Blum & Ebercon, 1981). Premachandra & Shimada (1988) indicated that CMS, measured by the PEG test, was significantly and positively correlated with leaf water potential, osmotic potential of leaf tissues, excised-leaf water retention, degree of leaf rolling, total plant weight and total root length under different soil moisture levels. Sullivan & Ross, (1979) found that membrane integrity and stability of sorghum plants subjected to drought correlated well with drought tolerance of other plant processes.

The aim of this study is to evaluate the physiological responses (leaf water potential, leaf diffusive resistance, relative water content, leaf proline content and cell membrane stability) of two cowpea cultivars to water stress and to determine the suitability of these parameters to distinguish between drought tolerant and drought susceptible cultivars when subjected to water stress.

## 2.2 Materials and Methods

Two cowpea (*Vigna unguiculata* (L.) Walp.) cultivars, Akkerbone Bechuana Wit and Akkerbone CH14, (ARC-Grain Crops Institute, Potchefstroom, RSA), were grown in 5-litre plastic bags filled with sterilized coarse sand in a temperature-controlled glasshouse (24/19°C day/night) during February 2003. The characteristics of the cultivars used are summarized in Table 2.1. The plants were grown from seeds pre-germinated for 48 hours in petri dishes containing filter paper moistened with distilled water. Three pre-germinated seeds were planted in each bag and then thinned to one plant per bag 7 days after planting.

From planting to 15 days after emergence (DAE) all the plants were irrigated and fertilized with a balanced nutrient solution. Water stress was induced from 16 to 30 DAE, by withholding water in the stress treatment. The well-watered plants were watered regularly to keep the soil moisture at field water capacity (FWC). The irrigation was done with a computerized system and the pulses were adjusted according to daily solar radiation. FWC was determined as the quantity of water needed to wet the soil till excess water started to drain from the bags.

Three samplings were done per plant, namely before the stress treatment started (15 DAE), at 22 and 30 DAE. At each sampling the following parameters were measured: leaf water potential ( $\Psi_w$ ), leaf diffusive resistance (LDR), relative water content of leaves (RWC), proline content of the leaves and leaf cell membrane stability (CMS).

Leaf diffusive resistance readings, in seconds per centimeter (s/cm), were made between 11:00 AM and 3:00 PM, using a LI-COR steady state porometer at the uppermost, fully expanded leaves. Directly after the diffusion readings, the leaf was detached and the water potential determined using a pressure chamber. Relative water content (RWC), was determined as described by Turner (1986); Premachandra *et al.*, (1995); Singh *et al.*, (2000); Bajji, Luttis & Kinet (2001); and Machado & Paulsen (2001) as follows: Fifteen leaf samples of approximately 1cm<sup>2</sup> in area, were weighed in a hermetically sealed container and then floated in distilled water for 24 hours until they were fully re-hydrated, then weighed, and dried for 48 hours at 80°C. Relative water content was calculated using the following formula:  $RWC = ((FM-DM)/(TM-DM))*100$ , where FM is fresh mass, TM is the mass of the leaves after re-hydration for 24 h at 10°C



in dark and DM is dry mass after drying at 80° for 48 h. The second set of fully expanded leaves of the main stems were used for this determination.

Proline content of the leaves was determined as follows (Bates *et al.*, 1973; Al-Karaki, Clark & Sullivan, 1996): About 0.5 g of fresh leaves from 'sun' fully expanded leaves were frozen in liquid nitrogen and then homogenized in mortar and pestle containing 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered through Whatman no. 2 filter paper. Two ml of filtrate were added to 2 ml acid ninhydrin and 2 ml of glacial acid in a test tube and left in reaction for 1 hour at 100°C where-after the reaction was terminated in an ice bath. The reaction mixture was then extracted with 4 ml toluene and mixed vigorously with a test tube stirrer for 15-20 sec. The chromophore 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 fresh weight basis as follows:  $[(\mu\text{g proline/ml} * \text{ml toluene}) / 115.5\mu\text{g}/\mu\text{mol}] / [(g \text{ sample})/5] = \mu\text{moles proline/g of fresh weight material.}$

Purified proline was used to standardize the procedure for quantifying sample values. Acid-ninhydrin was prepared by warming 1.25g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid, with agitation until dissolved and kept cool at 4°C for not more than 24 hours.

Cell membrane stability (CMS) was measured using the polyethylene glycol (PEG) test as described by Sullivan and Ross, (1979). Thirty leaf discs, obtained from the uppermost fully expanded leaves, were washed three times with de-ionized water in a test tube. The leaf discs were then submerged in 40 ml of 43% PEG 6000 solution (wt/vol.), (T1) or de-ionized water as a control (C1) and both were left for 24h at 10°C. The leaf discs were then quickly washed with de-ionized water twice and allowed to remain in 30 ml de-ionized water for another 24h at 10°C. The electrical conductivity (EC) of the liquid was measured afterwards. The leaf discs were boiled in hot water at about 100°C for 30 minutes to release all ions from the tissue, cooled again to 25°C where-after the EC was measured a second time (T2 and C2). The cell membrane stability was determined as the percentage of injury (PI), using the formula:  $PI = [(1 - (1-T1/T2)) / (1-C1/C2)] * 100.$

All treatments were replicated 8 times in a complete randomised block design arranged in a factorial combination of 2 water levels and 2 cultivars. The analysis of

variance (ANOVA) was done using SAS software. The Duncan Multiple Range Test was used to test for the significance differences ( $P = 5\%$ ) between treatment means.

**Table 2.1** Characteristics of the cowpea cultivars, Akkerbone Bechuana Wit and Akkerbone CH14, used.

Characteristics	Akkerbone Bechuana Wit (AB Wit)	Akkerbone CH14 (ACH14)
Seed colour	White	Dark Brown
Seed size	Small	Medium-large
100 seed weight (g)	14.3	19.2
Growth habit	Prostrate	Erect
Average time to flowering (days)	58	45

## 2.3 Results

Cultivar, water stress treatments and interaction responses with regard to physiological parameters are summarized in Table 2.2. From this Table it is clear that as expected no water stress effects were found before water stress was applied (15 DAE) while significant effects due to water stress treatments were found for most of the parameters at both 22 and 30 DAE.

No cultivar differences were found before water stress was induced while significant cultivar differences were shown at both 22 and 30 DAE. Significant cultivar  $\times$  stress interactions were found with regard to water potential only at 30 DAE and leaf diffusive resistance and proline content at both 22 and 30 DAE.

**Table 2.2** Cultivar (C), water stress (S) and cultivar  $\times$  water stress (C $\times$ S) interactions for physiological parameters at different sampling dates

Crop physiological parameters	Sampling time (DAE)								
	15			22			30		
	C	S	C $\times$ S	C	S	C $\times$ S	C	S	C $\times$ S
Leaf diffusive resistance	ns	ns	ns	*	*	*	*	*	*
Water potential	ns	ns	ns	*	*	ns	ns	*	*
Relative water content	ns	ns	ns	*	*	ns	*	*	ns
Proline content	ns	ns	ns	*	*	*	*	*	*
Cell membrane stability	ns	ns	ns	*	ns	ns	ns	*	ns

\* - Significant at P = 5%; ns = not significant; DAE = days after emergence

### **Leaf diffusive resistance (LDR)**

No significant differences in LDR between cultivars were found before water stress application (Figure 2.1a; Table 2.2). Under well-watered conditions, the LDR was as expected generally low, but it varied between sampling days due to the differences in daily solar radiation (Figure 2.1a). However, LDR proved to be a sensitive parameter to measure water stress conditions, since significant differences due to water stress treatment were found at 22 and 30 DAE. Water stress increased LDR in both cultivars at 22 and 30 DAE, but LDR of AB Wit was higher than that of ACH14 causing significant cultivar  $\times$  water stress interactions at both 22 and 30 DAE. LDR of stressed plants increased further with increasing time of exposure to water stress, indicating that the intensity of the stress was also increasing.

### **Water potential**

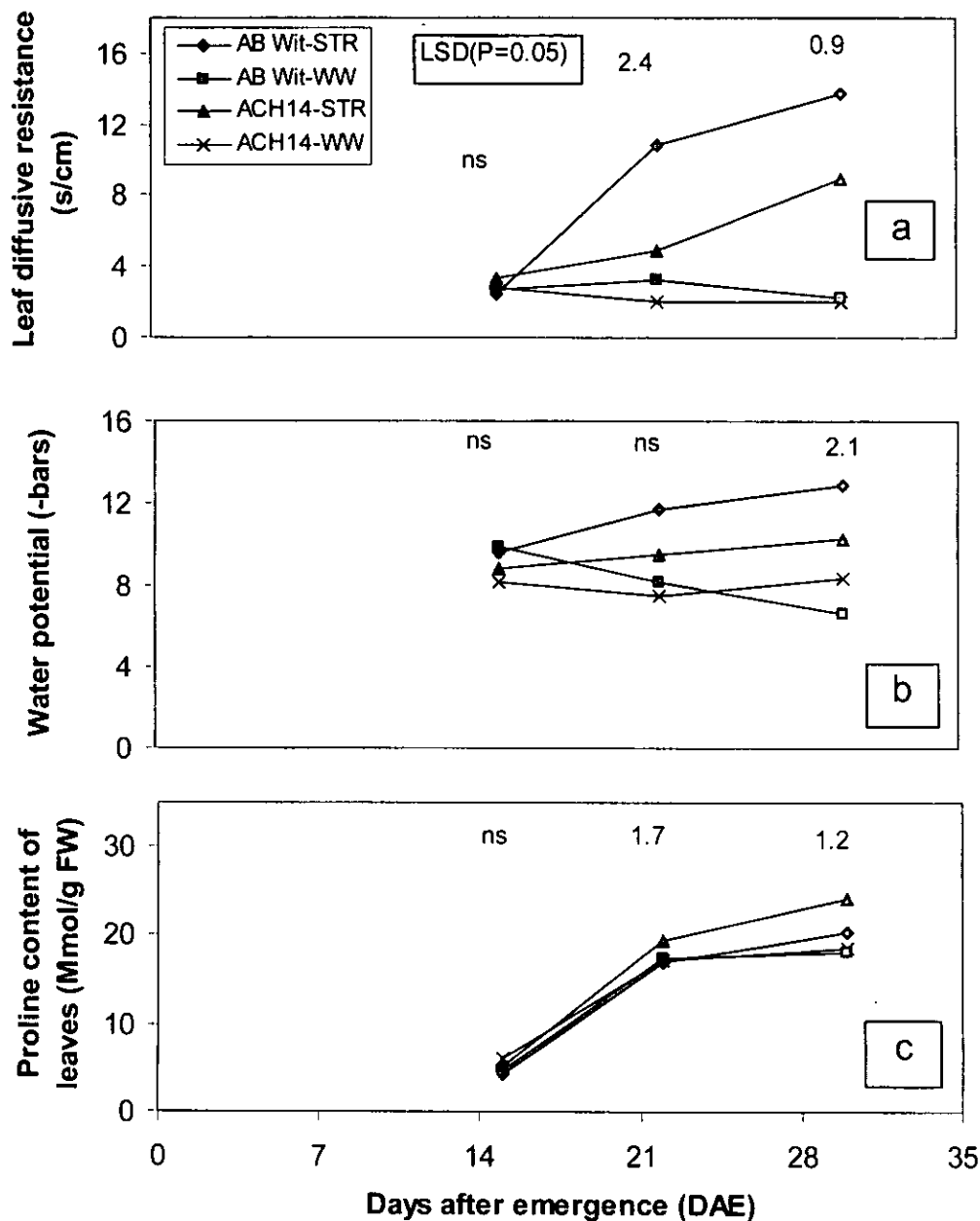
No significant differences were found between well-watered and water stressed plants with regard to water potential before water stress was induced (15 DAE) (Figure 2.1b). Water potentials of well-watered plants varied at different sampling days, probably due to differences in the daily transpiration rates (Figure 2.1b). At 22 DAE, water potential was reduced by water stress. When cultivars are compared, AB Wit showed a lower (more negative) water potential compared to ACH14. At 30 DAE, water potential was again reduced by water stress in AB Wit, but not in ACH14. Water potential of ACH14 was, therefore, higher than that of AB Wit under stressed conditions, but not under well-watered conditions, indicating that AB Wit was more sensitive to water stress than ACH14.

### **Proline content of the leaves**

As for all other physiological parameters, proline content of the leaves did not differ before water stress application (15 DAE) (Figure 2.1c). During the first 7 days of withholding water (22 DAE), proline content of the leaves increased in both cultivars and unexpectedly also in both watering treatments. However, proline content increased more in stressed than in the well-watered plants. At 22 DAE, water stress increased the proline content of ACH14, but not of AB Wit. At 30 DAE, although proline accumulated in both cultivars, the content of ACH14 was again higher. Under well-watered conditions, the two



cultivars did not differ with regard to their proline content at both 22 and 30 DAE. The significant interaction between cultivar and water stress observed on proline accumulation at both sampling dates (22 & 30 DAE) indicated that the two cultivars responded differently to water stress.



**Figure 2.1** Leaf diffusive resistance (a), leaf water potential (b) and proline content of the leaves (c) of two cowpea cultivars grown under well-watered and water stressed conditions. Values are means of 8 plants.

## Relative water content (RWC)

Relative water content, an indication of leaf turgidity, did not differ at the onset of the stress period (15 DAE), but was reduced by water stress at 22 and 30 DAE (Table 2.3). Relative water content of ACH14 was higher than that of AB Wit at both 22 and 30 DAE, but no significant cultivar  $\times$  stress interactions were found at any sampling date.

**Table 2.3** Relative water content of leaves of two cowpea cultivars grown under well-watered and water stressed conditions.

Water stress (S)	Cultivar (C)	Sampling time (DAE)		
		15	22	30
Well-watered	AB Wit	82.6	77.4	71.7
	ACH14	81.6	82.6	74.3
Mean		82.1	80.0	73.0
Stress	AB Wit	83.6	68.9	63.8
	ACH14	82.7	72.0	67.6
Mean		83.2	70.5	65.7
LSD <sub>s</sub> (P=0.05): S		ns	2.6	3.1

AB Wit = Akkerbone Bechuana Wit; ACH14 = Akkerbone CH14. DAE = days after emergence. Values are means of 8 plants.

## Cell membrane stability (CMS)

Cell membrane stability, measured by percentage of injury, as for all other physiological parameters, did not differ before water stress application (Table 2.4). At 22 DAE, the CMS of AB Wit was higher compared to ACH14, because the percentage of injury was lower. However, no water stress effects were found. At 30 DAE, CMS was increased by water stress, but no significant cultivar differences were found. This increase in CMS by water stress indicated that the plants acclimatized to water stress.

**Table 2.4** Cell membrane stability of two cowpea cultivars grown under well-watered and water stressed conditions.

Water stress (S)	Cultivar (C)	Sampling time (DAE)		
		15	22	30
Well-watered	AB Wit	71.9	72.0	73.4
	ACH14	55.3	77.9	75.9
Mean		63.6	74.9	74.7
Stress	AB Wit	61.2	62.3	59.7
	ACH14	61.2	74.5	66.7
Mean		61.2	68.4	63.2
LSD <sub>s</sub> (P=0.05): S		ns	ns	5.0

AB Wit = Akkerbone Bechuana Wit; ACH14 = Akkerbone CH14. DAE = days after emergence. Values are means of 8 plants.

## 2.4 Discussion

In this study, water stress was induced by withholding water for 14 days at the vegetative growth stage of two cowpea cultivars grown in a temperature-controlled glasshouse. The results show that both the cultivars tested were affected by water stress, but the response and adaptive mechanisms were different.

In general water stress reduced relative leaf water content (RWC) and water potential. As a result leaf diffusive resistance (LDR) increased with an accumulation in proline content. After 14 days of water stress (30 DAE), cell membrane stability of stressed plants increased due to the acclimation of these plants.

Leaf diffusive resistance (LDR) increased in both cultivars tested, but the responses were different. LDR of stressed AB Wit plants was higher compared to ACH14 plants. This rapid increase in LDR of AB Wit plants may indicate an earlier stomatal closure to reduce water loss. Jones (1974) and Maroco *et al.* (1997) suggested that cultivars showing rapid stomatal closure might be more drought tolerant, because rapid stomatal closure is associated with the conservation of water for later use and maintenance of higher leaf water potential. Blum (1974) on the other hand, suggested that low LDR values due to insensitive stomata might lead to drought avoidance under semiarid conditions.

Because leaf water potential was reduced by water stress in AB Wit, but not in ACH14, it is clear that the above mentioned more rapid increase in LDR of AB Wit was due to a higher transpiration loss as the result of a larger leaf area (results not shown). This indicates that AB Wit was in fact more drought susceptible than ACH14 and not more tolerant. Levitt, (1972) also reported that drought tolerant cultivars have the ability to maintain higher water potentials compared to the drought susceptible cultivars. These results show that water potential may be a useful tool to distinguish between drought tolerant and susceptible cultivars.

Although no significant interactions were found, higher relative water content (RWC) found for ACH14 confirmed the superiority with regard to drought tolerance of this cultivar compared to AB Wit. Earlier studies (Schonfeld *et al.*, 1988; Matin *et al.*, 1989) not only showed that the maintenance of a higher RWC during water stress conditions was

associated with drought tolerance in wheat and barley, but also found that RWC is a reliable tool to distinguish between drought tolerant and susceptible cultivars.

Proline increased in the leaves of stressed plants, but the rate of accumulation was higher in ACH14 than in AB Wit. The higher proline content in the leaves of stressed ACH14 plants also indicated that this cultivar is more drought tolerant than AB Wit, since high proline accumulation was found to be associated with drought tolerance in wheat (Van Heerden & De Villiers, 1996) and mulberry (Ramanjulu & Sudhakar, 2000).

Cell membrane stability (CMS) has been suggested to be a good indicator of drought tolerance in many crop plants such as sorghum (Sullivan, 1972; Sullivan & Ross, 1979; Premachandra *et al.*, 1992), wheat (Premachandra & Shimada, 1988) and maize (Premachandra, Saneoka & Ogata, 1989). CMS of AB Wit was higher compared to that of ACH14 at 22 DAE, but at 30 DAE no significant cultivar differences were found. For this reason CMS is not regarded as a useful parameter to differentiate between the drought tolerance of the two cowpea cultivars tested.

The changes in plant water relations with increasing time of exposure to water stress seems to be closely related to the decrease in relative water content, since the increase in LDR and proline accumulation of both cultivars and decrease in water potential of AB Wit followed the trends showed by relative water content (RWC). In this study rapid increases in LDR were not as expected (Jones, 1974) an indication of drought tolerance, but of drought susceptibility. Blum (1974) also found that most susceptible cultivars of sorghum showed higher leaf diffusive resistance compared to more tolerant ones. For this reason, as also found by Matin *et al.* (1989) LDR is not considered to be a reliable tool to differentiate between drought tolerant and susceptible cultivars.

Earlier studies showed that proline accumulated as a response to a decrease in water potentials (Handa *et al.*, 1986; Ober & Sharp, 1994; Bussis & Heineke, 1998) and or RWC (Rajagopal *et al.*, 1977; Argandona & Pahlich, 1991). This response in proline content caused some osmotic adjustment (Handa *et al.*, 1986; Bussis & Heineke, 1998), which enabled the plant to re-acquire turgidity under water shortage. In this study proline accumulated more rapidly in the drought tolerant cultivar (ACH14), which shows that proline is a usable parameter to distinguish between drought tolerant and susceptible cultivars.



## 2.5 Conclusions

The aim of this study was to evaluate the physiological responses (leaf water potential, leaf diffusive resistance, relative water content, leaf proline content and cell membrane stability) of two cowpea cultivars to water stress and to determine the suitability of these parameters to distinguish between drought tolerant and susceptible cultivars when subjected to water stress.

From the results it is clear that:

- 1) Water stress affected plant water relations due to an increase in leaf diffusive resistance and proline content and a decrease in relative water content in both cultivars. Water potential was only reduced in AB Wit.
- 2) Proline accumulates as a response to the decrease in RWC rather than a decrease in water potential.
- 3) ACH14 was more drought tolerant than AB Wit, because its water potential was not reduced by water stress and it accumulated more proline. The more rapid accumulation of proline by ACH14 seems to be the main mechanism responsible for its drought tolerance.
- 4) Water potential, relative water content and proline accumulation were the only physiological parameters that allowed to distinguish between the cultivars tested with regard to their drought tolerance. No significant interactions were found with regard to relative water content. LDR is not considered a reliable tool to identify the drought tolerance of the tested cultivars because its response to water stress was not associated with the responses of other physiological parameters.

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### CHAPTER 3

## EFFECT OF WATER STRESS ON GROWTH, YIELD AND GRAIN PROTEIN CONTENT

### Abstract

Water stress is the main constraint that affects the production of field crops in the arid and semi-arid areas of sub-Saharan Africa. Knowledge of responses and adaptive mechanisms that enable the crops to withstand water stress may help to improve the management practices and strategies for the areas subjected to drought and to develop drought resistant cultivars for these areas. A water stress experiment was conducted in a temperature-controlled glasshouse (24/19°C day/night) to evaluate the morphological responses (shoot growth, yield, and grain protein content) of two cowpea cultivars, Akkerbone Bechuana Wit (AB Wit) and Akkerbone CH14 (ACH14). Water stress treatments started at 21 days after emergence (DAE) and continued to maturation by reducing the water supply of the stressed plants to 50% of the amount given to well-watered plants. Well-watered plants were watered regularly to keep the soil water content at field water capacity. Water stress reduced plant growth by reducing the number of leaves, leaf area, leaf dry mass, and the dry mass of stems. Leaf area was reduced because water stress decreased both the number of leaves and the leaf expansion rate, but the number of leaves was the most important parameter that affected the leaf area. Leaf area reduction affected both biomass production and seed yield. Seed yield was reduced as a direct result of the reduction in the number of pods. Different mechanisms to tolerate water stress were exhibited by these cultivars. While ACH14 closed the stomata earlier to reduce water losses, AB Wit reduced the leaf area to minimize water losses by transpiration. In general ACH14 was the most drought tolerant cultivar because most of the parameters tested were not affected by stress.

**Keywords:** Cowpea; growth; protein content; yield; water stress.

### 3.1 Introduction

Water stress is the most important environmental factor limiting the production of cowpeas in the semi-arid areas of the tropics and sub-tropics (Summerfield, Huxley & Dart, 1976; Turk, Hall & Asbell, 1980; Akyeampong, 1986). The Cowpea is grown under rain-fed conditions and is subjected to water stress due to low rainfall and irregularity of the rain. Drought tolerant cultivars, able to withstand dry periods are therefore very important for these areas.

Crop growth and seed yield are often limited by water stress, whose effects on growth and seed yield are determined by the growth stage of the crop and the intensity of the stress (Turk *et al.*, 1980; Gardner, Pearce & Mitchell, 1985; Hale & Orcutt, 1987). Water stress during flowering and pod filling has been reported to reduce seed yield, while the effect of water stress during the vegetative stage may depend on the intensity of the stress. Mild water stress, for example, may hasten and severe water stress may delay flower initiation (Gardner *et al.*, 1985).

Turk *et al.* (1980), Turk & Hall (1980) and Akyeampong, (1986) found that cowpea seed yield was reduced by stress during flowering and pod filling, but not during vegetative growth stages. They argued that seed yield reduction was the result of flower abscission, lower pod density and smaller seed (Turk *et al.*, 1980).

Water stress during vegetative growth has been reported to affect plant growth parameters (Hsiao, 1973) such as root growth (Malik, Dhankar & Turner, 1979), stem elongation (Wien, Littleton & Ayanaba, 1979) and leaf expansion due to the sensitivity of cell growth to water stress (Akyeampong, 1986).

Water stress also reduces leaf production and promotes senescence and abscission resulting in decreased total leaf area per plant (Turk & Hall, 1980b; Akyeampong, 1986). Reduction of leaf area reduces crop growth and thus biomass production (Akyeampong, 1986). Because leaf area is positively correlated with seed yield (Turk & Hall, 1980), its reduction due to water stress may also reduce seed yield.

The effects of water stress on yield components also depend on timing and intensity of the stress (Wien *et al.*, 1979; Turk *et al.*, 1980). Turk *et al.* (1980) reported that water stress during pod filling reduced the number of pods due to abscission and grain size but not the number of seeds per pod.

Although water stress reduces seed yield, the grain protein content of stressed plants was found to be higher compared to well-watered plants in both cowpeas (Wien *et al.*, 1979) and wheat (Agenbag & De Villiers, 1995).

For the cowpea to be used as food or fodder crop in the arid and semi-arid areas, drought resistance is a very important characteristic. At present little is known with regard to cultivar differences and the drought resisting mechanisms involved. Knowledge about drought resisting mechanisms may contribute to the development of drought resistant cultivars. The aim of this experiment was to study the morphological responses (shoot growth, yield and grain protein content) of two cowpea cultivars to induced water stress to evaluate the drought tolerance of the tested cultivars.



### 3.2 Materials and Methods

Two Cowpea (*Vigna unguiculata* (L.) Walp.) cultivars, Akkerbone Bechuana Wit and Akkerbone CH14 (ARC-Grain Crops Institute, Potchefstroom, RSA), were grown in 15-litre pots filled with sterilized coarse sand in a temperature-controlled glasshouse (24/19°C day/night) on 14 October 2002. The characteristics of the cultivars used are summarized in Table 3.1. The plants were grown from seeds, pre-germinated for 48 hours in petri dishes containing filter paper moistened with distilled water. Three pre-germinated seeds were planted in each pot and then thinned to one plant per pot 7 days after planting.

From planting to 20 days after emergence (DAE) all the plants were irrigated and fertilized with the same balanced nutrient solution. The composition of nutrient solution was as follows: 13.2 mmol N l<sup>-1</sup> as nitrate, 7 mmol K l<sup>-1</sup>, 1.8 mmol P l<sup>-1</sup> as KH<sub>2</sub>PO<sub>4</sub>, 4 mmol Ca l<sup>-1</sup> as Ca(NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O; 1.5 mmol Mg l<sup>-1</sup> and 1.5 mmol S l<sup>-1</sup> both as MgSO<sub>4</sub>·7H<sub>2</sub>O; and 40 B, 39 Fe as FeHEDTA, 15 Mn, 3 Zn, 1 Mo and 0.8 Cu in μmol l<sup>-1</sup> (adapted from Al-Karaki, Clark & Sullivan, 1996).

Two water stress treatments were applied from 21 DAE to maturation stage, by watering the pots to field water capacity (FWC) and 50% FWC, to obtain no stress and water stress, respectively. The irrigation was done by a computerized system and the pulses were adjusted according to daily solar radiation and the crop growth stage, maintaining the same ratio between treatments. FWC was determined as the quantity of water needed to wet the soil till excess water started to drain from the pots. To supply the same amount of nutrients to both treatments, the concentration of the nutrient solution of the well-watered plants was 50% of that for the stressed plants.

Eight plants per treatment were harvested before the stress treatment started (20 DAE) and thereafter more plants were harvested at 20 days intervals, giving a total of 5 harvests. During the pre-maturity harvests, fresh weight of stems, leaves and pods were determined individually and in total (shoot growth). The dry mass (DM) was determined after drying for 48 hours at 80 °C. Dry mass was used to determine growth rates. The leaf water content (LWC) was determined on dry weight basis according to Turner, (1986). Leaf diffusive

resistance and water potential readings were made between 11:00 AM and 3:00 PM, using a Li-COR steady state porometer and pressure chamber, respectively. The relative growth rate (RGR) of leaf, stem and shoot DM were calculated using the following formula:  $(RGR = (\ln W_2 - \ln W_1) / T_2 - T_1)$ ; where  $W$ =plant DM;  $T_2 - T_1$  = time intervals (days) between successive samplings (Hunt, 1982; Gardner *et al.*, 1985; Poorter, 1989; França *et al.*, 2000).

The leaf area was measured using a leaf area meter (Model 3100 LI-COR Inc., USA) and the relative leaf area expansion rate (RLAER) was determined as the slope of the linear regression of transformed data (natural logarithm) of the leaf area against time according to Lynch, Lauchli & Epstein (1991). The specific leaf area (SLA = plant leaf area divided by total leaf dry mass) was calculated.

Drought stress tolerance index (DSTI) was calculated using the following formula:  $DSTI = \text{crop parameter under water stress} / \text{crop parameter under well-watered conditions}$  (Maiti *et al.*, 1996).

Seed yield and yield components were determined during the final harvest while the protein content of the grain was determined by the use of a technikon infralyzer 400 calibrated against Kjeldahl nitrogen content.

All treatments were replicated 8 times in a complete randomised block design arranged in a factorial combination of 2 water levels and 2 cultivars. The analysis of variance (ANOVA) was done using SAS software. The Duncan Multiple Range Test (DMRT) was used to test for significant differences ( $P=5\%$ ) between treatment means.

**Table 3.1** Characteristics of Akkerbone Bechuana Wit and Akkerbone CH14, the cowpea cultivars used.

Characteristics	Akkerbone Bechuana Wit (AB Wit)	Akkerbone CH14 (ACH14)
Seed colour	White	Dark Brown
Seed size	Small	Medium-large
100 seed weight (g)	14.3	19.2
Growth habit	Prostrate	Erect
Average time to flowering (days)	58	45

### **3.3 Results**

In this experiment no significant differences with regard to shoot growth parameters were found between cultivars tested during early growth stages and before water stress was induced at 21 DAE (Table 3.2). At 40, 60, 80 and 100 DAE significant differences between cultivars and water stress treatments were found for several parameters. Although significant cultivar and water stress interactions were only found for a few parameters at 60, 80 and 100 DAE, these interactions may indicate differences in drought resistance between the cultivars tested.

#### **General trends for growth and development**

Water stress affected crop growth and development by reducing the number of leaves, leaf area, leaf and stem dry mass, but did not influence the time to 50% flowering (data not shown). The number of leaves, leaf area and leaf dry mass declined, from 60 days after emergence (DAE) in the earlier cultivar (ACH14), because it stopped to produce new leaves and senescence and abscission occurred as a result of remobilisation and redistribution of carbohydrates and minerals to the reproductive parts. In contrast, the later maturing cultivar, AB Wit, continued producing new leaves and leaf senescence and abscission started between sampling at 80 DAE and 100 DAE only. Water stress also affected seed yield because it reduced pod number.

**Table 3.2** Significance of cultivar (C) and water stress (S) responses as well as cultivar × water stress interactions (C×S) for growth parameters, yield and protein content of two cowpea cultivars at different sampling dates

Crop growth and yield parameters	Sampling time (DAE)														
	20			40			60			80			100		
	C	S	C × S	C	S	C × S	C	S	C × S	C	S	C × S	C	S	C × S
No. of leaves	ns	ns	ns	ns	ns	ns	*	*	ns	*	*	ns	*	*	ns
Leaf size	ns	ns	ns	*	ns	ns	ns	ns	*	ns	ns	*	ns	ns	ns
Leaf area	ns	ns	ns	*	*	ns	*	*	ns	*	*	ns	*	*	ns
Leaf dry mass	ns	ns	ns	ns	*	ns	*	*	*	*	ns	ns	*	*	ns
Leaf water content	ns	ns	ns	*	ns	ns	*	*	*	*	*	*	*	*	*
Stem dry mass	ns	ns	ns	*	*	ns	ns	*	ns	*	*	ns	*	*	ns
Shoot dry mass	ns	ns	ns	*	*	ns	ns	*	*	*	*	ns	ns	*	ns
RGR of Leaf	ns	ns	ns	ns	ns	ns	*	*	ns	ns	*	ns	*	ns	ns
RGR of Stem	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns	ns	ns
RGR of Shoot	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
RLAER	ns	ns	ns	*	ns	ns	ns	*	ns	*	ns	ns	ns	ns	ns
Specific leaf area	-	-	-	*	ns	ns	*	ns	ns	ns	*	ns	*	*	ns
Number of pods	-	-	-	-	-	-	-	-	-	-	-	-	*	*	ns
No. of seeds per pod	-	-	-	-	-	-	-	-	-	-	-	-	*	ns	*
100-seed weight	-	-	-	-	-	-	-	-	-	-	-	-	*	*	ns
Seed yield	-	-	-	-	-	-	-	-	-	-	-	-	*	*	ns
Pod dry mass	-	-	-	-	-	-	-	-	-	-	-	-	ns	*	ns
Harvest index	-	-	-	-	-	-	-	-	-	-	-	-	*	*	*
Grain protein content	-	-	-	-	-	-	-	-	-	-	-	-	*	*	*

\* - Significant at P= 5%; ns = not significant; DAE = days after emergence

## Physiological responses to water stress

Leaf water potential (WP), as measured by the pressure chamber, was affected by water stress (Table 3.2). At 30 DAE, water stress decreased WP by 23% (Table 3.3). At 60 DAE a significant cultivar and water stress interaction, however, indicated differences in drought tolerance reactions between cultivars because WP decreased significantly in AB Wit, but not in ACH14. When expressed as a percentage of the well-watered plants, WP of AB Wit was 79% lower compared to 21% of ACH14.

Measurements of leaf diffusive resistance (LDR) during both the vegetative (30 DAE) and reproductive (60 DAE) growth stages, showed an increase in LDR in both cultivars due to the water stress treatment (Table 3.3). Water stress therefore resulted in the closure of stomata in both cultivars. Although no significant differences with regard to LDR were found between cultivars, the response (stressed value as % of the control) of ACH14 was almost double that of AB Wit at both 30 DAE and 60 DAE.

**Table 3.3** Leaf water potential (WP) and leaf diffusive resistance (LDR) of two cowpea cultivars grown under well-watered and water stress conditions.

Water stress (S)	Cultivar (C)	WP (bar)	WP (%)	LDR (cm/s)	LDR (%)
Vegetative stage (30 DAE)					
Well-watered	AB Wit	-11.2	100	9.3	100
	ACH14	-10.4	100	5.9	100
Mean		-10.8	100	7.6	100
Stressed	AB Wit	-14.6	130	15.5	167
	ACH14	-12.1	116	19.1	323
Mean		-13.3	123	17.3	228
LSD(P=0.05)					
C		ns		ns	
S		2.1		4.1	
C × S		ns		ns	
Reproductive stage (60 DAE)					
Well-watered	AB Wit	-6.6	100	3.6	100
	ACH14	-8.5	100	1.8	100
Mean		-7.5	100	2.7	100
Stressed	AB Wit	-11.2	179	8.5	236
	ACH14	-10.3	121	8.6	438
Mean		-10.7	143	8.6	319
LSD(P=0.05)					
C		1.3		ns	
S		1.3		2.1	
C × S		1.9		ns	

AB Wit = Akkerbone Bechuana Wit; ACH14 = Akkerbone CH14. DAE = days after emergence. Values are means of 8 plants.

### **Relative growth rate and relative leaf area expansion rate**

Relative growth rate (RGR) of leaves, stems and shoots are presented in Table 3.4. Water stress reduced RGR of leaves by 35% between 40 and 60 DAE and RGR of stems and shoots by 24% and 25%, respectively between 40 and 60 DAE, without any interaction with the cultivar. When cultivars are compared, the small seeded AB Wit showed a significantly higher RGR of leaves between 40 and 60 DAE and 60 and 80 DAE and a higher RGR of stems between 60 and 80 DAE (Table 3.2).

Relative leaf area expansion rate (RLAER) was affected by water stress only between 40 and 60 DAE, where a reduction of 69% was found (Table 3.4). RLAER of ACH14 was lower compared to AB Wit between 20 and 40 DAE and between 60 and 80 DAE (Table 3.2). No significant interactions between cultivars and water stress were found with regard to RLAER (Table 3.2).

**Table 3.4** Relative growth rate (RGR) of shoot, leaves and stems and relative leaf area expansion rate (RLAER) of two cowpea cultivars grown under well-watered and water stress conditions

Water stress (S)	Cultivar (C)	RGR (g.g <sup>-1</sup> .day <sup>-1</sup> )			RLAER (cm <sup>2</sup> .g <sup>-1</sup> .day <sup>-1</sup> )
		Shoot	Leaves	Stem	
Vegetative stage (0 – 20 DAE)					
Well-watered	AB Wit	0.73	0.71	0.66	1.10
	ACH14	0.73	0.72	0.67	1.11
Mean		0.73	0.72	0.67	1.11
Stress	AB Wit	0.72	0.71	0.66	1.10
	ACH14	0.72	0.71	0.66	1.10
Mean		0.72	0.71	0.66	1.10
LSD(P=0.05): S		ns	ns	ns	ns
Vegetative stage (20 – 40 DAE)					
Well-watered	AB Wit	0.124	0.110	0.150	0.111
	ACH14	0.121	0.104	0.146	0.097
Mean		0.123	0.107	0.148	0.104
Stress	AB Wit	0.111	0.098	0.138	0.103
	ACH14	0.120	0.098	0.145	0.090
Mean		0.116	0.098	0.142	0.097
LSD(P=0.05): S		ns	ns	ns	ns
Reproductive stage (40 – 60 DAE)					
Well-watered	AB Wit	0.064	0.060	0.070	0.034
	ACH14	0.046	0.033	0.056	0.018
Mean		0.055	0.047	0.063	0.026
Stress	AB Wit	0.041	0.035	0.050	0.008
	ACH14	0.041	0.026	0.046	0.009
Mean		0.041	0.031	0.048	0.009
LSD(P=0.05): S		0.011	0.010	0.013	0.014
Reproductive stage (60 – 80 DAE)					
Well-watered	AB Wit	0.024	-0.004	0.016	0.003
	ACH14	0.021	-0.011	-0.006	-0.021
Mean		0.023	-0.008	0.005	-0.009
Stress	AB Wit	0.035	0.013	0.020	0.010
	ACH14	0.023	0.004	0.001	-0.004
Mean		0.029	0.009	0.011	0.003
LSD(P=0.05): S		ns	0.011	ns	ns
Reproductive stage (80 – 100 DAE)					
Well-watered	AB Wit	0.003	0.006	0.020	-0.025
	ACH14	0.018	0.038	-0.005	0.005
Mean		0.011	0.022	0.008	-0.010
Stress	AB Wit	0.004	0.010	0.016	-0.031
	ACH14	0.015	0.021	0.001	-0.011
Mean		0.010	0.016	0.009	-0.021
LSD(P=0.05): S		ns	ns	ns	ns

AB Wit = Akkerbone Bechuana Wit; ACH14 = Akkerbone CH14. DAE = days after emergence. Values are means of 8 plants.

### Leaf growth parameters

The results of leaf growth parameters are shown in Table 3.5, Figure 3.1 & Figure 3.2. The number of leaves produced was affected by both cultivar and water stress from 60 DAE to 100 DAE, but no significant interaction was found between the two factors (Table 3.2). The number of leaves produced was reduced by 48%, 35% and 33%, at 60, 80 and 100 DAE, respectively, due to water stress (Table 3.5). During this period, the cultivar AB Wit produced more leaves compared to ACH14.

Leaf size of AB Wit was higher compared to ACH14 at 40 DAE, but no water stress effects were found (Figure 3.1). At other sampling days no significant cultivar and water stress effects were found (Table 3.2). Significant cultivar and water stress interactions were found at 60 and 80 DAE, because in contrast to AB Wit, leaf size of ACH14 was not significantly affected by water stress. These results indicated different responses of the tested cultivars to water stress (Figure 3.1).

Leaf area per plant, was affected by both cultivar and water stress from 40 to 100 DAE, but no significant interactions were found (Table 3.2). Leaf area of stressed plants was reduced by 20%, 49%, 33% and 39% at 40, 60, 80 and 100 DAE, respectively. AB Wit showed a higher leaf area compared to ACH14 during this period (Table 3.5).

Leaf dry mass was affected by cultivar from 60 to 100 DAE and water stress at 40, 60 and 100 DAE (Table 3.2). In general AB Wit produced more dry mass at all sampling times. Leaf dry mass of stressed plants was reduced by 26% and 19% at 40 and 100 DAE, respectively. Under well-watered conditions AB Wit produced more leaf dry mass than ACH14. At 60 DAE, water stress reduced the leaf dry mass of AB Wit, but not of ACH14. Under well-watered conditions, the cultivar AB Wit produced higher leaf dry mass compared to ACH14 (Figure 3.2). This significant interaction between cultivar and water stress treatment indicated different responses of the two cultivars to water stress.

Specific leaf area (SLA) was affected by cultivar at 40, 60 and 100 DAE and by water stress at 80 and 100 DAE. No significant interaction between cultivar and water stress treatments was found at any sampling date (Table 3.2). Water stress reduced SLA by 31% and 20%, at 80 and 100 DAE, respectively (Table 3.5). AB Wit showed a higher SLA compared to ACH14.



Leaf water content was affected by cultivar at 40 DAE, and by both cultivar and water stress as well as significant interactions between cultivar and water stress from 60 to 100 DAE (Table 3.2). At 40 DAE, the leaf water content of AB Wit tended to be higher than that of ACH14 (Figure 3.3). From 60 DAE to 100 DAE, a significant cultivar and water stress interaction was found because the water stress reduced leaf water content of the cultivar AB Wit, but not of ACH14. Under well-watered conditions the leaf water content of AB Wit was higher than ACH14, but under stressed conditions the two cultivars did not differ. This significant interaction between cultivar and water stress indicated different responses by the cultivars tested.

**Table 3.5** Number of leaves, leaf area and specific leaf area (SLA) of two cowpea cultivars grown under well-watered and water stressed conditions

Water stress (S)	Cultivar (C)	Leaf growth parameters				SLA (cm <sup>2</sup> g <sup>-1</sup> )	SLA (%)
		No. of leaves (no./plant)	No. of leaves (%)	Leaf area (cm <sup>2</sup> /plant)	Leaf area (%)		
Vegetative stage (20 DAE)							
Well-watered	AB Wit	4.1	100	380.4	100	NA	
	A CH 14	4.3	100	444.6	100	NA	
Mean		4.2	100	412.5	100		
Stress	AB Wit	3.9	95	370.8	97	NA	
	A CH 14	4.1	95	381.5	86	NA	
Mean		4.0	95	376.2	91		
LSD(P=0.05): S		ns		ns			
Vegetative stage (40 DAE)							
Well-watered	AB Wit	22.4	100	3399.3	100	259.0	100
	A CH 14	22.9	100	2956.9	100	244.2	100
Mean		22.7	100	3178.1	100	251.6	100
Stress	AB Wit	19.3	86	2777.3	82	266.6	1003
	A CH 14	18.8	82	2282.7	77	248.5	1002
Mean		19.1	84	2530.0	80	257.6	102
LSD(P=0.05): S		ns		191.9		ns	
Vegetative stage (60 DAE)							
Well-watered	AB Wit	46.5	100	6801.2	100	221.4	100
	A CH 14	33.8	100	4278.7	100	187.9	100
Mean		40.2	100	5539.9	100	204.7	100
Stress	AB Wit	25.6	50	3265.3	48	205.4	93
	A CH 14	16.3	49	2424.7	57	184.9	98
Mean		21.0	52	2845.0	51	195.2	95
LSD(P=0.05): S		6.3		910.2		ns	
Reproductive stage (80 DAE)							
Well-watered	AB Wit	61.6	100	7235.7	100	359.3	100
	A CH 14	23.3	100	2648.9	100	370.0	100
Mean		42.5	100	4942.3	100	364.7	100
Stress	AB Wit	36.1	59	4449.2	61	267.5	74
	A CH 14	16.0	69	2210.9	83	237.1	64
Mean		26.1	61	3330.1	67	252.3	69
LSD(P=0.05): S		9.1		1275.7		50.5	
Reproductive stage (100 DAE)							
Well-watered	AB Wit	45.3	100	5215.6	100	294.5	100
	A CH 14	24.3	100	2389.0	100	155.7	100
Mean		34.8	100	3802.3	100	225.1	100
Stress	AB Wit	30.0	66	2805.1	54	212.1	72
	A CH 14	17.0	74	1800.8	75	146.3	94
Mean		23.5	68	2303.0	61	179.2	80
LSD(P=0.05): S		7.8		987.2		40.1	

AB Wit = Akkerbone Bechuana Wit; ACH14 = Akkerbone CH14. DAE = days after emergence. NA = not applied. Values are means of 8 plants.

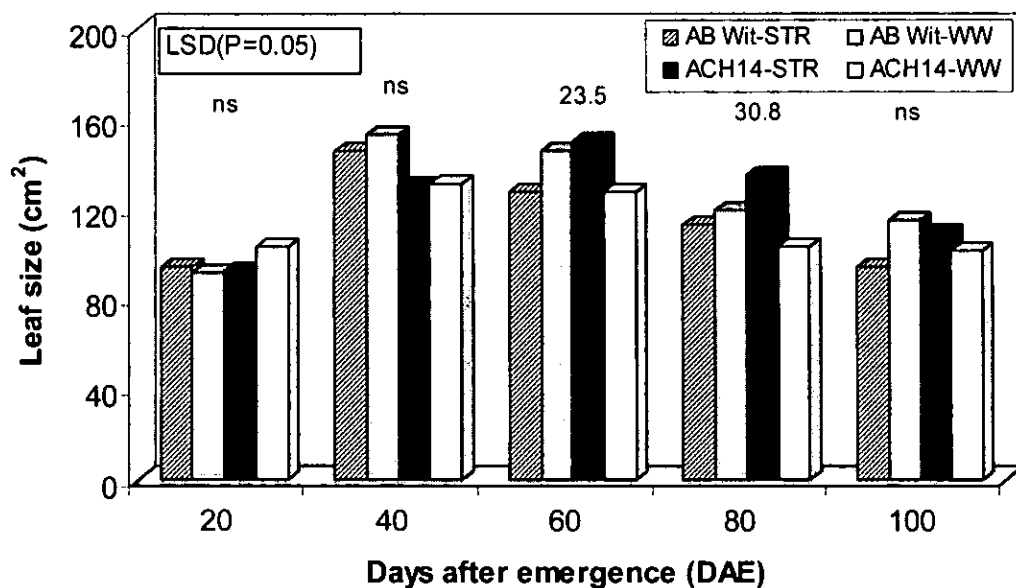


Figure 3.1 Leaf size of two cowpea cultivars, Akkerbone Bechuana Wit (AB Wit) and Akkerbone CH14 (ACH14), grown under well-watered (WW) and water stressed (STR) conditions. Values are means of 8 plants.

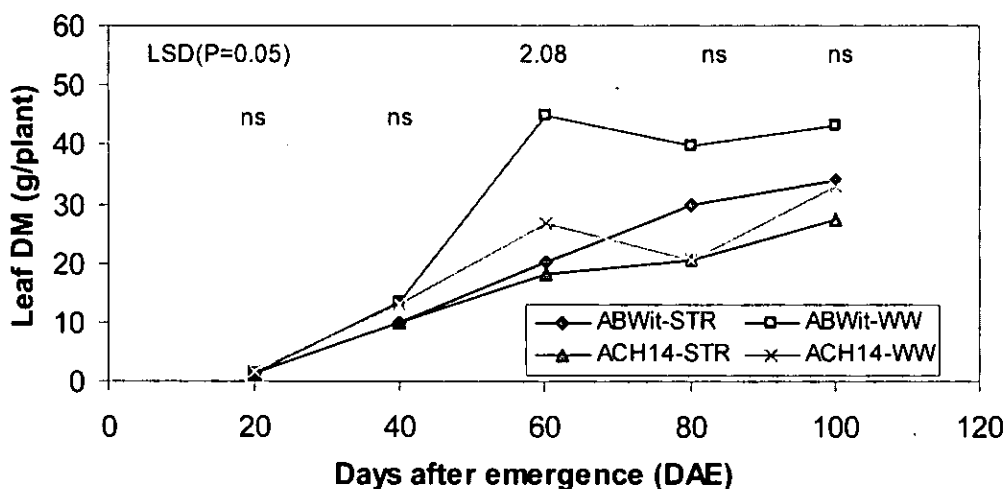
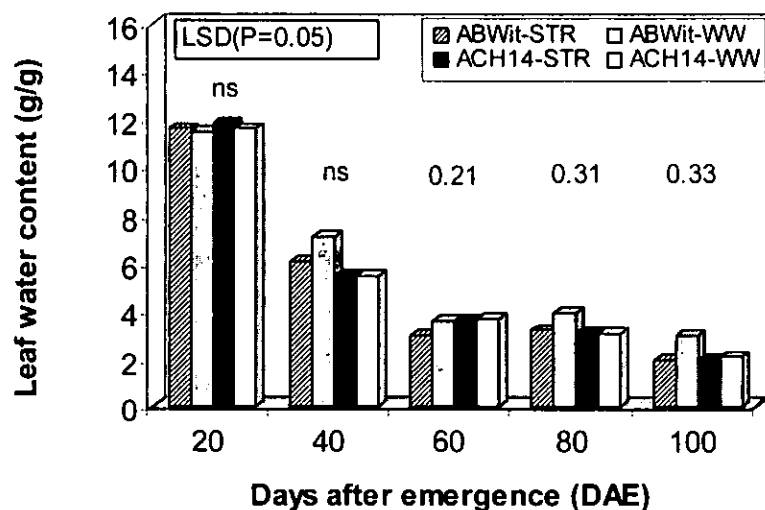


Figure 3.2 Leaf DM of two cowpea cultivars, Akkerbone Bechuana Wit (AB Wit) and Akkerbone CH14 (ACH14), grown under well-watered (WW) and water stressed (STR) conditions. Values are means of 8 plants.



**Figure 3.3** Leaf water content of two cowpea cultivars, Akkerbone Bechuana Wit (AB Wit) and Akkerbone CH14 (ACH14), grown under well-watered (WW) and water stressed (STR) conditions. Values are means of 8 plants.

### Stem growth

The results of stem dry mass are presented in Tables 3.2 and 3.6. The stem dry mass was affected by both cultivar and water stress at 40, 80 and 100 DAE and by water stress at 60 DAE, but not cultivar. However, no significant cultivar and water stress interactions were found at any sampling date (Table 3.2). Stem dry mass of stressed plants was reduced by 24%, 47%, 42% and 39% compared to well-watered plants at 40, 60, 80 and 100 DAE, respectively. When cultivars are compared, ACH14 produced higher stem dry mass at 40 DAE and less at 80 and 100 DAE, while at 60 DAE the two cultivars did not differ (Table 3.6).

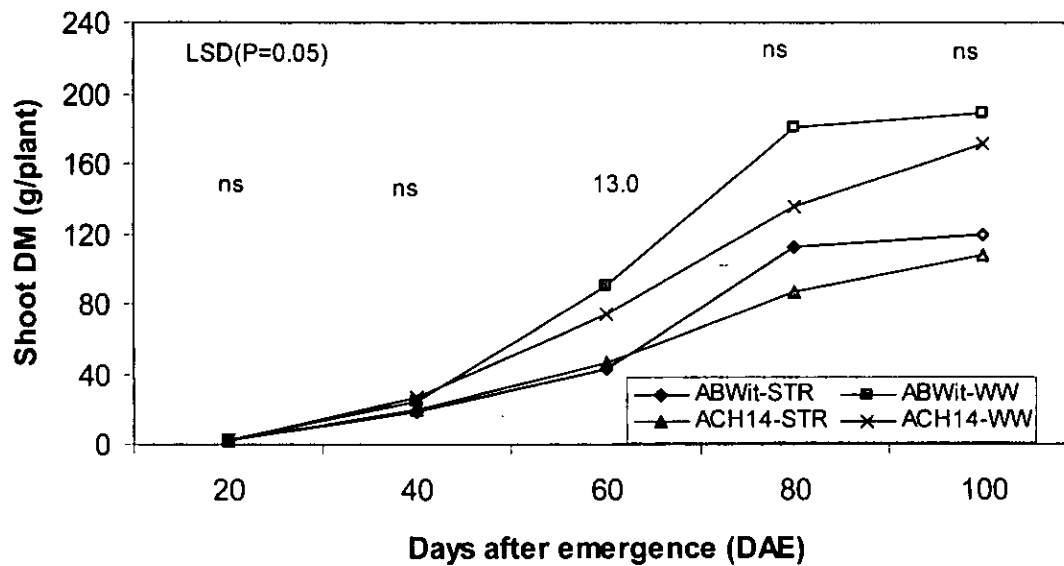
**Table 3.6** Stem dry mass of two cowpea cultivars grown under well-watered and water stressed conditions.

Water stress (S)	Cultivar (C)	Sampling time (DAE)				
		20	40	60	80	100
Well-watered	AB Wit	0.55	10.7	44.9	76.9	70.5
	ACH14	0.78	13.5	46.2	44.4	53.2
Mean		0.67	12.1	45.6	60.7	61.9
Stress	AB Wit	0.59	8.2	22.3	40.6	39.5
	ACH14	0.60	10.2	26.4	29.7	36.4
Mean		0.60	9.2	24.4	35.2	38.0
LSD(P=0.05): S		ns	0.9	4.6	12.7	9.5

AB Wit = Akkerbone Bechuana Wit; ACH14 = Akkerbone CH14. DAE = days after emergence. Values are means of 8 plants.

## Shoot growth

Shoot dry mass was affected by cultivar at 40 DAE and 80 DAE and water stress at 40, 60, 80 and 100 DAE (Table 3.2). Shoot dry mass of ACH14 was higher than that of AB Wit at 40 DAE and lower at 80 DAE, while water stress reduced the shoot dry mass by 24%, 46%, 37% and 37% at 40, 60, 80 and 100 DAE. At 60 DAE, the two cultivars did not differ under stressed conditions, but under well-watered conditions AB Wit produced more shoot dry mass. These results indicated differences in the reactions of the tested cultivars to water stress (Figure 3.4).

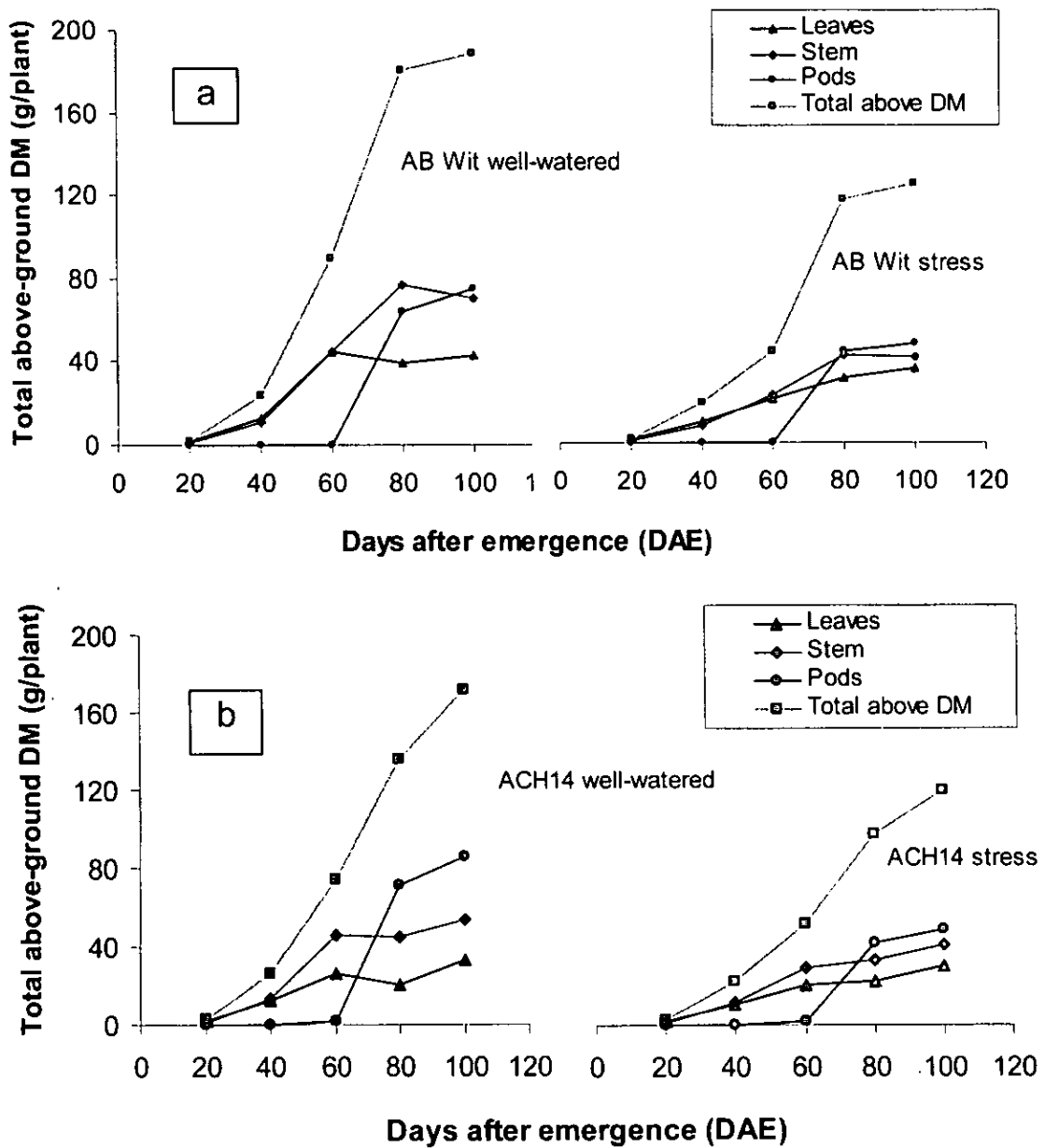


**Figure 3.4** Shoot dry mass of two cowpea cultivars, Akkerbone Bechuana Wit (AB Wit) and Akkerbone CH14 (ACH14), grown under well-watered (WW) and water stressed (STR) conditions. Values are means of 8 plants.

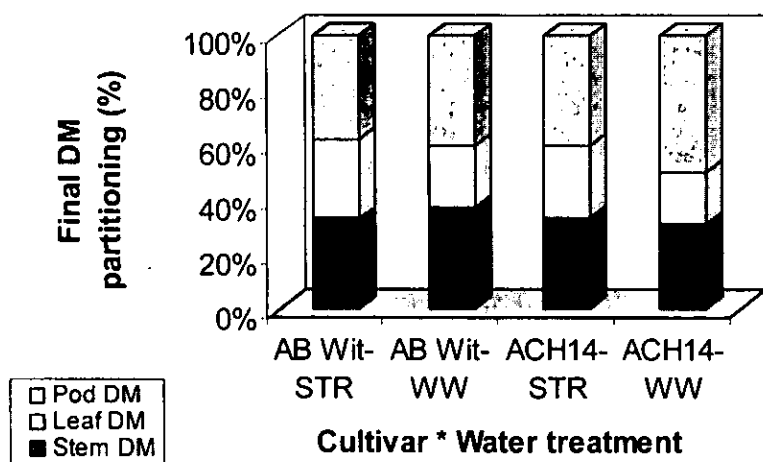
### **Total above-ground dry mass accumulation and partitioning**

The accumulation of above-ground dry mass of cowpea cultivars was affected by water stress (Figure 3.5a, b). Stressed plants accumulated less dry mass compared to the well-watered plants in both cultivars and pod DM was lower compared to the well-watered, which indicates that seed yield of stressed plants may be reduced due to the effect of water stress on biomass accumulation.

The results of dry mass partitioning between vegetative (leaves and stems) and reproductive (pods and seed) parts of cowpea cultivars are presented in Figure 3.6. The cultivar ACH14 allocated more dry mass to the pods (50%) compared to AB Wit (40%), under well-watered conditions, but under stressed conditions the two cultivars did not show significant differences (41% in ACH14 against 38% in AB Wit). Leaf dry mass of stressed plants was higher compared to well-watered plants in both cultivars, while stem dry mass did not differ (Figure 3.6).



**Figure 3.5** Total above-ground dry mass accumulation of two cowpea cultivars, Akkerbone Bechuana Wit (a) and Akkerbone CH14 (b) grown under well-watered and stressed conditions. Values are means of 8 plants.

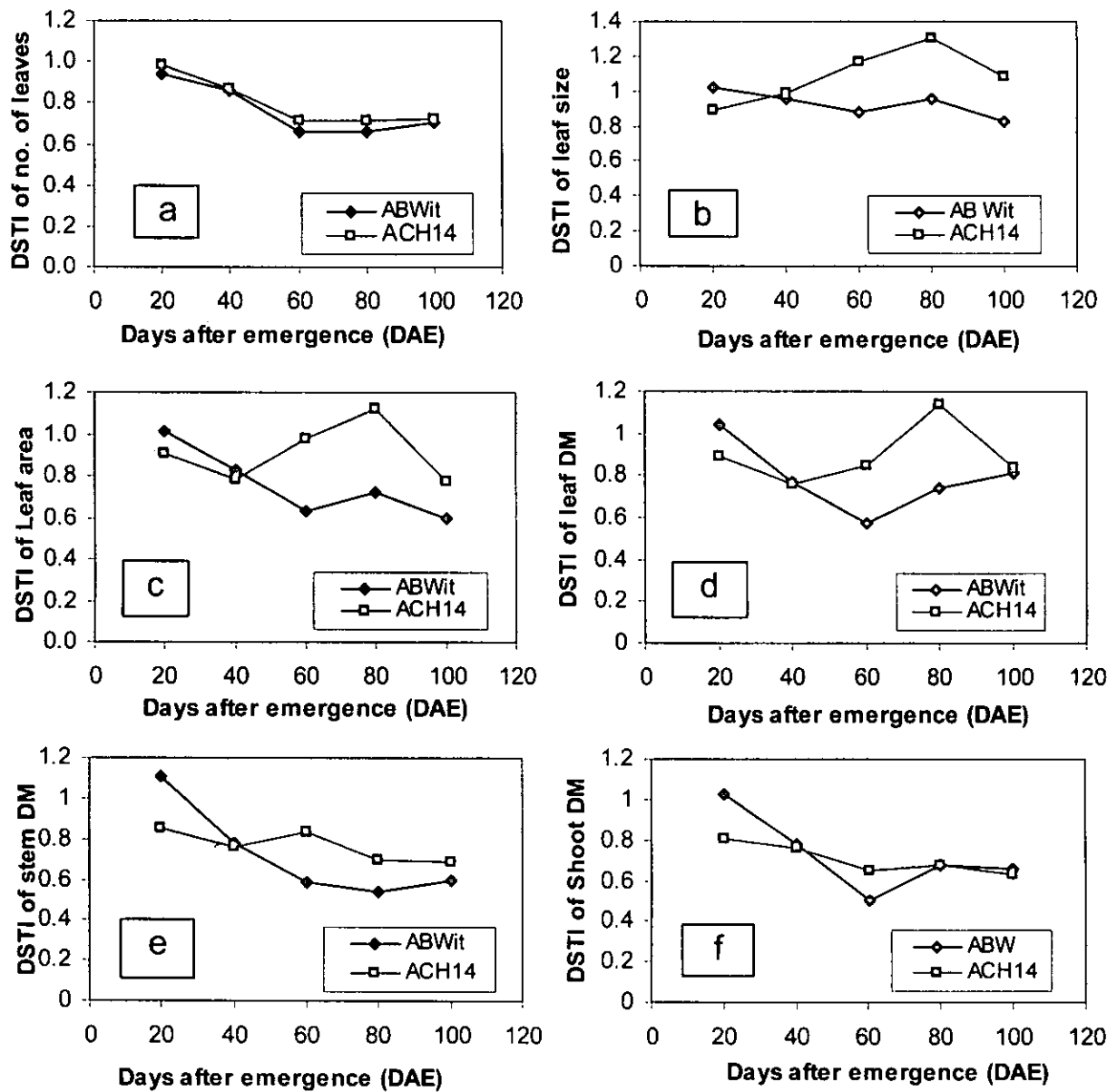


**Figure 3.6** Final above-ground dry mass partitioning of two cowpea cultivars, Akkerbone Bechuana Wit and Akkerbone CH14 grown under well-watered and stressed conditions. Values are means of 8 plants.

#### Effect of water stress on drought stress tolerance index (DSTI)

To summarize morphological responses the DSTI for different parameters was calculated (Figure 3.7 a, b, c, d, e, f). The DSTI changed with plant growth and increased time of exposure to water stress. From emergence to 60 DAE, the DSTI of AB Wit decreased for all parameters, but from that period to maturation (100 DAE), DSTI generally became stable. For ACH14, the DSTI did not show any definite trend because it decreased for some parameters and increased or stayed constant for others. A comparison between the cultivars shows that ACH14 is more tolerant to water stress than AB Wit, because most of the parameters measured showed higher response than in AB Wit.





**Figure 3.7** Drought tolerance indexes of the number of leaves (a), leaf size (b), leaf area (c), leaf dry mass (d), stem dry mass (e) and shoot dry mass (f) of two cowpea cultivars grown under water stressed or well-watered conditions.

### Seed yield and protein content of the grain

The results of seed yield and protein content of the grain are shown in Table 3.7. Seed yield was affected by both cultivar and water stress, but no significant interaction between cultivar and water stress was found (Table 3.2). Seed yield of stressed plants was 39% lower than that of well-watered plants (Table 3.7). When cultivars are compared it is clear that ACH14 produced a significantly higher seed yield than AB Wit.

Water stress increased grain protein content of AB Wit, but not of ACH14. Protein content of stressed plants, however, did not differ between cultivars (Table 3.7). This significant cultivar x water stress interaction indicated differences in cultivar responses.

**Table 3.7** Seed yield and protein content of the grain of two cultivars grown under well-watered and stressed conditions.

Water stress (S)	Cultivar (C)	Seed yield (g/plant)	Seed yield (%)	Grain protein (%)
Well-watered	AB Wit	58.3	100	17.3
	ACH14	84.1	100	20.3
Mean		71.2	100	18.8
Stressed	AB Wit	39.5	68	20.4
	ACH14	47.0	56	21.2
Mean		43.3	61	20.7
LSD(P=0.05): S		9.2		1.2

AB Wit is Akkerbone Bechuana Wit and ACH14 is Akkerbone CH14. Values are means of 8 plants.

## Yield components

The results of the number of pods, number of seeds per pod, 100-seed DM and pod DM are presented in Table 3.8. Water stress reduced both the number of pods per plant and pod DM, but not the number of seeds per pod. In contrast, water stress increased 100-seed DM. The number of pods per plant was the most important parameter that determined seed yield, since a strong correlation was found between the number of pods per plant and seed yield ( $r=0.82$ ;  $P<0.05$ ). These results suggest that the reduction in the number of pods per plant determined the seed yield reduction by water stress.

When cultivars are compared it is clear that ACH14 produced a significantly higher number of pods and 100-seed DM, but pod DM did not differ between the cultivars. In contrast, the number of seeds per pod of AB Wit was higher. A significant cultivar x water stress interaction was observed with regard to the number of seeds per pod because water stress decreased the number of seeds per pod in ACH14, but not in AB Wit.

**Table 3.8** Number of pods, seed number per pod, 100-seed DM and pod DM of two cultivars grown under well-watered and stressed conditions.

Water stress (S)	Cultivar (C)	Pod (no./plant)	Seed (no./pod)	100-seed DM (g)	Pod DM (g)
Well-watered	AB Wit	51.6	9.4	12.3	75.6
	ACH14	65.5	7.5	17.4	85.4
Mean		58.6	8.5	14.9	80.5
Stressed	AB Wit	26.3	10.3	15.0	45.3
	ACH14	43.8	6.0	18.9	43.5
Mean		35.1	8.2	17.0	44.4
LSD(P=0.05): S		5.0	1.5	1.6	11.6

AB Wit is Akkerbone Bechuana Wit and ACH14 is Akkerbone CH14. Values are means of 8 plants.

### 3.4 Discussion

In this experiment, two cowpea cultivars were subjected to water stress to examine the growth responses, yield and grain protein content in a temperature-controlled glasshouse, from the vegetative to the reproductive growth stages. The results showed that water stress treatments affected all the growth parameters as well as yield and grain protein content, but interactions with cultivars affected a few parameters.

Water stress reduced the number of leaves, leaf area, leaf dry mass, stem dry mass and total above-ground biomass production. Leaf area was reduced because water stress reduced both the number of leaves and the leaf expansion rate, but the number of leaves was the most important parameter that affected leaf area, since the number of leaves was more affected than the leaf expansion rate. A correlation of  $r=0.96$  was found between leaf area and leaf number. The reduction in the number of leaves and leaf expansion rate was probably due to the sensitivity of cell growth to water stress, as reported by Akyeampong (1986); Blum, Sullivan & Nguyen (1997). Because dry mass production in crops depends on the efficiency of leaves to use photosynthetic active radiation (PAR) to produce carbohydrates, leaf area reduction by water stress resulted in lower biomass production, as found by Akyeampong (1986). The lower biomass production due to water stress during vegetative and reproductive growth stages affected the production of seed yield. A correlation of  $r=0.85$  was found between seed yield and total above-ground dry mass. Seed yield was reduced due to a reduction in pod number per plant rather than seed dry mass per pod, since a significant positive correlation was found between seed yield and pod number ( $r=0.82$ ). Turk *et al.* (1980), Turk & Hall (1980) and Akyeampong (1986) also reported that seed yield reduction due to water stress was the result of low number of pods and small seed, but not to low number of seeds per pod. Although the number of seeds per pod was not affected by water stress, AB Wit had more seeds per pod compared to ACH14.

Cultivars displayed different responses to water stress. Stressed plants allocated more protein to the grain than well-watered plants in AB Wit, but not in ACH14. Wien *et al.* (1979) and Agenbag & De Villiers (1995) also showed an increase in grain protein content due to water stress in cowpeas and wheat, respectively. AB Wit, which outyielded ACH14 in most of the growth parameters tested under well-watered conditions, was more affected

by water stress compared to ACH14. In contrast to that of AB Wit, relative growth rate (RGR), leaf area expansion rate (RLAER) and leaf size of ACH14 were not affected by water stress. Leaf area and biomass production were also less affected than those of AB Wit. Differences in drought tolerance between the two cultivars were associated to both plant water relations and growth. Leaf diffusive resistance was not different between the cultivars, but that of ACH14 increased by 223% and 338% due to water stress at 30 DAE and 60 DAE, respectively. In AB Wit, water stress increased LDR by only 67% and 136% on these dates. Leaf water potential and leaf water content were reduced in AB Wit but not in ACH14. AB Wit showed a relatively higher leaf area at well-watered conditions, which could contribute to a higher transpiration demand per plant causing a relatively larger reduction in water potential and leaf water content compared to the relatively small ACH14 plants. Variation in leaf area was therefore probably the most important factor driving the respective differences in plant water status between the two cultivars. Blum *et al.* (1997) suggested that large and faster growing plants could be more susceptible to water stress because these plants exhibit higher rates of physiological activity, cell division and expansion, which can only be maintained in the absence of stress conditions. Tan & Halloran (1982) also found that high growth rates of wheat seedlings subjected to water stress correlated well with drought susceptibility.

The mechanism involved in drought tolerance in ACH14 seems to be related to the ability of this cultivar to close the stomata earlier, as shown by changes in LDR, which could help to maintain a higher water potential and leaf water content under water stress conditions, while for AB Wit the mechanism used to tolerate water stress seems to be associated to its ability to reduce the leaf area as mechanism to reduce the transpiration demands.

To summarize, it can be said that leaf water potential, leaf water content, RGR, leaf area expansion rate, seed dry mass and grain protein content of ACH14 was in contrast to that of AB Wit, not affected by water stress. These results suggested that ACH14 is more drought tolerant than AB Wit.

### 3.5 Conclusions

The aim of this experiment was to evaluate the morphological responses (shoot growth, yield and grain protein content) of two cowpea cultivars to induced water stress and therefore to identify the drought tolerance of the tested cultivars.

From the results it is clear that:

- 1) Water stress affected growth, yield and yield components of both cowpea cultivars.
- 2) Water stress reduced plant growth by reducing both the number of leaves, leaf area, leaf dry mass and the dry mass of stems. Leaf area per plant was reduced because of the reduction in both number of leaves and leaf expansion rate, but the number of leaves was the most important parameter that affected leaf area. Leaf area reduction by water stress reduced biomass production, which in turn affected seed yield.
- 3) Seed yield was reduced because water stress decreased the number of pods.
- 4) Protein content of the grain was increased by water stress only in AB Wit.
- 5) The two cultivars exhibit different mechanisms to tolerate water stress. While ACH14 plants closed their stomata more rapidly to reduce water loss and maintained water potential, AB Wit reduced leaf area to reduce the transpiration demand.
- 6) In general ACH14 seemed to be the most drought tolerant cultivar, since most of the parameters tested were not affected by water stress.

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## CHAPTER 4

# DROUGHT-INDUCED TOLERANCE OF COWPEA PLANTS AS A RESPONSE TO INCREASED PHOSPHORUS SUPPLY

### Abstract

Water stress is the main constraint that affects the production of cowpeas in the arid and semi-arid areas of sub-Saharan Africa. Knowledge of drought tolerance mechanisms induced by phosphorus nutrition may contribute to improve the management practices for areas subjected to drought. A water stress experiment was conducted in a temperature-controlled glasshouse (24/19°C day/night), to evaluate the plant growth responses and water relations (leaf diffusive resistance and proline accumulation in the leaves) of both well-watered and water stressed cowpea plants grown at high and low phosphorus levels. Phosphorus treatments were applied from planting to the end of the experiment by giving 1.80 mmol l<sup>-1</sup>, as high-P level and 0.10 mmol l<sup>-1</sup>, as low-P level. Water stress was applied from 16 to 30 days after emergence (DAE) by withholding water to the stressed plants. Well-watered plants were watered regularly to keep the soil moisture content at field water capacity (FWC). After the water stress period, the stressed plants were re-watered to FWC to evaluate the capability of plants to recover from the stress. High-P levels accelerated leaf appearance and plant growth during earlier stages, induced drought tolerance and resulted in more rapid recovery after re-watering compared to low-P plants. Water relations were improved by quicker stomatal closure in high-P plants reducing water losses by transpiration. Plant growth was higher in high-P level plants because the higher P increased root growth, which resulted in an increased leaf expansion rate and thus a greater leaf area, which intercepted more radiation and produced more biomass. After re-watering, all plant growth parameters of stressed plants recovered, but only the leaf expansion rate reached the level of the well-watered plants. Rapid recovery was due to better root growth and leaf expansion and most probably due to an increase in water uptake. Differences in drought tolerance between P levels indicated that management practices such as increased P supply should be applied in semi-arid areas.

**Keywords:** Cowpea; drought tolerance; phosphorus; re-watering; water stress.

#### 4.1 Introduction.

Cowpeas (*Vigna unguiculata* (L.) Walp.) are grown under low and erratic rainfall conditions in most of the arid and semi-arid areas of sub-Saharan African countries. Cowpea production in these areas is limited by the susceptibility of the plants to the dry conditions.

Recent findings suggested that plants could tolerate periods of drought if they were well supplied with phosphorus. For example, Singh, Sale & McKenzie (1997) found that high-P level white clover plants survived successive periods of soil drying and recovered better on re-watering than low-P level plants. High-P level plants extracted more water per unit time, were able to maintain higher leaf water potentials ( $\Psi_w$ ) in the dry soil than low-P level plants, and when defoliated frequently, displayed minimal water stress symptoms in dry soil. Singh & Sale (1997) found similar results. In a glasshouse experiment, clover plants supplied with high-P levels had turgid petioles and leaflets in the dry soil and frequent defoliation had no effect. Sing & Sale (1998) and Sing & Sale (2000) are of the opinion that high-P level plants are able to tolerate drought stress due to better water and P uptake as a result of a reduced resistance to water flow and an increased hydraulic conductivity.

Other literature also indicate that drought tolerance in terms of plant growth and improved water relations of some field crops can be enhanced by increasing P supply to the plants (Radin, 1984; Radin & Eidenbock, 1984). Benefits in cotton include increased plant growth, leaf conductance (Radin, 1984), root hydraulic conductivity, leaf expansion rate, leaf water potential and transpiration rate (Radin & Eidenbock, 1984).

More recent studies provided evidence that drought tolerance induced by high-P supply is associated with high proline concentrations (Al-Karaki, Clark & Sullivan, 1996; Sing *et al.*, 2000) and leaf expansion rates (Sing *et al.*, 2000). Al-Karaki *et al.* (1996) suggested that high accumulation of proline in the leaves of water stressed plants grown at high-P levels might be an adaptive response to drought for sorghum while Sing *et al.* (2000) suggested that increased proline accumulation and leaf expansion rates were directly associated with the ability of plants to recover from water stress.

Plant recovery from water stress after re-watering is an important aspect of drought tolerance (Huang, Duncan & Carrow, 1997); for example, recovery of root growth is important for rapid exploitation of water and nutrients following rainfall or irrigation (BassiriRad & Caldwell, 1992; Brady *et al.*, 1995).

Despite the sensitivity of cowpeas to water stress and their need for high P levels to maintain high growth rates and high yields, little is known with regard to the effect of P supply on the drought tolerance of cowpeas.

This study was conducted to test the hypothesis that high-P levels may improve drought tolerance in cowpeas and to examine the ability of the high-P plants to recover from stress.

## 4.2 Materials and Methods

The cowpea (*Vigna unguiculata* (L.) Walp.) cultivar Akkerbone Bechuana Wit (ARC-Grain Crops Institute, Potchefstroom, RSA) was grown for 45 days in 5-litre plastic bags filled with sterilized coarse sand in a temperature-controlled glasshouse (24/19°C day/night) during February 2003. The plants were grown from seeds pre-germinated for 48 hours in petri dishes containing filter paper moistened with distilled water. Three pre-germinated seeds were planted in each bag and then thinned to one plant per bag 7 days after planting.

Phosphorus treatments were applied to the plants from planting to the end of the experiment by using 1.8 mmol P l<sup>-1</sup> as high-P level and 0.10 mmol P l<sup>-1</sup> as low-P level, both as KH<sub>2</sub>PO<sub>4</sub>. The other nutrients that were applied were 13.2 mmol N l<sup>-1</sup> as nitrate, 7 mmol K l<sup>-1</sup>, 4 mmol Ca l<sup>-1</sup> as Ca(NO<sub>3</sub>)<sub>2</sub>·2H<sub>2</sub>O; 1.5 mmol Mg l<sup>-1</sup> and 1.5 mmol S l<sup>-1</sup> both as MgSO<sub>4</sub>·7H<sub>2</sub>O; and 40 B, 39 Fe as FeHEDTA, 15 Mn, 3 Zn, 1 Mo and 0.8 Cu in μmol l<sup>-1</sup>. For the low-P level nutrient solution (0.1 mmol P l<sup>-1</sup>), the rest of the anions were proportionally increased to 14.6 mmol N l<sup>-1</sup> and 3.3 mmol S l<sup>-1</sup> to maintain the same ratio between the anions while the levels and ratio between amount of the cations remained the same as used for the high-P level nutrient solution.

Water stress was applied from 16 to 30 days after emergence (DAE), by withholding water to the stressed plants. The well-watered plants were watered regularly to keep the soil moisture at field water capacity (FWC). From 31 to 45 DAE when water stress was terminated, stressed plants were re-watered to FWC to evaluate the capability of plants to recover from water stress. The irrigation was done by a computerized system and the pulses were adjusted according to daily solar radiation. FWC was determined as the quantity of water needed to wet the soil until excess water started to drain from the bags after irrigation.

Eight plants per treatment were harvested before the stress treatment started (15 DAE) and at 30 and 45 DAE. During each harvesting, fresh weight of leaves, stems and roots were determined individually and in total (shoot and total). The dry mass (DM) was determined after drying for 48 hours at 80 °C. Dry mass was used to determine root: shoot ratio. Leaf diffusive resistance (LDR) readings were done between 11:00 AM and 3:00 PM, using a Li-COR steady state porometer. Proline content determination was done as

described by Bates, Waldren & Teare (1973). The leaf area was measured using a leaf area meter (Model 3100 LI-COR Inc., USA) and the relative leaf area expansion rate (RLAER) was determined as the slope of the linear regression of transformed data (natural logarithm) of the leaf area against time according to Lynch, Lauchli & Epstein (1991).

All treatments were replicated 8 times in a complete randomised block design arranged in a factorial combination of 2 phosphorus levels and 2 water levels. The analysis of variance (ANOVA) was done using SAS software. The Duncan Multiple Range Test (DMRT) was used to test for significant differences ( $P=5\%$ ) between treatment means.

### 4.3 Results

Treatment effects for different parameters are summarized in Table 4.1. Phosphorus (P) as main factor affected most of the measured parameters in all sampling dates. Although some parameters showed significant effects due to stress treatments at 15 DAE, these results must be due to experimental error, because all plants were well-watered up to this stage. At the end of the stress treatment (30 DAE) almost all parameters were affected by water stress, and after re-watering (at 45 DAE) only a few recovered to the level of well-watered plants. Significant phosphorus  $\times$  stress interactions showed that for some parameters high-P levels might reduce the effect of water stress. This tendency may indicate P-induced drought tolerance.

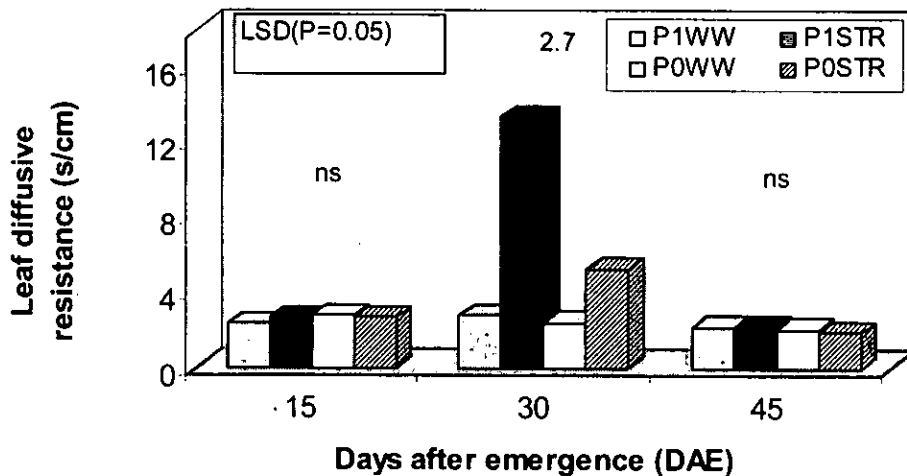
**Table 4.1** Significance of phosphorus (P) and water stress (S) responses as well as phosphorus  $\times$  water stress interactions (P  $\times$  S) for growth and physiological parameters at different sampling dates.

Crop growth and physiological parameters	Sampling date (DAE)								
	15			30			45		
	P	S	P $\times$ S	P	S	P $\times$ S	P	S	P $\times$ S
Leaf diffusive resistance	ns	ns	ns	*	*	*	ns	ns	ns
Proline content of leaves	ns	ns	ns	ns	*	ns	ns	ns	ns
RLAER	*	ns	ns	*	*	ns	ns	ns	ns
Root: Shoot ratio	ns	*	*	ns	ns	ns	*	*	*
Number of leaves	*	ns	ns	*	*	ns	*	*	ns
Leaf size	*	ns	ns	*	*	ns	*	*	ns
Leaf area	*	ns	ns	*	*	*	*	*	*
Leaf dry mass	*	*	*	*	*	*	*	*	*
Stem dry mass	*	ns	ns	*	*	*	*	*	*
Number of shoots	-	-	-	*	*	*	*	*	ns
Shoot dry mass	*	*	*	*	*	*	*	*	*
Root dry mass	*	ns	ns	*	*	ns	*	*	ns
Total dry mass	*	*	*	*	*	*	*	*	*

\* - Significant at P= 5%; ns = not significant; DAE = days after emergence

### Leaf diffusive resistance (LDR)

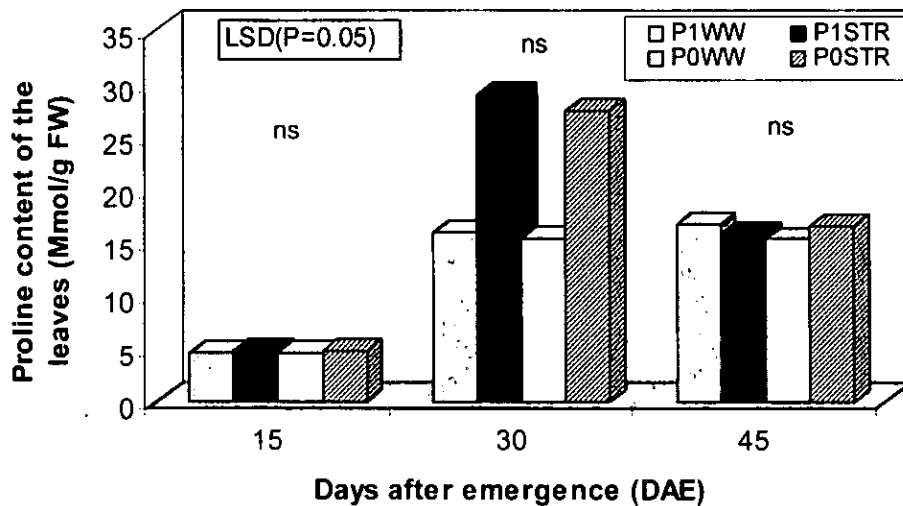
At the time that water was withheld (15 DAE), no significant differences in LDR were found due to either phosphorus or water stress treatment (Figure 4.1). After water stress was applied (30 DAE), LDR of stressed plants increased in both P levels, but was higher in plants receiving high-P than low-P treatment. High levels of P, therefore, caused plants to close their stomata more rapidly when subjected to water stress than plants grown under low-P levels. Under well-watered conditions, LDR did not differ due to P levels. After re-watering (45 DAE), the LDR of stressed plants decreased to the level of well-watered plants and there were no significant differences between P levels.



**Figure 4.1** Leaf diffusive resistance of well-watered (WW) and stressed (STR) cowpea plants grown under high-P (P1) and low-P (P0) levels. Values are means of 8 plants.

### Proline content of the leaves

Proline content of the leaves was not different at the onset (15 DAE) of the water stress (Figure 4.2). After water stress was applied (30 DAE), proline content of the leaves increased, but no significant phosphorus effect was found (Table 4.1, Figure 4.2). After re-watering (45 DAE), proline content of stressed plants decreased to the level of well-watered plants, but again with no significant differences between phosphorus levels. No significant interaction between phosphorus and water stress treatments was found at any sampling date.



**Figure 4.2** Proline content of well-watered (WW) and stressed (STR) cowpea plants grown under high-P (P1) and low-P (P0) levels. Values are means of 8 plants.



### **Number of leaves, leaf size, leaf area per plant and relative leaf area expansion rate**

The effects of the treatments on the number of leaves, leaf size, leaf area and relative leaf area expansion rate (RLAER) are presented in Table 4.2 and Figure 4.3. A significant interaction between phosphorus and water stress treatments was observed in the leaf area, but not in the number of leaves, leaf size and relative leaf area expansion rate (Table 4.1).

At 15 DAE, the number of leaves was increased by high-P levels (Table 4.2), but as expected no significant water stress effects were found (Table 4.1). After water stress was applied (30 DAE), the number of leaves of stressed plants was 23% lower compared to that of the well-watered plants. When P levels are compared, high-P levels resulted in a higher number of leaves compared to low-P levels. After re-watering (45 DAE), the number of leaves of stressed plants was 58% lower than that of well-watered plants. High-P levels again resulted in a higher number of leaves compared to low-P levels.

Leaf size was also increased by high-P levels at 15 DAE (Table 4.2), without any water stress effects (Table 4.1). Water stress reduced leaf size by 36% (30 DAE), but high-P level plants still had a higher leaf size compared to low-P level plants. After re-watering (45 DAE), leaf size of stressed plants was 35% lower than that of well-watered plants and leaf size of plants grown at high-P levels was still higher compared to that of plants grown at low-P levels.

Leaf area was reduced by low-P levels at 15 DAE, without any interaction with water stress (Table 4.1). At this stage, water stress did not affect leaf area (Table 4.1). After water stress was applied (30 DAE), leaf area was reduced by 56% and 65% at high and low-P levels, respectively, but high-P levels resulted in a higher leaf area compared to low-P levels in both water treatments (Figure 4.3). After re-watering, the leaf area of stressed plants were 50% and 70% lower than that of the well-watered plants with high and low P levels, respectively. High-P levels, therefore, resulted in higher leaf area in both well-watered and water stressed conditions. The interaction between phosphorus and water stress found at 30 and 45 DAE, indicated P-induced drought tolerance.

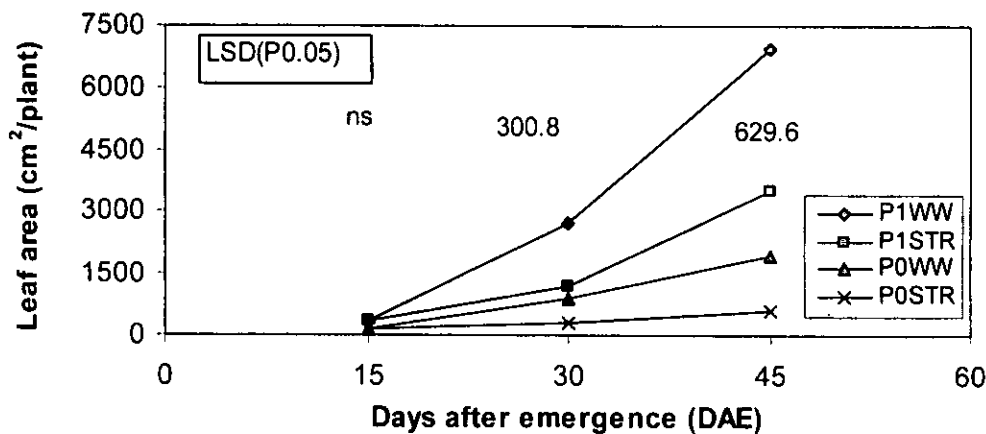
Relative leaf area expansion rate (RLAER) was reduced by low-P levels at 15 DAE (Table 4.2). After water stress was applied (30 DAE), RLAER of stressed plants was 33% lower than that of well-watered plants. When P levels are compared, high-P levels resulted

in higher RLAER compared to low-P levels. After re-watering (45 DAE), RLAER of stressed plants recovered, becoming similar to that of well-watered plants and no significant phosphorus effects or phosphorus × stress interactions were found (Table 4.1).

**Table 4.2** Number of leaves per plant, leaf size and relative leaf area expansion rate (RLAER) of well-watered and stressed cowpea plants grown under high and low phosphorus levels

Phosphorus levels (P)	Water stress (S)	No. of leaves	%	Leaf size (cm <sup>2</sup> )	%	RLAER (cm <sup>2</sup> .g <sup>-1</sup> .day <sup>-1</sup> )	%
15 DAE							
High	Well-watered	3.6	100	93.3	100	1.000	100
	Stressed	3.6	100	91.7	98	0.990	99
Mean		3.6	100	92.5	100	0.995	100
Low	Well-watered	2.8	100	52.8	100	0.940	100
	Stressed	3.0	107	51.4	97	0.950	101
Mean		2.9	81	52.1	56	0.945	95
LSD(P=0.05): P		0.3		8.7		0.012	
30 DAE							
High	Well-watered	17.4	100	155.2	100	0.140	100
	Stressed	10.9	63	109.9	71	0.090	64
Mean		14.2	100	132.6	100	0.120	100
Low	Well-watered	8.3	100	106.8	100	0.120	100
	Stressed	4.4	53	68.3	64	0.040	33
Mean		6.4	77	87.6	66	0.080	67
LSD(P=0.05): P		1.3		7.6		0.016	
45 DAE							
High	Well-watered	33.6	100	207.9	100	0.065	100
	Stressed	23.2	69	149.1	72	0.070	108
Mean		28.4	100	128.5	100	0.068	100
Low	Well-watered	16.3	100	126.6	100	0.030	100
	Stressed	7.3	45	81.7	65	0.045	150
Mean		11.3	42	104.2	81	0.038	66
LSD(P=0.05): P		2.0		14.9		ns	

DAE = days after emergence. Values are means of 8 plants.



**Figure 4.3** Leaf area of well-watered (WW) and stressed (STR) cowpea plants grown under high-P (P1) and low-P (P0) levels. Values are means of 8 plants.

#### Leaf DM, stem DM, root DM and root: shoot ratio

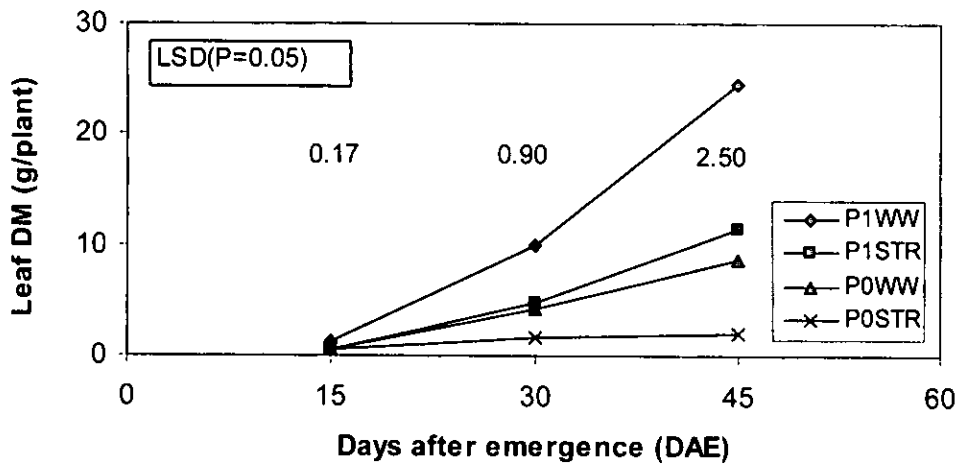
Leaf DM was affected by phosphorus and water stress treatments at all sampling dates (Table 4.1). Although significant effects due to water stress and phosphorus and stress interactions were found at 15 DAE (Table 4.1), these effects as already mentioned must be due to experimental error as no stress treatments were applied at this stage. After water stress was applied (30 DAE), leaf DM of stressed plants was reduced by 48% and 60% at high and low-P levels, respectively (Figure 4.4). When P levels are compared, it is clear that high-P level plants produced more leaf DM than low-P level plants. After re-watering (45 DAE), leaf DM of stressed plants remained lower compared to that of well-watered plants by 53% and 76%, at high and low-P levels, respectively. High-P level plants again produced higher leaf DM compared to low-P level plants, for both well-watered and water stressed conditions. The significant interaction between phosphorus and water stress treatments found at 30 and 45 DAE, indicated P-induced drought tolerance.

Stem DM was affected by phosphorus at 15 DAE and by phosphorus and water stress treatments at 30 and 45 DAE, and a significant interaction between phosphorus and water stress treatments was found at 30 and 45 DAE, but not at 15 DAE (Table 4.1). These interactions indicated P-induced drought tolerance. At 15 DAE, stem DM of high-P level plants was higher compared to the low-P level plants, but as expected no water stress effects were found (Table 4.1). After water stress was applied (30 DAE), stem DM of stressed plants was reduced by 51% and 58% compared to that of well watered plants at

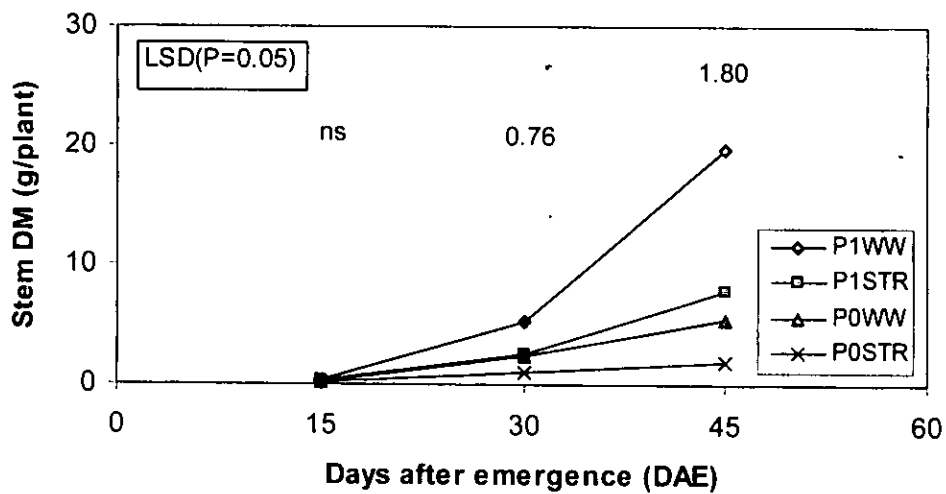
high and low-P levels, respectively. Stem DM of high-P level plants was higher compared to low-P level plants for both water treatments. After re-watering (45 DAE), stem DM of stressed plants remained lower than that of well-watered plants, by 60% and 65%, at high and low P levels, respectively. Stem DM of high-P level plants again was higher compared to that of low-P level plants for both water treatments.

Root DM was affected by phosphorus supply at 15 DAE and by phosphorus and water treatments at 30 and 45 DAE, but no significant interactions between phosphorus and water stress treatments were found at any sampling date (Table 4.1). At all sampling dates root DM of plants grown at high-P levels was higher compared to low-P levels (Table 4.3). After water stress was applied (30 DAE), root DM of stressed plants was 44% lower compared to that of well-watered plants. After re-watering (45 DAE), the root DM of stressed plants remained 55% lower than that of well-watered plants.

Root: shoot ratio was not affected by P levels at the time of onset of water stress (15 DAE). The significant water stress effects found at 15 DAE were due to already mentioned experimental error as no water stress treatments were applied up to this stage. A significant interaction between phosphorus and water stress treatments at 45 DAE, indicated differences in drought tolerance. After water stress was applied, root: shoot ratio was neither affected by phosphorus nor water stress treatments. After re-watering, the root: shoot ratio of stressed plants grown at low-P levels increased, but no such ratio increase took place at high-P levels. As a result low-P level plants showed higher root: shoot ratios than high-P level plants under stressed conditions, but not under well-watered conditions.



**Figure 4.4** Leaf DM of well-watered (WW) and stressed (STR) cowpea plants grown under high-P (P1) and low-P (P0) levels. Values are means of 8 plants.

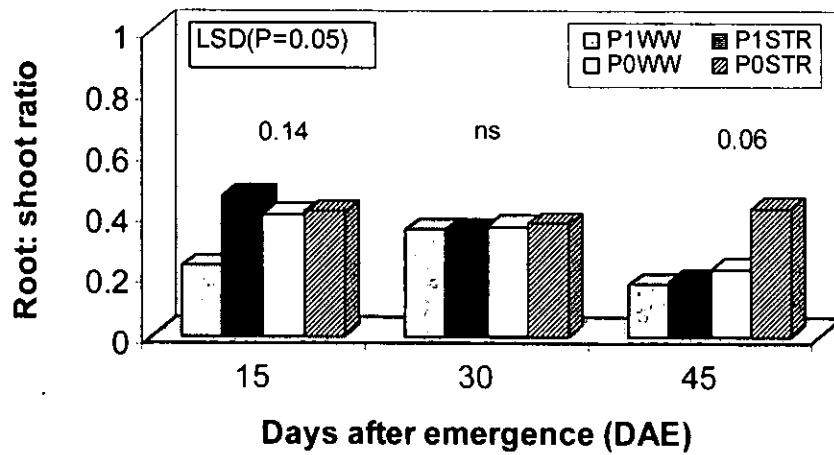


**Figure 4.5** Stem DM of well-watered (WW) and stressed (STR) cowpea plants grown under high-P (P1) and low-P (P0) levels. Values are means of 8 plants.

**Table 4.3** Root growth (g/plant) of well-watered and stressed cowpea plants grown under high-P and low-P levels.

Phosphorus level (P)	Water stress (S)	Sampling time (DAE)					
		15		30		45	
		DM	%DM	DM	%DM	DM	%DM
High	Well-watered	0.35	100	1.8	100	7.2	100
	Stressed	0.45	129	1.4	78	3.5	49
Mean		0.40	100	1.6	100	5.4	100
Low	Well-watered	0.29	100	1.2	100	3.1	100
	Stressed	0.33	114	0.6	50	1.7	55
Mean		0.31	77	0.9	56	2.4	45
LSD(P=0.05): P		0.07		0.2		1.4	

DAE = days after emergence. Values are means of 8 plants; DM = dry mass

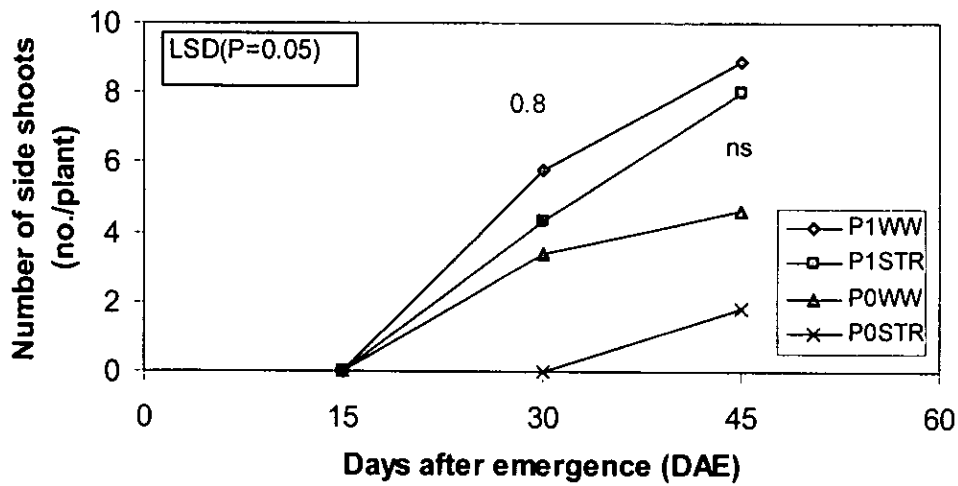


**Figure 4.5** Root: shoot ratio of well-watered (WW) and stressed (STR) cowpea plants grown under high-P (P1) and low-P (P0) levels. Values are means of 8 plants.

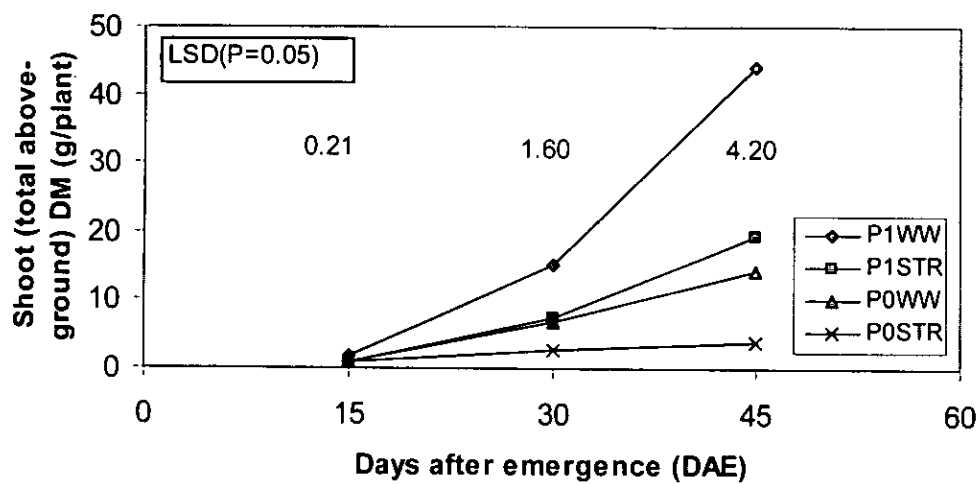
### **Number of side shoots, shoot (total above-ground) DM and total DM**

From emergence to 15 DAE, no side shoots were produced at both phosphorus levels. Side shoots started to emerge between 16 and 30 DAE (Figure 4.6). After water stress was applied (30 DAE), the production of side shoots was reduced by 26% and 100% at high and low P levels, respectively. When P levels are compared, it is clear that high-P levels caused more side shoots to emerge compared to low-P levels for both water stress treatments. After re-watering, the number of side shoots of stressed plants was 10% and 61% lower than that of the well-watered plants. High-P levels again resulted in a higher number of side shoots compared to low-P levels. The significant interaction between phosphorus and water stress treatment found at 30 DAE, indicated P-induced drought tolerance.

Shoot (total above-ground) and total DM were affected by phosphorus and water stress treatments at all sampling dates. The significant water stress effects and phosphorus  $\times$  stress interaction found at 15 DAE, were due to already mentioned experimental error, as no water stress was applied up to this stage (Table 4.1). At 15 DAE, shoot and total DM of high-P level plants were higher compared to low-P level plants (Figure 4.7 & 4.8). Shoot and total DM of high-P level plants were higher compared to low-P level plants. After the water stress was applied, shoot and total DM of stressed plants were reduced by 52% and 60%, and 48% and 59% at high and low P levels, respectively. When P levels are compared, high-P levels resulted in a higher shoot and total DM compared to low-P levels for all water stress treatments. After re-watering, shoot and total DM of stressed plants remained lower than that of well-watered plants by 56% and 72%, and 55% and 67% with high and low P levels, respectively. High-P levels again resulted in a higher shoot and total DM compared to the low-P levels. The significant interaction between phosphorus and water stress treatments found at 30 and 45 DAE, indicated P-induced drought tolerance.

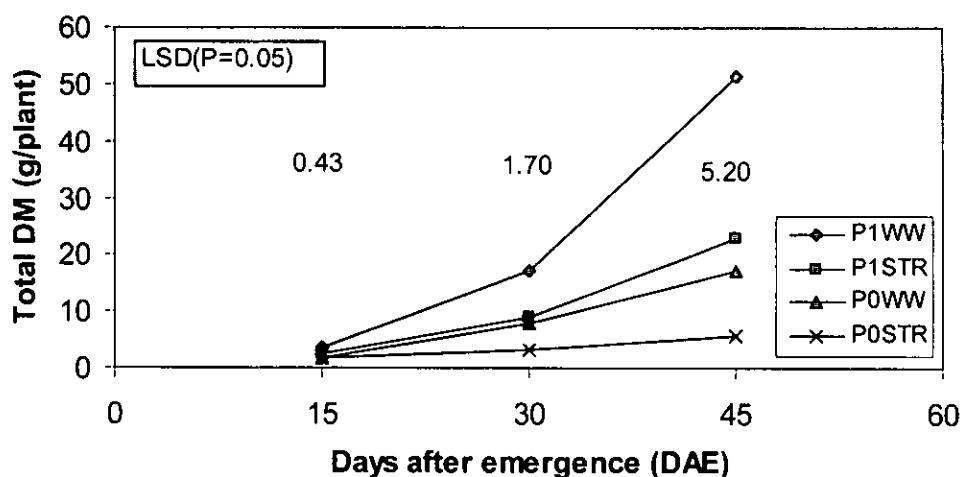


**Figure 4.6** Number of side shoots of well-watered (WW) and stressed (STR) cowpea plants grown under high-P (P1) and low-P (P0) levels. Values are means of 8 plants.



**Figure 4.7** Shoot (total above-ground) DM of well-watered (WW) and stressed (STR) cowpea plants grown under high-P (P1) and low-P (P0) levels. Values are means of 8 plants.





**Figure 4.8** Total DM of well-watered (WW) and stressed (STR) cowpea plants grown under high-P (P1) and low-P (P0) levels. Values are means of 8 plants.

#### 4.4 Discussion

In this study, phosphorus was fertigated from planting to the end of the experiment while a water stress was imposed from 16 to 30 DAE by withholding water to stress the plants. Water stress was terminated at 30 DAE by re-watering the stressed plants to field water capacity to examine the ability of plants to recover from the stress.

High-P levels accelerated leaf appearance as also reported by Radin & Eidenbock (1984); Lynch *et al.* (1991); Elliot *et al.* (1997); Gutierrez-Bloem & Thomas (1998) and Chiera, Thomas & Rufty (2002) and plant growth during earlier stages compared to low-P levels. Rapid leaf appearance most probably was the result of an increased rate of cell division as found by Chiera *et al.* (2002). Vigorous plant growth during earlier stages with increased P levels was the result of rapid leaf area development and increased root growth. Borges & Mallarino (2000) also reported an increase in early plant growth due to an increased P supply. Leaf area developed earlier as the result of earlier leaf appearance, increased leaf size, and leaf expansion rate, but leaf size seemed to be the most important contributing factor.

Water stress affected both water relations and plant growth. Leaf diffusive resistance (LDR) and proline content increased while leaf area, number of side shoots, leaf dry mass, shoot (above-ground) dry mass and root growth decreased. Leaf area and root growth were the most important parameters that affected plant growth. Better root growth

promotes more soil exploitation for water and nutrient uptake and large leaf areas increases radiation interception, which is used to produce carbohydrates.

Leaf area was reduced because water stress reduced the number of leaves, leaf size, leaf expansion rate and the number of side shoots. The leaf expansion rate and the number of side shoots were the parameters that affected leaf area more markedly than leaf size and number of leaves. A reduced leaf size and leaf expansion rate could be associated with a reduced rate of cell division (Chiera *et al.*, 2002) and expansion (Hsiao, 1973; Akyeampong, 1986). The reduction in root growth and leaf area development by the water stress affected shoot (total above-ground) and total biomass production.

Phosphorus supply generated different responses to water stress. High-P levels increased LDR. Rapid stomatal closure in high-P level plants was the result of induced water shortages due to increased water uptake and higher losses by transpiration because of better root growth and larger leaf area. For this reason, high-P level plants were the first to show stress symptoms, but recovered more rapidly when re-watered. Stomatal closure might limit the potential of carbon uptake during the water stress period, however, this strategy insured plant survival until re-watering and therefore, more rapid recovery. Al-Karaki *et al.* (1996) and Gutiérrez-Bloem & Thomas (1998) found that stomatal resistance was higher in low-P level plants than in high-P level plants. The contrast between these findings and the results of this study should be related to the volume of soil available per plant. Small bags (5 litre) and sand soil used in this experiment limited the water holding capacity of the soil available per plant. More vigorously growing plants (high-P) depleted the available water more rapidly. High-P level plants therefore seemed to be more drought tolerant. Singh *et al.* (1997) and Singh *et al.* (2000) showed that high-P supply might also induce drought tolerance due to the increased ability of water extraction, maintenance of higher water potential and rapid recovery after water re-establishment.

It is well known that proline increases in leaves of many plant species grown under water stress conditions (Handa *et al.*, 1986; Ober & Sharp, 1994; Bussis & Heineke, 1998) and that this increase in proline is seen as mechanism to improve drought tolerance. Although Al-Karaki *et al.* (1996) and Singh *et al.* (2000) found that high levels of P enhanced the accumulation of proline in sorghum and white clover, phosphorus level did not have any effect on proline content of the leaves in this study. Because Al-Karaki *et al.*

(1996) also found that proline content of beans did not respond to P level, this response might be plant specific.

High-P levels improved the growth of water stressed plants because it increased both leaf area, leaf size, leaf expansion rate, dry mass of leaves, shoot and roots compared to low-P levels. However, correlation values of  $r=0.99$  between leaf area and shoot DM as well as leaf area and total DM showed that leaf area was the most important parameter that promoted plant growth. More vigorous leaf growth is most probably the result of better and more efficient root growth at high-P levels. Increased biomass accumulation with increasing leaf area may be due to increased interception of photosynthetic active radiation (Mollier & Pellerin, 1999; Colomb, Kiniry & Debaeke, 2000), while higher root growth increased biomass accumulation because it increased the soil exploitation for nutrients and water uptake. High-P levels induced drought tolerance due to increased water and P uptake as a result of reduced resistance to water flow and increased root hydraulic conductivity (Singh & Sale, 1997). Singh & Sale (1997) suggested that reduced resistance to water flow was due to increased xylem diameter.

Leaf area increased in high-P level plants because of an increase in the number of leaves, leaf size; leaf expansion rate and number of shoots. Singh & Sale (1997) reported that higher leaf area in high-P level plants was due to an increased number of leaves and leaf size rather than the leaf expansion rate. In this study leaf area increased due to increased rate of leaf expansion, which was probably due to the ability to maintain cell turgor, since high-P level plants showed the tendency to maintain water content by closing the stomata earlier. These results support the findings of Radin & Eidenbock (1984); Singh *et al.* (2000) and Chiera *et al.* (2002) who observed that high-P levels increase leaf expansion rate due to an increased hydraulic conductivity and resultant higher leaf-tissue turgor pressure (Radin & Eidenbock, 1984) in combination with the maintenance of a higher turgor pressure, enhanced cell-wall extensibility (Singh *et al.*, 2000) and increased cell division (Chiera *et al.*, 2002).

After re-watering, LDR and proline content of stressed plants decreased to the level of well-watered plants. However, high-P level plants recovered more rapidly after re-watering and the visual stress symptoms disappeared in a shorter time. Rapid recovery after re-watering in high-P level plants was most probably due to the increased root

growth and leaf expansion rate. The leaf expansion rate of stressed plants increased to the level of well-watered plants. The rapid leaf expansion rate recovery was probably due to already mentioned P effects on the rate of cell division (Chiera *et al.*, 2002), cell expansion and cell extensibility (Singh *et al.*, 2000).

Root: shoot ratio is an indication of the partitioning of photosynthetic products between roots and above-ground tissue. In this study, water stressed low-P level plants showed a higher root: shoot ratio after re-watering than high-P level plants, while no differences were found between well-watered plants. This indicated, as was also found by Nielsen, Eshel & Lynch (2001) that a larger percentage of the biomass was allocated to the roots of stressed low-P level plants compared to high-P level plants. The reduced root and shoot DM of low-P plants at 45 DAE clearly showed that high-P level plants still outyielded low-P level plants during the water stress conditions. High-P plants may therefore still be regarded as more drought tolerant than low-P plants.

To summarize, high-P levels promoted earlier leaf appearance and plant growth, induced drought tolerance by improving both water relations and plant growth during the water stress period, and promoted rapid recovery on re-watering. These responses were the result of increased root growth and water and nutrient uptake and increased leaf expansion rate. However, this seemingly better tolerance to water stress with increased P supply may change with increasing time of exposure to drought conditions since larger plants are associated with larger extraction of water from the soil and greater losses by transpiration.

## 4.5 Conclusions

This study was conducted to test the hypothesis that P supply may alter the drought tolerance of cowpea plants and their ability to recover from stress.

From the results it was clear that:

- 1) High-P supply accelerated leaf appearance and plant growth at earlier stages.
- 2) High-P supply induced drought tolerance in cowpea plants by improving both water relations and plant growth during the stress period and by promoting rapid recovery after re-watering.
- 3) Water relations were improved by the more rapid stomatal closure, which resulted in a reduced rate of water loss by transpiration.
- 4) High-P level improved plant growth because it enhanced root growth, which may have improved water uptake resulting in a higher leaf expansion rate. A higher leaf expansion rate increased leaf area, which intercepted more radiation and produced more biomass.
- 5) After re-watering, high-P level plants recovered more rapidly compared to low-P level plants. All plant growth parameters of stressed plants increased after re-watering, but only leaf area expansion rate reached the level of well-watered plants.

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## CHAPTER 5

### SUMMARY

The term 'water stress', as used in this study, refers to an important constraint, which limits the production of cowpeas due to their sensitivity to water-limited conditions. This constraint affects plant water relations, which result in both reduced plant growth, biomass accumulation, seed yield and in some cases induced changes in plant quality. An important characteristic of crop production is the ability of crop plants to withstand water stress and to grow and yield satisfactorily under these conditions. This ability to withstand water stress may enable farmers to produce cowpeas in areas subjected to periodic droughts.

The aims of this study were the following; to evaluate the responses of physiological parameters (water potential, leaf diffusive resistance, relative water content, proline accumulation and cell membrane stability) of two cowpea cultivars to water stress; to identify those parameters which can be used to distinguish between drought tolerant and susceptible cultivars of cowpeas; to use some morphological growth responses, yield and grain protein content of two cowpea cultivars subjected to water stress to evaluate their drought tolerance and; to test the hypothesis that increased phosphorus supply may improve the tolerance of cowpea plants to water stress and the ability to recover from stress.

The results indicated that all the physiological parameters were affected by water stress in the cultivar AB Wit, but not in cultivar ACH14. Water potential, relative water content and proline content were the only parameters that responded in such a way that they could be used to distinguish between drought tolerant and susceptible cowpea cultivars. In both cultivars, changes in water relations seemed to be associated with changes in relative water content. However, these cultivars exhibited different responses to water stress. In ACH14, water potential was not affected by water stress and accumulated more proline. The more rapid proline accumulation in ACH14 induced osmotic adjustment that helped to maintain higher water potentials. For this reason, ACH14 is regarded as a more drought tolerant cultivar than AB Wit.

Because of the above mentioned effects of water stress on plant water relations, plant growth, biomass accumulation, seed yield and grain protein content, the two cowpea cultivars were also evaluated when subjected to water stress. The results clearly showed that water stress affected the plant growth parameters, yield and grain protein content of the tested cultivars. Both plant growth and yield were reduced because water stress reduced leaf area per plant as the result of a reduction in the number of leaves and leaf expansion rate. The number of leaves was, however, the most important parameter that determined leaf area reduction. Leaf area reduction by water stress reduced the photosynthetic active radiation received by the plant canopy and thus the biomass production. Because leaf area is positively correlated with seed yield, leaf area reduction also reduced seed yield. Seed yield reduction was the direct result of a reduced number of pods per plant. Protein content of the grain was affected by water stress in AB Wit only. The two cultivars showed, as already mentioned, different responses to water stress. The cultivar AB Wit, which performed better under well-watered conditions, was more affected by water stress. The susceptibility of AB Wit was associated with its higher leaf area, which resulted in excessive water loss. Under water stress conditions this cultivar reduced its leaf area as a mechanism to reduce water loss. For ACH14 most of the growth parameters were not affected by water stress. For this reason ACH14 is again regarded as more drought tolerant than AB Wit. The mechanism involved in drought tolerance by ACH14 seemed to be the earlier stomatal closure that may maintain high water potentials. As shown in the first experiment, this ability was most probably due to osmotic adjustment caused by proline accumulation.

Because both plant water relations and morphological growth parameters were found to be severely affected by water stress in AB Wit, another experiment was conducted to investigate if an agronomic practice such as increased phosphorus supply may improve the tolerance of this cultivar to water stress. From the results it became clear that increased phosphorus supply changed the response of cowpea plants to water stress. High-P level plants showed higher root growth, which resulted in more water uptake and larger leaf area compared to the low-P level plants during a water stress period. As a result high-P level plants produced more biomass than low-P level plants. More important, high-P level plants recovered quicker compared to the low-P level plants on re-watering. The rapid recovery from stress was the result of enhanced root growth and leaf expansion rates. This could ensure rapid water and nutrient uptake and higher rates of photosynthesis following

irrigation. Therefore, the induced drought tolerance with increased P supply indicated that P may be used in areas subjected to periodic droughts to help plants to withstand water stress.

Although this study clearly showed the responses and adaptive mechanisms of the cultivars tested, more studies involving these and other cultivars should be done under different environmental conditions to get a better understanding of the responses under these environments. Because the hypothesis that high P supply may induce drought tolerance in cowpeas was confirmed, more studies should be done testing different levels of phosphorus to determine the appropriate rate of application for future recommendation to the farmers. This study also showed that morphological parameters such as leaf area as well as physiological parameters such as water potential, relative water content, and proline content, were very important to determine the water stress of cowpeas, therefore, these parameters could be used as criteria to screen for drought tolerance in cowpeas.