EVALUATION OF PHYSIOLOGICAL AND MORPHOLOGICAL BASIS FOR DROUGHT RESISTANCE IN MAIZE AND SORGHUM

by

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Abstract

Drought stress is often the most limiting factor to maize and sorghum production in the semi-arid areas. This study evaluates the physiological (water relations, gas exchange characteristics, membrane leakage), biochemical (antioxidant protection mechanisms and photosynthetic pigment compositions) and seed viability and quality response of maize (cv Melkassa-2) and sorghum cv Macia) after exposure to and recovery from pre and post-flowering dehydration in plants grown in a controlled environment growth chamber under constant environmental conditions (12/12h day/night, 28-32/17 ^{0}C day/night temperature, 60-80% RH and PPFD of 1200-1400 µmol m-^{2} s^{-1}), at the Department of Botany, University of Cape Town.

Soil water contents and relative water contents during both pre and post-flowering dehydration declined steadily for both species but the decline in these parameters during both developmental stages were more rapid in maize than in sorghum. Maize displayed leaf rolling at very early stages of pre-flowering dehydration whereas sorghum delayed leaf rolling for extended period during both pre and post-flowering dehydration. Both species employed reduced g_s in response to dehydration to reduce water loss over a range of relative water contents during both developmental stages which was reflected in all other gas exchange characteristics (P_n, transpiration, respiration rates and water use efficiency). In maize, stomata appeared to be closed earlier and completely, while partial stomatal closure at relatively higher relative water contents appeared to have occurred in sorghum. g_s recovered to the control level in both species only following pre-flowering rehydration. Dehydration during pre and post-flowering stages resulted in decreased pigment (chlorophyll (a+b) and carotenoid (x+c)) contents of both species, but was more marked in maize than sorghum. Sorghum also exhibited better ability to restore chlorophyll and carotenoid level upon rehydration. Dehydration also led to a decrease in Fv/Fm ratios and increased rates of electrolyte leakage as compared to the control plants in both species. Both species, however, exhibited similar rates

of Fv/Fm ratios and rates of electrolyte leakages during pre and post-flowering dehydration. Fv/Fm ratios and electrolyte leakages rates appeared to be affected more during post than pre-flowering dehydration in both species. Fv/Fm ratios of both species recovered following pre-flowering rehydration but only maize recovered from post-flowering rehydration, respectively. Membrane reorganization was complete in maize during both pre and post-flowering rehydration, whereas in sorghum, full recovery was restored only following pr-flowering rehydration.

The present study on the response of antioxidant protection systems revealed that dehydration during both pre and post-flowering stages resulted in increased activities of enzymatic and in contents of non-enzymatic antioxidant protection mechanisms. There were differences between species in the type and extent of enhanced developmentally-induced and dehydration-induced antioxidant activities. Differences were also noticed between them in the relative water contents at which changes occurred. Under dehydration conditions, generally sorghum was found to have relatively higher antioxidant activities, providing it a better protection against oxidative stress by minimizing the level of lipid peroxidation.

Lipid peroxidation measured as malondialdehyde contents were increased in both species during pre and post-flowering dehydration, but the increase was greater in maize than in sorghum during both developmental stages. Sorghum appeared to be able to reduce malondialdehyde on rehydration but maize had only 85% less malondialdehyde content in rehydrated as compared to the control following pre-flowering rehydration. During post-flowering rehydration, neither species were able to decrease the malondialehyde contents to the control level.

Examination of standard germination test indicated that sorghum seeds harvested from both pre and post-flowering dehydration were not affected by drying but maize seeds had reduced % germination. Only sorghum seeds harvested from post-flowering dehydration significantly decreased vigor after accelerated ageing. With the exception of starch, dehydration during both pre and post-flowering stages resulted in reduced protein, lipid and soluble carbohydrates (sucrose, glucose and fructose) content in both species as compared to the control seeds. The species differed in the extent of reduction in those reserves.

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Chapter 1.

1. General Introduction

Over 35% of the world's land surface is considered to be arid and semi-arid, experiencing rainfall that is inadequate for agricultural crop production (Le Houerou, 1996). In such areas, the occurrence of recurrent and extended periods of drought is not uncommon. The problem is wide spread in areas where agriculture has been extended in to marginal rainfall areas and where subsistence agriculture is the main stay of the economy (McWilliams, 1989). As a consequence, in the developing countries such as in parts of sub-saharan Africa, much of Asia and North America, during the past several years a reduction in agricultural crop production has been a frequent occurrence. There are also increasing evidence indicating a change in the climatic zone of the earth (Idso, 1987; Sundquist, 1987; Kimball et al, 2001; Hillel and Rosenzweig, 2002; Pathak et al, 2003). The global atmospheric CO_2 has been observed to be rising steadily and is expected to double its current level before the end of this century, as a result of human activities (Jarvis, 1993, Keeling et al., 1995, Morison and Lawlor, 1999; Kimball et al, 2001). The rise in atmospheric CO_2 concentration is likely to affect global climate and to cause regional changes in air temperature and humidity, length of growing season, rainfall pattern and evaporation all of which will have considerable impact on plant water relations (Daie, 1988; Jarvis, 1993). Thus, water deficits will remain the most important limiting factor for crop production.

In the semi-arid areas of Africa, where much of the potentially arable land is located, maize and sorghum are important cereal food crops. In these areas, water deficit stress is the major environmental constraint to sustain crop production. The main feature of climate in most of these areas is that, for very short periods only, rainfall is adequate for crop growth, and for most of the growth period rainfall frequency and amounts often are quite variable (Ceccarelli 1984). Moreover, prevailing high diurnal temperatures and evaporation also aggravate the effects of soil moisture deficit. Despite their importance, production of these crops in many of the semi-arid areas is almost entirely under rain fed conditions; as a result the crops invariably suffer from water deficits of varying degrees and duration at any of their growth stages. Due to these fluctuations in water availability, yields of maize and sorghum are very low and

vary from one year to the other and from region to region (Maiti et al, 1996; Rosenow et al, 1997).

The response of plants to water deficits has both economic and evolutionary importance directly affecting crop productivity in agriculture and plant survival in the natural environment. For the past several years, improvement of the yield performance of agricultural crops in stressful environments has been addressed through traditional plant breeding by selecting suitable genotypes and/or cultivars with the ability to withstand the effects of water deficit and produce high and stable yield across locations and years. Many methods have also been employed to identify crop cultivars that are productive and drought tolerant. Some studies used mathematical models to compare the changes in grain yield between stress and non-stress (optimum) environments. For example, Fischer and Maurer (1978) developed a drought susceptibility index based on the ratio of yield of individual lines under stress and non-stress conditions to the ratio of line means across stress and non-stress conditions. This index has been widely used in identifying genotypes adapted to drought. Bidinger et al. (1978) developed a drought response index to provide an indicator of drought tolerance that was independent of phenology and yield potential in favourable environments. Although these strategies have been widely practiced in developing drought tolerant crop varieties, selection for grain yield under drought stress as compared to selection under non-stressed conditions has often been considered less efficient. A major reason for the slow progress in developing drought resistant crop varieties under drought stress conditions is the incidence of large genotype by environment (GxE) interactions, which result from a combination of differences in genotypic adaptation and the heterogeneous environments within the target areas (Blum, 1988; Johnson and Geadelmann, 1989; Nguyen et al., 1997). Ramanathan (1988) proposed, based on predictions of global environmental changes, that developing crops and crop cultivars which are more tolerant to water deficits while maintaining productivity will become a critical requirement in the early part of the 21st century. It is believed that the use of secondary traits can increase selection efficiency under environmental stress, provided that traits have a clear adaptive value under drought stress, have a relatively high heritability, have a significant genetic correlation with grain yield, and are easy to measure. Blum (1988) and Ludlow and Muchow (1990) proposed that the judicious incorporation of secondary traits within a breeding program for drought resistance in crop plants requires the identification of

relevant morphological and physiological traits as selection criteria. Studies of mechanisms of drought tolerance have suggested a wide range of selection criteria and most contemporary drought research assumes that tolerance results from interactions of a series of mechanisms (Blum, 1988). More detailed understanding of the physiological and biochemical adaptations, enabling superior performance of crop plants under water deficit stress is therefore needed, to identify reliable indices of drought resistance to complement conventional crop improvement programs.

1.1. Crop adaptation to drought stress

For different disciplines and investigators, the term drought stress is defined in different ways. For example, hydrologists may define drought stress as a naturally occurring phenomenon that exists when rainfall has been significantly below normal recorded levels causing hydrological imbalance that adversely affects land resource production systems (Williams and Balling, 1994). From the agricultural and ecological point of view, however, WMO's (1975) drought is taken. That is: 'A deficit of rainfall in respect to the long term mean, affecting a large area for one or several seasons or years, that drastically reduces primary production in natural ecosystems and rain fed agriculture'.

Plants are exposed to many types of environmental stresses, but drought stress constitutes the most serious limitation to plant growth, productivity and distribution (Boyer, 1982). Mechanisms for maintaining plant growth, productivity and development under such environments are complex and not fully understood. However, plants have a wide range of strategies to survive and reproduce under drought stress that occurs at various periods during the growth cycle. Their responses are expressed at morphological, physiological and cellular levels. A variety of adaptation mechanisms to water deficit exist in plants and has been extensively reviewed (Blum, 1988; Levitt, 1980). Turner (1986) indicated that in natural plant communities many of these attributes of drought resistance may be more important for survival than for high productivity, but there are also mechanisms that confer advantages for improved crop production under conditions of drought stress. Three general adaptive strategies for plant survival in drought stress environments have been proposed: drought escape, avoidance, or tolerance (Turner, 1986; Ludlow and Muchow, 1990) and drought resistance can be conferred by any one or a combination

of the three mechanisms. Each adaptive mechanism includes several traits which are being used for breeding drought resistance crop cultivars.

1.1.1. Drought escape

Mechanisms of drought escape usually involves early maturity before soil moisture becomes limiting. The most effective strategy of a drought-resistant crop should be to match the most sensitive phenological growth stage to the peak soil moisture availability (Richards, 1996), and drought escape has been the most reliable strategies of drought resistance for conditions of terminal drought stress. Development of short season varieties has provided beneficial where early season rainfall or soil moisture is reasonably predictable. In semi-arid regions where terminal drought is a common characteristic, extension of crop production has been accompanied by the development of early maturing cultivars that enable the crop to escape severe soil moisture deficits (Turner, 1986). In such environments, selection for a shorter time to flowering has been successful in improving the drought resistance of various crop species. Early flowering improved yield performance, greater yield stability and improved harvest index, if it enables the crop to escape drought during the critical reproductive stage (Ludlow and Muchow, 1990; Turner, 1997). The most notable benefit of drought escape which has resulted in improved yield under drought is that provided by shortening of the anthesis to silking interval of tropical maize (Bolanos and Edmeades, 1993). In their experiment, maize population went eight cycles of recurrent full sib selection under drought stress conditions where water supply was managed by irrigation. They found that by reducing the interval between anthesis and silking there was an increase in ear biomass and increased kernel number which was solely attributed to the increased partitioning of assimilates to the ear (Bolanos et al, 1993). The results of Winkel et al, (1997) also demonstrated a drought escape mechanisms in pearl millet grown in the sahelian environment (dryland). The major disadvantage in earliness of anthesis in marginal rainfall environments is, however, that yield decreased linearly with earliness by reducing dry matter at anthesis and due to shortened remobilization/translocation times for grain filling (Turner, 1986).

It is proposed that genotypes with a developmental plasticity may be more beneficial than earliness in flowering. Developmental plasticity is the mechanism whereby the duration of the growth period of a genotype varies depending on the extent of water deficits (Ludlow and Muchow, 1990). In this case, drought induced early maturity

may be advantageous during drought period, however, the plant is still able to respond to longer seasons and produce greater yields during long rainy seasons. In durum wheat subjected to different timing of water deficit, phenological development was affected differently (Simane et al, 1993) such that pre-anthesis water deficits delayed phonological development, whereas post-anthesis stress accelerated it.

1. 1. 2. Drought avoidance

Drought (dehydration) avoidance is some times referred to as drought tolerance at high water potential. This is an alternate mechanism for avoiding low water status in the plant tissues even under limited soil moisture conditions. This mechanism typically involves water conservation at the whole plant level (Tuinstra, et al, 1997). Drought avoidance is accomplished by either decreasing water loss from the shoot or by more efficiently extracting moisture from the soil. Traits such as reduced leaf area and stomatal regulation help reduce water loss from the shoot or deep root system enhance water uptake from the soil (Turner, 1986; Ludlow and Muchow, 1990). Therefore the mechanism that condition drought avoidance function at the whole plant level involving morphological and physiological responses.

One of the most obvious morphological changes following water deficit stress is a marked reduction in leaf area development through its effect on the rate of new leaf emergence and/or the rate of individual leaf expansion, or by reducing the number of leaves. It is well documented that reduction in leaf area has long been recognized as an adaptive mechanism of many crop species in response to water deficit (Hsiao, 1973; Levitt, 1980; Turner, 1986). Under water deficit conditions, reduction in leaf expansion rate occurs before any reduction in photosynthesis (Turner et al, 1986c, Saab and Sharp, 1989). The sensitivity of leaf growth to water deficits is a mechanism for reducing water loss, since below a leaf area index of about 3, crop transpiration rate is reduced linearly with leaf area (Turner, 1986; Ludlow and Muchow, 1990). In this case, evaporative demand can be controlled by a decrease in leaf growth, which is usually the first symptom of mild water deficits (Saab and Sharp, 1989). This response could enhance survival by reducing transpirational water loss, but on the contrary is detrimental to crop productivity upon relief from dehydration. As a result, maintenance of leaf area is considered as a trait contributing to yield under water limiting conditions (Ludlow and Muchow, 1990). Ludlow and Muchow (1990)

suggested that leaf area maintenance would improve yield stability in intermittent water deficit stress situation due to better radiation interception when water is available, whereas during post-anthesis water deficit, harvest index may be decreased as leaf area maintenance would increase the rate of water use and exhaust soil water more rapidly before maturity. Decreases in leaf area in response to water deficit stress has been reported in several crop species including wheat (Passioura, 1988); maize (Michelena and Boyer, 1982; Saab and Sharp, 1989); lupin (Jensen et al, 1998), sorghum (Rosenow et al, 1997) and rice (Bano et al, 1993). Moderate water deficits can induce considerable reductions in green leaf area as a consequence of senescence (Tardieu, 1996). As a result a decrease in water flux through the plant occurs, thereby contributing to the avoidance of rapid declines in leaf water potential and leaf turgor. In addition to reduced rates of leaf expansion, leaf rolling also occurs as a result of water deficit stress. This in turn reduces the total leaf area available for light interception (Hsiao, et al, 1984, Cruz, et al, 1986,) and reduces leaf temperature and water loss, with consequent increases in avoidance of drought (Ludlow and Muchow, 1990). The relative benefit of leaf rolling during pre-flowering dehydration may be enhanced survival until the next rainfall, however, no benefit is observed during post-flowering dehydration where it will only reduce the rate of water loss and delay the time until the water runs out (Ludlow and Muchow, 1990). In sorghum, leaf rolling is considered as a symptom of pre-flowering drought stress susceptibility and tolerance to pre-flowering drought stress is indicated by the alternative condition (Rosenow et al, 1997). Leaf rolling is a well recognized symptom of water stress in cereals (Hsiao et al, 1984) and is used extensively in drought resistance studies (Williams et al, 1987; Rosenow, 1993; Rosenow et al, 1983), since considerable genotypic variability under water deficit exists between and within crop species. Turner et al (1986) found that leaf rolling began at higher midday leaf water potentials and turgor pressures in upland rice than in low land adapted cultivars. The greater sensitivity of leaf rolling to water deficit in the upland cultivars delayed development of severe water deficit by 1-3 days. Lilley and Fukai (1994) have also reported genotypic variation in rice in sensitivity of leaf rolling to water deficit. Differences in the sensitivity of leaf rolling were accounted for by cultivar differences in water extraction. Transpirational water loss could also be regulated by the presence or absence of leaf traits such as epidermal conductance and leaf reflectance which enhances dehydration avoidance and thus, will promote survival of leaves and assists stability of grain yield. Moreover, early

seedling establishment and early vigor are important in order to reduce evaporation. These traits have been suggested as valuable drought resistance mechanisms at both intermittent and terminal stresses of cereals (Ludlow and Muchow, 1990).

Water deficit stress influences root growth profoundly. A severe water deficit usually reduces root growth and little or no root growth occurs in soils dried out to the permanent wilting point (Kramer and Boyer, 1995). Mild soil water deficits, however, promote root growth and often reduce shoot growth before root growth is reduced, resulting in increased root to shoot ratios. Several mechanisms are believed to contribute to the increase in root elongation. Mild water deficits which reduce leaf growth provide surplus carbohydrates that can be used for root growth (Aguirrezabal et al,1994). Since reduction in leaf expansion occurs before any reduction in photosynthesis, leaves of water stressed plants have higher concentrations of carbohydrates and this probably makes more carbohydrate available for root growth. The occurrence of osmotic adjustment which enhanced root cell expansion (Sharp et al, 1990), also resulted absolute root growth in stressed plants than non stressed plants (Jupp and Newman, 1987). Maize plants have a substantial capacity for osmotic adjustment and this correlated well with continued growth of the roots at low water potentials (Sharp and Davies, 1979; Westgate and Boyer, 1985). Increased ABA concentrations in the root has been implicated to play a role in maintaining cell expansion in the root while inhibiting cell expansion in the leaf under water deficit conditions (Saab et al, 1990; Saab et al. 1992). Sharp et al. (1994) have shown that accumulation of ABA is required for the maintenance of maize primary root elongation at low water potential.

Drought adapted plants are characterised by deep and vigorous root systems (Monneveux and Belhassen, 1996) and hence an increase in root length and density is considered as a major mechanisms of drought avoidance (Turner, 1986). Successful penetration of roots deep in the soil profile in drying soil may play an important role of increased uptake of water thereby resulting in the avoidance of water deficits at critical growth stages and increased harvest index. Sharp and Davies (1985) showed that root density could be considered as an alternative trait to screen for drought resistant lines. They observed that deep roots of the water stressed maize plants exhibited very high soil water uptake rates per unit root length relative to roots of well watered plants. Similar results have also been reported for sorghum (Blum and Arkin,

1984). In the semiarid areas where crops are grown in stored soil water, greater rooting depth could lead to improved stability in grain yield. However, extensive root depth and density at the early stage of plant development could be a disadvantage in semi-arid areas, as it may exhaust the available water prematurely and expose the plant to a critical and terminal drought later on (Passioura, 1996). Ludlow and Muchow (1990) suggested that in intermittent drought stress conditions, greater rooting depth could enhance yield stability by reducing the incidence and slowing of the development of water deficits. However, the risks of exhausting water before maturity would make greater rooting depth and density undesirable during post-anthesis water deficits. Genetic variation between plant species and within a species in root length density, root dry weight and root:shoot ratios are considerable and well established. Genetic variability for root length density have been reported for sorghum (Jordan and Miller, 1980), wheat (O'Brien, 1979), oats (Murphy and Nelson, 1982) and rice (Ekanayaka et al, 1985). Many of the root growth studies under water deficit conditions are performed in a controlled environment and/or greenhouse. This is because determination of root length and root depth in the field is time consuming. Moreover, due to interactive effects of other soil related stress factors (soil compaction and/or high soil temperature), it is subjected to year to year variability and inconsistency. As a result it is not feasible to screen large number of genotypes for rooting depth and density under field conditions.

Plants balance water loss with gas exchange through stomatal openings. Stomata allow atmospheric CO_2 to enter leaves for carbon fixation and oxygen to escape. Water evaporates from mesophyll and diffuses through open stomata. Stomatal water loss is specific to the leaves of vascular plants, in which, under non-stressed conditions, stomatal transpiration represents approximately 90% of total water loss (Monneveux and Belhassen, 1996). Shoot dehydration influences stomatal behaviour resulting from reduced turgor in the guard cells. Under water deficit, stomatal closure is the earliest response in crop plants and is a powerful tool for reducing water loss by adjusting the evapotranspirational demand to the water supplying capacity of the roots and thereby maintaining turgor (Sinclair and Ludlow, 1986). Stomatal sensitivity to water deficit may improve yield stability and internal water status and lowers the probability of exhausting the soil water before maturity, but it will reduce yield potential, as it has been shown to decrease net assimilation as a consequence of

reduced CO_2 influx. However, the degree of yield reduction due to stomatal closure is not yet clear. Since there is genetic variability of stomata behaviour in various crop species including maize and sorghum, genetic manipulation of this trait may be possible.

The reaction of stomata to water deficit stress varies with the developmental stages at which the stress occurs. As leaves grow older the stomata often becomes less responsive and may open only partly, even at midday (Kramer and Boyer, 1995). Ackerson and Kreig (1977) demonstrated that stomata of maize and sorghum closed when they were exposed to water deficit during vegetative stage but do not close in similar conditions during the reproductive stage. Garrity et al (1984) also observed a lack of stomatal response to water deficit after flowering in grain sorghum. It is therefore doubtful that during reproductive and grain filling stages when drought stress often occurs and is of greatest practical importance, that stomata play a significant role in regulating crop water loss (Garrity et al, 1984).

It has long been assumed that stomata respond uniformly to water deficit over the entire leaf. However, leaves of droughted plants exposed to ^{14}C show a heterogeneous distribution of fixed ^{14}C indicating that some stomates are closed completely where as others keep their aperture open (Downton et al., 1988). According to Beyschlag and Pfanz (1990) leaves of plants that reduce stomatal conductance during the middle of the day may only close some of their stomata, while others remain open. This non uniform reaction of stomata may occur only when plants are rapidly exposed to drought, whereas stomata may respond in a more uniform manner when the rate of the development of drought is more slowly (Gunasekara and Berkowitz, 1992).

It has been found that stomata of different species respond to soil drying differently (Tardieu et al., 1996). Leaves of isohydric species such as maize, cowpea and lupin, which control gas exchange in such a way that day time leaf water status is unaffected by soil drying, must control stomatal conductance by messages arriving from the root. This means that stomatal conductance declines before any adverse effects of water deficit arise in the leaves (Tardieu and Simonneau, 1998). In contrast to isohydric species, anisohydric species such as sorghum, sunflower, barley and wheat, both the leaf water potential (ψ_L) and stomatal conductance (g_s) decline with decreasing soil water potential. In isohydric species, the stress hormone ABA is the predominant message arriving from roots in contact with drying soil (Davies et al., 1994) while in anisohydric species both root sourced ABA and leaf water status regulate stomatal

conductance. The spectrum in stomatal strategies between isohydric and anisohydric plants is determined by the degree of influence of leaf water status on stomatal control for a given concentration of ABA in the xylem. Correlation between leaf conductance and leaf water status are only observed in plants where leaf water status has no controlling action on the stomata (Tardieu et al., 1996).

In higher plants, osmotic adjustment refers to the maintenance of turgor by lowering of tissue osmotic potential, arising from the net accumulation of compatible solutes in the cell in response to low water potential (Morgan, 1984; Turner, 1986; Zhang et al, 1999). Plant cells contain many dissolved substances known as solutes. Under water deficit conditions the cells and tissues of some crop plants increase their solute concentration. The increase in the concentration of solute inside the cell cause to dilute the internal water as compared to the outside. This assists the maintenance of cell turgor, because water is drawn back in to the cell, rather than being flowing out (Morgan, 1984; Kramer and Boyer, 1995). The maintenance of turgor pressure as the plant water potential declines is detrimental for cell enlargement, growth, stomatal opening and related physiological, morphological and biochemical processes (Morgan, 1984). Greater osmotic adjustment arises from the reduction in leaf growth rate at higher ψ_L than photosynthesis, thereby leading to a passive accumulation of solutes as the production of assimilates exceeds the demand for growth (McCree 1986; Quick, 1992; Zrenner and Stiff, 1991) and from hydrolysis of carbohydrates such as fructans (Spollen and Nelson, 1994).

Varieties of compatible solutes have been reported to accumulate in plant tissues under water deficit conditions. The compatible solutes differ between plant species and with in species. Many of these are sugars, organic acids, amino acids such as proline and glycine betaine, sugar alcohols like mannitols and other low molecular weight metabolites (Morgan, 1984; Bental et al, 1988; Voetberg and Sharp, 1991; Grumet and Hanson, 1988) The compatible solutes which have been used for osmotic adjustment may be used for regrowth under favourable conditions (McCree et al, 1984; Kramer and Boyer, 1995).

Munns (1988) questions the value of osmotic adjustment on the growth and yield performance of crop plants under water deficit conditions. She states that there are no reported cases where plants that adjust osmotically during stress grew more than those that did not adjust. However, there is increasing evidence that osmotic adjustment is

an effective adaptive mechanism against water deficits in a number of crop species (Westgate and Boyer, 1985; Ober and Sharp, 1994; Zhang et al, 1999). It is increasingly recognized in several crop plants as an effective component of drought resistance with a positive direct or indirect effect on plant productivity under conditions of drought stress (Ludlow and Muchow, 1990; Zhang et al, 1999). With the development of osmotic adjustment, there is development of cellular turgor, the result of which has a general effect on leaf rolling and delay of leaf death (Hsiao et al, 1984; Cruz et al, 1986). Leaf rolling and leaf death have been used as a reliable index of turgor loss in cereal crops by a reduction in leaf water potential (O'Toole and Cruz, 1980; Williams et al., 1987). Under field conditions, genotypes with low osmotic adjustment may show early leaf rolling because of loss of turgor in response to low water potential while genotypes with higher osmotic adjustment may show delayed leaf rolling because of turgor maintenance. Osmotic adjustment has been demonstrated to maintain root growth in response to low water potentials (Sharp and Davies, 1979; Westgate and Boyer, 1985a; Matyssek et al., 1991; Voetberg and Sharp, 1991; Ober and Sharp, 1994). Osmotic adjustment has also been shown to maintain stomatal opening and photosynthesis during water deficit (Downton, 1983; Ludlow et al, 1985; Seeman et al, 1986).

Several results have reported that ample genotypic variation exists for osmotic adjustment within a species under water deficit (Morgan, 1984; Turner, 1986). Genotypic variability under water deficit stress has been reported in cereals such as wheat (Morgan, 1983, 1996; Morgan et al, 1986); sorghum (Santamaria et al, 1990; Tangpremsri et al, 1991; Premachandra et al, 1992); barley (Blum, 1989); rice (Turner et al, 1986); pearl millet (Henson, 1982) and grain legumes such as chick pea (Morgan et al, 1991); field peas (Rodriguez-Maribona et al, 1992); pigeon pea (Flower and Ludlow, 1987). The existence of genotypic variation in osmotic adjustment under water deficit stress has a positive effect on growth and yield in a number of crop species and the mechanism by which osmotic adjustment affect yield are better understood for sorghum (Blum, 1988). For example, several field experiments using commercial hybrids with contrasting degrees of osmotic adjustment have shown clear yield advantages of hybrids with high osmotic adjustment when prolonged water deficit develops before anthesis or during grain filling (Ludlow et al, 1990; Santamaria et al, 1990). Grain yield achieved under water deficit conditions relative to that under well watered conditions increased linearly

with extent of maximum osmotic adjustment of the hybrids. Santamaria et al (1990) have also clearly shown that field grown sorghum genotypes with high osmotic adjustment had higher yields under water deficit conditions than those genotypes with low osmotic adjustment. The yield advantage of genotypes with high osmotic adjustment ranged between 15-34% higher than those genotypes with low osmotic adjustment when they were exposed to water deficit before anthesis. Tangpremsri et al (1991) have also demonstrated that in sorghum osmotic adjustment was positively associated with green leaf area retention during grain filling stage and to root length density. Thus genotypes with high osmotic adjustment used more water, as a result total dry matter was well related to osmotic adjustment during grain filling. Wheat genotypes with higher osmotic adjustment were able to produce a yield advantage of up to 50% over genotypes with low osmotic adjustment (Morgan et al, 1986). Osmotic adjustment may be very effective to compare genotypes for their drought resistance under the conditions that the rate of development of water deficit is slow. Osmotic adjustment requires time, and fast reduction in plant water status does not allow time for osmotic adjustment (Blum, 1996). It has been reported that osmotic adjustment is under the control of single or a few genes (Morgan, 1983) and that the trait is simply inherited.

1.1. 3. Drought tolerance

Drought tolerance is the mechanism by which plants stabilize and protect cellular and metabolic integrity even at low water potentials. Mechanisms that condition drought tolerance, therefore, function at the tissue or cellular level. Passioura (1996) indicated that no traits are known that confer global drought tolerance and short term response to water stress at the cellular and sub-cellular level alone may not contribute to yield under conditions of water deficit.

1.1.3.1 Water deficit-induced damages

Water deficit stress is implicated in causing damages to cell membranes and its effects are the result of the duration and severity of stress (Ristic et al, 1992). Dehydration in sensitive plants is usually accompanied with membrane injury resulting in the leakage of cytoplasmic solutes (McKersie and Tomes, 1980). In addition, rehydration following dehydration has been observed leading to further deterioration of membrane structure (Leopold et al, 1981; Ristic et al, 1992), often measured by the leakage of

solutes from cells as an indicator of membrane stability and survival. Leakage substances include various electrolytes, amino acids, sacharides, organic acids, hormones, phenolics and fluorescent materials (Blum, 1988). It has been proposed that the critical feature of tolerance to dehydration depends on the abilities of the plant to limit membrane damage during water deficit and to regain membrane integrity and membrane bound activities quickly upon rehydration (Tripathy et al, 2000). Blum and Ebercon (1981) suggested that cell membrane stability can be a reliable measure of drought and heat resistance in crop plants. Enormous genotypic variability for cell membrane stability has been found in various crop species including sorghum (Blum and Sullivan, 1986; Flower et al, 1990; Premachandra et al, 1992), wheat (Premachandra and Shimada, 1988), maize (Premachandra et al, 1989) and *Eragrostis tef* (Belay and Baker, 1996). Increases in cell membrane stability with increasing water deficit have been reported in only a few species, specifically cereals such as wheat (Blum and Ebercon, 1981); maize (Premachandra et al, 1989) and sorghum (Premachandra et al, 1992).

Water deficit stress is known to cause various physiological and biological effects on plants. The reduction in photosynthesis (Kaiser, 1987; Lawlor, 1995), stomatal closure (Chaves, 1991), and increased osmotic adjustment (Nelson and Orcutt,1996) appear to be typical plant responses to water deficit in the early stages. When plants are subjected to water deficits of various intensities, photosynthesis is inhibited primarily by stomatal closure or directly by the effects of water deficit depending on the intensity and magnitude of the stress (Smirnoff, 1993, 1995). This inhibition of photosynthesis could alter the balance between electron transport and CO_2 fixation, thereby a large proportion of the electron flux of the photosynthetic system could be diverted from CO_2 assimilation to O_2 reduction (Smirnoff, 1993, 1995). As a result excess reactive oxygen species (ROS) are accumulated in the cell (Moran et al. 1994). The mitochondria and chloroplasts are important intercellular generators of ROS. In chloroplasts, ROS are produced by excess transfer of energy from triplet excited chlorophyll to oxygen ($^1O_2$) or photoreduction of oxygen (formation of superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH)) (Asada, 1999; McKersie and Lesham, 1994). ROS are highly reactive and in the absence of any protective mechanisms they can seriously lead to peroxidation and breakdown of thylakoid lipids, proteins, polysaccharides and nucleic acids (Davies, 1987; Elstner, 1991;

McKersie and Lesham, 1994). To minimize the damaging effects of ROS, plants possess intrinsic antioxidant defence mechanisms (Acar et al, 2001; Bor et al, 2003; Meloni et al, 2003). Basically, this antioxidant defence systems fall into three general classes: 1) enzymatic antioxidants such as superoxide dismutase, catalase and ascorbate peroxidase, 2) the water soluble reductants such as ascorbate and glutathione, and 3) the lipid soluble, membrane associated antioxidants such as α–tocopherol and β– carotene (Smirnoff, 1995; Zhang and Kirkman, 1994). It has been proposed that the formation of reactive oxygen species is an inherent consequence of metabolism and that control of their levels is essential for normal functioning. The toxicity of an externally imposed biotic or abiotic stress can be partly attributed to the over riding of existing resistance mechanisms. Only when those mechanisms are overwhelmed would injury occur (Smirnoff, 1993; Zhang and Kirkham, 1996). This indicates that the strengthening of the protective mechanisms, through enhancing functions of their components such as ascorbic acid, superoxide dismutase and β–carotene may reduce or prevent oxidative damage and improve drought resistance of plants. Selection for genotypes with increased antioxidant defence systems in the cell may help the plant to detoxify active oxygen species, and minimize drought induced damage.

Cellular water deficit causes several structural changes. A change in cell volume is one of the alterations taking place under severe dehydration conditions (Poljakoff-Mayber, 1981; Bray, 1997). It has been proposed that small cells are more tolerant of dehydration (Iljin, 1957) and that they enhance osmotic adjustment and turgor maintenance (Cutler et al, 1977). High tissue elasticity is believed to assist in volume maintenance by reducing the change in volume per unit of change in turgor (Ludlow and Muchow, 1990). There are reports which indicated that plants subjected to dehydration may avoid reduced water potential and maintain turgor by reduction of their turgor-loss volume via tissue shrinkage associated with elastic adjustment of their cell walls (Buxton et al, 1985; Eze et al, 1986; Levitt, 1986; Fan et al, 1994). According to Blum (1988) the association between structural changes and cellular function under dehydration is not well understood. For crop improvement purposes neither the cost nor the value of the trait has been investigated and no genetic variability has been identified (Ludlow and Muchow, 1990), and thus the possibility

for practical solution to breeding for dehydration tolerance in cultivated crop plants appears to be far remote.

1.1.3.2 Synthesis of abscisic acid (ABA)

ABA is recognised as a stress hormone and is thought to regulate physiological processes in response to water deficit in plants. ABA is synthesized through the carotenoid biosynthetic pathway. ABA is suggested to be produced in roots. Several workers have provided evidence that ABA is produced in the roots (Cornish and Zeevart, 1985; Zhang and Davies, 1987; Bano et al, 1993) and has been shown to move in the xylem sap to the leaves in which it induced stomatal closure (Davies et al, 1994). However, other workers were also able to show that not all ABA in the xylem is produced only in the root. Working with salt stressed *Lupinus albus,* Wolf et al (1990) found a 10 fold increase of ABA in the xylem sap and about which 50% of the ABA was produced in the leaves and was transported to the roots in the phloem. When plants are exposed to water deficit, ABA levels increase as a result of an increase in the rate of synthesis and/or release of the hormone sequestered in organelles (Zeevart and Creelman, 1988). Munns and King (1988) working on wheat, Bano et al., (1993) on rice and Trejo and Davies (1991) on *Phaseolus vulgaris* have provided evidence that the concentration of ABA in the xylem sap rises with increasing soil water deficits. Several workers also supported this condition while showing no clear perturbation in leaf water relations (Zhang and Davies, 1990a; Schurr et al., 1992). Genetic differences in accumulation of ABA in response to water deficit has been demonstrated in several crops such as wheat (Quarrie and Jones, 1979); millets (Mahalakshmi et al.; 1983; Henson et al.; 1981); sorghum (Durley et al.; 1983) and for various plant parts such as leaves (Pekic and Quarrie, 1987); roots (Sharp et al., 1994; Ribaut and Pilet, 1991); grain tissues of cereals (Ober et al., 1991) and xylem sap (Tuberosa et al., 1994).

An increase in the level of ABA in response to water deficit leads to many changes in growth, development and physiology. Most significantly, an increase in the concentration of ABA in response to water deficit is believed to play a role as a signal for the induction of processes involved in adaptation to water deficit and other environmental stresses (Bray, 1993; Turner, 1997).

1.1.3.3 Expression of water deficit-induced proteins and genes

Water deficit has been shown to induce stress proteins and genes that are potentially useful in conferring stress tolerance to plants. There are two general types of molecules that are potentially useful in conferring stress tolerance to plants. The first type includes high molecular weight compounds such as late embryogenesis abundant (LEA) proteins which are highly accumulated in the embryos at the late stage of seed development (Shinozaki and Yamaguchi-Shinozaki, 1997). Synthesis of dehydrins, a class of LEA proteins appears to be a common component of stress response that constitutes changes in cell metabolism. These molecules are suggested to be responsive to environmental stresses such as drought stress and to accumulation of ABA. Dehydrins are an immunologically distinct group of plant proteins which typically accumulate in the late stage of embryogenesis or in response to ABA application, or any environmentally imposed dehydrative conditions (Close, 1996). Under dehydrative conditions, dehydrin proteins often comprise up to 1-10% of the total soluble proteins produced (Close, 1996). A variety of genes have been identified in response to environmental stresses such as drought, salt stress, high/low temperatures, etc. in various crop species (Shinozaki and Yamaguchi-Shinozaki, 1997). Dehydrins are highly expressed during late stages of seed development at normal growth conditions and are also accumulated in vegetative tissues when plants are exposed to water deficit (Cellier et al, 1998; Riccardi et al, 1998). The function of the dehydrins is not clearly defined yet, but they are considered to have cellular protective function. Dehydrins are proposed to be located in the cytoplasm (Dure et al, 1989). Thus, they are thought to function in promoting cellular tolerance of dehydration through protective functions in the cytoplasm, alteration of cellular water potential to promote water uptake, control of ion accumulation, and further regulation of gene expression under water deficit (Bray, 1993). Xu et al (1996) have presented experimental evidence showing that over expression of a dehydrin gene resulted in enhanced drought and salt tolerance. Positive correlations were also reported for species tolerant to various stresses that have a dehydrative component such as salt stress (Galvez et al., 1993; Moons et al., 1995); freezing and cold stress (Arora and Wisniewski, 1995; Close, 1996). Physiological investigations associated with the varietal differences in tolerance have been reported in rice (Moons et al, 1995) and maize (Reccardi et al, 1998). Other studies suggested that dehydrin proteins control

some of the agronomic important traits. For example, the maize *dhn1* locus positioned within QTL (quantitative trait loci) intervals control a number of significant agronomic traits including anthesis-silking interval and yield under drought stress conditions (Ribaut et al, 1996).

Another class of stress inducible gene is the biosynthetic gene involved in water retention. When plants are exposed to stress that have a dehydrative component such as water deficits and salt stress, many compounds that decrease intercellular water potential accumulate (Bohnert et al, 1995). These osmoprotectants, known as compatible osmolytes include amino acids (proline, ectoine); quaternary amines (glycine betaine) and sugars (mannitol) (Shinozaki and Yamaguchi-Shinozaki, 1997). The genes have been demonstrated to be inducible by both drought and salt stress. Unlike sodium and other ions, these compounds are compatible with the function of cellular enzymes at high concentration and several water deficit inducible genes are known to encode enzymes in path ways that lead to compatible solutes. For example, genes encoding enzymes for betaine biosynthesis are activated in response to water deficit (Mullet and Whitsitt, 1996). In dehydrated pea leaves a gene that encodes an aldehyde reductase that may be involved in osmotic adjustment has been induced (Guerrero et al, 1990). The involvement of compatible solutes in osmoregulation and protection of proteins against dehydration induced denaturation is now well established. For example, proline accumulation lowers the generation of free radicals under NaCl stress, in this way reducing oxidative membrane deterioration (Losch, 1996).

1.2. Justification

Drought tolerance in crop plants is a complex trait and the mechanisms for expression and inheritance are not well understood. For the past several years attempts have been made to combine physiological and morphological studies in various crop plants including maize and sorghum, to develop effective screening methods for drought tolerance. In both maize and sorghum, reduction in yield due to drought depend on the developmental stages at which drought occurs. Therefore, for several years, studies on the drought tolerance of these crop species at pre and post-flowering stages has been the subject of interest. Phenotypic variations in tolerance to drought stress during pre

and post-flowering stages has been observed for maize (Bolanos et al. 1993) and sorghum (Rosenow et al, 1997; Sowder et al. 1997). However, consistent relationships between variation of physiological and morphological traits and stress tolerance at pre-flowering or post-flowering stages have not been observed. Researchers have consistently reported the significant role of biochemical protection mechanisms against tissue injuries following dehydration. Attempts to incorporate biochemical protection mechanisms as a selection tool in maize and sorghum for drought tolerance are very scant. Furthermore, there is a lack of information whether physiological, morphological and biochemical traits are useful as selection criteria during recovery upon rehydration after exposure to pre and post-flowering dehydration. A better understanding of a species' physiological, morphological and biochemical options for drought tolerance and in depth insight on the ability to resume physiological and biochemical activities upon rehydration should provide a foundation for more efficient water management and for exploiting genetic variability. Given the difficulties stated above and the lack of information on mechanisms of recovery from drought stress, the current study was undertaken.

1.2.1. General objectives

The general aim was to compare the drought tolerance of supposedly drought resistant cultivars of maize and sorghum; to characterize their physiological and biochemical responses to dehydration during pre and post-flowering growth stages and their ability to recovery upon rehydration.

1.2.2. Specific objectives

a) To evaluate the drought adaptive strategies of maize and sorghum in terms of changes in water relations, gas exchange, cell membrane integrity, antioxidant defence systems and pigment concentrations,

b) Identify the contribution of individual antioxidants to the drought resistance of maize and sorghum during pre and post-flowering dehydration

c) To evaluate agronomically important traits of seed viability and quality (total carbohydrates, proteins and lipid) in maize and sorghum,

d) Characterize drought tolerance mechanisms in terms of resumption of activities of the above mentioned physiological and biochemical traits and plant survival during rehydration.

2. Chapter 2

Water relations, gas exchange characteristics and membrane leakage in maize *and* sorghum after exposure to and recovery from pre and post-flowering dehydration.

2.1. Introduction

Water deficit is the most common adverse environmental factor limiting crop production in the dryland areas of the world. In these areas, maize and sorghum are the most important staple crops. They are grown across a range of agro-ecological zones where shortages of water resulting from low and erratic rainfall is a major constraint for crop production (Maiti et al, 1996; Rosenow et al, 1997). Crop growth and productivity in such environments depend on the amount of water stored in the soil, the water requirements of the crop, and the efficient use of the limited water supply (Scott e al, 1987).

When crop plants are exposed to water deficit, in order to survive, they under go physiological, morphological and biochemical changes. The changes that occur at various levels of organization (cellular, molecular, etc.) of the plant in response to drought stress are considered to be adaptation mechanisms (Turner, 1986). Drought resistance is controlled by multiple genes and thus several traits must be considered for adaptations to water deficit (Blum, 1997; Pimentel, 1999). Several workers have examined the response of different crops to water deficits and have identified various traits that confer drought resistance in cereals (Blum, 1989; Ludlow and Muchow, 1990; Morgan, 1984; Turner and Begg, 1981; Turner, 1997). These include maintenance of high water potential, control of stomatal behaviour and osmotic adjustment under drought conditions (Blum, 1988; Ludlow and Muchow, 1990). The changes in membrane permeability following exposure to drought stress can also be used to estimate drought tolerance in crop plants (Blum and Ebercon, 1981).

Water deficit is implicated in causing damages to membranes. It has been proposed that the critical feature of tolerance to dehydration depends on the abilities to limit membrane damage during dehydration and to regain membrane integrity and membrane bound activities quickly upon rehydration (Tripathy et al, 2000). Furthermore, genotypes differ in their ability to recover upon rehydration, and the

ability of a genotype to recover from stress is closely related to its hydration status prior to recovery (Malabuyot et al, 1985).

Genetic variation in leaf water potential, stomatal conductance and photosynthetic rate have been reported in several crop species (Austin, 1989; Peng and Kreig, 1992). These traits might be used to select superior cultivars or crop species with the ability to maintain high plant water status, high stomatal conductance and maintenance of photosynthetic rate under water deficit conditions. The selection of such physiological traits in the improvement programs of crop plants, however, requires the establishment of significant association between various traits and drought resistance.

More detailed understanding of the physiological adaptations, enabling superior performance of crop species and/or genotypes under drought stress and/or required for the maintenance of physiological activities for growth and productivity during periods of recovery from stress upon rehydration will ultimately help in the selection and promotion of drought tolerant crop species. This is particularly relevant for crops such as sorghum and maize, predominantly grown in marginal rainfall regions of the world.

In this chapter, the study aimed at investigating whether there are differential responses in some of the physiological traits of drought resistance and recovery upon rehydration of maize and sorghum. Measurements were taken on plant water relations, gas exchange characteristics, cell membrane leakage and growth characteristics of maize and sorghum after exposure to and recovery from pre and post-flowering dehydration.

2.2. Materials and Methods

2.2.1. Growth conditions and treatment

Seeds of maize *cv* Melkassa-2 and sorghum *cv* MACIA were obtained from the National Maize Improvement program, Melkassa Agricultural Research Centre, Ethiopia, and ICRISAT Centre, Bulawayo, Zimbabwe, respectively. The maize variety obtained from Ethiopia was chosen on the basis of its better yield performance under water limited environments in Ethiopia. The sorghum variety was recommended by ICRISAT Centre, Bulawayo, Zimbabwe, for its drought resistance stay green trait. Both varieties are medium maturing type.

The experiment was conducted in a controlled chamber under constant environmental conditions (12/12 h day/night, 28-32/17 ^{0}C day/night temperature, 60-80% RH and PPFD of 1200-1400 μ mol $m^{-2}s^{-1}$) at the Department of Botany, University of Cape

Town. The following was done for both species. To ensure emergence, five seeds were sown in plastic pots, each 31 cm deep with an internal diameter of 18 cm. About 10 kg of sandy loam soil was used for each pot. Emergence occurred 5-7 days after planting. Twenty days after emergence, the pots were thinned to two seedlings of uniform size per pot. Plants were watered frequently to avoid the development of any moisture deficit. At 60 (pre-flowering) and 90 (post-flowering, grain filling stage) days after emergence, two watering treatments were applied: either maintained fully hydrated (control) or dehydrated treatments. Control plants were regularly watered to field capacity (F.C) to avoid any development of water stress and the dehydration was induced by withholding water for 20 days at each growth stages. At the end of each dehydration treatment, plants were rehydrated by soil watering (as for the control plants) for another 20 days and their recovery was studied. Five different samples were taken during the dehydration period at each different growth stage and during recovery, respectively. Since maize and sorghum do not grow to seed set stage in the Western Cape Climate, all the experiments had to be done in a phytotron. Size limitations of this facility forced reduced plant numbers, which did not allow for meaningful statistical examination of the data (Yohannes, personal communication, 2004). Each pot was given P and N at the rate of 0.80 g/pot (150 kg/ha) and 1.1 g/pot (200 kg/ha), respectively. Single superphosphate and lime ammonium nitrate were used as source of P and N, respectively.

At regular interval during the entire cycle (pre and post-flowering dehydration) the following parameters (detailed below) were measured. The same measurements were performed on control plants which remained hydrated throughout.

2.2.2. Soil water status

Soil water status was assessed by measuring soil water content and soil water potential (ψ_s). Soil water content (SWC) was measured by taking about 30 to 40g of soil samples at 10 to 15cm depth in the pot. Fresh soil weight was measured immediately after sampling, oven dried at 105 ^{0}C for 96 hr and weighed. Soil water content was determined by the formula [(FW – DW)/DW] x 100.

Soil samples were also taken from each pot for the determination of ψ_s using an AquaLab (water activity meter, Series 3, Decagon Devices, Inc).

2.2.3. Plant water relations

Water status of both species was determined by measuring the relative water content (RWC) and leaf water potential (ψ_L) (-MPa).

Samples of the youngest fully expanded leaf, opposite to those leaves used for the measurement of gas exchange were used. ψ_L was determined with a pressure chamber (PMS Instrument Co., Corvallis, Oregon, USA).

For the construction of pressure-volume curve the $1/\psi_L$ was plotted against relative water content and apoplastic water content was calculated by extrapolation of the linear region of the relationship between $1/\psi_L$ and relative water content. Relative water content was calculated using the method of Henson et al. (1981) as

$$RWC\ (\%) = [(FW-DW)/(TW-DW)] \times 100$$

Where, FW represented fresh weight, DW: dry weight and TW: turgid weight. Turgid weight was determined after floating leaf segments in distilled water in sealed vials for 24h at room temperature, and oven dried at 70^0C for 48h.

2.2.4. Gas exchange parameters

At each growth stages during dehydration, and rehydration the gas exchange physiology (stomatal conductance ($\mathbf{g_s}$), photosynthesis ($\mathbf{P_n}$), respiration (R), transpiration (E) and internal CO_2 concentrations (Ci)) of the youngest fully expanded intact leaves of upper canopy were recorded using a portable Infrared Gas Analysis (IRGA) system. (LCA-3, the Analytical Development Corporation, Hoddeston, England).

Measurements of chlorophyll fluorescence were made on those leaves used for the measurement of gas exchange, with a modulated portable fluorometer (OS-500: Opti-sciences, USA) at various stages of dehydration and recovery during rehydration. Before measurements were made, leaves were dark adapted for 20 min by attaching the leaves with specially designed, light proof clips. The initial, F_0, and maximum fluorescence, F_M, using a saturating light intensity of approximately 4 μ mol photons $m^{-2}s^{-1}$ and a duration of 1s, was measured. F_v was obtained by subtracting F_0 from F_M and F_v/F_M was calculated. This ratio gives an estimate of the yield of PSII photochemistry.

2.2.5. Water Use Efficiency

The ratio of photosynthesis to transpiration rates were used for the determination of water use efficiency and was expressed as mol CO_2 $mol^{-1}H_2O$ x 10^3

2.2.6. Determination of chlorophyll and carotenoid

Chlorophyll and carotenoid contents were determined from 0.25g leaf tissues. The leaf segments were cut into small pieces and extracted in 1 ml of 100% acetone for 48h at 4^0C. The absorbance of the extracts were measured spectrophotometrically (Beckman DU 650, U.S.A) at 661.6 nm, 644.8 nm and 470 nm. Chlorophyll (a+b) and Carotenoid (x+c) contents were calculated as described by Lichtenthaler (1987) and expressed as mg $ml^{-1}g^{-1}$ DW.

2.2.7. Electrolyte leakage

Damage to the cell membrane was evaluated by measuring the rate of electrolyte leakage from leaves of dehydrated plants at different growth stages. Leakage was also measured from control and rehydrated plants. Individual leaf segments of approximately 1cm x 1cm of maize and sorghum leaf segments were used for each treatment. These were placed in wells filled with 3ml double distilled (milli-Q) water and rate of leakage was read at 1 min intervals for 60 min using a conductivity meter (CM 100 conductivity meter, Reid and associates cc). Leakage rate was calculated as the slope of the line (generated from the time course of leakage) to the leaf dry weight and was expressed as $\mu S\ g^{-1}$ DW min^{-1}.

2.2.8. Growth characteristics

At the end of each dehydration and rehydration period, green leaf area was measured using an area meter (Li-3100, U.S.A). The same leaves used for the measurement of leaf area were oven dried at 70 ^{0}C for 48h and weighed. Above ground total dry weight (leaf and stem including leaf sheath), were also oven dried for 72 h at 70 ^{0}C and weighed. At maturity, grain yield from each pot was harvested and air dry weight recorded.

In order to give detailed insight, the data in this chapter are presented in two ways. The main figure gives physiological changes with changes in relative water contents. Charts inset provide time course of the changes of each measured parameters for both

control and dehydrated treatments. Since there was relatively little change in relative water contents for control plants, only data for dehydration treatment is presented.

2.3. Results

2.3.1. Soil water status

The initial soil water contents of both control and dehydrated treatments in both maize and sorghum during pre and post-flowering stages were about 25% (Fig. 2.1a). Except in the well watered maize grown pots where there was an increase during the late phase at pre-flowering stage, the soil water contents in the control pots of sorghum at both pre and post-flowering stages and in maize at post-flowering stage remained similar to the initial values throughout the duration of the experiment.

When the dehydration treatment began, soil water contents in both species at both pre and post-flowering stages declined below that in the control pots within 5 days of withholding water. The two species differed in response to pre and post-flowering dehydration with time (Fig. 2.1). Under dehydrating conditions, during the early phase (for the first 5 days) after imposition of dehydration treatment, soil water contents of maize pots during pre and post-flowering were markedly lower than that of sorghum. The decrease in soil water contents, in maize pots was by 82% and 79% during pre and post-flowering dehydration whereas, the reduction in soil water contents of sorghum pots were by 66% and 36% during pre-flowering and post-flowering dehydration, respectively. With increase in the duration of dehydration, the differences in soil water contents between both species and growth stages disappeared. In maize, there was no difference in the patterns of changes in soil water contents between pre and post-flowering dehydrated pots with time. Sorghum, on the other hand exhibited a remarkable difference between pre and post-flowering dehydrated pots. Soil water contents of pre-flowering dehydrated pots declined faster than post-flowering dehydrated pots, probably coincident with increased water demand as a result of maximum leaf area development. Soil water contents in the dehydrated pots were reduced to the mean value of 1.8% and 1.4% in maize and 2.5% and 2.2% in sorghum, during pre and post-flowering stages, respectively at the end of the dehydration cycle.

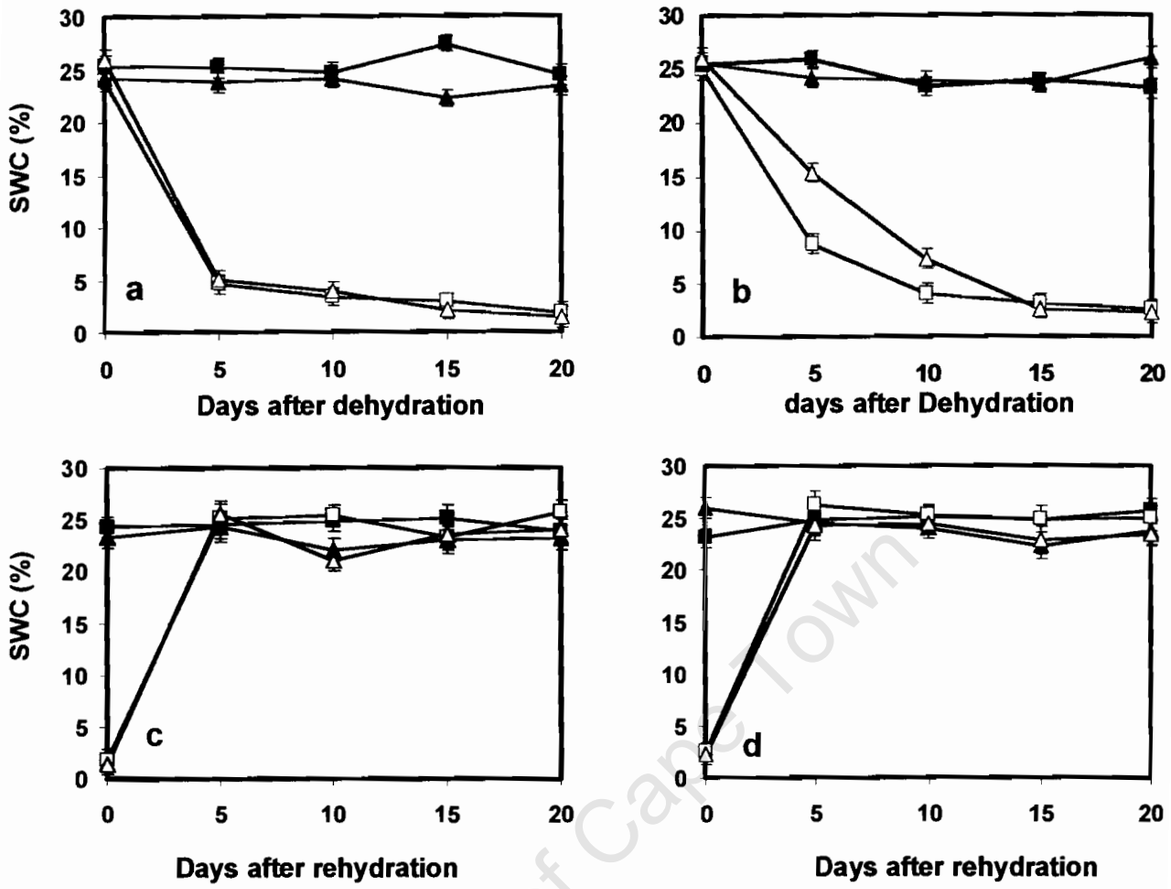

Figure 2.1 Time course of the changes in soil water content (%) of control vs dehydrated maize (a, c) and sorghum (b, d) during pre (■, □) and post-flowering (▲, Δ) dehydration (a, b) and rehydration (c, d), respectively. ■, ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

Like soil water contents, ψ_s in the control pots was maintained between the range of 0 and -0.12 MPa in both maize and sorghum (Fig. 2.2a and b) throughout the duration of the experiment. Under well watered conditions, there was no difference in ψ_s between the two species. Dehydration during pre and post-flowering stages markedly decreased ψ_s of both species. Under dehydration conditions, sorghum had higher ψ_s than maize during both pre and post-flowering stages.

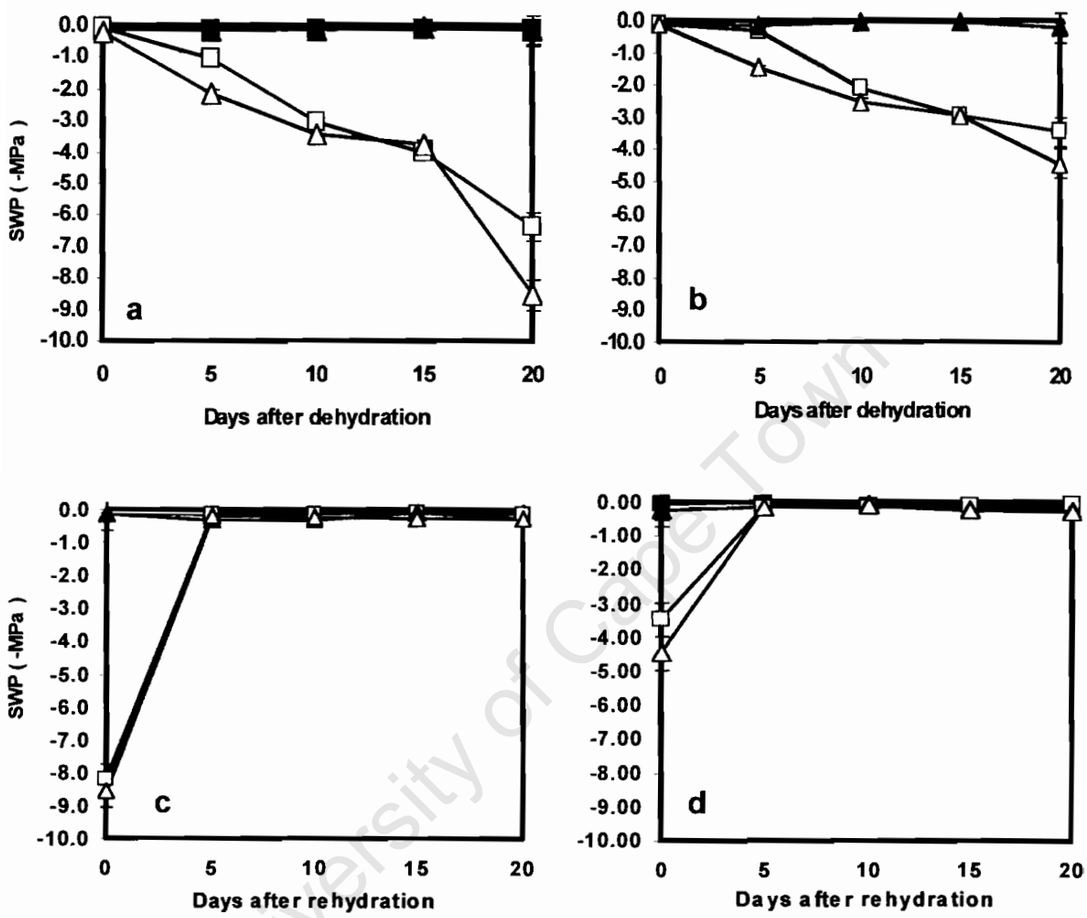

Figure 2.2 Time course of the changes in soil water potential (-MPa) of control vs dehydrated maize (a, c) and sorghum (b, d) during pre (■, □) and post-flowering (▲, Δ) dehydration (a, b) and rehydration (c, d), respectively. ■, ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

There were differences in the pattern of decline in ψ_s between maize and sorghum during the course of pre and post-flowering dehydration. In maize, during pre-flowering dehydration a dramatic decline in ψ_s occurred within 5 days of withholding water but the decline was slower and continual throughout the duration of the experiment. During post-flowering dehydration, the decline in ψ_s was rather rapid until 10 days of imposition of dehydration. Between 10 and 15 days of withholding water, there was no difference in ψ_s between pre and post-flowering dehydrated maize grown soils, but thereafter, a marked and rapid decline in ψ_s was observed during post-flowering dehydration.

By contrast, ψ_s in pre-flowering dehydrated sorghum grown pots was not different from the control during the first 5 days of withhold water but thereafter a steady decline was noticed whereas during post-flowering dehydration a marked decline in ψ_s occurred within 5 days of withholding water. Differences in ψ_s between pre and post-flowering dehydration also occurred in both species. In maize differences in ψ_s between pre and post-flowering dehydration occurred within 5 days and at 20 days after imposition of dehydration where the post-flowering stage had a markedly lower ψ_s than the pre-flowering stage. In sorghum, a difference in ψ_s between pre and post-flowering dehydration was noticed up to day 10 of withholding water. During pre-flowering dehydration, the ψ_s in maize decreased to -6.38 MPa and that of post-flowering stage was -8.53 MPa where as in sorghum, the decline was in the order of -3.46 MPa and -4.44 MPa during pre and post-flowering dehydration, respectively.

Upon rehydration both SWCs and ψ_s recovered to the control level within 5 days of rehydration in both species during pre and post-flowering stages (Fig. 2.1c and d and Fig. 2.2c and d).

2.3.2. Plant water relations

The response of maize and sorghum to pre and post-flowering dehydration and rehydration is given in Figure 2.3. Under control conditions, there was no difference in relative water contents between pre and post-flowering maize plants, whereas in sorghum relative water contents of pre-flowering control plants had slightly higher relative water contents than post-flowering control plants. Dehydration during pre and post-flowering stages caused a decrease in relative water contents of both species. The

sequence of changes in relative water contents also differed between both species with increase in the duration of the stress. The difference between the two species was observed during the early phase (0-10 days) after withholding water (Fig. 2.3a and b). Relative water contents of both pre and post-flowering dehydrated maize plants declined rapidly for the first 10 days and then slowly but steadily.

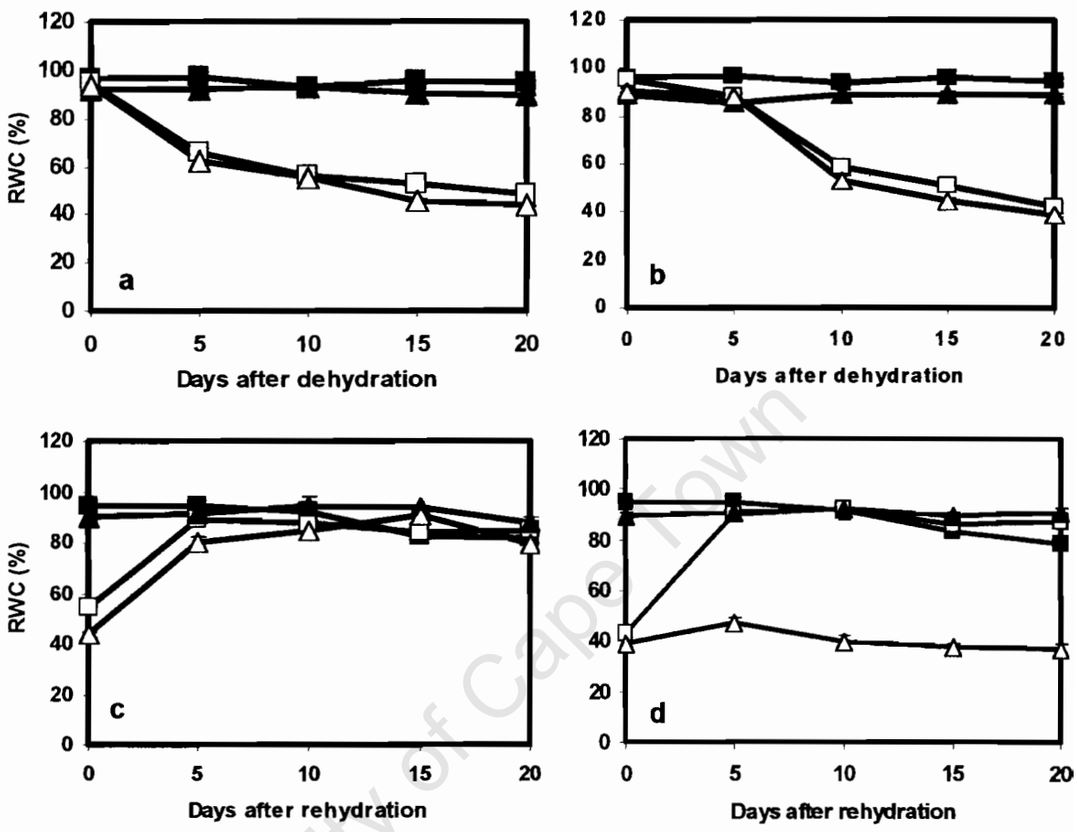

Figure 2.3 Time course of the changes in relative water content (%) of control vs dehydrated maize (a, c) and sorghum (b, d) during pre (■, ▲) and post-flowering (□, Δ) dehydration (a, b) and rehydration (c, d), respectively. ■, ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

In contrast to maize, there was almost no change in relative water contents of sorghum plants for the first 5 days under the prevailing dehydration conditions but thereafter there was a sharp and sharp decline to a level equivalent to that of maize plants. Mean relative water contents during dehydration was reduced from the initial full turgor value of 95% and 93% to 48% and 43% in maize at the end of dehydration period at pre and post-flowering stages, respectively. Relative water contents in sorghum declined from 95% and 90% to 43% and 39% at the end of the dehydration period at pre and post-flowering stages, respectively. Patterns of changes in relative water contents in pre-flowering dehydrated plants were similar to that of post-flowering dehydrated plants in both species.

Relative water contents in maize recovered to the control level following both pre and post-flowering dehydration (Fig. 2.3c). Full recovery also occurred in sorghum during pre-flowering rehydration as opposed to that of post-flowering which showed no change to that measured at the end of the dehydration period (Fig. 2.3d).

Pressure volume curves of maize and sorghum leaves as related to changes in relative water contents during pre and post-flowering dehydration are shown in Figure 2.4. In maize, since the data points are few it was difficult to determine the point at which turgor was lost.

However, the range of points at which turgor loss occurred was estimated between -2.3 MPa and -2.6 MPa during pre-flowering and -2.3 MPa and -2.7 MPa during post-flowering dehydration. This corresponds to the relative water contents between 82% and 65% and 80% and 62% within 5 days after dehydration commenced during pre and post-flowering stages, respectively. During pre-flowering dehydration, after turgor loss there was a period during which ψ_L fluctuated between relative water contents of 65% and 48%. During post-flowering dehydration a linear relationship between the inverse of ψ_L and relative water contents was observed.

In pre and post-flowering dehydrated sorghum turgor loss occurred at -1.75 MPa and -1.95 MPa, respectively corresponding to 88% 5 days after dehydration treatment began. During both pre and post-flowering dehydration a linear relationship between the inverse of ψ_L and relative water contents was observed in sorghum. In both species, however, turgor loss was not age dependent but differed between species. Under dehydration, maize leaves displayed leaf rolling at relative water contents of

82% (corresponding to ψ_L of -1.8 MPa) within 3 days of pre-flowering dehydration, with progressive dehydration leaf rolling tightened. In contrast, no sign of turgor loss was observed in sorghum for at least 12 days after initiation of dehydration and then leaf rolling was initiated at approximately relative water contents of 70% or ψ_L of -2.6 MPa. During post-flowering dehydration leaf rolling was not displayed in either species.

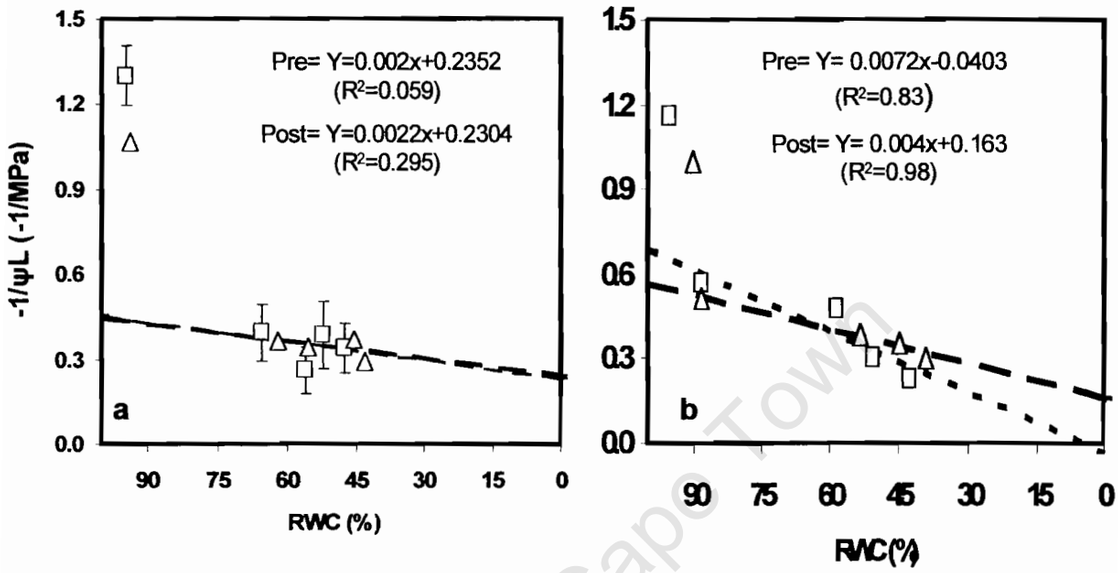

Figure 2.4 Pressure volume curves of maize (a) and sorghum during pre (□) and post-flowering (Δ) dehydration, respectively. Broken lines indicate linear regression for the pre-flowering dehydration and dotted lines indicate linear regression for post-flowering dehydration. Linear regression line for pre and post-flowering dehydrated maize are similar. Vertical bars denote standard errors (3=n).

2.3.3. Gas exchange characteristics

2.3.3.1. Stomatal conductance

Stomatal conductance (g_s) of the two species during dehydration and rehydration is presented in Figure 2.5. While g_s of pre-flowering well watered plants in both species were generally constant throughout the experimental period, g_s of post-flowering plants at later growth stages slightly increased (Fig. 2.5 inset).

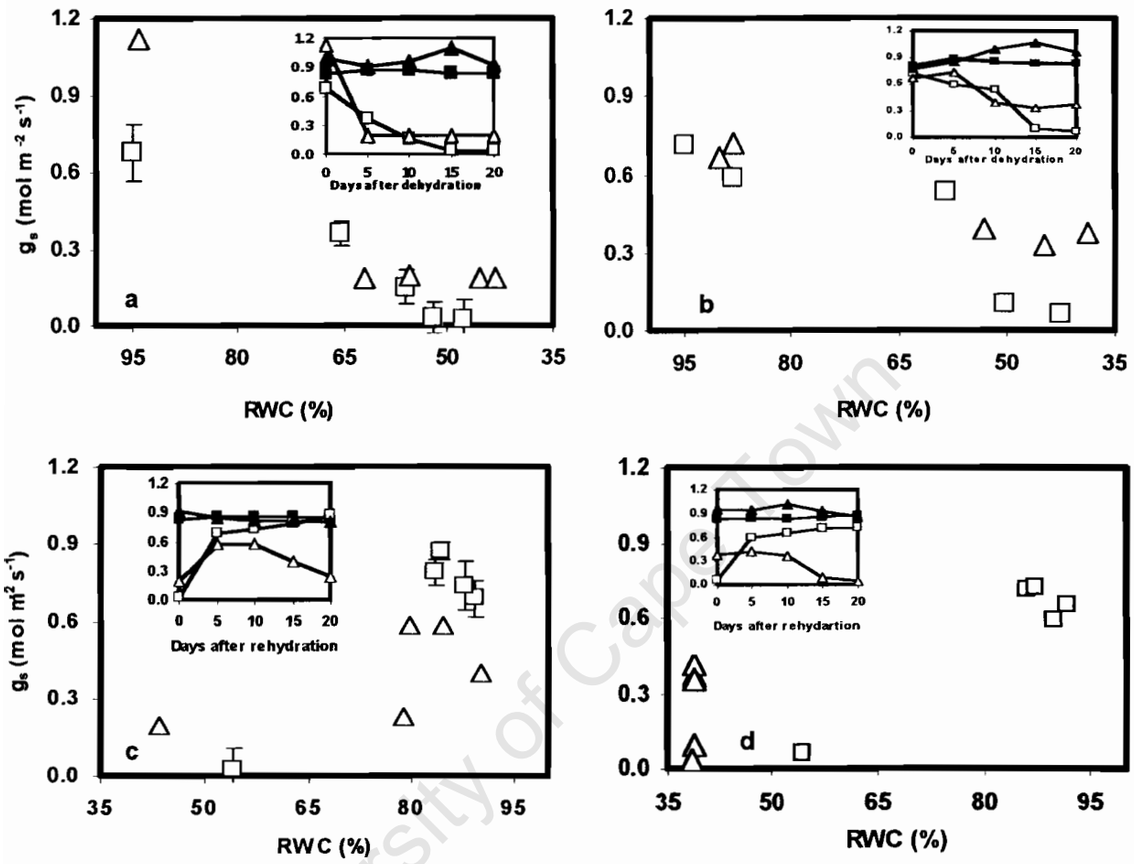

Figure 2.5 Changes in mean stomatal conductance (mol m^{-2} s^{-1}) of maize (a, c) and sorghum (b, d) during pre (■, □) and post-flowering (▲, Δ) dehydration (a, b) and rehydration (c, d) as related to RWCs, respectively. Insets refer to the same data expression in a time basis; ■ and ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

The two species had similar rates of conductance during well watered conditions ranging from 0.80 to 0.89 mol m^{-2} s^{-1} and 0.80 to 1.10 mol m^{-2} s^{-1} during pre and post-flowering stages, respectively. Once water was withheld and as relative water contents began declining, there was an immediate stomatal response in both species during pre and post-flowering stages. Difference in g_s of maize was observed between pre and post-flowering dehydrated plants. Their differences occurred at the beginning of the measurement period approximately at relative water contents of 95% and between relative water contents of 53% and 48% when conductance of post-flowering dehydrated plants was much higher than pre-flowering dehydrated plants. Differences in g_s of sorghum between pre and post-flowering dehydrated plants became evident between relative water contents of 58% and 39% at which point the post-flowering dehydrated plants showed significantly higher g_s values than pre-flowering dehydrated plants. Corresponding to the decline in relative water contents, the patterns of changes of g_s differed between species during pre and post-flowering dehydration. During pre-flowering dehydration, g_s of maize continually declined from its highest mean value of 0.68 mol m^{-2} s^{-1} to the lowest value of 0.03 mol m^{-2} s^{-1} at the end of the dehydration period. g_s of maize undergoing post-flowering dehydration showed a dramatic decline between relative water contents of 95% and 64% within 5 days of withholding water after which it remained without any significant change between relative water contents of 64% and 48% at the final stage of dehydration. In contrast to maize, after an initial decline approximately between relative water contents of 95% and 88%, g_s of sorghum plants during pre-flowering dehydration showed no remarkable change between relative water contents of 88% and 58% and then conductance declined most markedly until the end of the experiment. Between relative water contents of approximately 88% and 53% the decline in g_s of post-flowering dehydrated sorghum was rather gradual, and thereafter conductance showed little change until relative water contents reached 39% at final phase of dehydration.

By 5 days of rehydration, g_s of maize fully recovered at about 88% relative water contents following pre-flowering rehydration (Fig. 2.5c and inset). Recovery in the g_s of pre-flowering rehydrated sorghum was rather slow and full recovery occurred at approximately 90% of relative water contents at the final phase (20 days after rehydration began) of rehydration (Fig. 2.5d and inset). Approximately 55% of g_s in maize undergoing post-flowering rehydration resumed at 80% of relative water

contents 5 days after rehydration began but g_s showed a steady decline 10 days after post-flowering rehydration. There was no change in g_s of sorghum from that measured at the end of post-flowering dehydration for the first 10 days after rehydration commenced but thereafter conductance declined to almost zero at the end of rehydration period.

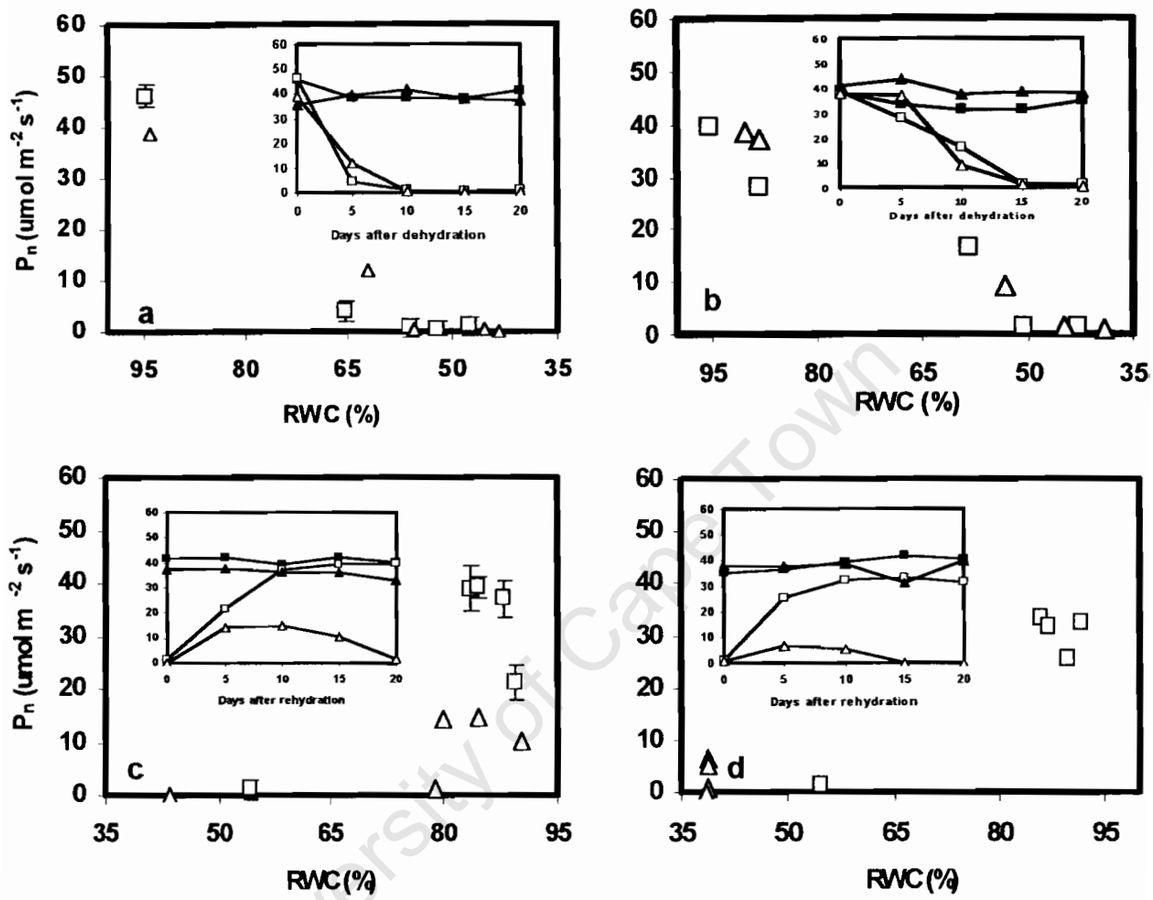

Figure 2.6 Changes in mean photosynthesis rate (μmol m^{-2} s^{-1}) of maize (a, c) and sorghum (b, d) during pre (■, □) and post-flowering (▲, Δ) dehydration (a, b) and rehydration (c, d) as related to RWCs, respectively. Insets refer to the same data expression in a time basis; ■ and ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

2.3.3.2. Photosynthesis rate (P_n) and Quantum efficiency of photosystem II (Fv/Fm)

It is clear that P_n of sorghum post-flowering control plants was higher than pre-flowering control plants throughout the duration of the experiment (inset in Fig. 2.6b). Dehydration during pre and post-flowering stages caused a considerable decrease in P_n rates of both species (Fig. 2.6a and b and inset). Little difference in the patterns of changes in P_n rates was noted between pre and post-flowering dehydrated plants with changes in relative water contents in both species. In maize, differences between pre and post-flowering dehydrated plants were evident approximately between relative water contents of 95% and 65% when P_n of pre-flowering dehydrated plants decreased more markedly than post-flowering dehydrated plants. In sorghum P_n of pre-flowering dehydrated plants declined continually until the end of the dehydration period whereas P_n of plants undergoing post-flowering dehydration was maintained without changes for the first 5 days of dehydration approximately between relative water contents of 93% and 88% and with further decrease in relative water contents, P_n was negligible after 10 days of dehydration. In both species, however as relative water contents dropped below 60% the differences in P_n between pre and post-flowering dehydrated plants disappeared. Species difference in the sequence of changes in P_n rates were also observed in response to pre and post-flowering dehydration. Differences were evident immediately after withholding water between 95 and 55% relative water contents from 5 to 10 days after exposure to dehydration. Concomitant with a sharp decline in the relative water contents of maize, P_n rates of pre and post-flowering dehydrated plants in this species decreased much faster than sorghum (Fig. 2.6a and b). However, as relative water contents dropped further P_n was also negligible. In maize, as relative water contents dropped from 95% to 55% with in 5 days after the initiation of dehydration, the decrease in P_n ranged from 46.2μmol m^{-2} s^{-1} to 0.7μmol m^{-2} s^{-1} during pre-flowering dehydration and from 39.0μmol m^{-2} s^{-1} to 0.4μmol m^{-2} s^{-1} during post-flowering dehydration. In contrast, at similar relative water contents the decrease in P_n of sorghum was from 39.2μmol m^{-2} s^{-1} to 16.3μmol m^{-2} s^{-1} and from 38.3μmol m^{-2} s^{-1} to 9.1μmol m^{-2} s^{-1} during pre and post-flowering dehydration, respectively.

There was a noticeable difference in the patterns of response in P_n rate between pre and post-flowering rehydrated plant in both species. P_n of pre-flowering dehydrated

maize fully resumed 10 days after rehydration began and that of post-flowering dehydrated plants showed an initial increasing trend following rehydration, but with further increase in relative water contents P_n rather declined until the end of the rehydration period. Sorghum on the other hand exhibited only 75% recovery until the end of rehydration period but post-flowering rehydrated plants did not show any noticeable change until the end of rehydration period (Fig. 2.6c and d).

Fv/Fm ratio was measured to examine the effect of dehydration on PSII photochemistry. Changes in Fv/Fm ratio of the species over a range of relative water contents for pre and post-flowering dehydrated maize and sorghum plants and the duration of the dehydration period for control and dehydrated treatments are presented in Figure 2.7a and b and inset, respectively. Under well watered conditions, both species had similar Fv/Fm ratios during both pre and post-flowering stages (inset in Fig. 2.7a and b) and no changes in the Fv/Fm ratio occurred for both species throughout the duration of the experiment.

Dehydration during pre and post-flowering stages caused a decrease in Fv/Fm ratio of both species as compared to the well watered plants, indicating that dehydration caused a direct effect on the PSII photochemistry.

There were slight differences in the patterns of the changes in Fv/Fm ratio between pre and post-flowering dehydration in both maize and sorghum. During pre-flowering dehydration, between 95 and 65% relative water contents there was a gradual but consistent decrease in Fv/Fm ratio of maize and thereafter no significant change was observed with further loss in water content. By contrast during post-flowering dehydration, once relative water contents declined below 65%, there was a large and much faster decrease. In pre-flowering dehydrated sorghum plants, a decrease in Fv/Fm ratio was noticed between relative water contents of 95% and 88%, 5 days after dehydration, but thereafter, up to 50% relative water content no change in Fv/Fm ratio occurred. Like maize, between relative water content of 55% and 39% post-flowering dehydrated sorghum plants showed a large and rapid decrease in Fv/Fm. The magnitude of the effect of pre and post-flowering dehydration on Fv/Fm ratio was similar for the two species. When Fv/Fm ratio was expressed relative to the initial value, there was 18% and 20% reduction during pre-flowering dehydrated maize and sorghum at the end of the dehydration cycle, respectively. By contrast, during post-

flowering dehydration, the reduction in Fv/Fm ratios was 60% and 56% in maize and sorghum at the end of the dehydration period, respectively.

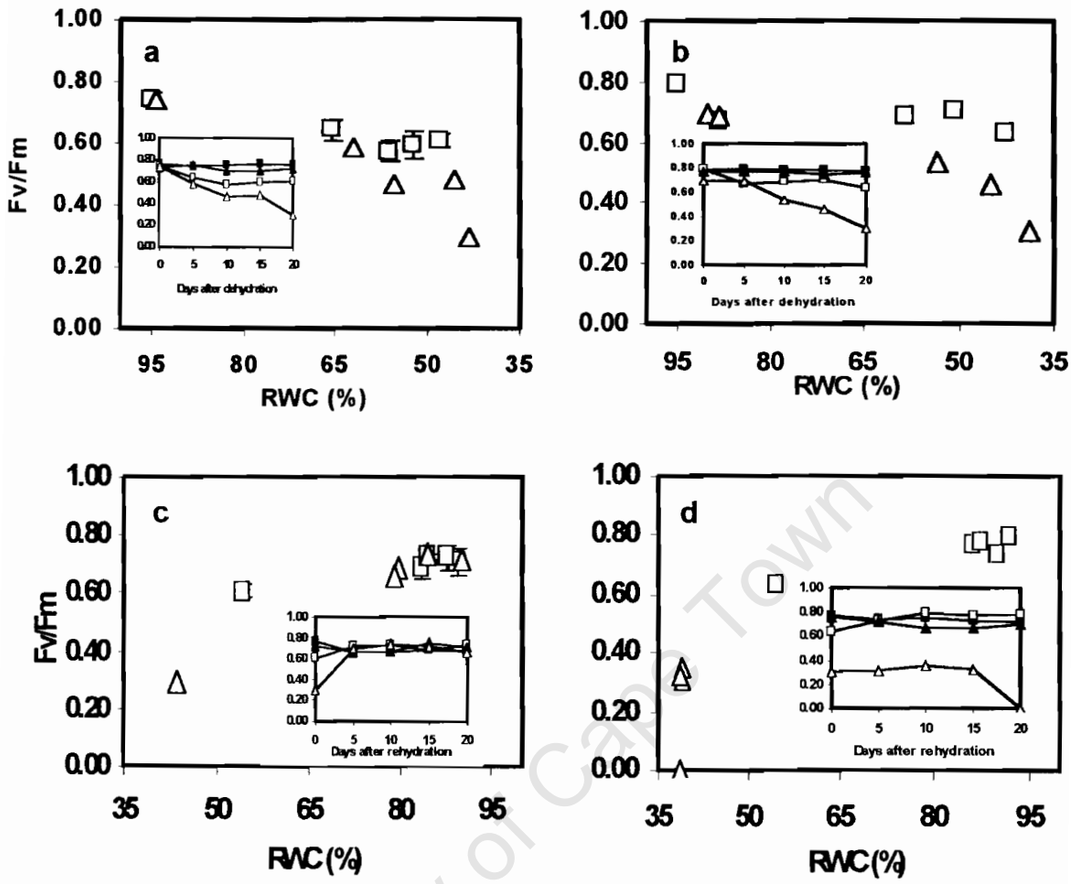

Figure 2.7 Changes in mean quantum efficiency of maize (a, c) and sorghum (b, d) during pre (■, □) and post-flowering (▲, Δ) dehydration (a, b) and rehydration (c, d) as related to RWCs, respectively. Insets refer to the same data expression in a time basis; ■ and ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

Maize recovered to the control level as soon as rehydration began during both pre and post-flowering rehydration (Fig. 2.7c and inset). Similar to maize, Fv/Fm of sorghum was restored to the pre water stress level within 5 days of pre-flowering rehydration, but there was no change in Fv/Fm ratio from that measured during post-flowering dehydration until days 15 and thereafter Fv/Fm rather declined with further rehydration (Fig. 2.7d and inset).

2.3.3.3. Transpiration rate

The influence of dehydration during pre and post-flowering stages on the transpiration rates (E) of both species is given in Figure 2. 8 and inset. Under control conditions, E rates of both species during pre and post-flowering stages were relatively constant throughout the study period. It can clearly be seen that under well watered conditions, E rates during post-flowering stage were generally higher than during pre-flowering stage in both species throughout the experimental period (inset in Fig. 2.8a and b). Dehydration during pre and post-flowering stage led to a large drop in E rates in both species. As relative water contents decreased, the magnitude of the effect of dehydration on E rates varied between pre and post-flowering dehydration in both species. In both maize and sorghum, the initial E rates of plants undergoing post-flowering dehydration were much higher than plants undergoing pre-flowering dehydration. In parallel to P_n, the rapid declines in relative water contents from 95% to 65% in maize led to a rapid and large decrease in E rates and were about only 22% of the control 5 days after pre-flowering dehydration began and thereafter there was no change until relative water contents reached 48% at the final phase of dehydration (Fig. 2.8a). In plants undergoing post-flowering dehydration, E rates declined gradually until relative water contents reached 62% and were 57% of the control. As relative water contents declined further, E rates dropped sharply until the end of the dehydration cycle. In contrast to maize, approximately between relative water contents of 95% and 88% (within the first 5 days) E rates of sorghum plants were not affected by pre and post-flowering dehydration as compared to their respective control plants (Fig. 2.8b inset). With further water loss, however, a steady decrease in E rates of both pre and post-flowering dehydrated plants were observed until the end of the dehydration cycle. Mean values of E rates in dehydrated maize plants ranged from a maximal value of 6.0 to 0.1 mmol m^{-2} s^{-1} and 9.7 to 0.1 mmol m^{-2} s^{-1} during pre and post-flowering dehydration, respectively. In sorghum, E rates of dehydrated

plants were in the order of 5.1 to 1.4 mmol m^{-2} s^{-1} during pre-flowering and 8.5 to 1.1 mmol m^{-2} s^{-1} during post-flowering stage.

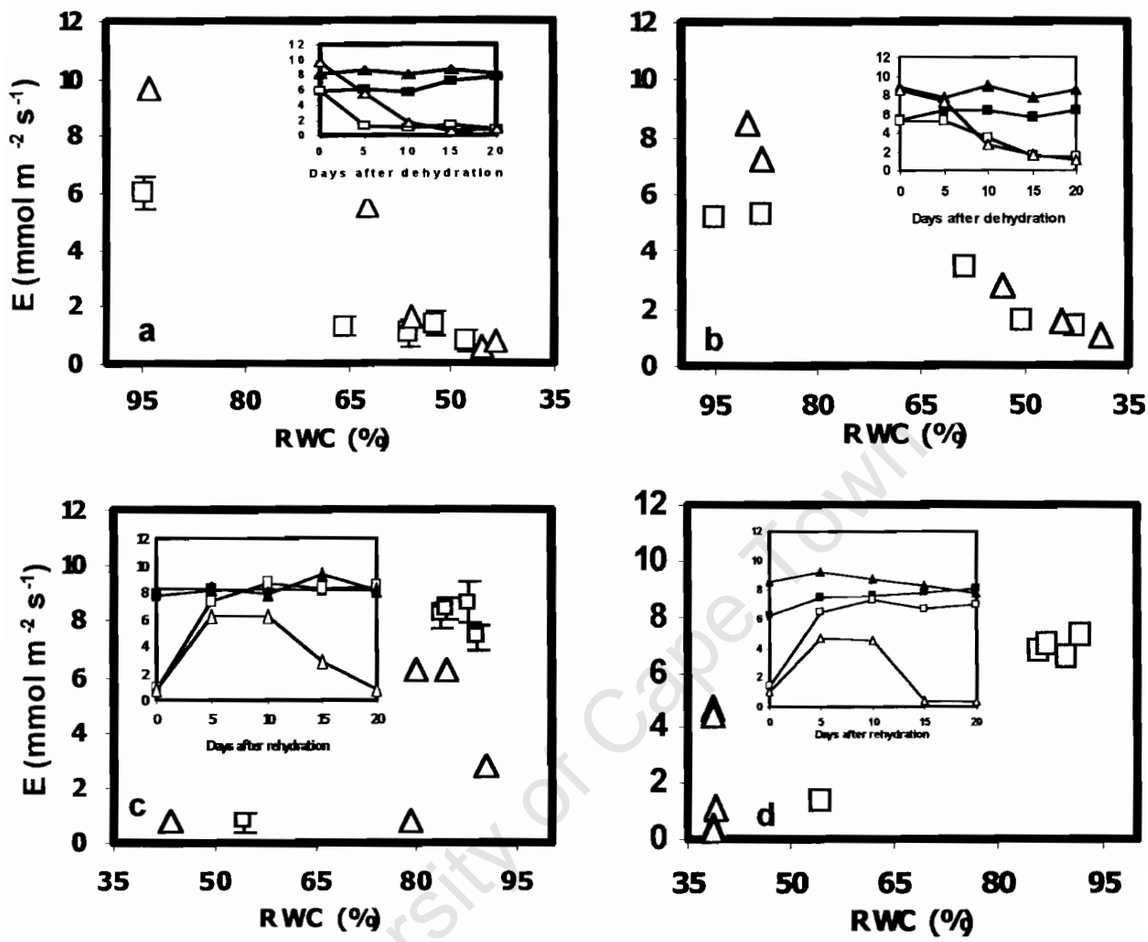

Figure 2.8 Changes in mean transpiration rates (mmol m^{-2} s^{-1}) of maize (a, c) and sorghum (b, d) during pre (■, □) and post-flowering (▲, Δ) dehydration (a, b) and rehydration (c, d) as related to RWCs, respectively. Insets refer to the same data expression in a time basis; ■ and ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

In maize plants dehydrated during the pre-flowering stage, E rates were fully restored as soon as rehydration began. In parallel to g_s, those dehydrated during post-flowering stage recovered approximately 60% within 10 days of rehydration, but as rehydration continued E rates declined again (Fig. 2.8c and inset). During pre-flowering rehydration, E rates of sorghum plants fully recovered within 10 days of rehydration and thereafter, transpiration declined to 86% of the control (Fig. 2.8d and inset). Like maize, E rates of plants undergoing post-flowering rehydration recovered only 55% and thereafter decreased to a level comparable to that measured at the beginning of rehydration.

2.3.3.4. Respiration rate

The response of respiration rates (R_d) in maize and sorghum during pre and post-flowering dehydration and rehydration is presented in Figure 2. 9 and inset. R_d of both pre and post-flowering control plants in both species did not show any notable change throughout the duration of the experiment. Pre-flowering control plants of both maize and sorghum had a markedly lower R_d rates as compared to the post-flowering plants. R_d rates in both maize and sorghum were sensitive to a decrease in relative water contents (Fig. 2.9a and b and inset). Similar to P_n, a large decrease in R_d rate occurred in both pre and post-flowering dehydrated leaves of both species as compared to the control leaves. Although the sequence of changes followed similar trends, there was a variation in R_d rates between pre and post-flowering dehydration in both species. As relative water contents dropped, decreases in R_d rates of plants undergoing pre-flowering dehydration was relatively faster in both maize and sorghum compared to those plants undergoing post-flowering dehydration. The response of both species was different for R_d rates during pre and post-flowering dehydration (Fig. 2.9). During dehydration at either of the growth stages, a decrease in the R_d rates of leaves of maize was much faster compared with those of sorghum. In maize, R_d rates declined from their peak values at the beginning of the dehydration to its minimal values by 95% and 97% at the end of pre and post-flowering dehydration, respectively. On the other hand the decline in sorghum was by 80% and 86% during pre and post-flowering dehydration, respectively.

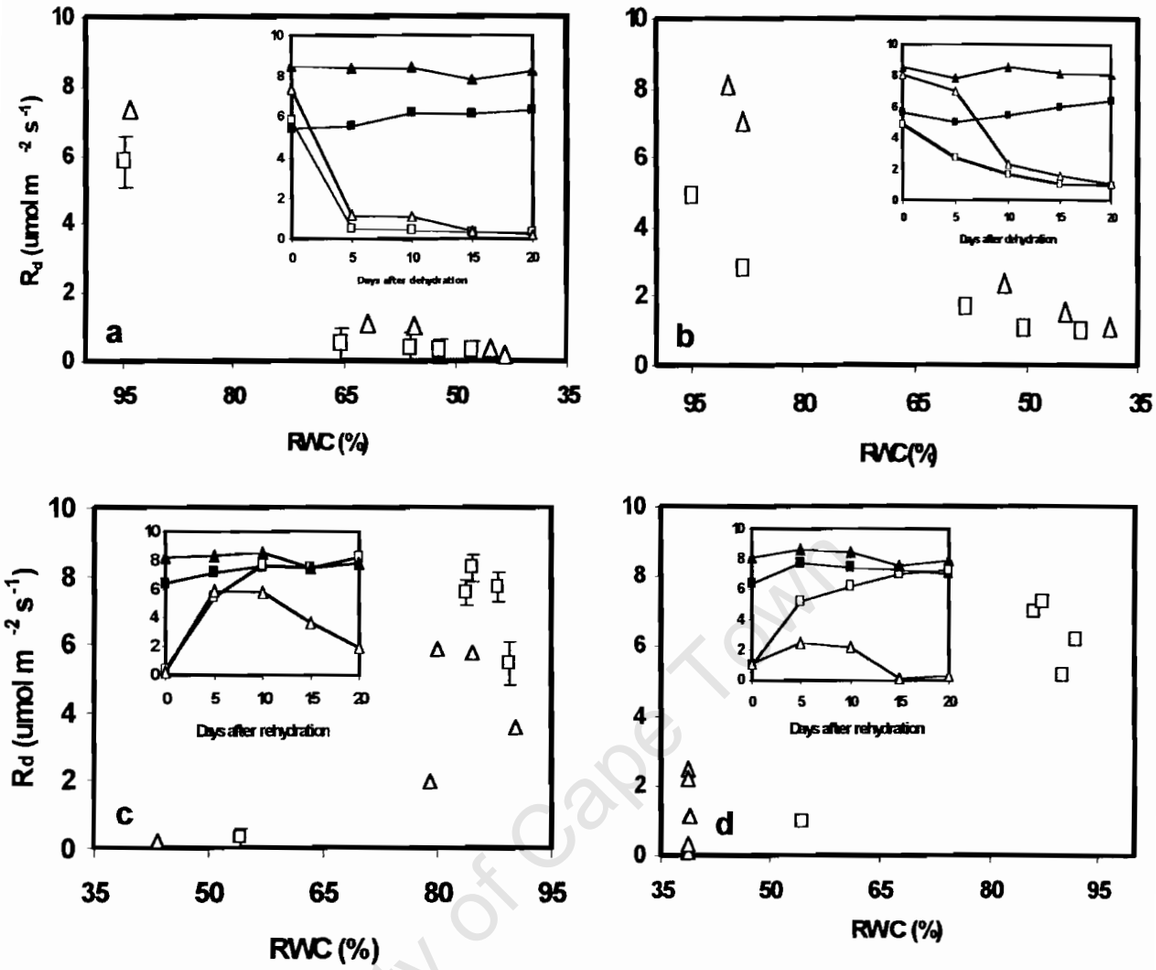

Figure 2.9 Changes in mean respiration rates (µmol m^{-2} s^{-1}) of maize (a, c) and sorghum (b, d) during pre (■, □) and post-flowering (▲, Δ) dehydration (a, b) and rehydration (c, d) as related to RWCs, respectively. Insets refer to the same data expression in a time basis; ■ and ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

In maize, full recovery of R_d rate was restored 10 days after pre-flowering rehydration began, corresponding to 85% of relative water contents, while during post-flowering, R_d rate tended to recover but declined again irreversibly to a level comparable to that measure at the end of the dehydration period (Fig. 2.9c and inset). In pre-flowering rehydrated sorghum, recovery of R_d rate was rather slow and full recovery was attained 15 days after rehydration began. In contrast to maize, there was no change in R_d rates of sorghum following post-flowering rehydration (Figure 2.9d and inset).

2.3.3.5. Intercellular $C0_2$ concentration

The response of intercellular CO_2 concentration (C_i) in maize and sorghum to pre and post-flowering dehydration and rehydration is given in Figure 2.10 and inset. Dehydration during both pre and post-flowering stages led to an increase in C_i of both species as compared to the control (Fig. 2.10a and b and inset). Time course of the changes in C_i indicated that a variation between pre and post-flowering dehydration in both species occurred only 10 days after dehydration began. Maximal increases of C_i were observed at pre-flowering dehydration at relative water contents of 56% in maize and 58% in sorghum as compared to post-flowering dehydration. During pre-flowering dehydration, once water was withheld, there was a linear increase in C_i of both species after which there was a fluctuation below relative water contents of 55%. During post-flowering dehydration, as relative water contents declined there was a more gradual increase in C_i until relative water contents reached to 65% in maize and 55% in sorghum, and then C_i remained without any notable changes until the end of the dehydration cycle.

C_i in maize was restored to the control level as soon as rehydration began during both pre and post-flowering stage (Fig. 2.10c and inset). C_i of pre-flowering dehydrated sorghum also fully recovered within 5 days of rehydration whereas C_i did not recover upon relief from stress following post-flowering dehydration (Fig. 2.10d and inset).

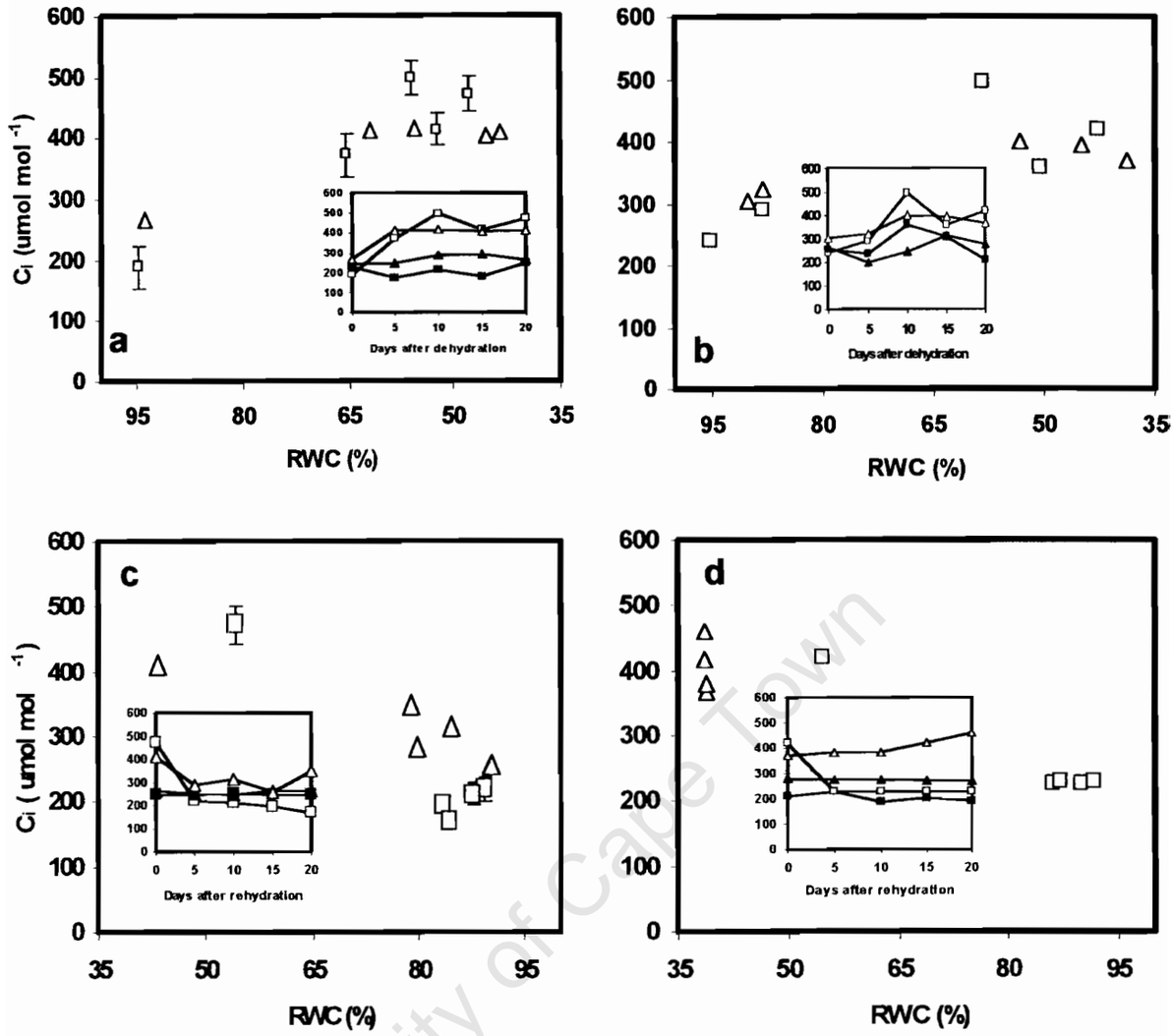

Figure 2.10 Changes in mean intercellular CO_2 concentration (µmol mol^{-1}) of maize (a, c) and sorghum (b, d) during pre (■, □) and post-flowering (▲, Δ) dehydration (a, b) and rehydration (c, d) as related to RWCs, respectively. Insets refer to the same data expression in a time basis; ■ and ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

2.3.4 Water use efficiency

Changes in water use efficiency of maize and sorghum with changes in relative water contents and with time during pre and post-flowering dehydration and rehydration are given in Figure 2.11 and inset). There was a reduction in photosynthetic water use efficiency with changes in relative water contents in both species at both pre and post-flowering dehydration. The decrease in water use efficiency appeared to be due to greater dehydration induced reduction in P_n than transpiration (see Fig. 2.6 and Fig. 2.8). The patterns of the changes in water use efficiency in a range of relative water contents over the duration of dehydration period differed between pre and post-flowering stages in both species. During the first 10 days of dehydration (between relative water contents of 95% to 56%), pre-flowering dehydrated maize exhibited the greatest decrease in water use efficiency. During post-flowering dehydration, however, the decrease was more gradual and only when relative water contents dropped between 62% and 56% was there a sharp decrease in water use efficiency. During the first 10 days of dehydration (between relative water contents of 95% to 58%) water use efficiency of pre-flowering dehydrated sorghum plants was comparable to that of control plants, although there was a gradual declining trend in both cases. However, below relative water contents of 58% concomitant to the large decrease in soil water contents, water use efficiency showed a precipitous decrease until the end of dehydration cycle. During post-flowering dehydration, water use efficiency showed an increase for the first 5 days of dehydration (between relative water contents of 90% to 88%) and when relative water contents dropped below 88% a large decrease in water use efficiency was observed in sorghum. Under control conditions, water use efficiency of pre-flowering stage in both species was always higher than that of post-flowering stage (Fig. 2.11 inset).

During pre-flowering rehydration, water use efficiency of maize was restored to the control level after 10 days of rehydration, while during post-flowering, approximately 80% of water use efficiency recovered within 5 days of rehydration. Thereafter water use efficiency stabilized until 15 days of rehydration after which there was a decline (Fig. 2.11c and inset). In sorghum full recovery of water use efficiency occurred immediately after the initiation of pre-flowering rehydration (Fig. 2.11d and inset). Unlike pre-flowering stage, there was no change in water use efficiency of sorghum

during post-flowering rehydration, although there was an increasing trend during the late phase of rehydration.

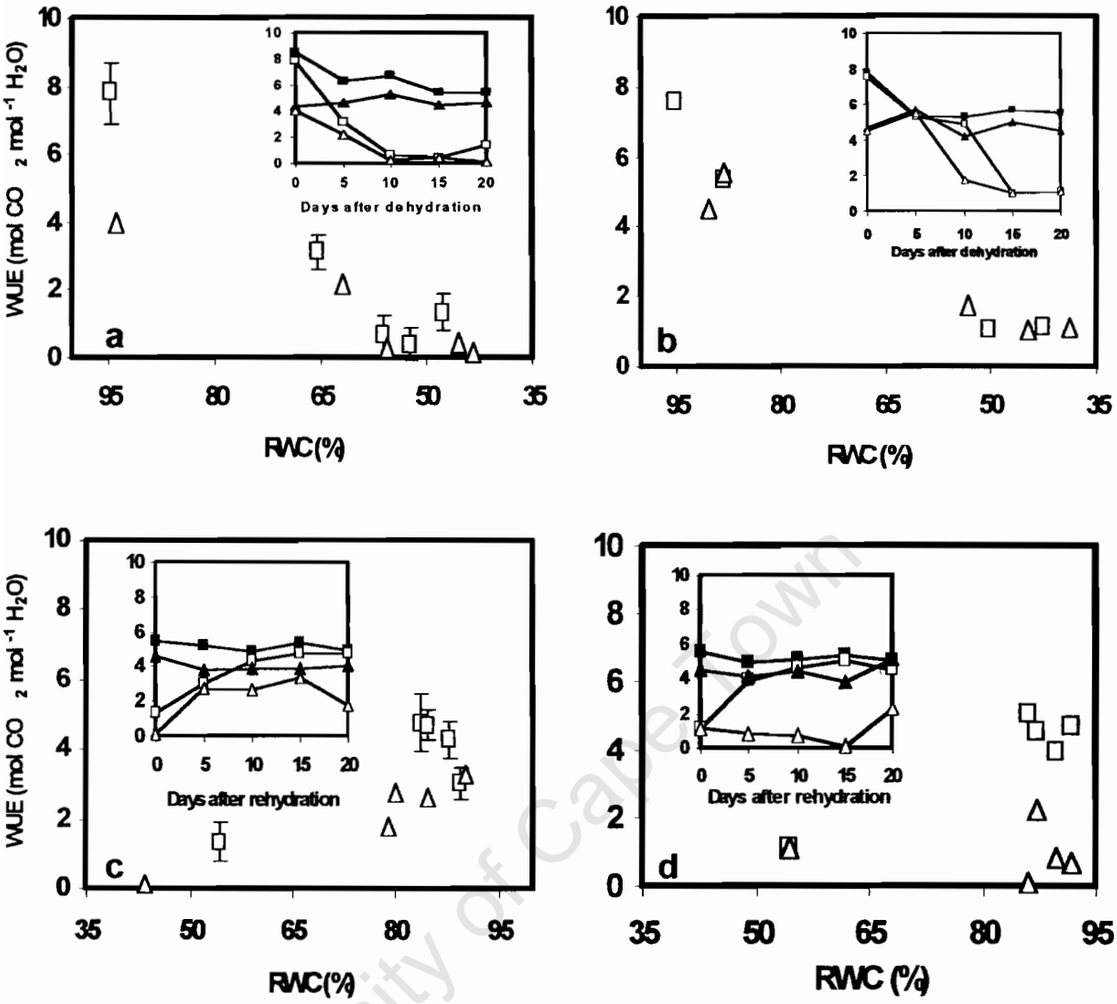

Figure 2.11 Changes in mean water use efficiency (mol CO_2 $mol^{-1}H_2Ox10^3$) of maize (a, c) and sorghum (b, d) during pre (■, □) and post-flowering (▲, Δ) dehydration (a, b) and rehydration (c, d) as related to RWCs, respectively. Insets refer to the same data expression in a time basis; ■ and ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

2.3.5 Photosynthetic pigment composition

2.3.5.1 Chlorophyll

Chlorophyll contents were generally high in sorghum than in maize control leaves during both pre and post-flowering stages (Fig. 2.12a and b and inset). In both species, high concentrations of chlorophyll were observed during pre than post-flowering stage. Chlorophyll contents of control leaves were continually decreasing during pre and post-flowering stages in maize and during post-flowering stage in sorghum, during "dehydration" and "rehydration" treatment periods. During pre-flowering treatment period, an increase in sorghum chlorophyll contents until 10 days was followed by a decrease with time (Fig. 2.12b inset). It was found that the extent of chlorophyll degradation was greater in maize than in sorghum during both pre and post-flowering "dehydration" and "rehydration" treatment period.

Figure 2.12 presents chlorophyll contents of maize and sorghum during pre and post-flowering dehydration. Dehydration caused a decrease in chlorophyll contents of maize and sorghum during both pre and post-flowering dehydration. Plotted patterns of changes in chlorophyll contents suggested variation between species with changes in RWCs. Once water became limiting, loss of chlorophyll contents were faster in maize than in sorghum during both developmental stages. The data also revealed that chlorophyll content was generally higher in sorghum than in maize over the duration of both pre and post-flowering dehydration. In maize, once water became limiting chlorophyll contents decreased and its contents continued to decrease until the end of the dehydration cycle during both pre and post-flowering dehydration. It was, however, observed that the trend of chlorophyll degradation differed between maize leaves undergoing pre and post-flowering dehydration. Differences occurred between relative water contents of 95% and 62% when there was a rapid decline from 111% to 46% in leaves undergoing post-flowering dehydration. During pre-flowering dehydration, chlorophyll contents were rather retained with in the ranges of 84% and 70% until relative water contents reached 56% after which there was a gradual decrease with further decline in relative water contents. In sorghum, the patterns of changes in chlorophyll contents also differed between plants undergoing pre and post-flowering dehydration.

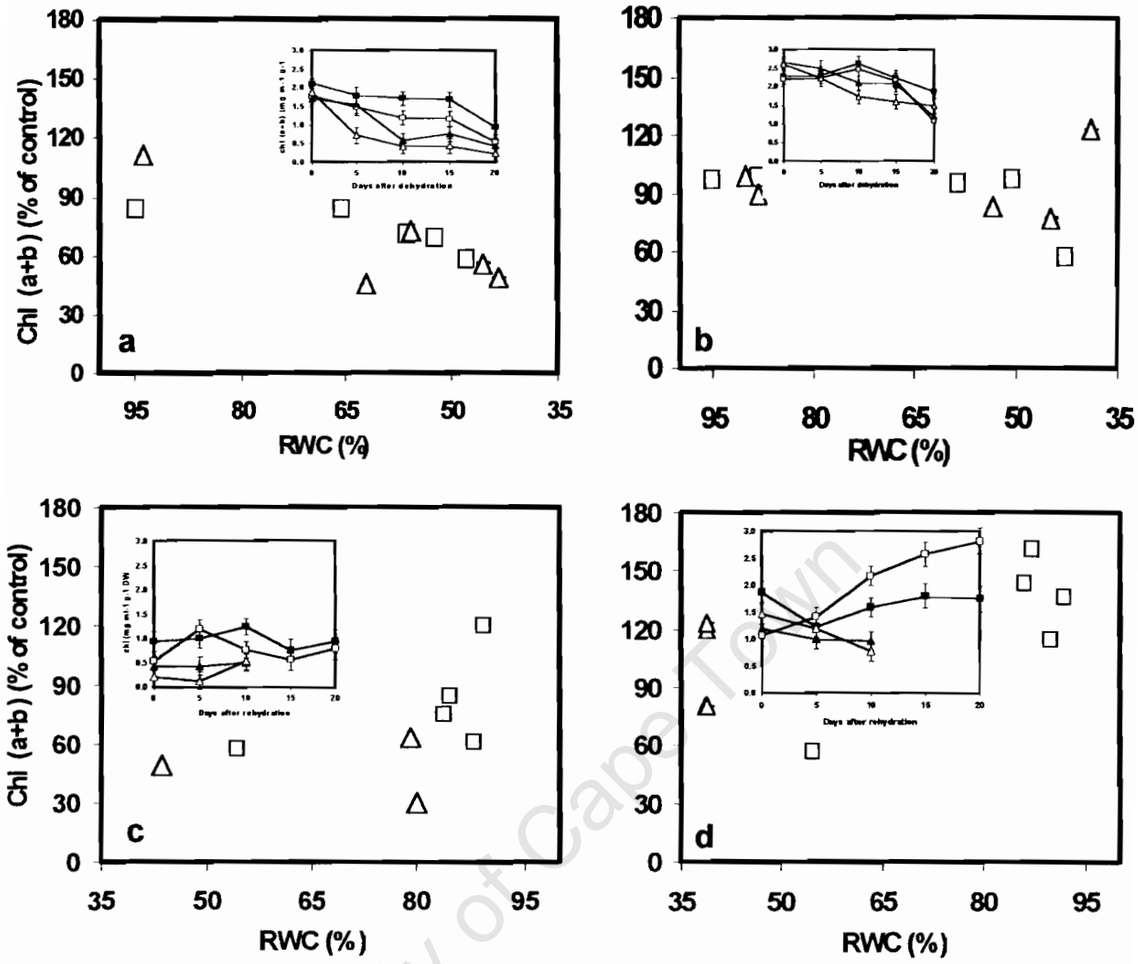

Figure 2.12 Changes in mean chlorophyll (a+b) content (% of control) of maize (a, c) and sorghum (b, d) during pre (■, □) and post-flowering (▲, Δ) dehydration (a, b) and rehydration (c, d) as related to RWCs, respectively. Insets refer to the same data expression in a time basis; ■ and ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

During pre-flowering dehydration, chlorophyll was retained between 98% and 96% within a range of 95% and 51% of relative water contents. In plants undergoing post-flowering dehydration, there was a gradual and continual decrease of chlorophyll contents with a corresponding decrease in relative water contents.

Full recovery of chlorophyll contents in pre-flowering rehydrated maize was followed by a loss of chlorophyll 5 days after rehydration began. During post-flowering rehydration, there was no change to that measured at the end of dehydration period. Sorghum fully regained its chlorophyll contents well above the control level following pre-flowering rehydration (Fig. 2.12c and d and inset). During post-flowering rehydration, chlorophyll contents did not recover, rather there was a continual decline.

2.3.5.2 Carotenoid

Carotenoid contents of control maize and sorghum at pre and post-flowering stages during the dehydration and rehydration treatment period is presented in inset in Figure 2.13. Carotenoid concentrations of control maize leaves declined at 20 and 15 days after treatment began during pre and post-flowering stages, respectively. Sorghum retained relatively constant carotenoid contents throughout the duration of both pre and post-flowering treatment period. In both maize and sorghum, carotenoid contents of control plants were generally higher during the pre than the post-flowering stage (Fig. 2.13a and b inset). In addition carotenoid contents in control maize and sorghum were higher during dehydration than during rehydration treatment period. Dehydration during both pre and post-flowering stages caused a steady decrease in carotenoid content in both maize and sorghum (Fig. 2.13a and b). Parallel to chlorophyll contents, there was a difference between species for carotenoid contents during both pre and post-flowering dehydration. Carotenoid contents of both pre and post-flowering dehydrated sorghum was markedly higher than maize throughout the duration of the dehydration period. Patterns of changes in carotenoid content as related to changes in relative water contents in both species varied between pre and post-flowering growth stages, indicating that the response of carotenoid content to dehydration is crop age dependent. In maize, carotenoid content was retained between 75% and 88% of the control until relative water contents reached 52% and thereafter it declined to 48% during the final stage of pre-flowering dehydration at 47% relative

water contents. During this period a decrease in the carotenoid content of control (fully hydrated) plants was also observed. In plants subjected to post-flowering dehydration, carotenoid content continued to decrease to 40% until relative water contents reached 56%.

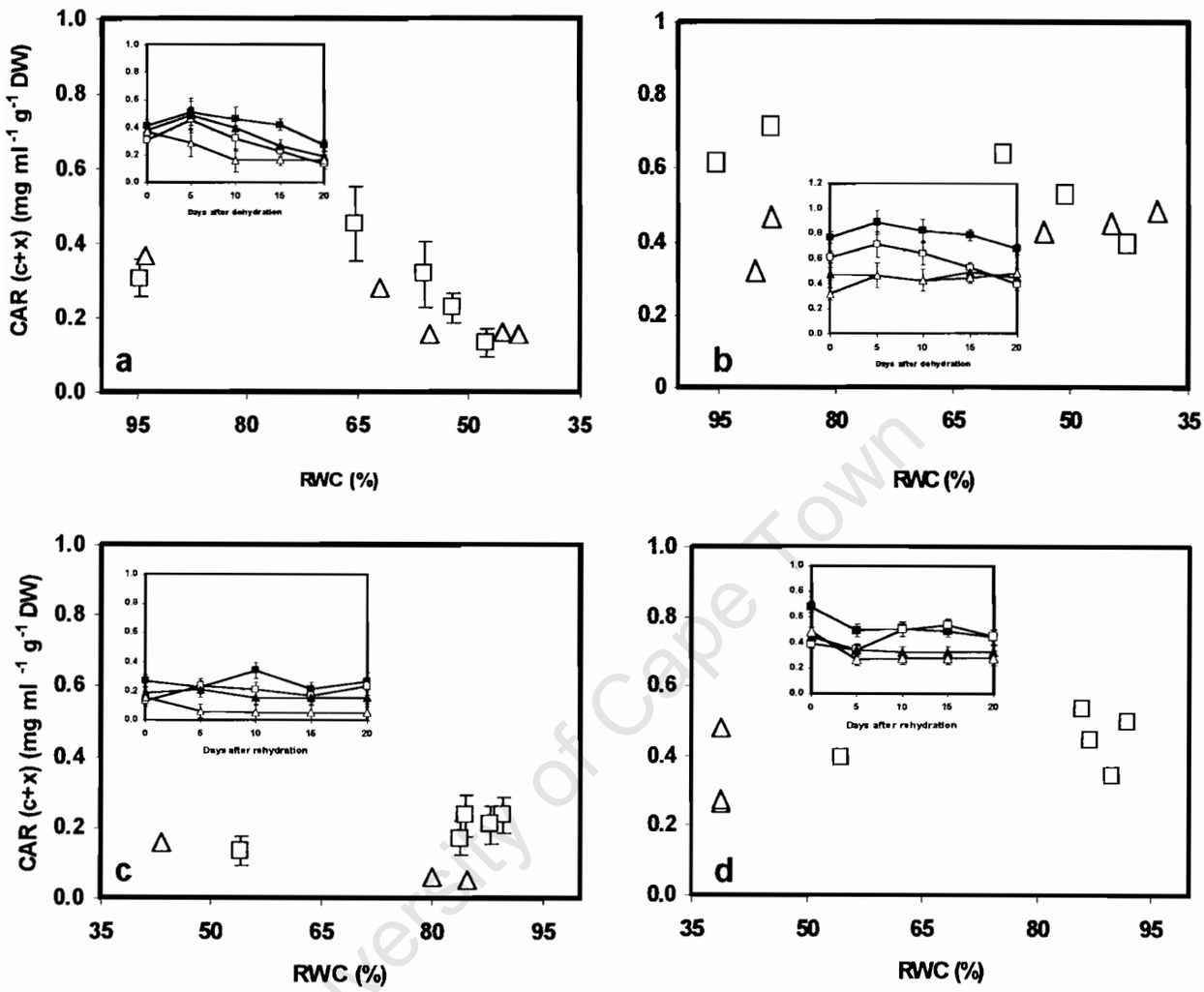

Figure 2.13 Changes in mean carotenoid (c+x) content (% of control) of maize (a, c) and sorghum (b, d) during pre (■, □) and post-flowering (▲, Δ) dehydration (a, b) and rehydration (c, d) as related to RWCs, respectively. Insets refer to the same data expression in a time basis; ■ and ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

During this period, there was an extensive lose of carotenoid content of control plants. Sorghum plants subjected to pre-flowering dehydration retained 83% to 100% of carotenoid content until relative water contents reached 50% and thereafter there was a sudden loss at 43% relative water content. In plants undergoing post-flowering dehydration, between approximately 95% and 88% relative water content the carotenoid content of sorghum plants increased to 100% and was retained with out noticeable changes until 40% relative water content at the final phase of dehydration.

There was no change in carotenoid contents of pre-flowering rehydrated maize to that measured at the end of dehydration period, whereas in sorghum full recovery occurred 10 days after rehydration began (Fig. 2.13c and d and inset). During post-flowering, there was no noticeable change upon rewatering in carotenoid contents of both maize and sorghum.

2.3.6. Electrolyte leakage

Dehydration during pre and post-flowering stages increased the rate of electrolyte leakage as compared to the control treatment (Fig. 2.14a and b and inset). The rate of increase in electrolyte leakage during pre and post-flowering dehydration was similar between both species (Fig. 2.14a and b and inset). During both pre and post-flowering dehydration, high rates of electrolyte leakage occurred below relative water contents of 55% in both species. There was, however, a different trend in post-flowering dehydration compared with pre-flowering plants in electrolyte leakage in both species, indicating that dehydration induced rate of electrolyte leakage was crop age dependent. Between 52% and 48% of relative water contents in maize and between 51% and 43% in sorghum, rate of electrolyte leakage during pre-flowering dehydration increased by about 3 fold, whereas during post-flowering the increase was by more than 12 fold.

In maize, there was a full recovery of electrolyte leakage during both pre and post-flowering rehydration, whereas in sorghum, full recovery was restored only during pre-flowering rehydration (Fig. 2.14c and d and inset). During post-flowering rehydration there was some initial decrease in the rate of leakage, but with increase in the duration of rehydration the rate of electrolyte leakage increased to the level recorded at the end of the dehydration period.

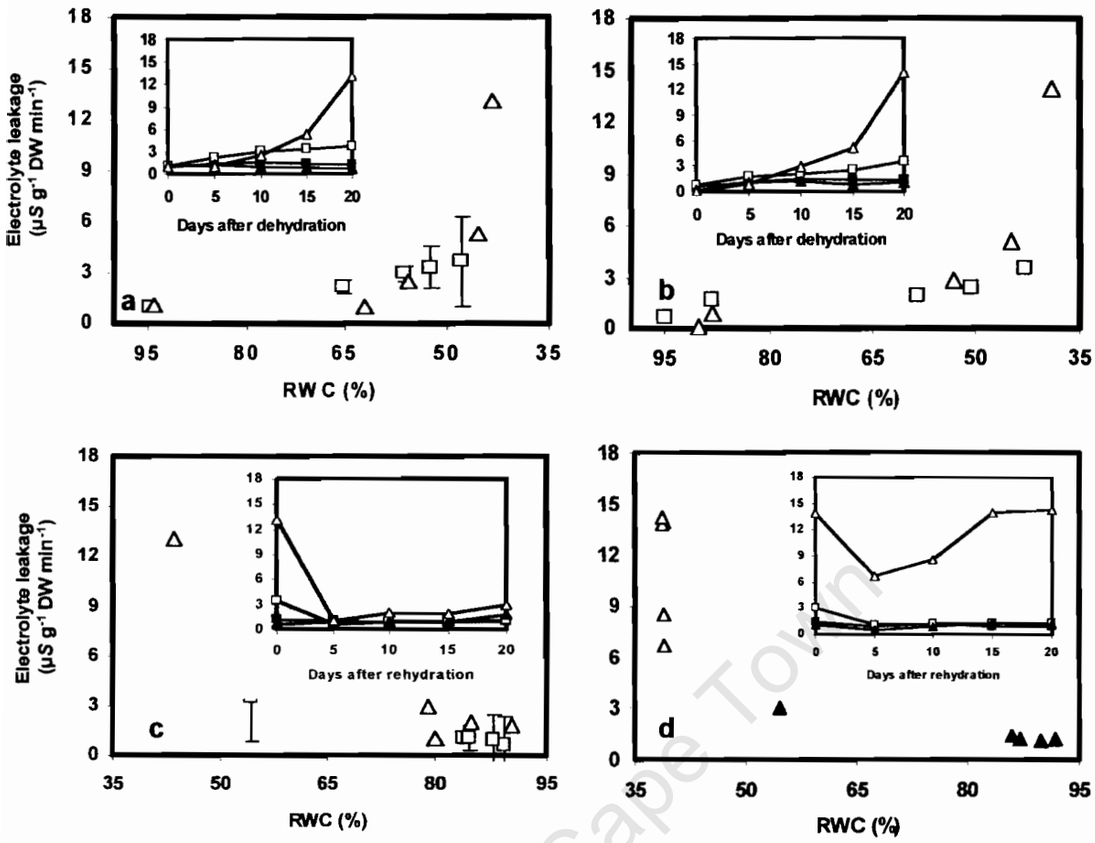

Figure 2.14 Changes in mean rates of electrolyte leakage (µS g^{-1} DW min^{-1}) of maize (a, c) and sorghum (b, d) during pre (■, □) and post-flowering (▲, Δ) dehydration (a, b) and rehydration (c, d) as related to RWCs, respectively. Insets refer to the same data expression in a time basis; ■ and ▲ represent control and □, Δ represent dehydration/rehydration treatments. Vertical bars denote standard errors of means (n=3).

2.3.7. Growth Characteristics

Generally, there was a noticeable reduction in mean leaf area, total dry weight and final grain yield due to the effect of dehydration. Dehydration at pre and post-flowering stage resulted a marked reduction ($P<0.05$) in mean leaf area of maize and sorghum relative to the control plants (Fig. 2.15a).

Under dehydrated conditions, the extent of reduction in mean leaf area of maize at post-flowering stage was much higher (about 87% reduction) than pre-flowering stage (about 50% reduction).

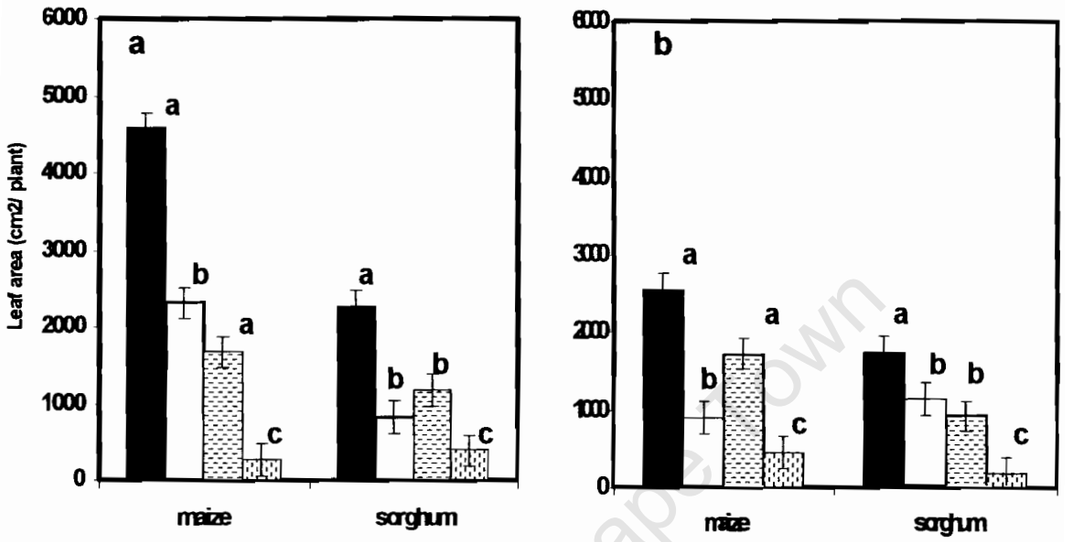

Figure 2.15 Leaf area (cm^2/plant) of maize and sorghum at pre and post-flowering dehydration (a) and rehydration (b), respectively. Vertical bars denote standard error of means (n=3). Solid and horizontal dashed bars are pre and post-flowering control, open and vertical dashed bars are pre and post-flowering dehydrated/rehydrated treatments. Letters followed by different letters are significantly different at $P<0.05$.

However, dehydration at pre (64% reduction) and post-flowering (66% reduction) stage had a similar ($P<0.05$) effect on the mean leaf area of sorghum. Control plants of maize at pre-flowering stage showed a significant reduction in mean leaf area during rehydration. However this was not the case in sorghum. The reason for this could be the effect of high light intensity and temperature.

There was a significant ($P<0.05$) decrease in mean leaf area of pre-flowering dehydrated maize plants upon rehydration, but sorghum did not show any noticeable change. In both species, plants dehydrated during post-flowering stage did not exhibit a significant ($P<0.05$) change upon rehydration from that measured during dehydration.

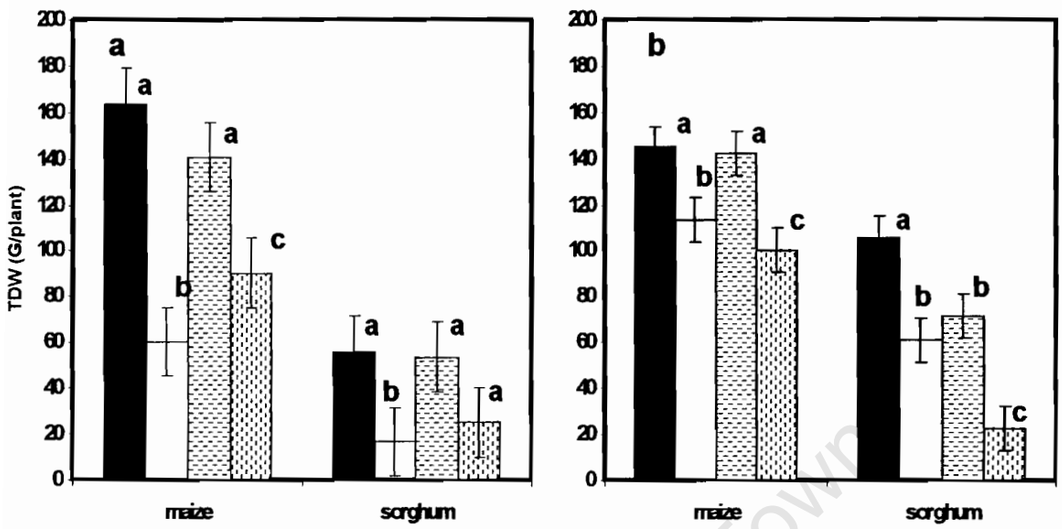

Figure 2.16 Total dry weight (g/plant) of maize and sorghum at pre and post-flowering dehydration (a) and rehydration (b), respectively. Vertical bars denote standard error of means (n=3). Solid and horizontal dashed bars are pre and post-flowering control, open and vertical dashed bars are pre and post-flowering dehydrated/rehydrated treatments. Letters followed by different letters are significantly different at $P<0.05$.

In contrast to leaf area, the reduction of mean total dry weight in both species were greater at pre-flowering than post-flowering dehydration and the percent reduction were by 63% and 36% in maize and 69% and 53% in sorghum at pre and post-flowering dehydration, respectively (Fig. 2.16a).

Approximately 78% and 58% of mean total dry weight recovered during pre-flowering rehydration in maize and sorghum, respectively (Fig. 2.16b). As expected, rehydration at post-flowering stage, however, did not change mean total dry weight in both species.

Similar to leaf area, the greatest reduction in grain yield occurred at post-flowering than pre-flowering dehydration in both species (Fig. 2.17). The percent reduction in grain yield due to the effect of dehydration at pre and post-flowering stage was in the order of 58% and 72% in maize and 48% and 86% in sorghum, respectively.

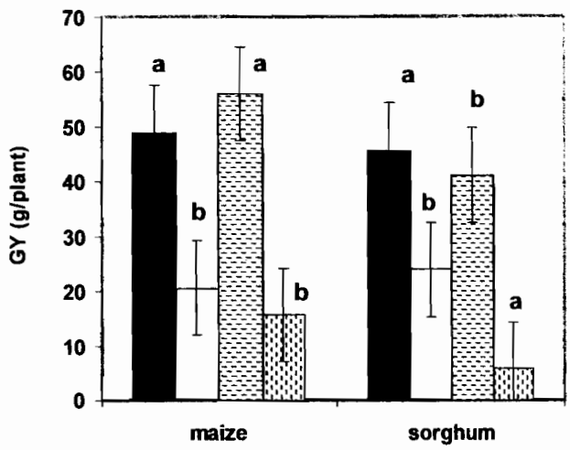

Figure 2.17 Grain yield (g/plant) of maize and sorghum harvested from plants dehydrated during pre and post-flowering stage. Vertical bars denote standard error of means (n=3). Solid and horizontal dashed bars are pre and post-flowering control, open and vertical dashed bars are pre and post-flowering dehydrated treatments. Letters followed by different letters are significantly different at P<0.05.

2.4 Discussion

2.4.1 Differences in the environmental stress condition between treatments

2.4.1.1 Soil water status

The soil water status under which maize and sorghum were grown through out the period of experimentation was evaluated by two variables, soil water contents and ψ_s. The two species differed in soil water demand, in a way that soil water deficits developed relatively slowly for sorghum compared with maize (Fig. 2.1a and b). This difference was attributed primarily to the substantially lower rate of water consumption by sorghum than maize. Plant size is a morphological characteristic

influencing water loss which contributes to the maintenance of ψ_L (Ludlow and Muchow, 1990; Lilley and Fukai, 1994). Sorghum had a smaller leaf area and plant stature at the beginning of the dehydration treatment, and this may be a factor which contributed to a lower water requirement than maize (Fig. 2. 15a). Larger leaf areas increased the rate of water use and increase the probability of the crop running out of water before maturity (Ludlow and Muchow, 1990), a possible reason why maize at the beginning of the treatment during both pre and post-flowering dehydration had a greater demand for water.

2.4.1.2 Light and Air temperature

Initially both maize and sorghum were grown in constant environmental conditions as indicated in the materials and methods section of this chapter. Since maize had overall greater plant size than sorghum, when the dehydration treatment began during pre and post-flowering stages the upper leaves and tassel were already in contact with the ceiling. As a result maize was exposed to high light (PPFD=1400μmol m^{-2} s^{-1}) and temperature (32/20 ^{0}C day/night) than sorghum throughout the duration of the experimentation. It is therefore suggested that exposure of maize plants to high light and temperature might be one possible factor contributing for the early on set of leaf rolling, decreased g_s (Fig. 2. 5) and loss of pigment compositions (loss of chlorophyll and carotenoid) (Fig. 2. 12-Fig. 2. 13).

2.4.2 Differences in physiological responses to dehydration between maize and sorghum

The two crops differed in the rate of change in relative water contents immediately after withholding water. According to Blum (1996) and Monneveux and Belhassen (1996) water loss at the plant level largely depends upon the size of the evaporating areas. As evapotranspiration from the limited volume of soils in the pots of maize became high there was a sharp decrease in the soil water contents and the leaves experienced a shortage of water supply, and relative water contents declined quickly both during pre and post-flowering dehydration (Fig. 2.3a and b). The fast rate of development of plant water deficits in maize than sorghum induced early leaf rolling (data not shown) and stomatal closure (Fig. 2. 5) in the former than the latter. Under conditions of water deficits, leaf rolling is a common response in grasses (Fernandez and Castrillo, 1999) and is a desirable drought avoidance mechanism to reduce

radiation incident on leaves, reduce leaf temperatures and water loss. Early leaf rolling may also be initiated due to lower leaf osmotic adjustment and higher leaf temperature (Fernandez and Castrillo, 1999). This result is in contrast to the finding of Fernandez and Castrillo (1999) who reported leaf rolling initiation in pot grown maize was at the 9th day of dehydration period. Lilly et al (1996) mentioned that while leaf rolling is a dehydration avoidance mechanism, it is also an indication of leaf water status and generally plants with greater leaf rolling are classified as drought sensitive. Leaf rolling would seem to be a desirable trait in intermittent stress environments, as it may only enhance survival until the next rainfall (Ludlow and Muchow 1990). The observed delay of leaf rolling in sorghum may suggest that some osmotic adjustment may have been occurred.

Variation in g_s is a more sensitive indicator for selecting desirable cultivars and crop species. Many studies have demonstrated that stomata close over a range of leaf water potentials, of generally less that -0.5 MPa, resulting in the concept of a threshold leaf water potential for stomatal closure (Begg and Turner, 1976). The present study indicated that both maize and sorghum employed reduced g_s to reduce water loss continuously over a range of relative water contents (ψ_L) and duration of pre and post-flowering dehydration. However, there was a difference in the extent of response of g_s between maize and sorghum with changes in relative water contents. During both pre and post-flowering dehydration drought resistance in maize is achieved through complete stomatal regulation, while partial stomatal closure at relatively higher values of relative water contents appeared to be occurred in sorghum (Fig. 2.5a and b and inset). Dehydration-induced stomatal regulation in maize has also been reported earlier (Sharp and Davies, 1985; Premachandra et al, 1992; Stikic and Davies, 2000). However, despite complete stomatal regulation and early onset of leaf rolling, maize exhibited fast rate of decline in relative water contents and ψ_L. Under dehydration conditions, stomatal closure could be triggered by both changes in chemical signalling and/or hydraulic status of the plant (Tardieu and Davies, 1992). Chemical messages, mainly ABA originating from roots and transferred to the leaf through the transpiration stream may cause stomatal closure regardless of the ψ_L of the plant (Davies and Zhang, 1991).

Decreases in g_s should improve the stability of yield, because it reduced water loss and lowers the probability of exhausting soil water before maturity. Alternatively, since stomata controls gas exchange, dehydration avoidance achieved through stomatal closure in plants reduces productivity (Ludlow and Muchow, 1990). The parallel decrease in gas exchange characteristics (P_n, E and R_d) in both species at approximately between relative water contents of 93% and 65% during both pre and post-flowering dehydration strongly support stomatal closure as the major factor in reducing photosynthesis (Chaves, 1991; Cornic, 1994). The greater decrease in P_n, (Fig. 2.6a and b), E (Fig. 2.8a and b) and R_d (Fig. 2. 9a and b) of maize was probably attributed to the much faster decrease in relative water contents which induced early leaf rolling and effective stomatal closure. Sorghum on the other hand was able to use water more efficiently by maintaining relatively higher relative water contents, delayed leaf rolling for extended period and stomata remain partially open altogether allowing a relatively higher P_n, E, and R_d. Stomata are well known to be sensitive to plant water deficit and stomatal closure may affect photosynthesis by reducing CO_2 fixation by increasing diffusional resistance of CO_2 into the leaf. Stomatal closure is considered to be responsible for the decline in P_n in several crop species exposed to moderate water deficits (Cornic, 1994) and there was no indication of damage to chloroplast reactions (Sharkey and Seemann, 1989).

Under more sever dehydration, however, reduced P_n is generally considered to be due to non-stomatal factors (Kaiser, 1987; Lawlor, 1995; Lawlor and Cornic, 2002). The decline in P_n of both maize and sorghum to almost zero below relative water content of approximately 65% during the late phase of dehydration period, and subsequent lack of recovery upon rehydration may suggest that factors other than stomatal limitation might have been involved.

To distinguish whether the decrease in photosynthesis is due to stomatal or non-stomatal factor, several studies suggest the ratio of Fv/Fm gives a direct estimate of the yield of PSII photochemistry (Kicheva et al, 1994; Massacci and Jones, 1990; Liang et al, 1997). A sustained decrease in Fv/Fm is believed to indicate the occurrence of photoinhibitory damage, in response to many environmental stresses including water deficit stress (Maxwell and Johnson, 2000). In this study, the observed decrease in the ratio of Fv/Fm in pre and post-flowering dehydrated maize

and sorghum (Fig. 2.7a and b and inset) supported the idea that dehydration during both developmental stages in maize and sorghum had a direct effect on the PSII photochemistry. The results of this study was in agreement with the findings of Massacci et al (1996) who reported that the inhibition of P_n by dehydration was non-stomatal factor in field grown sweet sorghum. Pankovic et al (1999) have also reported the possibility of long term drought stress to cause a non-stomatal limitation of P_n in leaves of sunflower plants grown in the field. There are reports that show the inhibition of P_n caused by dehydration was mainly due non-stomatal factor when the plants were rapidly dehydration (Saccardy 1996). It is possible that the inhibition of P_n due to non-stomatal factor in both pre and post-flowering dehydrated maize plants could be attributed to the relatively faster rate of development of dehydration (Fig. 2.3a and b and inset). There are contradictory reports of the direct effects of drought stress on PSII photochemistry function. Some suggested that under mild drought stress PSII photochemistry is not affected (Jefferies, 1994), while under sever drought stress damage occurs to PSII photochemistry (Meyer and De Kouchkovsky, 1993). Others showed that alterations of PSII activity under drought stress are related to photoinhibition rather than to a direct damage to PSII photochemistry (Baker and Bowyer, 1994). Lawlor (1995) suggested that contradictory results about the limitation of P_n by stomatal (Cornic and Briantais, 1991; Brestic et al, 1995; Lal and Edwards, 1996) as opposed to metabolic regulation (Graan and Boyer, 1990; Tezara and Lawlor, 1995; Kanechi et al, 1995, 1996) in drought stressed leaves could be caused by different plant species being investigated under different environmental conditions.

At the single-leaf level, the relationship between production and water use can be expressed as the intrinsic water use efficiency (P_n/transpiration) (Blum, 1988; Condon et al, 2002). Stomatal regulation controls the exchange of water and carbon between the leaf and the atmosphere and thus affects water use efficiency (Blum, 1988). In this study, the faster rate of decline observed in the water use efficiency (Fig. 2. 11) of maize may have been attributed to the early and complete stomatal closure which in turn resulted to a greater decrease in P_n than transpiration. It has been proposed that enhanced transpiration efficiency is an important component of maintenance of green leaf area under dehydration conditions. Genotypes containing stay-green trait during water deficit stress maintain more photosynthetically active leaves compared with

genotypes not containing this trait (Rosenow et al, 1983; Borell et al, 2000). Sorghum by retaining green leaf area for an extended period than maize during pre and post-flowering dehydration (Fig. 2.12) was better able to maintain relatively higher water use efficiency than maize. In this case, the higher water use efficiency was due to increased photosynthetic rate during both pre and post-flowering dehydration (Fig 2.6). During leaf senescence, the photosynthetic apparatus is dismantled and nutrients are exported to young tissues or storage organs. In maize, the observed extensive dehydration-induced senescence during both pre and post-flowering dehydration could be one possible reason why maize had lower water use efficiency than sorghum. Therefore, in the present study water use efficiency which indicate the tissue water relation of a species suggests differences in adaptation strategies between the two species to dehydration.

A decrease in relative water contents also elicited a parallel decrease in R_d rate of pre and post-flowering dehydrated maize and sorghum with the greatest decrease occurring in maize than sorghum (Fig. 2.9a and b and inset). This decrease in R_d rates was attributed to a greater decrease in the growth rates of dehydrated than corresponding control maize and sorghum plants. The results of this study are in agreement with that of McCree et al, (1984) who reported a decrease in the R_d rates of controlled-environment grown grain sorghum with decrease in the predawn water potential which was attributed to a lower rate of biomass synthesis compared with the decrease in photosynthesis, and may have been the result of a greater emphasis on storage of recent photoassimilate. The greater decrease in R_d rates during the early phase of dehydration in maize may be associated with a faster decrease in relative water contents which in turn induced a reduction in g_s and extensive leaf senescence as opposed to that of sorghum which retained its green leaf area and g_s remained partially opened during the same period. However, as the duration of dehydration progressed R_d rates of both maize and sorghum reached to their minimum values as compared with the control plants. Under such conditions the decreased in R_d rates were unlikely to be a result of reduced growth of leaves. There are reports indicating direct mitochondrial alterations caused by severe plant water deficits which resulted in reduced respiratory metabolism (Sells and Koeppe, 1981; Schmitt and Dizengremel, 1989). The greater decrease as dehydration became more sever combined with the observation that dehydrated leaves of maize and sorghum did not

fully recovered following rehydration, suggest some disruption of the respiratory machinery.

As compared with hydrated plants, dehydration decreased chlorophyll contents of both species during pre and post-flowering stages (Fig. 2.12a and b and inset). Decrease in chlorophyll contents due to drought stress have also been reported in wheat (Baisak et al, 1994), sunflower (Quartacci and Navari-Izzo, 1992) and sunflower and sorghum (Zhang and Kirkham, 1996). Retaining chlorophyll contents have been suggested as a drought resistance mechanism in sorghum (Thomas and Howarth, 2000). It is believed that stay-green characteristics enhanced radiation use efficiency and transpiration efficiency, which enable the plant to set a higher yield potential by anthesis, ultimately leading to higher grain yield (Borrel and Hammer, 2000). The presence of higher chlorophyll contents might partly explain why sorghum maintains higher photosynthetic rate than maize during both pre and post-flowering dehydration. In maize, chlorophyll loss was faster than sorghum during both pre and post-flowering dehydration (Fig. 2. 12). Chlorophyll loss is undesirable trait and is a negative consequence of stress. However, it has also been considered as an adaptive feature in plants grown under adverse climatic conditions, usually exposed to an excess of excitation energy (Maslova and Popova, 1993; Munne-Bosch and Alegre, 1999). Chlorophyll loss reduces the amount of radiation intercepted by leaves and at the same time reduces the possibility of further damage to the photosynthetic machinery by the formation of reactive oxygen species under high light (Munne-Bosch and Alegre, 1999). The observed recovery of Fv/Fm ratio in maize during post-flowering rehydration (Fig. 2. 7c) could probably be due to the protective effect of loss of chlorophyll.

Carotenoid contents play a role in protecting the photosynthetic apparatus against destructive effects of excess light and O_2 and collects light energy for photosynthesis processes (Siefermann-Harms, 1987). The observed decrease in carotenoid contents of both species during pre and post-flowering dehydration as compared to the control (Fig. 2.13a and b and inset), indicates enhanced oxidative stress in senescing chloroplasts (Munne-Bosch et al, 2001). Since carotenoid play an important role as an antioxidant against reactive oxygen species under environmental stress, their comparative levels in a particular crop species may determine its relative tolerance.

Dehydration in sensitive plants is often accompanied by membrane damage, resulting in the leakage of solutes (Blum and Ebercon, 1981; Blum, 1988). In the species studied here the most dramatic changes in the rate of electrolyte leakage were noticed during the late phase of post-flowering dehydration when relative water contents decreased below 65% (Fig. 2.15a and b and inset). The greater rate of electrolyte leakage as dehydration became severe may be related to visual signs of leaf senescence characterized by chlorosis and leaf drying which caused membrane damages that induces an increase in membrane permeability in both species. Results of the present study are in contrast to that was found in maize (Premachandra et al, 1992) and sorghum (Premachandra et al, 1992) where increased cell membrane stability with increase level of drought stress has been reported. The ability to increase membrane stability in response to severe water deficit is characteristic of a drought tolerance strategy (Turner, 1986). Maize and sorghum appear to tolerate moderate dehydration as has been observed by the maintenance of the rate of electrolyte leakage to the control level between 90 and 65% relative water contents, but as relative water contents dropped below 65% their membrane structure appeared to be damaged and thus, increased membrane permeability.

The major effect of dehydration during pre and post-flowering stages in this study was a general reduction on plant growth characteristics. This was observed in the form of decreased leaf area, total dry weight and final grain yield (Fig. 2. 15a, 2.16a and 2.17, respectively). The observed reduction in grain yield, leaf area and total dry weight are typical responses of crop plants when subjected to dehydration (Kramer, 1983). Extended periods of drought in plants may decrease the photosynthetically active leaf area (Ludlow and Muchow, 1990). Both species showed reduced leaf area under pre and post-flowering dehydration conditions, an important drought avoidance mechanism to reduce transpirational water loss. In maize, the decrease in leaf area was due to rapid and extensive leaf senescence at both pre (50%) and post-flowering (84%) dehydration (Fig. 2. 12a). Dehydrated-induced accelerated senescence observed in maize during both pre and post-flowering stages might have been exacerbated due to the prevailing high light and temperature conditions, since the loss of chlorophyll in this species may be a strategy in response to excess light and high temperature. By contrast, sorghum leaf expansion rate was inhibited and possessed

few leaves during both pre and post-flowering dehydration relative to the control plants while maintaining green leaf area. As with leaf area, dehydration induced decrease in grain yield was also found in both species at pre and post-flowering stages. In maize, grain yield reduction resulted from a decrease in seed number rather than individual seed weight, while in sorghum there was a general decrease in seed size. This was in agreement with the works of Ouattar et al (1987) in maize and Tuinstra et al (1997) in sorghum.

2.4.3 Differences between pre and post-flowering growth stages in response to dehydration in maize and sorghum

The expression of drought tolerance in crop plants is dependent on the stage of development at which the stress occurs (Rosenow et al, 1983; Blum, 1988, Tuinistra et al, 1997). For example in sorghum developmentally specific patterns of drought tolerance have been identified and symptoms of susceptibility during each stage have been characterized (Rosenow and Clark, 1981; Rosenow et al, 1997). Leaf rolling is a symptom of pre-flowering drought stress susceptibility in cereal crops (O'Toole and Cruz, 1980; Rosenow et al, 1997) and tolerance to pre-flowering dehydration is indicated by the alternative condition. It was noticed that leaves of maize decreased transpiration by early leaf rolling as soon as relative water content drops below 90% but sorghum did not display until relative water content reached to 70%. Early leaf rolling in maize may be associated with fast rate of decline in relative water contents and higher leaf temperature.

It has been proposed that growth stage had a major effect on stomatal sensitivity to dehydration and this has been demonstrated in maize and sorghum (Ackerson and Krieg, 1977); sorghum (Garrity t al, 1984) at which stomatal response was totally insensitive during the reproductive stage. This change in stomatal sensitivity with crop age was suggested to be due to osmotic adjustment which would allow the plant to maintain cell turgor and open stomata under low leaf water potentials (Ackerson and Krieg, 1977). However, the results of the present study indicated that stomatal response of both maize and sorghum at both pre and post-flowering dehydration were sensitive with decrease in the relative water contents over the duration of dehydration cycle (Fig. 2.5). This is in agreement with Massacci et al, (1996) who reported that

stomata did not show decreasing sensitivity to drought stress during plant development in field grown sweet sorghum.

Dehydration may reduce leaf R_d in an indirect manner by reducing growth and concomitant energy demand or by reducing substrate availability (Collier and Cummins 1996). The data of the present study revealed that dehydration induced decrease in R_d rates during the early phase of pre-flowering stage was greater than that of post-flowering stage in both species (Fig. 2. 9). It is well known that pre-flowering stage is characterized by a rapid leaf expansion and with a decrease in RWC, reduction in leaf expansion rate occurs before any reduction in photosynthesis (Turner et al, 1986c; Saab and Sharp, 1989). This decrease in leaf expansion rate combined with a rapid onset of leaf senescence may have been responsible for the greater decline in the R_d rates during pre-flowering dehydration. However, as the stress became more severe, the decline in R_d rates may have been attributed to the lack of substrate availability. Since mature leaves were used for post-flowering dehydration, decline in the R_d rates were unlikely to be due to a reduced growth. It is, therefore, suggested that the decrease in R_d rates during post-flowering stage is associated to substrate limitation, as much of the carbohydrates are translocated to the reproductive organs.

So far, the influence of dehydration on Fv/Fm ratio has usually been examined in the early developmental stages of plants and experimental data are scarce for comparison with the results from dehydration at pre and post-flowering stages, but the available reports indicate that the way Fv/Fm changes with dehydration strongly depend with plant age (David et al, 1998; Massacci et al, 1996; Pankovic et al, 1999). In the present study, dehydration during pre and post-flowering stage exerted differing effects on the Fv/Fm ratio of both species with the effect being much more pronounced below relative water contents of 65% during the late phase of post-flowering dehydration (Fig. 2.7a and b). The result reported in this study is consistent with the findings of Massacci et al, 1996. Previous reports have shown that drought stress may accelerate leaf senescence in several species (Aparicio-Tejo and Boyer, 1983; O'Neill, 1983; David et al, 1998). In the present study, in response to dehydration the photosynthetic pigments (chlorophyll and carotenoid contents) decreased in both species when compared with the control but the decrease was

relatively greater during post-flowering stage than pre-flowering stage. Hence the contribution of non-stomatal factors to explain drought induced depression in photosynthesis may be expected to increase with plant age, and our results are in accordance with this hypothesis.

The influence of dehydration on electrolyte leakage is most commonly examined using a polyethylene glycol (PEG) test in several crop species including sorghum (Sullivan, 1972); wheat (Blum and Ebercon, 1981; Premachandra and Shimada, 1987) and maize (Premachandra et al, 1989) and usually at the seedling stages and experimental data are scarce for electrolyte leakage in response to dehydration at pre-anthesis and grain filling stages. But the limited available reports indicate that cell membrane stability increased with increasing water deficits and with plant age in four sorghum cultivars (Premachandra et al, 1992) and tobacco (Riga and Vartanian, 1999). Our results also indicated that the rate of electrolyte leakage during dehydration increased with plant age (Fig. 2.14a and b and inset). The greater rate of electrolyte leakage during post-flowering dehydration was related to plant age which was characterized by greater leaf burning, loss of leaf turgidity and drying which resulted to increased membrane permeability.

Crop age dependent response to dehydration was observed for leaf area in maize. The greater reduction of leaf area during post-flowering stage was a result of extensive leaf senescence associated to translocation of reserve carbohydrates to the growing reproductive organs (Fig. 2. 12a). Maintenance of chlorophyll in sorghum was particularly observed during post-flowering dehydration. Maintenance of green leaf area, commonly known as stay green is a desirable post-flowering drought resistance trait in sorghum (Rosenow et al, 1997). Evidently, terminal post-flowering dehydration resulted in an abbreviated period of grain development and therefore remarkably reduced grain yield in both species (Fig. 2. 17) (Tuinstra et al, 1997). The greater reduction in total dry matter accumulation during pre than post-flowering dehydration in both species could probably be due to extensive leaf senescence and reduced leaf area extension in maize and sorghum, respectively. On the other hand the decrease in total dry matter accumulation during post-flowering dehydration was compensated for development of reproductive organs (grain yield).

2.4.4 Differences in the physiological responses to rehydration between maize and sorghum

Despite the fact that rehydration following pre and post-flowering dehydration is determinant to stabilize grain yield in cultivated crop plants, there is a lack of literature concerning the effect of rehydration on the physiological response of crop plants at pre and post-flowering stages. Our understanding of the recovery of gas exchange characteristics (g_s, P_n, E and R_d) and the processes which controls it is, therefore, poorly understood at different developmental stages in general and at pre and post-flowering stages in particular at which water deficits exerts the greatest loss of grain yield. Our rehydration experiment indicates that the ability of g_s to recover after stress relief decreases with plant age. As shown in Figure 2. 5, although maize attained full recovery, the rate of recovery of g_s during pre-flowering rehydration was slow and sorghum did not attain full recovery. These findings are in accordance with the findings of Ludlow et al, (1980) who reported that recovery of g_s upon rewatering was slow and incomplete. During post-flowering stage, although there was an initial recovery, rehydration accentuated the negative effect of dehydration on g_s of both maize and sorghum (Fig.2 5). These results indicate that under the condition of dehydration prevailing in the present work, rehydration of the species under investigation at the later reproductive stage is apparently deleterious than pre-flowering stage. It appears that except during post-flowering rehydration in sorghum where relative water content did not improve upon rehydration, the rate of recovery of g_s was not determined by the rate at which relative water contents recovered during pre and post-flowering rehydration in maize and pre-flowering rehydration in sorghum, since full recovery of relative water contents have been attained during these periods. According to Ludlow et al (1980) the slow rate of recovery of g_s to the level of control plants has been called the 'after effect' of water deficits which results from accumulation of abscisic acid. This could be the reason why there was a slow rate of recovery in both species during pre-flowering rehydration. The absence of recovery during post-flowering rehydration in both maize and sorghum may be associated with the harmful effect of rehydration at the maturity stage of both species. The lack of recovery of g_s has also exerted an influence on the recovery of P_n (Fig. 2. 6); E (Fig. 2. 8); R_d (Fig. 2. 9) and water use efficiency (Fig. 2. 11).

During pre and post-flowering stage in maize and during pre-flowering stage in sorghum once plants are fully rehydrated the Fv/Fm ratio and electrolyte leakage returned to the control level and the photosynthetic apparatus and cell membranes have been repaired completely (Fig. 2.7c and d and Fig. 2. 14d). However after an apparent initiation of repair, the rapid decline in Fv/Fm ratio (Fig. 2.7d) and increase in electrolyte leakage (Fig. 2. 14d) during post-flowering rehydration in sorghum suggest that rapid rehydration may be as harmful to the photosynthetic apparatus and cell membranes as the dehydration itself during post-flowering stage. Exacerbation of membrane damage during recovery is believed to be a consequence of a series of irreversible changes in the structure of cell membranes under stress conditions (Ristic et al, 1992). Similarly, Ristic et al, (1992) reported occurrence of sever deterioration of membrane in drought sensitive maize variety after rehydration.

2.4.5 Conclusion

The results of this study pointed out the need for using integrated traits when evaluating drought resistance of plants. The results showed that the maize cv Melkassa-2 and sorghum cv MACIA present a remarkable array of contrasting behaviour in response to pre and post-flowering dehydration and rehydration. Differences in maintenance of relative water content may be related to performance under dehydration, particularly when crop species of different adaptation are compared under stress conditions of varying intensity. This has been observed particularly during moderate water deficits when sorghum exhibited relatively higher relative water contents during both pre and post-flowering dehydration. In conclusion, sorghum appeared to be more resistant to moderate pre and post-flowering dehydration than maize; this can be attributed to its greater capability to maintain relatively higher relative water contents and consequently delayed leaf rolling, maintain stomata partially open, delayed chlorophyll and carotenoid loss, maintenance of P_n at a reduced rate and relatively higher water use efficiency. Both species, however, were found to be susceptible to sever pre and post-flowering dehydration.

The ability of maize plants to achieve a complete repair of photosynthetic apparatus and cell membranes and recovery of chlorophyll and carotenoid contents of sorghum plants upon pre and post-flowering rehydration may suggest that selection for drought

resistance on the basis of the above parameters for maize and sorghum plants, respectively during pre and post-flowering rehydration could contribute in the development of drought resistant cultivars.

This study should help understand some adaptive mechanisms developed by maize and sorghum and contribute to identify useful traits for breeding programs. However, further studies are necessary under field conditions to clarify the adaptive responses in both maize and sorghum during pre and post-flowering dehydration and the capacity to return to normal physiology during post-stress rehydration.

Chapter 3.

Antioxidant defence mechanisms and pigment compositions of maize and sorghum after exposure to and recovery from pre and post-flowering dehydration.

3.1 Introduction

Plant growth and productivity is adversely affected by various environmental factors and water deficit stress is considered one of the most important causes of decreased grain yield in cultivated crops (Menezes-Benavente and Texeira, 2004). Genetic improvement for drought tolerance is, therefore, of particular importance to agricultural plants. Different environmental stresses that have a dehydrative component such as water deficits, temperature and salt stress are known to cause various physiological and metabolic effects on plants. One of the negative effects of water deficit stress in crop plants is the excess generation of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($O_2^{\cdot}$) (Foyer et al, 1994; Mittler, 2002). The excess production of reactive oxygen species during drought stress results from impaired electron transport processes in chloroplast and mitochondria (Smirnoff, 1993; Asada, 1999). These reactive oxygen species are highly reactive and cause lipid peroxidation, protein denaturation and DNA mutation (Bowler et al., 1992). To remove the enhanced reactive oxygen species, plant cells are equipped with an antioxidant defence mechanisms that protect cells against oxidative damage. These antioxidant defence mechanisms are represented by various scavenging systems consisting of low molecular weight antioxidants, such as ascorbate, α-tocopherol, glutathione and carotenoids as well as antioxidative enzymes. The antioxidative enzymes included superoxide dismutase which catalyses the dismutation of superoxide radicals to H_2O_2 and O_2 (Bowler et al, 1992; Scandalios, 1993; Smirnoff, 1993), ascorbate peroxidase (APX) and catalase which catalyses the change of H_2O_2 to water (Asada, 1992; Scandalios, 1994). In chloroplasts, H_2O_2 is also eliminated by the action of ascorbate glutathione cycle where glutathione reductase and ascorbate peroxidase are key enzymes (Foyer, 1993; Foyer et al., 1994). Carotenoids are also involved in the protection of the photosynthetic apparatus against photoinhibitory damage by singlet

oxygen which is produced by the excited triplet state of chlorophyll (Loggini et al., 1999). Carotenoids can directly deactivate singlet oxygen and can also quench the excited triplet state of chlorophyll, thus indirectly reducing the formation of singlet oxygen (Foyer and Harbinson, 1994). The damaging effects of an externally imposed biotic or abiotic stress can be partly attributed to the over riding of existing resistance mechanisms. Only when those mechanisms are overwhelmed would injury occur (Smirnoff, 1993; Zhang and Kirkham, 1996). This indicates that the strengthening of the defence mechanisms, through enhancing functions of their components such as superoxide dismutase, ascorbate peroxidase, glutathione and β–carotene may reduce or prevent oxidative damage and improve drought resistance of plants. It was noted that different species have different antioxidant activity responses in the face of water deficit (Smirnoff, 1993). Previous studies have indicated that under drought stress, increase in antioxidant activities was reported in drought stressed tomato (Perl-Treves et al., 1988), maize (Pastori and Trippi, 1993; Jiang and Zhang, 2002), sorghum (Jagtap and Bhargava, 1995), sorghum and sunflower (Zhang and Kirkham, 1996) and wheat (Loggini et al, 1999, Lascano et al, 2001, Sgherri et al, 2000), implying their role in the ameliorating, to some extent , reactive oxygen species generated by water deficit stress. Although several studies strongly suggest that the accumulation of antioxidant defence system play a drought tolerance role, there is very little information on the correlation of their expression and level of drought tolerance in maize and sorghum. Furthermore, since water deficit stress during pre and post-flowering developmental stages is determinant in the productivity of maize and sorghum, the role of antioxidant in their response to water deficit stress must be examined. This has not been reported on to date. Understanding of physiological and metabolic changes and tissue response to water deficit may offer an avenue in the selection of drought tolerant crop species, and elucidation of underlying control mechanisms.

This study is, therefore, aimed at investigating the accumulation of some of the antioxidant defence systems, compares the expression of these traits and the level of drought resistance in maize and sorghum after exposure to and recovery from pre and post-flowering dehydration.

3.2 Materials and Methods

Detailed information on cultivars of test species, methodology and environmental conditions used in this report has been given in chapter 2. Only a brief summary of the relevant experimental details is presented here. As described in Chapter 2, the size of the phytotron under which this experiment was conducted forced reduced plant numbers and this does not allow a meaningful statistical examination of the data (Yohannes, personal communication, 2004).

The accumulation of antioxidant defence mechanisms were measured, after exposure to and recovery from pre and post-flowering dehydration. A pot experiment was conducted in a controlled environment phytotron (12/12 h day/night, 28-30/17 ^{0}C day/night temperature, 60-80% RH and PPDF of 1200-1400 μ mol m^{-2} s^{-1}) in the department of Botany, University of Cape Town. Five plants were grown per 10kg dry soil capacity pot which was thinned to two seedlings of uniform size per pot twenty days after emergence. At 60 (pre-flowering) and 90 (post-flowering, grain filling stage) days after emergence, two moisture treatments were applied: either maintained fully hydrated (control) or dehydrated treatments. Control plants were regularly watered to field capacity (F.C) and the dehydration was induced by withholding water for 20 days at each growth stages. At the end of each dehydration treatment, plants were rehydrated by soil watering for another 20 days and their recovery was studied. At regular interval during the entire cycle (pre and post-flowering dehydration and rehydration) the following parameters (detailed below) were measured. The same were performed on control (fully hydrated) plants.

Leaf samples of approximately 1g fresh weight were taken randomly from 3 different plants of each treatment and species and at least 3 leaf samples were taken per plant for determination of enzymatic, non enzymatic antioxidant activities and MDA assays, immediately frozen in liquid nitrogen and stored at -80 ^{0}C until analysis.

3.2.1 Assays of antioxidant enzyme activities

Frozen leaf samples of 0.5g were ground into fine powder using chilled mortar and pestle under liquid N_2. Tissue was extracted by homogenizing the powder in 0.1M phosphate buffer (pH 7.8) containing 2mM DTT, 0.1mM EDTA and 1.25mM PEG 4000. Insoluble material was removed from the homogenate by centrifugation at 11500 rpm for 15 minutes at 4 ^{0}C. The extract was filtered by running through PD10 sephadex column, which has been equilibrated by 3 washes of 3mls of 0.1M

phosphate buffer (pH 7.8). The retained protein in the column was eluted with 3.5ml of 0.1M phosphate buffer (pH 7.8), collected and retained for the following antioxidant enzyme analysis.

3.2.1.1 Superoxide dismutase (SOD) activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Giannopolitis and Reis (1977). The 3ml reaction mixture contained 2.20ml of 0.1M phosphate buffer (pH 7.8), 0.25ml of 1.3µM riboflavin, and 0.25ml of 13mM methionine, 0.25ml of 63µM NBT and 50µl enzyme extract. Riboflavin was added last. Blank assays without extracts (water in place of sample) were prepared. The cuvettes containing the reaction mixtures and blanks were placed under high light intensity lamps. The reaction was initiated by switching on the light after which it was switched off after 15 minutes. A non-irradiated reaction mixture that did not develop colour served as control. The reaction mixture which lacked enzyme developed maximum colour as a result of maximum reduction of NBT. One unit of SOD activity was defined as the amount of enzyme necessary to inhibit the reduction of NBT by 50% as monitored spectrophotometrically (Beckman DU 650, USA) at 560nm. Results are expressed as units mg^{-1} protein min^{-1}.

3.2.1.2 Glutathione reductase (GR) activity was determined by following the oxidation of NADPH at 340 nm for 5 minutes at 25 ^{0}C in 400µl of an assay mixture containing 200µl of 0.1M phosphate buffer (pH 7.8), 50µl of 3mM $MgCl_2$, 25µl of 10mM GSSG, 25µl of 0.5mM NADPH and 100µl enzyme extract. Correction was made for the background absorbance at 340 nm, without NADPH (Schaedle and Bassham, 1977). Activity of GR was calculated from the rate of oxidation of NADPH by using the extinction coefficient of 6.22 mM^{-1} cm^{-1}. One enzyme unit is defined as µmol mg^{-1} protein min^{-1}.

3.2.1.3 Catalase (CAT) CAT activity was determined by following the consumption of H_20_2 at 240nm (spectrophotometer Beckman DU 650) for 5 minutes at 25 ^{0}C. The 3ml reaction mixture for the determination of catalase contained 2.55ml of 50mM phosphate buffer (pH 7.0), 250µl of 37.5 mM H_20_2 and 200µl of enzyme extract in a 3ml volume. One unit of enzyme activity was defined as the amount

necessary to decompose 1 µM of H_2O_2 min^{-1} at 25 ^{0}C, calculated from the extinction coefficient for H_2O_2 at 240nm of 0.0436 µmol^{-1} cm^2.

3.2.1.4 Ascorbate peroxidase (APX) Frozen leaf samples (0.5 g) were ground into fine powder using chilled mortar and pestle under liquid N_2. The fine powder was extracted by homogenizing in 10 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpyrrolidone (PVP), with the addition of 1 mM ascorbic acid (ASC). A reaction mixture of 1ml contained 500 µl of 50 mM potassium phosphate buffer (pH 7.0), 15 µl of 0.5 mM ASC, 15 µl of 0.1 mM H_2O_2, 200 µl of enzyme extract and 270 µl H_2O. The reaction was started by adding H_2O_2. The homogenate was centrifuged at 15000 g for 20 min at 4 ^{0}C. APX activity was determined by following the decrease at 290 nm, using extinction coefficient 2.8 mM^{-1} cm^{-1} for 1 min.

3.2.2 Determination of non-enzymatic antioxidants

3.2.2.1 Ascorbate (ASC) was extracted from 0. 2 g leaf segments with 2ml of cold 5% trichloroacetic acid (CTA) containing 80 mg insoluble PVP. The supernatant were centrifuged at 16000g for 10 minutes at 4 ^{0}C. The 1.2 ml reaction mixture for the determination of ascorbate contained 100% ethanol, 160 µl of 4.5M TCA, 120 µl of 30mM bathophenanthroline, 120 µl of 80mM phosphoric acid, 136 µl of 1.5mM $FeCl_3$, 80 µl sample extract and 64 µl water. The extraction solution was allowed to stand for 90 minutes at 30^{0}C for the Fe^{2+}-bathophenanthroline developed. The method was based on the reduction of ferric ion to ferrous ion with ascorbate in acid solution and then the formation of the red chelate between ferrous ion and bathophenanthroline, which absorbs at 530nm. A standard curve covering the range of 0-250 µmol ascorbate was used to calculate the concentration of ascorbate in the sample.

3.2.2.2 Total glutathione (GSH + GSSG) was extracted from 0.5g leaf segments with 10ml of 5% sulfosalicylic acid and centrifuged at 10000g for 5 minutes. Total glutathione and GSSG were determined by the 5, 5'-dithiobis-(2-nitrobenzoic acid)-GR recycling procedure (Griffiths 1980). The reaction mixture contained 100 mM sodium phosphate buffer (pH 7.5), 5 mM EDTA, 0.2 mM NADPH and 0.6 mM 5,5 dithiobis- (2-nitrobenzoic acid (DTNB). The reaction started by addition of 0.05 units

of glutathione reductase. Changes in absorbance of the reaction mixtures were measured with a spectrophotometer (Beckman DU 650, U.S.A) at 412 nm. To measure GSSG, GSH was masked by addition of 10 µl of 2-vinylpyridine to 500 µl sample extract. The extract was incubated for 60 min at room temperature. GSH was determined by subtraction of GSSG from the total glutathione content. Total GSH and GSSG content was calculated from a standard curve in the range of 20 to 200 ng GSH.

3.2.2.3 α-Tocopherol Leaf segments of 0.5g were ground in liquid N_2 using chilled pestle and mortar. Alpha-tocopherol was extracted from the powder by vigorously mixing for 1 minute with 8 ml *n*-heptane containing 1 µg ml-1 butylated hydroxytoluene (BHT) and using ultrasonication. After the samples were centrifuged at 15000g for 10 minutes, 4 ml of the upper *n*-heptane layer was carefully removed and evaporated to dryness using a desiccator. Samples were dissolved in 400 µl of methanol out of which 10 µl was injected into a HPLC apparatus using 100% and 15% methanol as buffer. α-Tocopherol was quantified through its absorbance at 290 nm. Pure α-tocopherol was used as a standard.

3.2.2.4 Anthocyanin contents were determined using 0.25g leaf material. The leaf segments were cut into small pieces to facilitate leaching of the pigment, and extracted in 1ml of acidified methanol (methanol: water: HCl [79:20:1]) for 48 hr at 4^0C. The absorbance were measured spectrophotometrically at 530 and 657 nm and the anthocyanin concentrations (A) were determined using the formula A= A530-(1/3A657). The result was presented as [A] per gram dry weight (Mancinelli et al; 1975).

3.2.3 Determination of lipid peroxidation The products of lipid peroxidation in leaf segments were determined by quantitating malondialdehyde (MDA) (a product of lipid peroxidation) content using the thiobarbituric acid reaction (Dhindsa et al, 1981). Approximately 0.5 g leaf sample was homogenized in 6 ml of 0.1% trichloroacetic acid. The homogenate was centrifuged at 10000 g for 10 min. To 1 ml of aliquot of the supernatant, 4 ml of 20% trichloroacetic acid containing 0.5% thiobarbituric acid was added. The mixture was heated at 95% ^{0}C for 30 min and then quickly cooled in an ice bath. After the tubes were centrifuged at 10000 g again for 10 min, the

absorbance of the supernatant was read spectrophotometrically (Beckman DU 650, U.S.A) at 532 nm. The value for the non specific absorption at 600 nm was subtracted from the 532 nm reading. The concentration of MDA was calculated using extinction coefficient of MDA of 155 mM^{-1} cm^{-1} (Heath and Packer, 1968).

3.2.4 Determination of total protein

Frozen leaf samples (0.5 g) were ground into fine powder under liquid N_2 using chilled mortar and pestle. Total protein was extracted by suspending the fine powder in 30mM TES (pH 7.5), 20mM NaCl and 1mM phenylmethanesulphonylfluoride (PMSF). The extracts were shaken vigorously for 15 min at 4 ^{0}C. Insoluble material was removed by centrifugating at 10000 rpm for 15 min at 4 ^{0}C. Total protein content of leaf samples was determined according to Bradford (1976) by using bovine serum albumin (BSA) as a standard.

Since both maize and sorghum plants died during the middle phase of post-flowering rehydration, biochemical analysis for the determination of enzymatic and non-enzymatic antioxidants and MDA contents were undertaken for only the first three periods of rehydration.

The data are presented in figures and these gives the changes in the activities of enzymatic and non enzymatic antioxidants and MDA contents as related to changes in RWCs (%). Since there were relatively little change in RWCs for control treatment over the duration of the experiment, the mean values measured at regular intervals during the treatment period are presented in Tables.

3.3 Results

3.3.1 Antioxidant enzyme activities

The superoxide dismutase activity during pre and post-flowering dehydration and rehydration of maize and sorghum as related to changes in relative water contents is presented in Figure 3.1. The results indicated that superoxide dismutase activity was enhanced by dehydration in both species during pre and post-flowering stages as compared with the control (Table 3.1). In maize, superoxide dismutase activity increased immediately after withholding water during both pre and post-flowering dehydration. There were no noticeable differences in the patterns of changes in superoxide dismutase activity between the two developmental stages in maize. An

increase in superoxide dismutase activity was gradual but continual during both pre and post-flowering dehydration and followed similar trends until the end of the evaluation period (Fig. 3.1a). When expressed as % of control, dehydration induced increase in superoxide dismutase activities ranged between 130% and 309% during pre-flowering and 149% and 355% during post-flowering dehydration. In sorghum, superoxide dismutase activity response varied between pre and post-flowering dehydration (Fig. 3.1b and Table 3.1). During pre-flowering dehydration, most marked increases in activity was observed between relative water contents of 95% and 58% which ranges between 111% and 202% of the control and then activity remained relatively constant with further loss in water content.

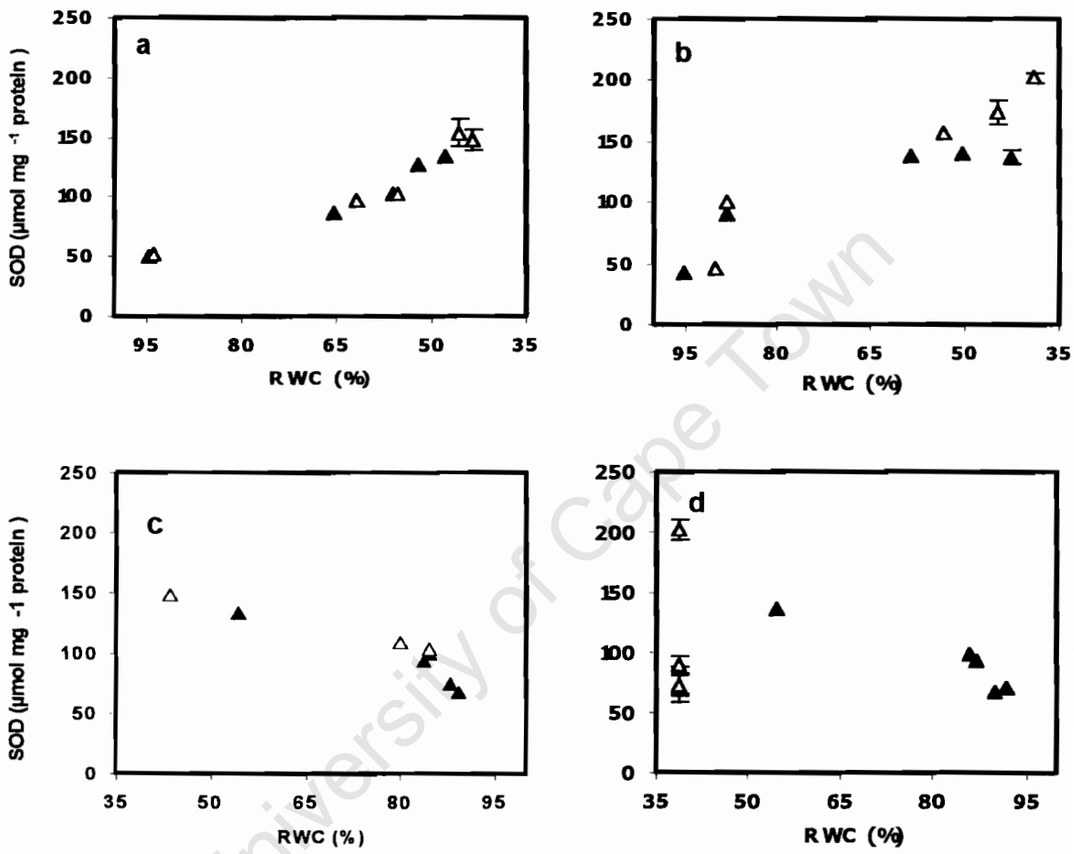

Figure 3.1 Superoxide dismutase activities (units mg^{-1} protein) in maize (a, c) and sorghum (b, d) during pre and post-flowering dehydration (a, b) and rehydration (c, d) as related to RWC (%), respectively. Vertical bars denote standard errors of means (n=3). ▲ and Δ symbols indicate pre and post-flowering dehydrated/rehydrated treatments, respectively.

During post-flowering dehydration, increase in superoxide dismutase activity was continual and peaked at 39% relative water contents. In both species, activity of superoxide dismutase was maximal during post-flowering dehydration at relative water contents of 45% (255% of control) and 39% (319% of control) in maize and sorghum, respectively.

On the other hand, the activity of superoxide dismutase in control plants of both species exhibited crop age dependent changes (Table 3.1). In maize, there was a continual decrease in activity during pre-flowering "dehydration" treatment period. Whereas during post-flowering "dehydration" treatment period, except at 15 days after treatment began, at which time there was a marked decrease, activity of superoxide dismutase was enhanced with time, and was maximal after 20 days of dehydration. In control plants of sorghum, activity of superoxide dismutase increased with time during both pre and post-flowering "dehydration" treatment period until day 15 after treatment began, after which there was a marked decrease in activity during the final stage of the treatment.

Table 3.1 Mean superoxide dismutase (unit mg^{-1} protein) activities of control maize and sorghum leaves at regular intervals (days) during pre and post-flowering dehydration and rehydration treatment periods, respectively. Numbers in parenthesis are SOD activities of dehydrated plants as % of control. Numbers followed by the same alphabets are not different.

Dehydration Treatment period (days)	Superoxide dismutase (units mg^{-1} protein)			
	Maize flowering		Sorghum flowering	
	pre	post	pre	post
0	64.2(80)	26.5(198)	38.7(111)	35.2(131)
5	66.9(130)	60.9(160)	68.8(132)	61.2(164)
10	61.5(167)	69.4(149)	68.1(202)	68.9(227)
15	50.4(252)	43.3(355)	69.6(201)	78.2(221)
20	43.1(309)	86.3(172)	53.5(255)	63.4(319)
mean	57.2(188)	57.3(207)	59.7(180)	61.4(212)
Rehydration Treatment period (days)				
0	43.1(309)	86.3(172)	53.5(255)	63.4(319)
5	60.4(112)	74.1(146)	60.9(109)	85.7(105)
10	55.5(135)	69.5(149)	74.9(95)	84.6(87)
15	70.9(132)	-	72.8(135)	-
20	67.8(148)	-	75.6(123)	-

Superoxide dismutase activities of control maize and sorghum during "rehydration" treatment period were generally higher than "dehydration" treatment period during both developmental stages. In addition superoxide dismutase activity was higher in sorghum than in maize during both "dehydration" and "rehydration" treatment periods of the two developmental stages.

When maize plants that had been dehydrated during both pre and post-flowering stage were rehydrated, only 50% recovered, which still represented a 1.5 fold induce compared to the control. In sorghum plants undergoing pre-flowering dehydration, approximately 75% of the superoxide dismutase activities recovered to the control level and those undergoing post-flowering dehydration decreased to the control level up on rehydration (Fig. 3.1c and d and Table 3.1).

Table 3.2 Mean glutathione reductase (μmol mg^{-1} protein) activities of control maize and sorghum leaves at regular intervals (days) during pre and post-flowering dehydration and rehydration treatment periods, respectively. Numbers in parenthesis are GR activities of dehydrated plants as % of control. Numbers followed by the same alphabets are not different.

Dehydration Treatment period (days)	Glutathione reductase (μmol mg^{-1} protein)			
	Maize flowering		Sorghum flowering	
	pre	post	pre	post
0	0.9(100)	2.9(61)	3.0(80)	2.2(75)
5	2.0(242)	2.5(131)	2.5(279)	2.2(125)
10	1.8(368)	3.4(101)	1.9(319)	2.1(200)
15	2.3(285)	1.5(154)	3.0(249)	1.5(216)
20	2.9(206)	0.9(205)	2.1(388)	1.5(241)
mean	1.9(240)	2.2(130)	2.5(263)	1.9(171)
Rehydration Treatment period (days)				
0	2.9(206)	0.9(205)	2.1(388)	1.5(241)
5	3.2(153)	1.4(90)	2.1(232)	1.1(298)
10	2.0(78)	0.9(70)	2.5(88)	0.6(446)
15	1.8(101)	-	3.2(89)	-
20	2.9(106)	-	2.2(164)	-
mean	2.6(129)	1.0(99)	2.4(192)	0.9(339)

There were differences in glutathione reductase activity of control plants between species during both pre and post-flowering "dehydration" treatment period (Table 3.2). During pre-flowering "dehydration" treatment period, control sorghum plants generally had higher glutathione reductase activities than maize, but was the reverse during post-flowering "dehydration" treatment period. There was also crop age dependent variation in glutathione reductase activities with time between control plants of the two species.

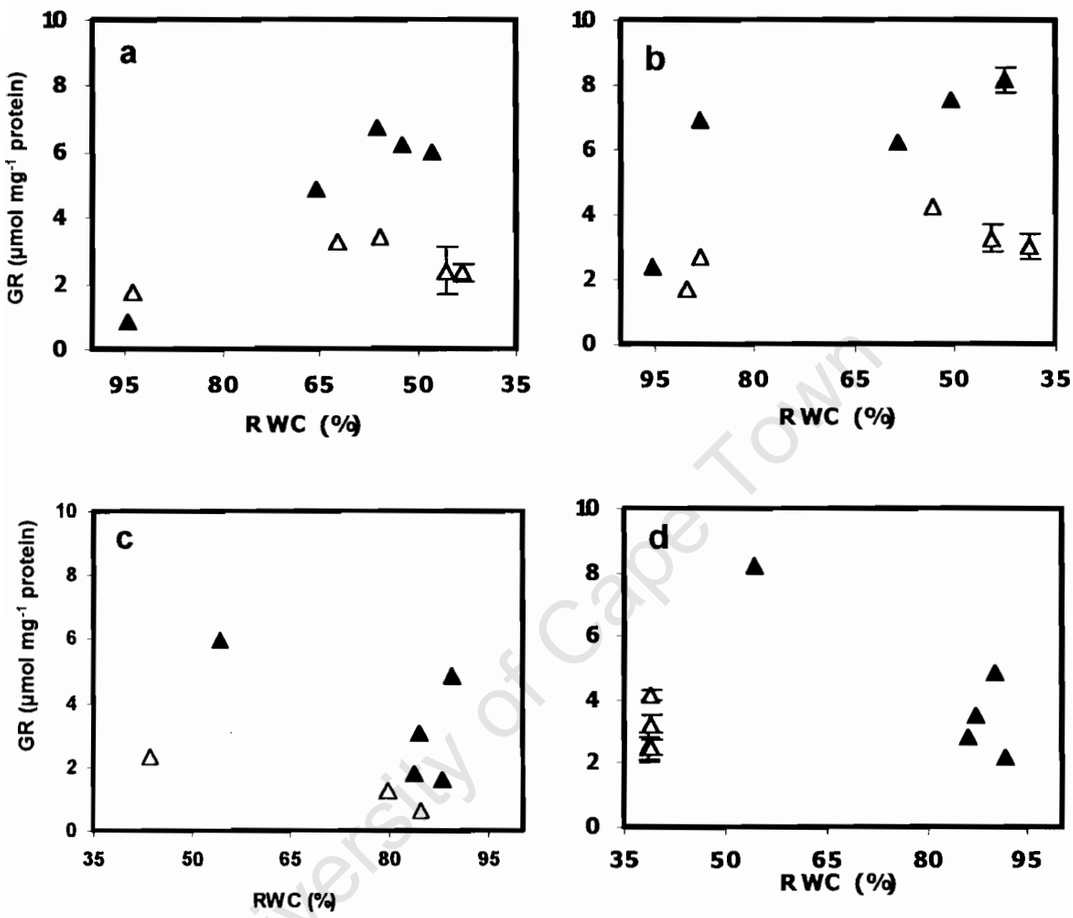

Figure 3.2 Glutathione reductase activities (µmol mg^{-1} protein min^{-1}) in maize (a, c) and sorghum (b, d) during pre and post-flowering dehydration (a, b) and rehydration (c, d) as related to RWCs (%), respectively. Vertical bars denote standard errors of means (n=3). ▲ and Δ symbols indicate pre and post flowering dehydrated /rehydrated treatments, respectively.

In maize, glutathione reductase activity increased during pre-flowering "dehydration" period and was maximal at 20 days after treatment began. After a slight decrease in glutathione reductase activity during post-flowering "dehydration", activity reached maximum 10 days after treatment and then it declined markedly with time. During pre-flowering treatment period, control sorghum plants did not have consistent glutathione reductase activities while those during post-flowering "dehydration" showed a consistent decrease (Table 3.2).

Dehydration also induced a marked increase in glutathione reductase activity of both species as compared with the control (Table 3.2 and Fig. 3. 2). In maize, glutathione reductase activity during pre-flowering dehydration peaked at relative water contents of about 56% and was followed by a decrease with increase in the intensity of dehydration until relative water contents of 48% (Fig. 3.2a).

Table 3.3 Mean catalase (μmol mg^{-1} protein min^{-1}) activities of control maize and sorghum leaves at regular intervals (days) during (μmol mg^{-1} protein) pre and post-flowering dehydration and rehydration treatment periods, respectively. Numbers in parenthesis are CAT activities of dehydrated plants as % of control. Numbers followed by the same alphabets are not different.

dehydration Treatment period (days)	Catalase (μmol mg^{-1} protein)			
	Maize flowering		Sorghum flowering	
	pre	post	pre	post
0	6.2(94)	7.6(72)	5.5(132)	6.9[a](90)
5	7.5(237)	7.4(117)	6.4(250)	8.8[a](125)
10	6.1(292)	8.4(165)	8.2(246)	7.2[a](231)
15	7.4(212)	7.7(102)	6.8(292)	9.6[a](210)
20	7.2(224)	5.1(102)	9.7(192)	8.5[a](207)
mean	6.9(212)	7.2(112)	7.3(222)	8.2(173)
rehydration Treatment period (days)				
0	7.2(224)	5.1(102)	9.7(192)	8.5(207)
5	3.9(195)	4.5(91)	8.3(93)	7.9(117)
10	9.2(109)	4.9(80)	10.5(103)	5.0(179)
15	9.3(64)	-	10.4(64)	-
20	5.5(101)	-	5.4(82)	-
mean	7.0(139)	4.9(91)	8.9(178)	6.3(166)

During post-flowering dehydration, there was a gradual increase in glutathione reductase activity until relative water contents reached to about 55%, and then like

pre-flowering dehydrated leaves, there was a declining trend in activities until approximately 43% relative water contents. Although activities showed a decrease, these values are still 2.9 fold and 2.1 fold higher than the pre and post-flowering control values, respectively. Unlike maize, glutathione reductase activity in sorghum was induced most markedly between relative water contents of 95% and 88%, followed by a slightly decreasing trend and then activity reached maximal at relative water contents of 43% during pre-flowering dehydration. When compared with the control, the increase in glutathione reductase activities during pre-flowering stage was by 388% at the end of the dehydration cycle (Table 3.2). The patterns of changes in post-flowering dehydrated sorghum leaves followed similar trends to its counter part maize (Fig. 3.2b), but activity was still 2.4 folds higher than its control counter part.

Glutathione reductase activities in both maize and sorghum, after attaining full recovery following pre-flowering rehydration, activity were enhanced again and peaked at relative water contents of approximately 90% (Fig. 3.2c and d). During post-flowering rehydration, glutathione reductase activity in maize was fully restored to a control level whereas; in sorghum an induction in glutathione reductase activity was observed as rehydration progressed (Table 3.2).

Over all mean catalase activities of control sorghum plants during both pre and post-flowering "dehydration" and "rehydration treatment" periods were higher than maize (Table 3.3). During "dehydration treatment" period, patterns of changes in catalase activities of control plants with time were not different between pre and post-flowering stages in both species. However, during pre-flowering "rehydration" treatment period catalase activities in both species show a marked increase at 10 and 15 days after treatment began. Catalase activities during post-flowering "rehydration" treatment period did not show a noticeable changes in either species.

Catalase activity in both maize and sorghum as related to changes in relative water contents during pre and post-flowering dehydration is shown in Figure 3.3. It was found that dehydration during pre and post-flowering stages remarkably increased catalase activity in both species as compared with their respective control. Catalase activity was generally higher in sorghum than in maize under dehydration condition. Differences were observed between species in catalase activity with changes in relative water contents during pre and post-flowering dehydration. In maize, between

relative water contents of 95% to 65%, there was a marked increase in catalase activity during pre-flowering dehydration and was maximal at 56% relative water contents (292% of the control) (Table 3.3). During post-flowering dehydration however, there was a more gradual increase in activity until RWCs reached to 62%, after which catalase activity peaked at relative water contents of 55% (165% of control).

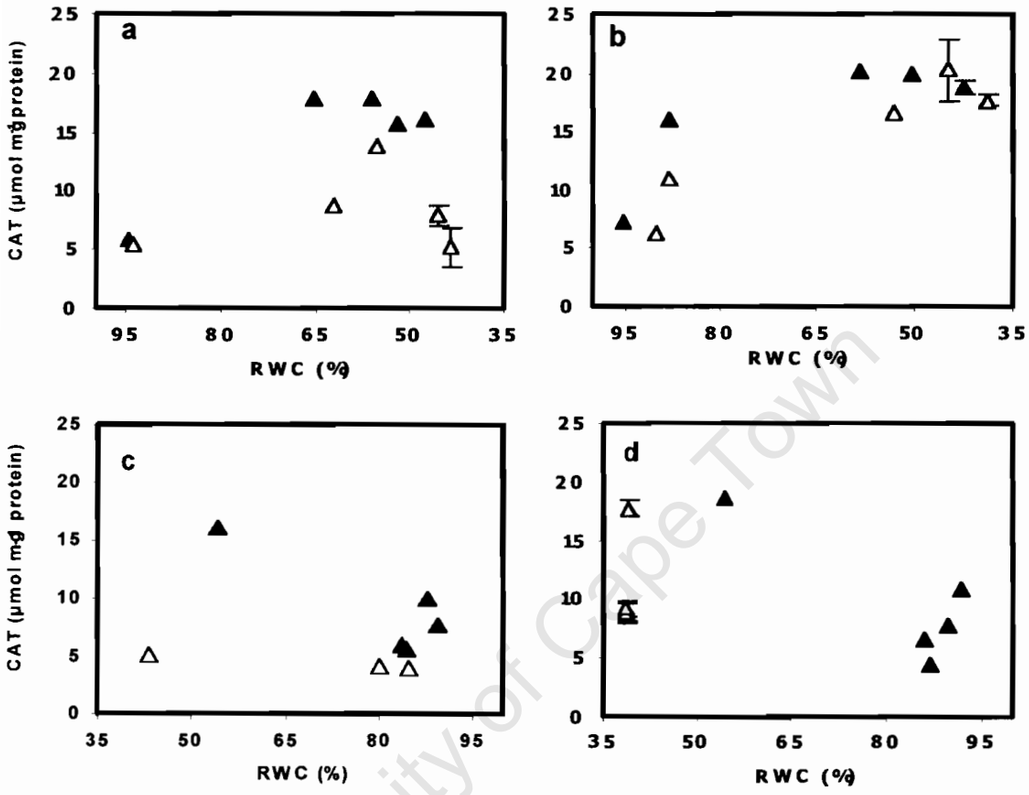

Figure 3.3 Catalase activities (µmol mg^{-1} protein) in maize and sorghum during pre and post-flowering dehydration (a, b) and rehydration (c, d) as related to RWC (%), respectively. Vertical bars denote standard errors of means (n=3). ▲ and Δ data points indicate pre and post flowering dehydrated /rehydrated treatments, respectively.

With further leaf water loss from approximately relative water contents of 55% to 43%, maize plants undergoing post-flowering dehydration exhibited a sharp decline in catalase activity and reached to the control level at the end of the dehydration cycle (Table 3.3). In sorghum, as water became limiting there was a corresponding increase in catalase activity during both pre and post-flowering dehydration. At the end of the dehydration cycle, catalase activities were 192% and 207% of the control during pre and post-flowering stage, respectively (Table 3.3). Catalase activity was maximal at 58% and 45% relative water contents during pre and post-flowering dehydration, respectively. In both species, catalase activity was markedly higher during pre than post-flowering dehydration.

The activities of catalase in maize and sorghum dehydrated during pre-flowering stages returned to control level at relative water contents of 84% and 87%, respectively. But upon further rehydration activity tended to increase again and peaked at relative water contents of 88% and 93%, respectively (Fig. 3.3c and d). Although Activity showed an increasing trend, it was still below the control level at the end of rehydration cycle in both species (Table 3.3). Maize leaves undergoing post-flowering rehydration fully recovered up on rehydration whereas that of sorghum remained elevated.

Under control conditions, ascorbate peroxidase activities differed between species at both pre and post-flowering treatment period (Table 3.4). During both pre and post-flowering dehydration treatment period, maize had consistently higher ascorbate peroxidase activities than sorghum. In both species, ascorbate peroxidase activities of control plants were relatively constant over the duration of the experiment during pre-flowering "dehydration" treatment period. However, during post-flowering "dehydration" treatment period, ascorbate peroxidase activities reached maximum in maize and sorghum at 20 and 5 days after treatment was initiated, respectively (Table 3.4). Figure 3.4 presents ascorbate peroxidase activity of maize and sorghum as related to changes in relative water contents during pre and post-flowering dehydration. When compared with their respective control plants, decreases in relative water contents induced an increase in ascorbate peroxidase activity in both maize and sorghum during pre and post-flowering dehydration, the increase being greater in sorghum during post-flowering dehydration than in maize (Table 3.4).

Table 3.4 Mean ascorbate peroxidase (μmol mg^{-1} protein min^{-1}) activities of control maize and sorghum leaves at regular intervals (days) during (μmol mg^{-1} protein) pre and post-flowering dehydration and rehydration treatment periods, respectively. Numbers in parenthesis are APX activities of dehydrated plants as % of control. Numbers followed by the same alphabets are not different.

dehydration Treatment period (days)	Ascorbate Peroxidase (μmol mg^{-1} protein)			
	Maize flowering		Sorghum flowering	
	pre	post	pre	post
0	5.7(116)	3.2(39)	4.2(77)	1.8(105)
5	4.4(205)	8.6(99)	4.1(142)	6.3(159)
10	4.8(195)	6.8(169)	4.7(121)	5.2(222)
15	5.4(201)	8.8(104)	4.1(234)	4.0(397)
20	4.2(298)	11.2(105)	4.6(229)	4.5(391)
mean	4.9(203)	7.7(103)	4.3(161)	4.4(255)
rehydration Treatment period (days)				
0	4.2(298)	11.2(105)	4.6(229)	4.5(391)
5	7.9(69)	12.4(107)	7.1(79)	7.2(134)
10	6.6(111)	11.8(81)	6.4(101)	6.1(156)
15	7.8(70)	-	5.8(79)	-
20	10.4(85)	-	9.6(55)	-
mean	7.4(110)	11.8(91)	6.7(97)	6.0(208)

In maize plants undergoing pre-flowering dehydration, as the intensity of dehydration became more severe, ascorbate peroxidase activity increased continually and reached maximum at relative water contents of 48% (298% of control). Whereas in those plants undergoing post-flowering dehydration, ascorbate peroxidase activity consistently increased until relative water contents reached 55% (169% of control) and then activity did not follow consistent trend with further decline in relative water contents. In contrast to maize, patterns of changes in ascorbate peroxidase activity of sorghum with changes in relative water contents followed similar trends during both pre and post-flowering dehydration, with the extent of increase being most marked during post than pre-flowering dehydration. During both pre and post-flowering dehydration the increase in ascorbate peroxidase activities reached 229% and 391% of the control at the end of the dehydration cycle, respectively.

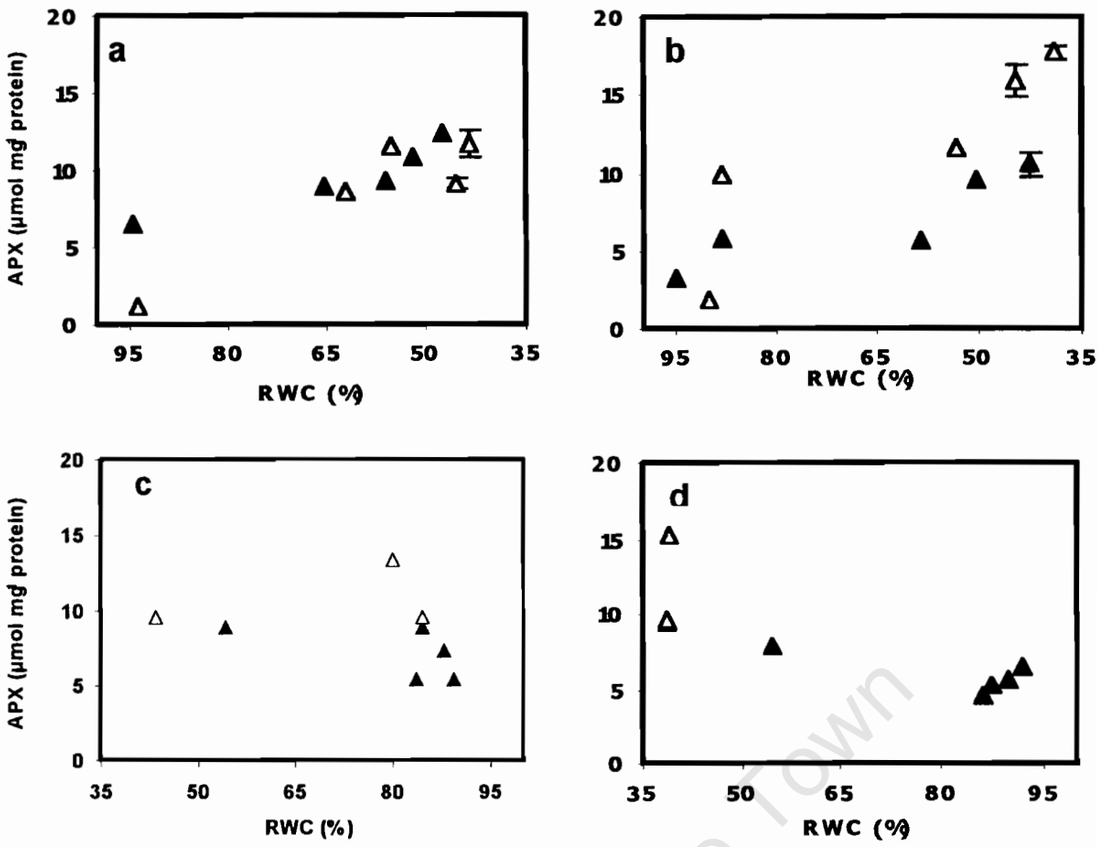

Figure 3.4 Ascorbate peroxidase activities (µmol mg^{-1} protein) in maize (a, c) and sorghum (b, d) during pre and post-flowering dehydration (a, b) and rehydration (c, d) as related to RWC (%), respectively. Vertical bars denote standard errors of means (n=3). ▲ and Δ symbols indicate pre and post flowering dehydrated/rehydrated treatments, respectively.

In maize plants that have been undergoing pre-flowering dehydration, ascorbate peroxidase activities fully recovered to the control level following rehydration whereas in sorghum ascorbate peroxidase activities after an initial recovery were slightly induced as rehydration progressed (Fig. 3. 4d). Approximately between relative water contents of 48% and 80% an induction of ascorbate peroxidase activities were observed in maize plants undergoing post-flowering rehydration after which there was a marked decline with further increase in relative water contents which still represented 80% of the control plants (Fig. 3.4c; Table 3.4). Sorghum

plants on the other hand achieved only 50% recovery in ascorbate peroxidase activities following post-flowering rehydration (Fig 3. 4d).

3.3.2 Non-enzymatic antioxidant contents

Under control conditions, overall mean ascorbate contents differed between species and was markedly higher in maize than in sorghum during both pre and post-flowering "dehydration" treatment period (Table 3.5).

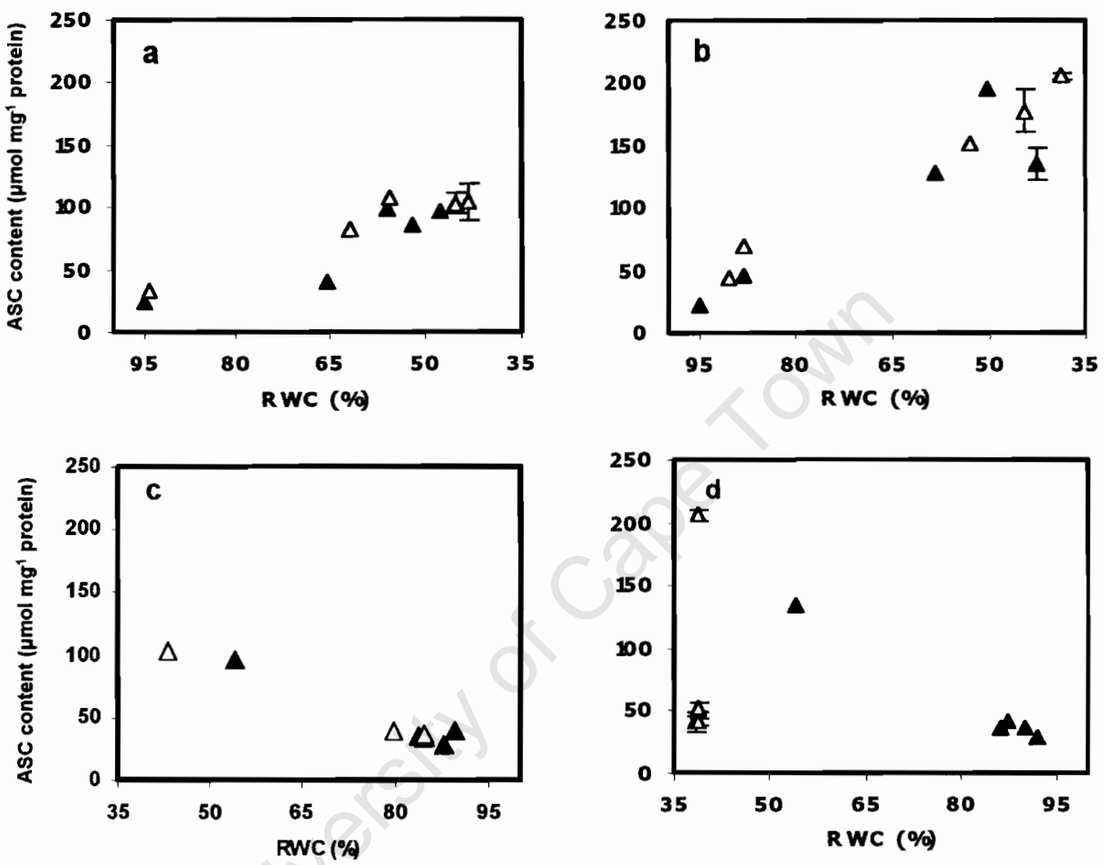

Figure 3.5 Ascorbate contents (µmol mg^{-1} protein) in maize (a, c) and sorghum (b, d) during pre and post-flowering dehydration (a, b) and rehydration (c, d) as related to RWC (%), respectively. Data represents mean values of samples of 3 replications. Vertical bars denote standard errors of means (n=3). ▲ and Δ data points indicate pre and post flowering dehydrated /rehydrated treatments, respectively.

During the course of "dehydration" treatment period, post-flowering control maize plants always had higher ascorbate contents than pre-flowering plants. However, in sorghum the differences between pre and post-flowering control plants occurred only at the beginning and at 15 and 20 days after treatment began. During late phase of (15 to 20 days) pre-flowering stages and throughout the duration of post-flowering treatment period, ascorbate contents of maize control plants were markedly higher than sorghum. In maize, ascorbate content continued to increase until the final stage of treatment during both pre and post-flowering developmental stages. Whereas, ascorbate contents in sorghum increased until the middle phase (10 days) of pre-flowering "dehydration" treatment period, after which there was a decrease. During post-flowering "dehydration" treatment period, sorghum plants had highest values at the later stage, 20 days after treatment was initiated. Overall mean ascorbate contents of control plants were also higher in maize than in sorghum during "rehydration" treatment period in both developmental stages.

Table 3.5 Mean ascorbate (µmol g^{-1} DW) contents of control maize and sorghum leaves at regular intervals (days) during pre and post-flowering dehydration and rehydration treatment periods, respectively. Numbers in parenthesis are ASC contents of dehydrated plants as % of control. Numbers followed by the same alphabets are not different.

Dehydration Treatment period (days)	Ascorbate (µmol g^{-1} DW)			
	Maize flowering		Sorghum flowering	
	pre	post	pre	post
0	23.0(100)	38.9(83)	22.2(99)	39.7(112)
5	25.3(158)	40.6(204)	28.4(158)	31.1(225)
10	35.3(278)	50.4(213)	39.2(327)	38.3(395)
15	39.5(218)	89.3(115)	33.5(585)	37.5(474)
20	47.2(203)	80.2(130)	26.2(512)	42.2(488)
mean	34.1(191)	59.9(149)	29.9(336)	37.7(339)
Rehydration Treatment period (days)				
0	47.2(203)	80.2(130)	26.2(512)	42.2(488)
5	42.9(92)	94.6(42)	42.8(84)	33.9(155)
10	35.7(80)	54.9(68)	30.8(95)	32.8(124)
15	40.2(89)	-	41.1(86)	-
20	35.1(97)	-	52.3(81)	-
mean	40.2(112)	67.9(80)	38.7(172)	34.9(256)

The changes in ascorbate content of maize and sorghum as related to changes in relative water contents during pre and post-flowering dehydration is given in Figure 3.5. When compared with the control ascorbate content increased in both species during pre and post-flowering dehydration, but the extent and patterns of increase as related to changes in relative water contents differed between species. Sorghum had consistently higher ascorbate contents than maize during both pre and post-flowering dehydration. In maize, there was a slight and more gradual increase and maximal ascorbate contents of 98μmol g^{-1} DW and 107 μmol g^{-1} DW was attained at relative water contents of 56% during pre and post-flowering dehydration, respectively (Fig. 3. 5a). When expressed as % of control this represented 278% and 213%, respectively (Table 3.5). In sorghum, ascorbate contents increased most markedly to a maximum value of 196μmol g^{-1} DW (585% of control) and 206μmol g^{-1} DW (488% of control) at relative water contents of 51% and 39% during pre and post-flowering dehydration, respectively (Fig. 3.5b and Table 3.5). However, in pre-flowering dehydrated sorghum leaves, a peak in ascorbate contents were followed by a marked decrease from its maximum value of 196 μmol g^{-1} DW to 134μmol g^{-1} DW during the last stage of dehydration, which still represented a 512% of the control.

With the exception of post-flowering rehydrated sorghum plants, ascorbate contents of both species returned to the control level immediately up on rehydration during both developmental stages (Fig. 3.5c and d; Table 3.5). Both species appear to have similar ascorbate contents at the end of rehydration period during pre and post-flowering stages.

The changes in α-tocopherol content with time in control maize and sorghum leaves during pre and post-flowering treatment period is presented in Table 3.6. In control plants, both maize and sorghum exhibited variable changes during pre and post-flowering "dehydration" treatment period over the duration of the experiment. During middle stage of pre-flowering "dehydration" treatment period, α-tocopherol content in maize slightly decreased but showed a much higher increase during later stage. During post-flowering "dehydration" treatment period, however, after a marked increase during the middle phase, α-tocopherol showed a marked decline with time. On the other hand, α-tocopherol in control sorghum leaves consistently increased with time during both developmental stages, the increase being much higher during post

than pre-flowering stage. During "rehydration" treatment period, maize did not show a consistent trend in α-tocopherol content during pre-flowering stage as opposed to post-flowering stage, during which time there was a consistent increase. Sorghum, on the other hand, only decreased its α-tocopherol contents at the final phase of treatment period during both pre and post-flowering stages.

The influence of dehydration on α-tocopherol contents in maize and sorghum during pre and post-flowering stages is given in Fig. 3.6; Table 3.6. Dehydration generally induced an increase in α-tocopherol contents of both species as compared with the control. Variation was observed in α-tocopherol contents between species in response to dehydration. It was observed that, the sequence of changes in α-tocopherol contents of maize and sorghum differed during the two developmental stages over the duration of the experiment.

Table 3.6 Mean α-tocopherol (μg g^{-1} DW) contents of control maize and sorghum leaves at regular intervals (days) during pre and post-flowering dehydration and rehydration treatment periods, respectively. Numbers in parenthesis are TOC contents of dehydrated plants as % of control. Numbers followed by the same alphabets are not different.

Dehydration Treatment period (days)	α-tocopherol (μg g^{-1} DW)			
	Maize flowering		Sorghum flowering	
	pre	post	pre	post
0	38.4(103)	25.2(130)	18.4(154)	47.2(110)
10	19.9(605)	100.9(89)	17.4(634)	88.8(122)
20	188.3(108)	69.2(150)	33.6(407)	150.9(79)
mean	63.4(272)	65.1(123)	23.1(398)	95.6(104)
Rehydration Treatment period (days)				
0	188.3(108)	69.2(150)	33.6(407)	150.9(79)
10	91.8(80)	82.9(63)	39.8(52)	206.4(52)
20	129.6(70)	118.6(63)	31.8(122)	169.9(70)
mean	136.6(86)	90.2(92)	35.1(194)	175.7(67)

Accordingly, maize exhibited most marked increase in α-tocopherol contents than in sorghum during pre-flowering dehydration. During post-flowering dehydration, both species followed similar trends with changes in relative water contents, with slightly higher α-tocopherol contents in sorghum than in maize. When expressed as % of

control, in maize increases in α-tocopherol ranged between 103% and 605% and 89% and 150% during pre and post-flowering dehydration, respectively. Sorghum on the other hand exhibited an increase ranging between 154% and 634% during pre-flowering and 79% and 122% during post-flowering dehydration.

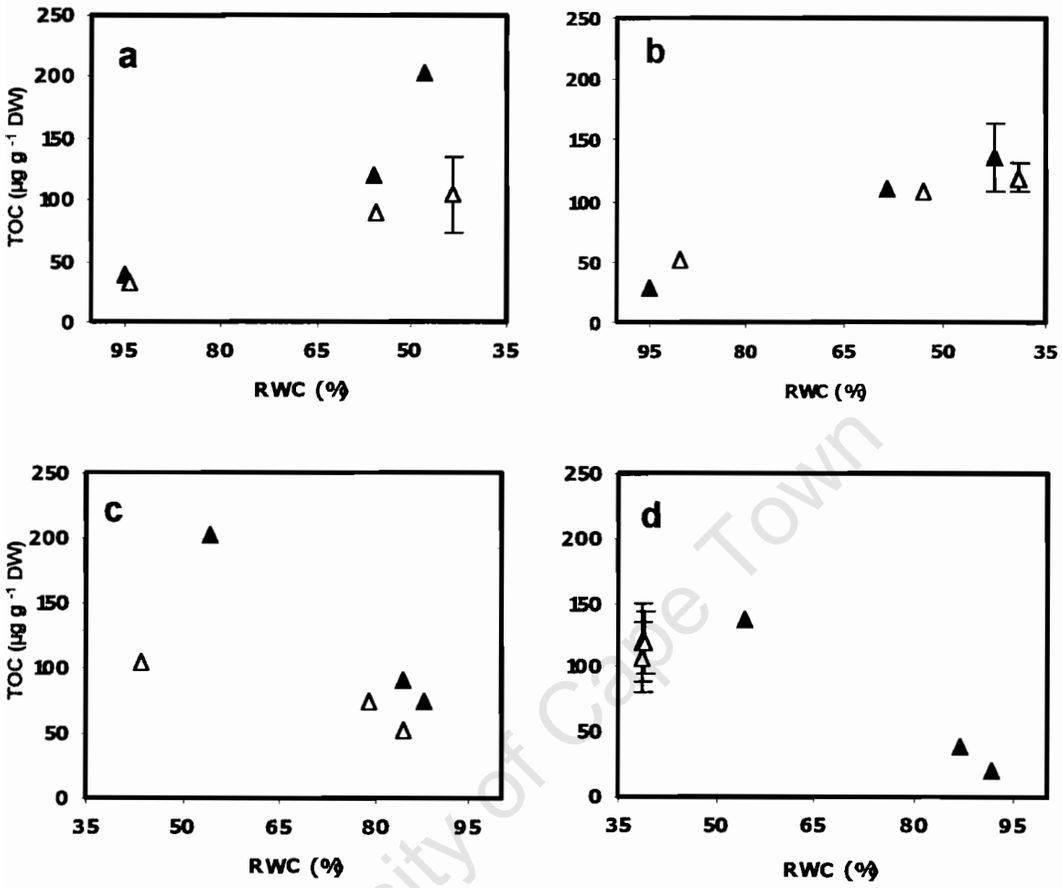

Figure 3.6 Alpha-tocopherol contents (µg g^{-1} DW) in maize (a, c) and sorghum (b, d) during pre and post-flowering dehydration (a, b) and rehydration (c, d) as related to RWC (%), respectively. Data represents mean values of samples of 3 replications. Vertical bars denote standard errors of means (n=3). ▲ and Δ data points indicate pre and post flowering dehydrated /rehydrated treatments, respectively.

Table 3.7 Mean reduced glutathione (ng g^{-1} DW) contents of control maize and sorghum leaves at regular interval (days) during pre and post-flowering dehydration and rehydration treatment periods, respectively. Numbers in parenthesis are GSH contents of dehydrated plants as % of control. Numbers followed by the same alphabets are not different.

Dehydration Treatment period (days)	Reduced Glutathione (ng g^{-1} DW)			
	Maize flowering		Sorghum flowering	
	pre	post	pre	post
0	312.9(104)	227.6(97)	390.0(77)	230.8(106)
5	352.9(130)	248.6(162)	331.1(113)	208.4(164)
10	325.4(120)	202.8(195)	235.3(215)	239.3(205)
15	198.6(222)	221.0(179)	287.4(199)	270.0(195)
20	217.5(216)	213.7(151)	335.8(196)	286.1(195)
mean	281.5(158)	222.7(157)	315.9(160)	246.9(173)
Rehydration Treatment period (days)				
0	217.5(216)	213.7(151)	335.8(196)	286.1(195)
5	334.1(146)	295.5(87)	351.4(129)	275.9(178)
10	282.3(153)	358.2(62)	238.4(125)	319.2(99)
15	292.2(95)	352.8(44)	302.4(129)	324.8(68)
20	426.3(60)	-	353.2(79)	-
mean	310.5(134)	314.6(86)	316.3(132)	306.2(135)

With the exception of post-flowering dehydrated sorghum, α-tocopherol contents fully recovered in both pre and post-flowering dehydrated maize and pre-flowering dehydrated sorghum plants (Fig. 3.6c and d). Sorghum leaves undergoing post-flowering dehydrated remained elevated until the end of post-flowering rehydration.

Data for reduced glutathione (GSH) and oxidized glutathione (GSSG) contents of control maize and sorghum during pre and post-flowering "dehydration" and "rehydration" treatment periods is presented in Table 3.7 and 3.8. Under control conditions, most glutathione in both species was in the reduced form (GSH). When expressed as percentage of total glutathione (GSH+GSSG), reduce glutathione contents in control maize ranged between 93% to 80% and 92% to 85% during pre and post-flowering treatment period, respectively. In sorghum, it ranged between 93% to 87% during pre-flowering and 90% to 85% during post-flowering treatment period.

The influence of dehydration on the reduce glutathione, oxidized glutathione contents and the ratio GSH:GSSG of maize and sorghum during pre and post-flowering stages are shown in figure 3.7, Figure. 3.8 and Figure 3.9, respectively. Data expressed as % of control is also shown in Table 3.7-Table 3.9. In both species, dehydration during pre and post-flowering stages increased reduced glutathione and oxidized glutathione contents as compared to the control (Table 3.7-Table 3.8). The ratio GSH:GSSG was also influenced in both species by both pre and post-flowering dehydration (Fig. 3.9), but the sequence of changes showed variable changes with changes in relative water contents.

Table 3.8 Mean oxidized glutathione (ng g^{-1} DW) contents of control maize and sorghum leaves at regular interval (days) during pre and post-flowering dehydration and rehydration treatment periods, respectively. Numbers in parenthesis are GSSG contents of dehydrated plants as % of control. Numbers followed by the same alphabets are not different.

Dehydration Treatment period (days)	Oxidized Glutathione (ng g^{-1} DW)			
	Maize flowering		Sorghum flowering	
	pre	post	pre	post
0	26.1(146)	23.1(85)	37.3(83)	23.0(105)
5	25.9(119)	26.2(100)	30.8(142)	20.7(110)
10	23.6(212)	25.7(178)	33.1(138)	23.1(156)
15	26.9(175)	28.5(113)	34.2(179)	23.8(176)
20	40.6(137)	23.9(83)	36.3(174)	21.7(195)
mean	28.6(158)	25.6(112)	34.3(143)	22.5(148)
Rehydration Treatment period (days)				
0	40.6(137)	23.9(83)	36.3(174)	21.7(195)
5	45.4(121)	27.9(54)	47.2(133)	27.5(104)
10	40.4(103)	29.7(60)	39.4(140)	27.9(41)
15	29.6(127)	24.9(37)	43.4(131)	23.2(31)
20	34.2(95)	-	37.8(119)	-
mean	38.0(117)	26.3(59)	40.8(139)	24.7(93)

Approximately between relative water contents of 95% and 64%, the GSH:GSSG in pre-flowering dehydrated maize showed a remarkable increase, suggesting that oxidized glutathione was reduced to reduced glutathione there by increasing the ratio

GSH:GSSG. But with further loss in relative water contents the GSH:GSSG decreased markedly until relative water contents reached 47% at the end of the dehydration cycle (Fig 3.9a) indicating a stronger increase in oxidized glutathione than reduced glutathione. During post-flowering dehydration, the GSH:GSSG did not show a consistent trend in a way that between relative water contents of 95% and 65% the GSH:GSSG increased which was followed by a marked decline.

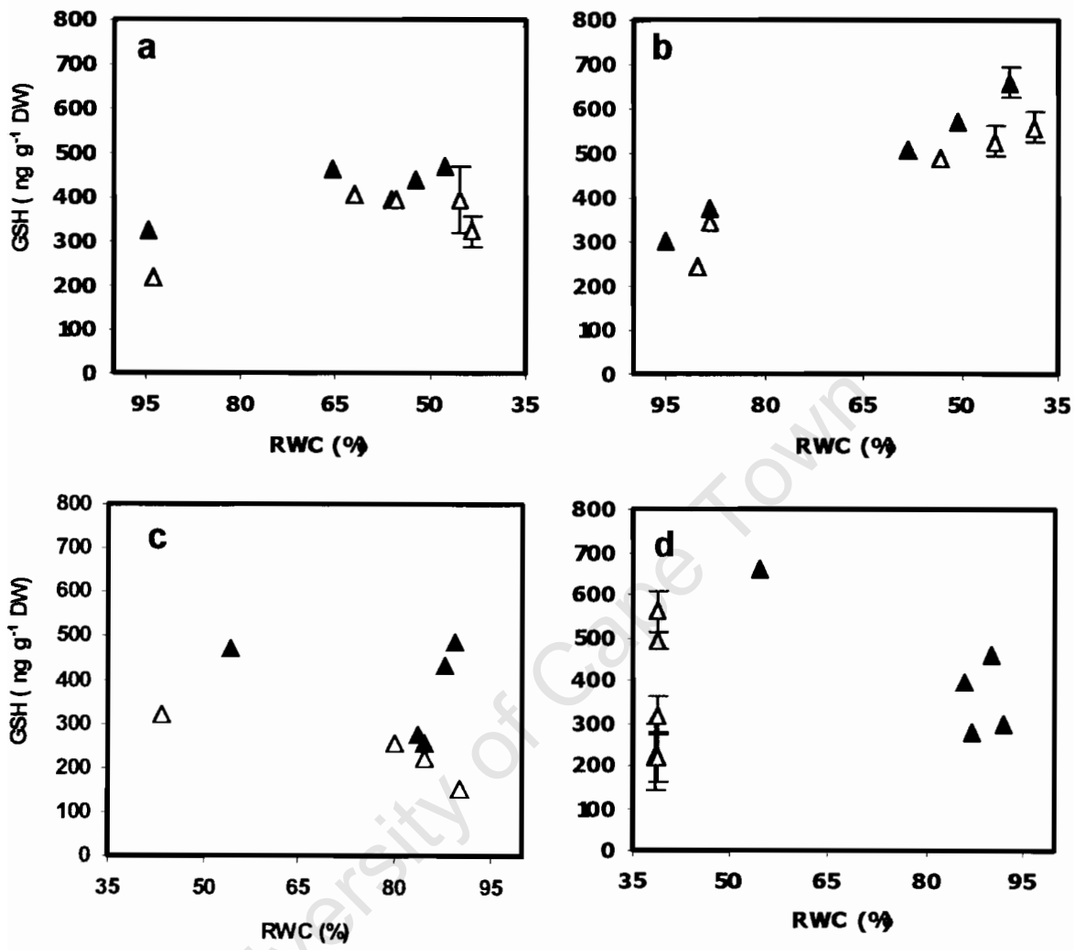

Figure 3.7 Reduced glutathione contents (ng g^{-1} DW) in maize (a, c) and sorghum (b, d) during pre and post-flowering dehydration (a, b) and rehydration (c, d) as related to RWC (%), respectively. Data represents mean values of samples of 3 replications. Vertical bars denote standard errors of means (n=3). ▲ and Δ data points indicate pre and post flowering dehydrated /rehydrated treatments, respectively.

Approximately between 50% and 43% relative water contents, however, the GSH:GSSG increased most markedly until the end of the dehydration cycle. Pre-flowering dehydration did not change the GSH:GSSG in sorghum, whereas during post-flowering dehydration, the GSH:GSSG increased within 5 days (between 90% and 88% relative water contents) after treatment began, but as the relative water contents decreased the GSH:GSSG showed a consistent and gradual decline until RWCs reach 39% at the end of the dehydration period (Fig 3.9b) suggesting oxidation of reduced glutathione to oxidized glutathione.

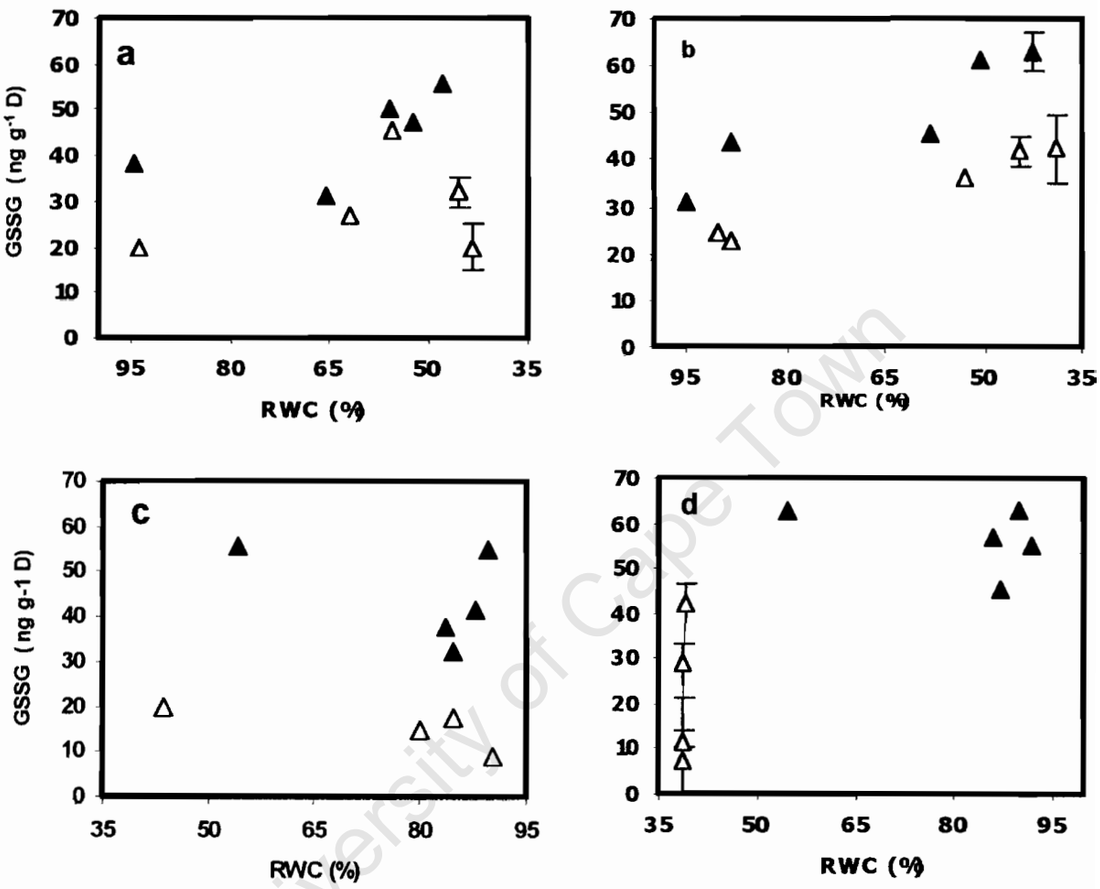

Figure 3.8 Oxidized glutathione contents (ng g^{-1} DW) in maize (a, c) and sorghum (b, d) during pre and post-flowering dehydration (a, b) and rehydration (c, d) as related to RWC (%), respectively. Data represents mean values of samples of 3 replications. Vertical bars denote standard errors of means (n=3). ▲ and Δ data points indicate pre and post flowering dehydrated /rehydrated treatments, respectively.

Differences in the GSH:GSSG between pre and post-flowering dehydration in maize occurred between relative water contents of 53% and 43% when the GSH:GSSG in post-flowering dehydrated plants markedly increased.Whereas in sorghum post-flowering dehydrated plants had markedly higher GSH:GSSG than pre-flowering dehydrated plants through out the duration of the experiment.

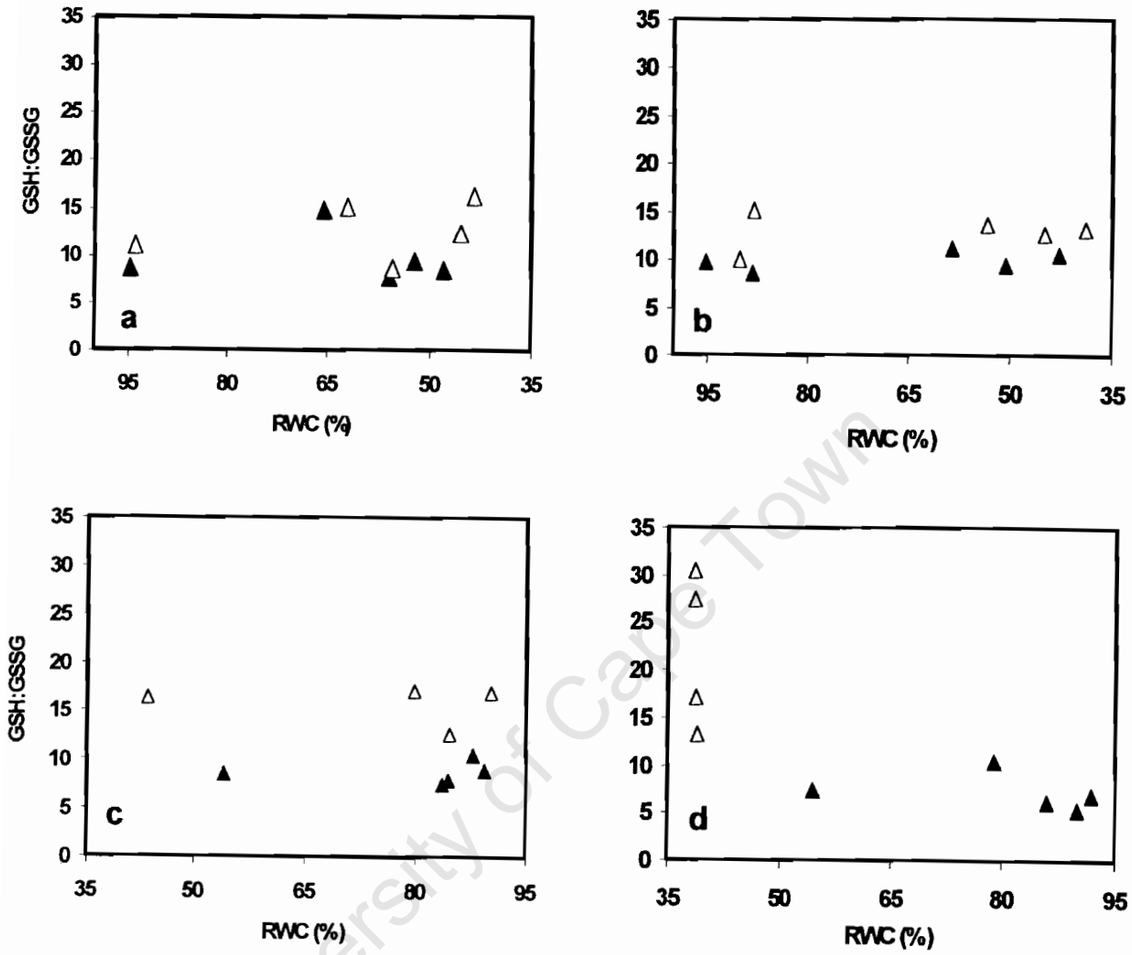

Figure 3.9 GSH:GSSG in maize (a, c) and sorghum (b, d) during pre and post-flowering dehydration (a, b) and rehydration (c, d) as related to RWC (%), respectively. Data represents mean values of samples of 3 replications. Vertical bars denote standard errors of means (n=3). ▲ and Δ data points indicate pre and post flowering dehydrated /rehydrated treatments, respectively.

When maize plants that had been dehydrated during pre-flowering stage were rehydrated, the GSH:GSSG did not show a noticeable change until relative water contents reached to 83%, but as relative water contents further increased to approximately 90%, the GSH:GSSG exhibited a marked increase (Fig 3.9c). Similar to pre-flowering rehydration, approximately between relative water contents of 43% and 80% there was no change in the GSH:GSSG during post-flowering rehydration, but between relative water contents of 80% and 85% the GSH:GSSG markedly declined and then was followed with a remarkable increase at 90% relative water contents. There was no change in the GSH:GSSG in sorghum plants rehydrated following pre-flowering dehydration (Fig 3.9d). By contrast, when sorghum plants that had been dehydrated during post-flowering stage were rehydrated, the GSH:GSSG was most markedly elevated.

Table 3.9 Mean anthocyanin (mg ml^{-1} g^{-1} DW) contents of control maize and sorghum leaves at regular interval (days) during pre and post-flowering "dehydration" and "rehydration" treatment periods, respectively. Numbers in parenthesis are anthocyanin contents of dehydrated plants as % of control. Numbers followed by the same alphabets are not different.

Dehydration Treatment period (days)	Anthocyanin (mg $ml^{-1}g^{-1}$ DW)			
	Maize flowering		Sorghum flowering	
	pre	post	pre	post
0	1.0(189)	0.6(70)	1.2(101)	0.3(173)
5	0.8(269)	0.9(227)	0.5(319)	1.1(203)
10	0.6(86)	1.5(109)	0.6(193)	1.0(171)
15	0.6(159)	0.7(221)	0.5(233)	0.9(214)
20	1.7(41)	0.8(153)	0.9(51)	1.0(187)
mean	0.9(149)	0.9(156)	0.7(179)	0.9(190)
Rehydration Treatment period (days)				
0	1.7(41)	0.8(153)	0.9(51)	1.0(187)
5	0.0(433)	1.5(88)	0.2(325)	1.5(142)
10	0.3(169)	1.9(42)	0.1(500)	2.0(60)
15	1.1(76)	-	0.3(172)	-
20	1.0(142)	-	1.0(113)	-
mean	0.8(172)	1.5(61)	0.5(232)	1.6(130)

Anthocyanin (mg ml^{-1} g^{-1} DW) contents of maize and sorghum under well watered condition during pre and post-flowering "dehydration" and "rehydration" treatment periods is presented in Table 3.9. Generally, both species had similar overall mean anthocyanin contents during pre and post-flowering "dehydration" and "rehydration" treatment period.

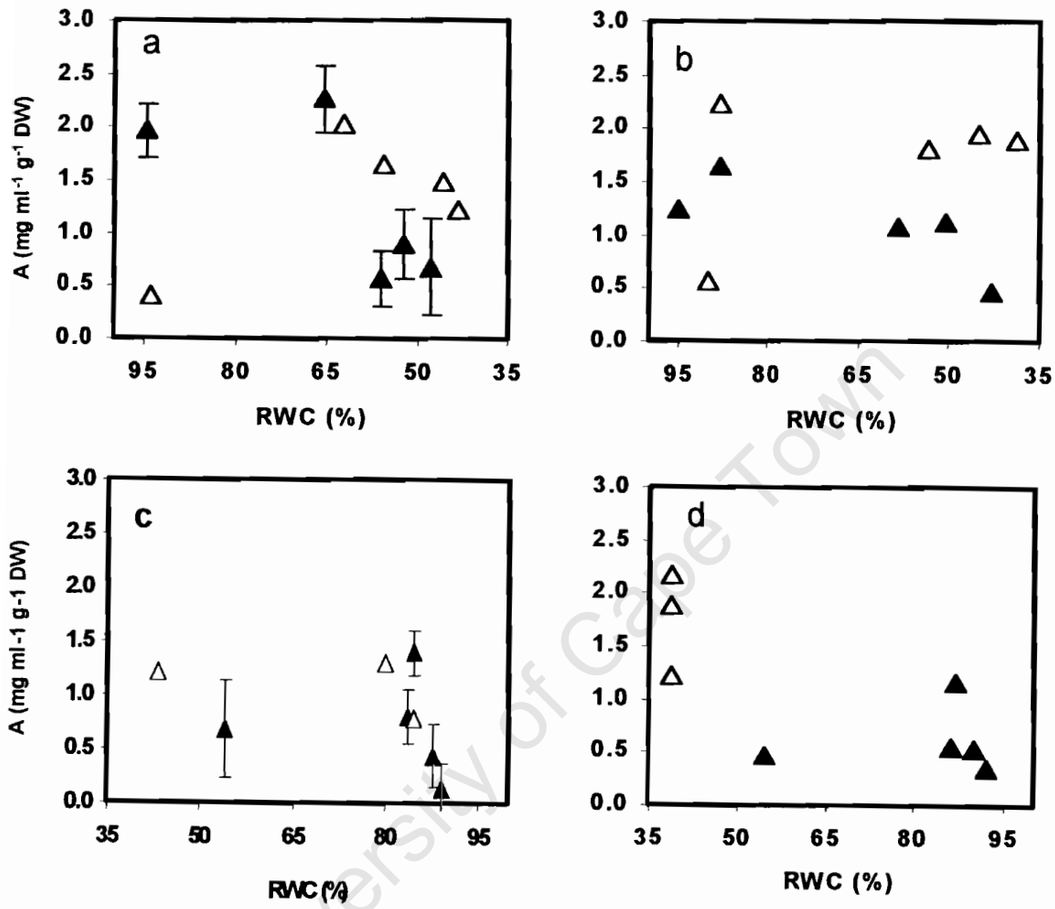

Figure 3.10 Anthocyanin contents (mg g^{-1} DW ml^{-1}) in maize (a, c) and sorghum (b, d) during pre and post-flowering dehydration (a, b) and rehydration (c, d) as related to RWC (%), respectively. Data represents mean values of samples of 3 replications. Vertical bars denote standard errors of means (n=3). ▲ and Δ data points indicate pre and post flowering dehydrated /rehydrated treatments, respectively.

Both species exhibited a decrease in anthocyanin contents during pre-flowering treatment period with time until 15 days after treatment began and at 20 days, anthocyanin contents in maize increased markedly. During post-flowering treatment period, maize exhibited a gradual increase until the middle phase of treatment period and then it declined again to values recorded at the beginning of the experiment. There was no change in anthocyanin contents of sorghum during post-flowering treatment period. During rehydration, mean anthocyanin contents in both species were higher during post than per-flowering treatment.

The influence of dehydration on the anthocyanin contents of maize and sorghum during pre and post-flowering dehydration is given in Figure 3.10 and Table 3.9. Anthocyanin contents increased during pre and post-flowering dehydration in both species as compared with the control (Table 3.9). Differences were observed in the patterns of changes in anthocyanin contents between species with changes in relative water contents during both developmental stages. In pre-flowering dehydrated maize, there was no change in anthocyanin content approximately between relative water contents of 95% and 65%, and as relative water contents decreased further it decreased remarkably. When expressed as % of control anthocyanin content ranged between 269% and 41% of the control (Table 3.9). Sorghum on the other hand, initially exhibited a marked increase (319% of control), after which there was a more gradual decrease until relative water contents reached 43% and represented only 51% of control at the end of the dehydration period. During post-flowering dehydration, anthocyanin content in maize increased most markedly and reached maximal at 63% relative water contents (227% of control) followed by a decrease between relative water contents of 63% and 43%, which still was 153% of the control. In sorghum, within a very short intervals in relative water contents (90% and 88%), anthocyanin content showed an exponential increase (203% of control), and between relative water contents of 88% and 53% a more gradual decrease after which it remained constant without a noticeable changes with further loss in relative water contents. In both maize and sorghum, increases in anthocyanin contents relative to the control were greater during post than pre-flowering dehydration (Table 3.9).

In both species, anthocyanin contents returned to the control level upon pre-flowering rehydration (Fig. 3.10c and d). During post-flowering rehydration, however,

approximately 70% of anthocyanin content in maize and 50% in sorghum recovered at the end of rehydration.

3.3.3 Lipid peroxidation

Table 3.10 presents mean malondialdehyde contents of control maize and sorghum leaves during pre and post-flowering treatment period. Mean malondialdhyde content was much higher in maize than in sorghum under control conditions. The data indicated that malondialdehyde content of control maize leaves during pre and post-flowering stages were consistently increasing until the end of the treatment. Except during post-flowering stage where there was an increase at the last stage of treatment period, control sorghum plants showed a consistent decrease in malondialdehyde content during both developmental stages (Table 3.10).

Table 3.10 Mean malondialdehyde (μmol g^{-1} DW) contents of control maize and sorghum leaves at regular interval (days) during pre and post-flowering "dehydration" and "rehydration" treatment periods, respectively. Numbers in parenthesis are MDA contents of dehydrated plants as % of control. Numbers followed by the same alphabets are not different.

Dehydration Treatment period (days)	MDA (μmol g^{-1} DW)			
	Maize flowering		Sorghum flowering	
	pre	post	pre	post
0	2.2(118)	2.0(111)	0.8(91)	1.6(96)
5	1.7(149)	1.8(163)	1.1(95)	1.6(179)
10	2.2(205)	2.3(152)	0.6(460)	0.8(296)
15	2.8(289)	3.8(151)	0.8(338)	1.0(284)
20	2.7(188)	4.2(153)	0.4(713)	1.8(167)
mean	2.3(190)	2.8(146)	0.7(339)	1.4(204)
Rehydration Treatment period (days)				
0	2.7(188)	4.2(153)	0.5(713)	1.8(167)
5	2.9(104)	4.7(91)	1.1(84)	1.8(122)
10	3.5(105)	3.7(117)	0.6(139)	2.1(126)
15	2.4(111)	-	1.1(107)	-
20	3.1(135)	-	1.1(88)	-
mean	2.9(129)	4.2(120)	0.9(226)	1.9(138)

During "rehydration" treatment period, mean malondialdehyde concentration of control maize and sorghum further increased much higher than during "dehydration" treatment period.

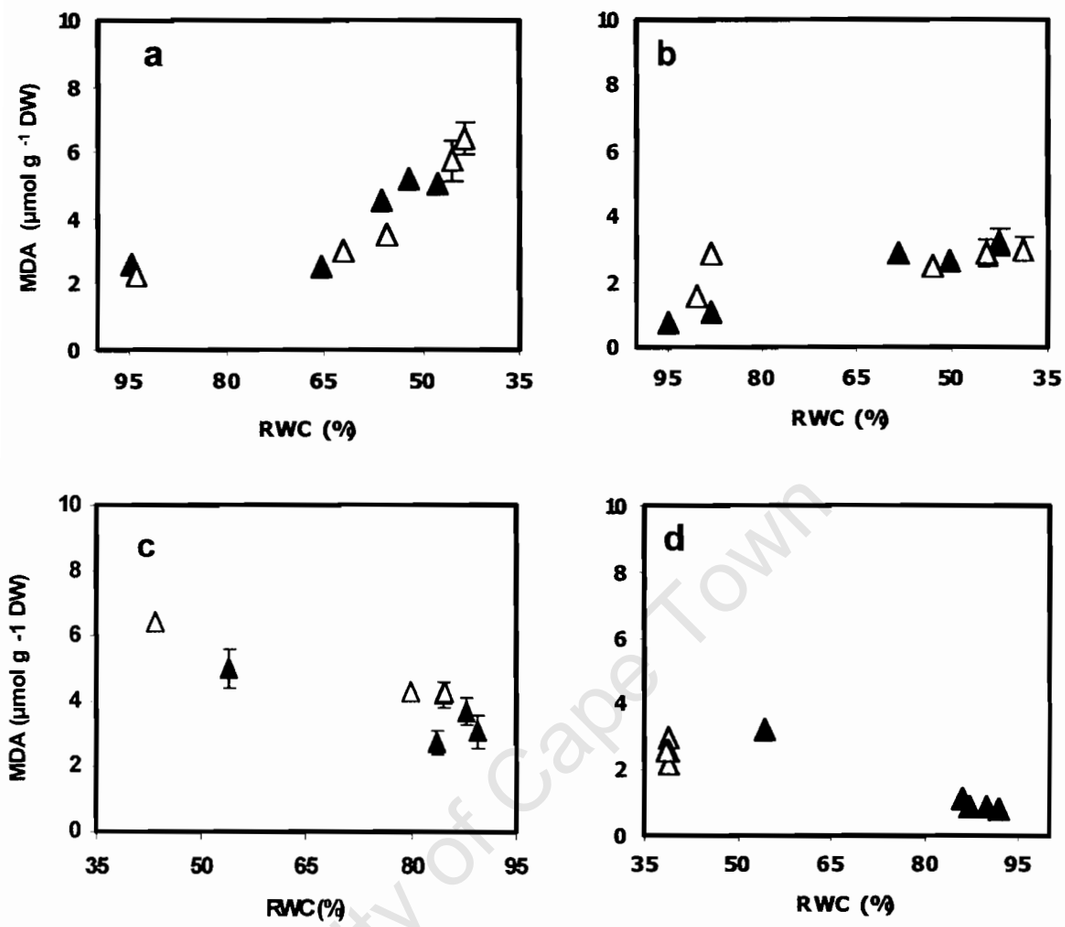

Figure 3.11 Malondialdehyde contents (µmol g^{-1} DW) in maize (a, c) and sorghum (b, d) during pre and post-flowering dehydration (a, b) and rehydration (c, d) as related to RWC (%), respectively. Data represents mean values of samples of 3 replications. Vertical bars denote standard errors of means (n=3). ▲ and Δ data points indicate pre and post flowering dehydrated /rehydrated treatments, respectively.

Dehydration during pre and post-flowering stages led to a remarkable increase in the level of malondialdehyde content in maize and sorghum (Fig. 3.11), the increase was most marked higher in maize than in sorghum indicating the prevalence of reactive oxygen species in maize than in sorghum tissues. There were differences between species in the response of malondialdehyde content to dehydration with plant age. In maize leaves dehydrated during pre-flowering stage, malondialdehyde contents were relatively constant until relative water contents reached approximately 65% and thereafter, malondialdehyde contents increased remarkably from 2.51μmol g^{-1} DW to a maximum value of 5.17μmol g^{-1} DW. During post-flowering dehydration, maize leaves exhibited a continual increase in malondialdehyde concentrations with further loss in relative water contents and peaked 6.42μmol g^{-1} DW at 43% relative water contents. When expressed as % of control dehydration induced increase in malondialdehyde contents in maize were between 118% and 289% during pre-flowering stage and between 111% and 163% during post-flowering stage. In sorghum, malondialdehyde contents also increased continually from the initial values of 0.71μmol g^{-1} DW to 3.21μmol g^{-1} DW during pre-flowering dehydration which represents 91% and 713% of the control, respectively. During post-flowering dehydration, after the initial increases from 1.57μmol g^{-1} DW to 2.89μmol g^{-1} DW between relative water contents of approximately 92% and 88%, malondialdehyde contents stabilized until the final stage of dehydration. During this stage the increase in malondialdehyde content ranged between 96% and 296% of the control. In both species dehydration induced increase in malondialdehyde contents relative to the control was higher during pre-flowering than post-flowering stage (Table 3.10).

In maize leaves undergoing pre-flowering rehydration, there was approximately 65% less malondialdehyde contents as compared with the control leaves, while in sorghum malondialdehyde contents were to the control level following pre-flowering rehydration, suggesting that the tissues damaged by reactive oxygen species were repaired (Fig. 3.11c and d). During post-flowering rehydration, there was approximately 85% and 75% less malondialdehyde contents in maize and sorghum leaves as compared with the control level upon rehydration.

3.3.4 Discussion

Drought stress may lead to stomatal closure, which reduces CO_2 availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn could increase the generation of reactive oxygen species and induce oxidative stress (Acar et al, 2001). The increased production of activated oxygen species in chloroplasts of plants under drought stress has been described (Smirnoff, 1993; Asada, 1999). This reactive oxygen species are potentially damaging and often results in an increase in lipid peroxidation measured here as malondialdehyde. To minimize the damaging effects of reactive oxygen species, plants have evolved various enzymatic and non-enzymatic defensive systems that can reduce oxidative stress by scavenging reactive oxygen species (Zhang and Kirkham, 1996). The metabolism of reactive oxygen species is, therefore, dependent on various functionally interrelated antioxidant defence systems. An enhancement of antioxidant defence mechanisms is considered to be an adaptive mechanism of plants to drought stress. The available studies suggest a correlation between stress tolerance and antioxidant defence capacity (Pastori and Trippi, 1993; Jagtap and Bhrgava, 1995; Loggini et al, 1999; Sgherri et al, 2000; Acar et al, 2001).

3.3.4.1 Antioxidant responses in maize and sorghum during pre and post-flowering dehydration.

In the present study, the major adaptive changes in terms of the level of enzymatic (superoxide dismutase, glutathione reductase, catalas and ascorbate peroxidase) and non-enzymatic antioxidants (ascorbate, alpha tocopherol, reduced glutathione and oxidized glutathione and GSH:GSSG), anthocyanin and the effect of dehydration on lipid peroxidation were examined in maize and sorghum after exposure to and recovery from pre and post-flowering dehydration. The results suggested that the diverse responses of enzymatic antioxidants (superoxide dismutase, glutathione reductase, catalase and ascorbate peroxidase) and non-enzymatic antioxidants (ascorbate, alpha tocopherol, reduce and oxidized glutathione) to dehydration during pre and post-flowering stages may be an influential component of environmental stresses in maize and sorghum.

Enhanced formation of reactive oxygen species under stress conditions induces both protective responses and cellular damage. The scavenging of $O_2^{\cdot-}$ is achieved through

an upstream enxyme, superoxide dismutase, which catalyses the dismutation of superoxide to H_2O_2. (Bowler et al, 1992; Blokhina et al, 2003; Menezes-Benavente, (2004). The enhancement of superoxide dismutase activities suggests its involvement in the detoxification process of $O_2^{\cdot-}$ in both maize and sorghum in response to pre and post-flowering dehydration (Fig. 3. 1). It is proposed that superoxide dismutase activity (both constitutive and induced) of dehydration tolerant species/varieties would be substantially higher as compared to the sensitive ones (Zhang and Kirkham, 1996; Pastori and Trippi, 1992). The fact that higher dehydration-induced superoxide dismutase activity in sorghum than in maize may indicate a major role of this enzyme in sorghum than in maize during both developmental stages (Fig. 3.1). Luna et al, 1985 have shown that maize exhibited higher levels of superoxide dismutase as compared to wheat. A correlation between drought resistance and increases in superoxide dismutase in maize varieties has also been reported by Malan, et al, (1990). Sorghum exhibited a much higher activity during post than pre-flowering dehydration, indicating that the contribution of superoxide dismutase activity increased with crop age (Fig. 3.1b). The finding of this study is in agreement with those of Zhang and Kirkham (1996) and Pastori and Trippi (1992) who observed increased superoxide dismutase in sunflower and sorghum, and maize, respectively. Previous reports indicated that superoxide dismutase responds to dehydration in diverse ways in different crop species (Smirnoff, 1993). In pea plants (Moran et al, 1994) there were unchanged superoxide dismutase activities. While there were increases in activity in sorghum (Jagtab and Barghava, 1995) and wheat (Baisak et al, 1994), there were also decreases in sunflower (Quartacci and Navari-Izzo, 1992) and wheat (Zhang and Kirkham, 1994).

Glutathione reductase is key enzyme in the ascorbate-glutathione cycle and is involved in scavenging the products of oxidative stress such as H_2O_2 (Bowler et al, 1992). Glutathione reductase plays a pivotal role in protection against oxidative stress by maintaining the GSH:GSSG ratio, which is required for the regeneration of ascorbate level (Bartoli et al, 1999). The elevated levels of glutathione reductase in both maize and sorghum during both developmental stages (Fig. 3.2) could help minimize oxidative stress by detoxifying reactive oxygen species and maintain high level of GSH:GSSH ratio (Fig. 3.9). Furthermore the observed increase in glutathione reductase may be able to increase the ratio of $NADP^+$/NADPH (data not shown), there

by resulting in the availability of $NADP^+$ to accept electrons from the photosynthetic electron chain (Baisak et al, 1994). Under such conditions, the flow of electrons to O_2 and, therefore, the formation of O_2^- would be minimized. The result of this study is in accordance with those of Zhang and Kirkham (1996) who observed increases in activities of this enzyme in sunflower and sorghum. The observed decrease in glutathione reductase activity in maize during both pre and post-flowering and in sorghum during post-flowering stages as the intensity of dehydration became more severe could probably be due to the fact that this enzyme is efficient against oxidative stress during moderate dehydration, but as the dehydration process became severe, production of reactive oxygen species might have overwhelmed and could impair the H_2O_2 scavenging system of cells and favours accumulation of H_2O_2 (Zhang and Kirkham, 1996). This decrease in glutathione reductase could be owing to an accelerated senescence induced by dehydration in maize during both pre and post-flowering and in sorghum during post-flowering dehydration.

Along with superoxide dismutase, catalase constitutes a front line defence against reactive oxygen species, converting H_2O_2 in to water (Menezes-Benavente and Texeira, 2004). Our results indicated that the activities of catalase increased above the control in both species in response to dehydration during both pre and post-flowering stages (Fig. 3.3a and b). This has also been reported in other crop species exposed to oxidative stresses such as rice (Vaidyanathan et al, 2003); tobacco (Van Rensburg and Kruger, 1994); cotton (Rajguru et al, 1999) and sugar beet (Bor et al, 2003). The high level of catalase activities during pre than post-flowering dehydration may suggest that its contributory role to decompose H_2O_2 is higher during pre than post-flowering dehydration. The decrease in catalase activities in maize during the late phase of pre and post-flowering dehydration (Fig. 3.3a) may be explained by the fact that the activity of catalase may be differentially regulated during this period in maize and sorghum. This result suggested that the protective action of catalase does not appear to be an efficient way of scavenging H_2O_2 under conditions of the present study that is experienced by maize during pre and post-flowering stages. Hence, other protective mechanisms located within the chloroplasts must dispose off the H_2O_2, which might have been formed by elevated levels of superoxide dismutase induced by drought treatment (Acar et al, 2001). Beside higher level of superoxide dismutase, the enhanced activities of catalase observed in sorghum during both pre and post-flowering dehydration than in maize aids in the rapid elimination of H_2O_2 which

could have been produced during the two developmental stages. Confirming the result of the present work, Jagtap and Bharagava (1995) in sorghum and Van Rensburg and Kruger (1994) in tobacco have reported an increase in catalase activities under drought stress conditions.

The enzyme ascorbate peroxidase, located in the cytosol and chloroplasts, breaks down H_2O_2 efficiently using ascorbate as the electron donor (Bor et al, 2003). Wang et al (1999) have shown that over expression of ascorbate peroxidase gene in plants increases protection against oxidative stress. Since increased activity of superoxide dismutase under dehydration during both pre and post-flowering stages was accompanied by increases in ascorbate peroxidase activity in leaves of both species (Fig. 3.4a and b), it may also be suggested that superoxide dismutase and ascorbate peroxidase are working more efficiently in concert to scavenge reactive oxygen species such as $O_2^{\cdot-}$ and H_2O_2 which might possibly be produced during dehydration. Similar results are also reported in other crop species such as wheat (Bartoli et al, 1999; Lascano et al, 2001; Sgherri et al, 2000); tobacco (Van Rensburg and Kruger, 1994); cotton (Meloni et al, 2003) and rice (Vaidyanathan et al, 2003). The pattern of change in ascorbate peroxidase activity as related to changes in relative water contents differed from that of glutathione reductase and catalase in both species during both pre and post-flowering dehydration, indicating the importance of ascorbate peroxidase activity under intense dehydration conditions as reactive oxygen species scavenging system. The role of ascorbate peroxidase appears to be equally important irrespective of crop age in maize whereas a higher activity exhibited during post than pre-flowering dehydration in sorghum (Fig. 3.4b and d), on the other hand, suggested that the contributory role may be greater during the former than the later.

Our results suggest that the critical water contents for stimulation of enzymatic antioxidant activities (fig. 3.1-3.4), and decrease in Fv/Fm (Fig. 2.7) and chlorophyll contents (Fig. 2.12) differed between maize and sorghum during both pre and post-flowering dehydration. The stimulation of enzymatic antioxidants in sorghum at early phase of dehydration (88% relative water contents) is associated with its capacity to scavenge the reactive oxygen species generated during the early phase of dehydration. This correlates with the delay in the decrease of Fv/Fm ratio and chlorophyll contents suggesting that the reactive oxygen species has been efficiently detoxified. However, in maize the critical water contents for the stimulation of enzymatic antioxidant activities occurred during sever dehydration (between 65% and 60% relative water

contents) which coincides with extensive loss of chlorophyll contents and decrease in Fv/Fm ratio indicating high prevalence of oxidative damage. Thus, in dehydrated maize plants extensive losses in chlorophyll concentration led to greater reduction in light absorption, which in turn might reduce the risk of over-excitation of reaction centres and thus photo-oxidative damage to chloroplast and caused damages to the photochemistry (Fv/Fm) during both pre and post-flowering dehydration. This has been evidenced by the bulk oxidative lipid metabolism resulting to larger increases in malondialdehyde contents of maize than sorghum.

These collective results suggested that reactive oxygen species formed in maize and sorghum during pre and post-flowering dehydration appeared to have been detoxified by increased activities of antioxidant enzymes (superoxide dismutase, glutathione reductase, catalase and ascorbate peroxidase) (Fig. 3.1-Fig. 3.4). In sorghum, there seems to be a co-ordinated response of all antioxidant enzymes in eliminating reactive oxygen species, as evidenced from the higher increases during both pre and post-flowering dehydration and lower level of malondialdehyde, as compared to maize (Fig. 3.11). Whereas, in maize although there was an increase in antioxidant enzymes, it appeared that it greatly depends on superoxide dismutase (Fig. 3.1a) and ascorbate peroxidase (Fig. 3.4a), as evidenced by decreases in activities of glutathione reductase (Fig. 3.2a) and catalase (Fig. 3.3a) with increase in intensity of dehydration during pre and post-flowering dehydration.

Increased levels of ascorbate have been reported to be correlated with a high tolerance to oxidative stress caused by adverse environmental conditions (Noctor and Foyer, 1998). In support of this view, upon exposure to pre and post-flowering dehydration, we observed an enhancement in the ascorbate of both species (Fig. 3. 5a and b). This is similar to other works on ascorbate under environmental stresses in other crop species such as wheat (Lascano et al, 2001); sunflower (Caretto et al, 2002) and rice (Vaidyanathan et al, 2003). Under both pre and post-flowering dehydration conditions, sorghum is able to produce a higher level of ASC contents (Fig. 3. 5b), which could be accounted for its higher ascorbate peroxidase activity (Fig. 3.4b). This may be considered an indication that sorghum has a higher capacity to scavenge reactive oxygen species more rapidly. On the other hand, since maize exhibited higher mean constitutive ascorbate contents than sorghum, the relatively low level of ascorbate in the former than in the later may indicate that it can rely on a large amount

of constitutive ascorbate contents to counteract the potentially harmful effect of dehydration (Table 3.5). Ascorbate is a powerful antioxidant and can directly scavenge $O_2^{\cdot-}$, $OH^{\cdot}$ and $^1O_2$ and reduce H_2O_2 to water via ascorbate peroxidase reaction (Noctor and Foyer, 1998; Blokhina et al, 2003). Ascorbate not only quenches reactive oxygen species but also regenerates α-tocopherol from tocopheroxyl radical providing membrane protection (Blokhina et al, 2003). Any of the routes for ascorbate oxidation listed above, could lead to the decrease in ascorbate content in severely pre-flowering dehydrated sorghum (Fig. 3. 5b). The decline in ascorbate contents in sorghum with further loss in relative water contents during the last phase of pre-flowering dehydration may suggest an overall degeneration of its capacity to withstand oxidative stress conditions. Smirnoff (1993) noted that reduction of ascorbate contents under water deficit may be due to the depletion by excess reactive oxygen species or utilization for recycling α-tocopherol. Similar decline in ascorbate contents have been reported under stress conditions in sunflower (Zhang and Kirkham, 1996) and wheat (Bartoli et al, 1999).

An increased concentration of α-tocopherol a lipid soluble antioxidant and concentrated in the chloroplasts, especially the thylakoid membranes, protects lipids and other membrane components by physically quenching and reacting chemically with $^1O_2$ (Munne-Bosch and Alegre, 2002). The same effect was observed in maize and sorghum leaves subjected to dehydration during pre and post-flowering stages (Fig. 3.6a and b). This result is consistent with previous works by Bartoli et al, (1999) who reported increased concentration of α-tocopherol under drought stress conditions. The increase of this antioxidant may be triggered by excess production of reactive oxygen species in the photosynthetic apparatus under dehydration. Lawlor (1995) observed that water deficit caused accumulation of reactive oxygen species in the chloroplasts. This may result in an enhanced level of α-tocopherol which quenches oxygen radicals within the membrane and terminates chain reactions that causes oxidative damage. Increased α-tocopherol levels may serve as an acclimation strategy of plants to tolerate dehydration (Munne-Bosch et al, 1999). Differences were observed in α-tocopherol contents of maize between pre and post-flowering dehydration with changes in relative water contents. It has been suggested that the increase of α-tocopherol under stress may be related to the degradation of chlorophyll, because phytol from chlorophyll degradation may be used for the synthesis of α-

tocopherol (Hess, 1993). Thus, the greater increase in α-tocopherol during pre than post-flowering dehydration may be related to the chlorophyll loss used for increased contents of α-tocopherol. On the other hand, the smaller amounts of α-tocopherol during post-flowering dehydration indicate an age-dependent increase in oxidative stress and also age-dependent decrease in antioxidant defences. An age-dependent decrease in α-tocopherol contents has also been reported by Munnes-Bosch and Alegre, (2002). In sorghum, no differences were noticed in the α-tocopherol contents between pre and post-flowering dehydrated leaves, suggesting that although the α-tocopherol concentration of leaves increased in response to both pre and post-flowering dehydration, sorghum was able to efficiently regenerate α-tocopherol regardless of plant age.

The glutathione pool plays a key role in drought tolerance and limited cellular damage against the toxic effects of reactive oxygen species by keeping the reactive oxygen species scavenging ascorbate in its reduced and, hence, active form by involvement in the ascorbate-glutathione cycle (Sgherri and Navari-Izzo, 1995). Dehydration markedly increased the reduce glutathione, oxidized glutathione contents and GSH:GSSG ration in both species during pre and post-flowering stages relative to the control (Fig. 3.7-Fig 3.9; Table 3.7-Table 3.8). This is in agreement with previous reports which indicate that under water deficit conditions reduce and oxidized contents increased above the control level (Sgherri and Navari-Izzo, 1995; Zhang and Kirkham, 1996; Lascano et al, 2001; Vaidyanathan et al, 2003). The increase in the GSH:GSSG ratio compared to the control in both species suggested that oxidized glutathione was in a reduced state and form increased levels of reduce glutathione (Fig. 3. 7). In pre-flowering dehydrated maize the decrease in the GSH:GSSG with further loss of relative water contents was due to a stronger increase of oxidized glutathione (Fig. 3.8a) compared to reduced glutathione. During post-flowering dehydration, however, reduced glutathione was oxidized initially and oxidized glutathione formed and reduced back to form reduced glutathione thereby increasing the GSH:GSSG (Fig. 3.9a). Generally, sorghum exhibited greater increases in reduced glutathione (Fig. 3. 7b) and oxidized glutathione (Fig. 3. 8b) contents with increase in the intensity of dehydration than maize during both developmental stages, possible reason why sorghum had relatively stable GSH:GSSG ratio through out the dehydration cycle (Fig 3.9b). The higher reduced glutathione and oxidized glutathione

content in sorghum may be related to its higher glutathione reductase activities during both developmental stages. Since glutathione reductase is involved in maintaining high ratios of GSH:GSSG, the increase in leaves undergoing pre and post-flowering dehydration could probably be due to the observed increase in glutathione content. Elevated levels of reduced glutathione and oxidized contents would be associated with increased in oxidative stress tolerance. Broadbent et al, (1995) found that transgenic plants of tobacco over expressing glutathione reductase had both elevated reduced glutathione and oxidized glutathione and increased tolerance to oxidative stress in leaves. Zhang and Kirkham (1996) have also observed an increase in reduced glutathione and oxidized glutathione content in sorghum with increase in the duration of water deficit stress.

Anthocyanins are most commonly located in the vacuoles of plant photosynthetic cells (Gould et al, 2000) and are proposed to play a protective role from photo-inhibition (Gould et al, 1995; Dodd, et al, 1998) and scavenging of reactive oxygen species under stressful environments (Yamasaki, 1997; Sherwin and Farrant, 1998). In the present study, the increase in anthocyanin contents in maize and sorghum relative to the control may protect them from high light intensity and reactive oxygen species produced under dehydration conditions during both pre and post-flowering stages. It was notice that in both maize and sorghum, the most vulnerable stage to the effect of dehydration was post-flowering stage as compared to pre-flowering dehydration (Fig. 3.10). During post-flowering dehydration, extensive loss of chlorophyll and the retention of elevated anthocyanin contents in both species with further water loss may be necessary for protection against light and oxidative damage caused by dehydration. During pre-flowering dehydration the decline in anthocyanin contents in both species may indicate that the anthocyanin synthesis is inhibited for an extensive period. During this stage, maize employed leaf rolling at very early stage and sorghum at later stage and this could benefit both species as an alternative protection from light induced damage and increase in anthocyanin contents might not have been required.

Considering overall responses of the non-enzymatic antioxidants (ascorbate, α-tocopherol, GSH:GSSG and anthocyanin) to pre and post-flowering dehydration, the result suggested that the adaptive changes of these antioxidants would provide

protection against oxidative stress in both maize and sorghum. However, since the comparative levels of each antioxidant differed among species and at different developmental stages, it appears that the protective capacity of these non-enzymatic antioxidants also differed between species, as shown from the variation in increased malondialdehyde contents during dehydration. High levels of ascorbate, α-tocopherol, GSH:GSSG and carotenoid have been reported to be correlated with a high tolerance to oxidative stress caused by adverse environmental conditions (Noctor and Foyer, 1998). Moreover, ascorbate and reduced glutathione are also known to be required for a normal progression of cell cycle, cell wall growth and cell expansion (Noctor et al, 1998). Results reported here suggested that in sorghum, the observed concerted response of all these antioxidants provided sufficient protection systems against reactive oxygen species, as evidenced from the higher increases during both pre and post-flowering dehydration as compared to maize. By contrast, in maize as dehydration became more severe during both pre and post-flowering stages, the level of the non-enzymatic antioxidants appeared to be degraded. This may impair the reactive oxygen species scavenging capacities of these antioxidants and favours excess production of reactive oxygen species.

Lipid peroxidation measured as malondialdehyde content in both maize and sorghum was used as an indication of reactive oxygen species damage due to dehydration. The high malondialdehyde content observed in maize than in sorghum (Fig. 3.11; Table 3.10) indicates the prevalence of reactive oxygen species in maize tissue than in sorghum. In the current study it was found that, in spite of a consistent increase of activities of antioxidant enzymes particularly superoxide dismutase and ascorbate peroxidase and non-enzymatic antioxidants ascorbate, α-tocopherol and GSH:GSSG, maize exhibited higher malondialdehyde during both pre and post-flowering dehydration. This indicated that the increases of these antioxidant enzyme activities and non-enzymatic antioxidant contents could not provide sufficient protection to the membrane against reactive oxygen species. Since a measured enzyme activity and non-enzymatic content is a result of both synthesis and degradation, any decrease in enzyme activity and non-enzymatic antioxidant content under dehydration may be considered to either reduced synthesis or degradation. Although maize exhibited enhanced activities of glutathione reductase, catalase, GSH:GSSG, there was also a declining trend with increase in the intensity of dehydration during both pre and post-

flowering dehydration. It is possible that the extensive senescence processes observed in maize suppressed the antioxidant defence systems due to diminished activities of these antioxidants and caused exposure of the tissues and cells to more oxidative stress. Sorghum, on the other hand exhibited an enhanced activities of enzymatic (superoxide dismutase, glutathione reductase, catalase and ascorbate peroxidase) and non-enzymatic antioxidant (ascorbate, α-tocopherol and GSH:GSSG) contents through out pre and post-flowering dehydration period. The coordinated action of these enzymatic and non-enzymatic antioxidants in sorghum might have contributed in providing effective protection against oxidative stress, enable to minimize lipid peroxidation under dehydration conditions better than maize. This suggests that this sorghum variety has a higher inherent and induced capacity under dehydration which provides it a better protection from oxidative damage. However despite better protection against reactive oxygen species in sorghum, the cooperative action of adaptive changes in enzymatic and non-enzymatic antioxidants were not sufficient to completely prevent oxidative stress since the malondialdehyde content still markedly increased as compared with the control (Fig 3. 11b).

3.3.4.2 Antioxidant responses in maize and sorghum during pre and post-flowering rehydration

Enzymatic antioxidants (superoxide dismutase, glutathione reductase, catalase and ascorbate peroxidase) appears to have an important role during the recovery of both maize and sorghum upon pre and post-flowering rehydration in providing additional protection against reactive oxygen species, which is a very important developmental stage for drought resistance in both species. The extent of their protective role however varied depending on developmental stages and species. The fact that the increase in superoxide dismutase, glutathione reductase, catalase and ascorbate peroxidase activities during the late phase of rehydration following pre-flowering dehydration and the retention of this enzymes elevated during post-flowering rehydration in sorghum as well as the decrease in the activities of superoxide dismutase and ascorbate peroxidase during pre-flowering rehydration and glutathione reductase and catalase during post-flowering rehydration in maize relative to their respective control plants (Fig. 3.1c and d-Fig. 3. 4c and) may suggest that this enzymes operates better in sorghum than maize. In sorghum, reactive oxygen species might be detoxified more efficiently, thereby lowering the extent of the exposure of

membrane lipids to high reactive oxygen species. The increase in glutathione reductase and catalase activities during the late phase of pre-flowering rehydration in maize may indicate that the operation of these enzymes appeared to be more efficient when maize is under moderate stress conditions. On the other hand, the retention of superoxide dismutase and ascorbate peroxidase activities high during post-flowering rehydration may be viewed as a key factor in controlling the oxidative stress that might have occurred during the rehydration process and providing additional protection against reactive oxygen species. The involvement of enzymatic antioxidants in scavenging reactive oxygen species was also revealed in other crop species such as spinach (Tanaka et al, 1985) and cotton (Foster and Hess, 1980).

The high concentrations of ascorbate in plants suggest that it has an important role as antioxidant defence mechanism (Smirnoff, 1995). In the present study the decline in ascorbate concentrations in both maize and sorghum upon pre and post-flowering rehydration (Fig. 3.5) may suggest degradation of ascorbate upon pre and post-flowering rehydration probably due to oxidative stress which might have been formed during rapid rehydration. While α-tocopherol content recovered in maize during pre and post-flowering and sorghum during pre-flowering rehydration, the retention of α-tocopherol contents up-regulated in sorghum during post-flowering rehydration may help sorghum plants to eliminate $^1O_2$ which are formed during rehydration as a result of excited triplet chlorophyll with oxygen in the pigment bed (Fryer, 1992) and minimize membrane damage.

The maintenance of elevated level of reduced glutathione, oxidized glutathione contents and GSH:GSSG ratio appear to have a particular advantage in both species during post-flowering rehydration (Fig.3.7c and d; Fig. 3.8c and d and Fig. 3. 9c and d). A possible explanation of this increase during rehydration in both crops could be seen as a defence mechanism to counter act the deleterious effects of an oxidative stress during rehydration. In maize where superoxide dismutase and ascorbate peroxidase during pre-flowering and glutathione reductase and catalase during post-flowering rehydration are decreased to the control level, reactive oxygen species formed at both developmental stages is readily available for membrane damage. Coupled with decreased ascorbate and α-tocopherol contents during rehydration at both developmental stages, maize might have been exposed to reactive oxygen species which resulted to higher malondialdehyde contents than sorghum.

Interestingly, sorghum have balanced its lower ascorbate and α-tocopherol contents with increased contents of GSH:GSSG ratio and a more efficient capability of enzymatic antioxidants (superoxide dismutase, glutathione reductase, catalase and ascorbate peroxidase). As a result reactive oxygen species might be detoxified more efficiently and this may be the reason why sorghum has less malondialdehyde contents during pre and post-flowering rehydration as compared with maize.

3.3.5 Conclusions

In conclusion, our results showed that activities of several enzymatic and contents of non-enzymatic antioxidants were elevated in both maize and sorghum plants during pre and post-flowering dehydration, which confirms that dehydration affects the antioxidant defence mechanisms and supports the hypothesis that dehydration increases the potential for oxidative stress in plants. The difference in activities of enzymatic antioxidants (SOD, GR, CAT and APX) and contents of non-enzymatic antioxidants (ascorbate, α-tocopherol and total glutathione and anthocyanin) between maize and sorghum could be ascribed to the difference in mechanisms underlying oxidative stress injury and subsequent tolerance to dehydration. Notably sorghum plants that have been subjected to pre and post-flowering dehydration exhibited consistent and comparatively higher increases in all activities of enzymatic and contents of non-enzymatic antioxidants than maize. Hence, antioxidant defence mechanisms in sorghum were concertedly regulated to ensure proper protection against reactive oxygen species generated after exposure to pre and post-flowering dehydration. This has been demonstrated by minimizing the extent of lipid peroxidation in sorghum than maize. However, adaptive changes in enzymatic and non-enzymatic antioxidants were not sufficient to prevent oxidative stress since contents of malondialdehyde remarkably increased in both species during pre and post-flowering dehydration.

The results discussed in the response of maize and sorghum during rehydration draw to a conclusion that rehydration enhanced a more efficient defence mechanism in sorghum than in maize against reactive oxygen species which might have been formed during the process of pre and post-flowering rehydration. The results suggest that the ability of sorghum to tolerate the effects of rehydration after exposure to pre and post-flowering dehydration are associated with the capacity to modulate the

activity of antioxidant defence mechanisms according to the prevailing generation rates of reactive oxygen species.

Chapter 4

Seed quality of maize and sorghum harvested from plants after exposure to and recovery from pre and post-flowering dehydration

4.1 Introduction

Sorghum and maize are important staple crops cultivated by many subsistence farmers in the semi-arid regions of the world. Hence, among the cereal crops, sorghum and maize are considered as the principal source of energy, protein, vitamins and minerals for millions of the poor people living in these regions (Buerkert et al, 2001; Duodu et al, 2003). Production of these crops in many of the semi-arid areas is almost entirely under rainfed conditions; as a result the crops invariably suffer from drought stresses of varying intensities and durations at any of their growth stages. In areas where supplemental irrigation to avoid the occurrence of drought at critical stages of crop growth is rarely practiced, resource poor farmers depend largely on those plants which survived drought stresses of the previous season for their food consumption and planting material. This situation severely threatens the rural household food security, leading to chronic under-nourishment of the people. Observations of several of the subsistence farmer's fields where sorghum and maize are grown in developing countries often show poor seed germination, stand establishment, mortality and retarded growth early in the seedling stage.

Poor germination and stand establishment are generally a result of several environmental and intrinsic seed factors. Vigour and germinability are important characteristics of seed quality. The germinability of a seed is defined as the ability to germinate or produce a normal seedling in a standard germination test, usually performed under optimal growth conditions (ISTA, 1985). The vigour test is an important component of seed testing and this kind of test can presumably reflect a seed's potential performance or vigour, where vigour is defined as the capacity of the seed to germinate under adverse and optimal conditions (AOSA, 1983). According to Gurmu and Naylor (1991) seed vigour reflects both the production history of the seed lot on the parent plant and the conditions under which it was subsequently harvested, handled and stored. Deterioration of seed quality (including vigour) is fastest under poor storage conditions.

4.1.1 Objectives

Since germinability and vigour are prerequisites for successful stand establishment and increased grain production, and quality of grain production insures food security for rural community, this study was carried out to achieve a better understanding of;

i) the effects of water deficit during pre and post-flowering stage on viability and food (storage reserve contents) quality.

ii) the effects of dehydration and rehydration cycles at critical growth stages on subsequent seed performance and production that could possibly lead to development of cultivars more able to efficiently partition assimilates to the reproductive organs in the field.

4.2 Materials and Methods

4.2.1 Plant material, growth conditions and Seed harvesting

Species and cultivars, growth conditions and drought treatments in this experiment were similar to those discussed in chapter 2. In this chapter, standard germination, vigour, carbohydrates (glucose, fructose and sucrose), lipid and protein contents of maize and sorghum seeds produced from plants after exposure to and recovery from pre and post-flowering dehydration were examined. Control seeds were harvested from plants grown under non-stressed conditions. For comparison, seeds of maize and sorghum grown under non-stressed conditions and with improved agronomic practices and management conditions in the field were included as original seeds. These seeds were supplied by Ethiopian Agricultural Research Organization, Melkassa Agricultural Research Centre and ICRISAT Centre, Bulawayo, Zimbabwe, respectively.

At physiological maturity (development of black-layer) (Tuinstra et al, 1997), seeds of maize and sorghum were harvested from each treatment. Seeds for standard germination and vigour tests were sun dried (25 to 27 ^{0}C) to a moisture content of 12% and those for sugar, lipid and total protein content analysis were freeze dried and kept until analysis, where triplicate extractions were performed from seeds of 3 separate plants.

4.2.2 Standard germination and vigor tests

Standard germination test was carried out using the method described by Association of Official Seed Analysis (1981). Except in the case of maize seeds of pre and post-flowering dehydration treatments where only three replicates of 25 seeds were used (no enough seeds were produced due to the effects of dehydration), four replicates of 25 seeds of both maize and sorghum were placed between a double layer of paper towels moistened with distilled water, gently rolled into a tube and covered with a plastic bag. The rolled tubes were kept upright in a dark germination chamber with a constant temperature of 25 ^{0}C for 7 days. To keep seeds moistened, the paper towel tubes were opened and watered three to four days after planting. Germination counts were taken 7 days after planting. Seeds were considered germinated when normal protrusion of coleoptiles and radicles from the point of attachment with the seed to the tip were visible. For the seed vigour test, seeds were subjected to accelerated aging (AA) by exposing them to adverse conditions of high temperature (43 ^{0}C) and high relative humidity. To create high relative humidity conditions, 40 mls of distilled water was placed in a plastic container on top of which four replicates of 25 seeds of maize and sorghum from each treatment were evenly distributed on a wire mesh, and the containers closed with a plastic lid. The AA boxes were then placed in a germination chamber adjusted to 43 ^{0}C in the dark for 72 hrs. After AA, seeds were allowed to germinate in a rolled moistened paper towel following the same procedures as that of the standard germination test. Due to lack of seed numbers, the AA test was not performed on maize pre and post-flowering dehydrated treatments. Both standard germination and seed vigour test counts were expressed as percentage germination.

4.2.3 Determination of soluble and insoluble carbohydrates

Freeze dried maize and sorghum seeds (0.25-0.50 g) were ground to fine powder in liquid nitrogen (N_2) using a pestle and mortar. Soluble sugars (sucrose, glucose and fructose) were extracted with 100 mM NaOH in 50% (v/v) ethanol. The reaction mixture was vigorously mixed during which tissue extracts were adjusted to pH 7-8 by adding 500 µl of 100 mM HEPES in 100 mM glacial acetic acid. The reaction mixture was centrifuged at 16000 rpm in a Beckman J2-21 centrifuge for 20 min at 4 ^{0}C. The remaining pellets

were re-extracted using the same procedure as described above and the two supernatants from each extracts combined.

Quantification of the soluble sugars was done enzymatically using Boehringer Mannheim sugar food analysis kit (Bergmeyer and Bernt, 1974). The amount of NADPH from the reaction of glucose-6-phosphate with NADP + glucose-6-dehydrogenase was measured spectrophotometrically at 340nm. Fructose was converted to glucose-6-phosphate in two steps using hexokinase and glucosephosphate isomerase, while sucrose was measured as the additional amount of D-glucose formed after inversion with b-fructosidase (D-glucose being measured as described above).

Starch concentration was colorimetrically determined using the method described by Buysse and Merckx (1993). Maize and sorghum seed samples (0.25 g) were ground to fine powder in liquid N_2 using pestle and mortar. After drying the sample used in sugar extractions, extraction of starch was carried out with 10 ml of 32% HCl. The sample extract was incubated in a boiling water bath for 3 hrs and centrifuged at 25000g in a Beckman J2-21 centrifuge for 5 min at 4 ^{0}C. Determination of starch concentration was made using a reaction mixture containing 1 ml of phenol, 5 ml H_2SO_4 and 0.5 ml sample. A blank (the appropriate phenol, H_2SO_4/ water without added sample) was measured at the beginning. A standard curve with glucose concentrations in the range of 20-80 µg/ml was established.

4.2.4 Determination of lipid

Approximately 1 g of maize and sorghum seeds was finely ground with a mortar and pestle using liquid N_2. A volume of 25-40 ml of chloroform: methanol (v/v) was added and homogenized with Ultra-Turox homogenizer. The sample extract was centrifuged and the clear supernatant was carefully removed using an automatic pipette into a measuring cylinder and the total volume made up to 60 ml with the extraction medium. To remove the polar contaminants, the extracts were transferred to a separating funnel and a folch wash was carried out comprising of 15 ml of 0.88% KCl solution. After shaking the contents of separating funnel together, the two phases were allowed to separate. The lower organic phase containing lipids was then removed by running it into

a preweighed glass vessel after which it was concentrated under a vacuum in a Savant SpeedVac SC110 rotary concentrator. Samples were stored in a desiccator and weighed daily until a constant mass was attained. The total lipid content was expressed as mg lipid/g dry weight.

4.2.5 Determination of total seed protein

Approximately 0.1 g of freeze dried maize and sorghum seeds were weighed and ground in to finely powder with a pestle and mortar in liquid N_2. A volume of 3 ml extraction buffer containing 30 mM TES (pH 7.5), 20 mM NaCl and 1mM PMSF was used for the extraction of total protein. The sample extract was shaken for 15 min and centrifuged at 10000 rpm for 15 min at 4 ^{0}C. A 40 µl of protein supernatant was then mixed with 2 ml of diluted Coomassie Blue G-250 reagent prepared according to the method of Bradford (1976) in 96% ethanol and 85% phosphoric acid. Absorbance was read at 595 nm in a Beckman DU650, USA spectrophotometer. The concentration of protein in the sample was calculated from a standard curve prepared from known masses of bovine serum albumine (BSA).

4.2.6 Statistical analysis

Statistical analyses were carried out using STATISTICA for windows Version 6.0, Statsoft, Inc, USA. The results presented were the mean of three replicates. In all figures, means were calculated and significances between treatments as well as between the two species were tested by factorial analysis of variance and Duncan's multiple range tests at the 5% level of significance. Standard errors are represented as vertical bars.

4.3 Results

4.3.1 Standard germination and Vigour

Original seeds of both maize and sorghum had similar percentage germination of 95% and 92%, respectively (Fig. 4.1). Seeds of both species from pre and post-flowering control plants had similar % germination. When compared to the original seeds, there was a slight reduction in percentage germination of maize seeds from both pre and post-

flowering control plants. Percent germination of maize seeds from pre and post-flowering control plants was 80% and 87%, respectively.

Sorghum seeds from pre and post-flowering control plants on the other hand showed 89% and 90% germination, respectively. The magnitude of decrease in percentage germination of seeds harvested from pre and post-flowering dehydrated plants varied between species. Percentage germination of maize seeds from plants dehydrated during both developmental stages were significantly ($P<0.05$) decreased as compared to the control and original seeds (Fig. 4.1a and b). Germination of sorghum seeds harvested from pre and post-flowering dehydrated plants were not significantly ($P<0.05$) affected. Percentage germination of sorghum seeds harvested from plants dehydrated during pre and post-flowering stages decreased to only 82% and 75% relative to the control, respectively. In contrast, percentage germination of maize seeds harvested from plants dehydrated during pre and post-flowering stages were reduced to 17% and 28% relative to the control, respectively.

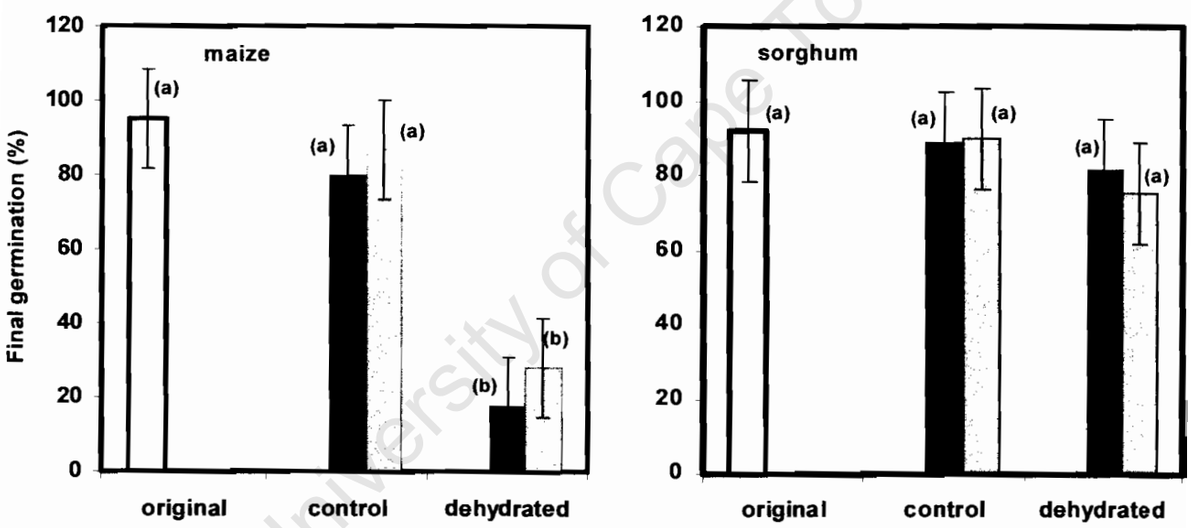

Figure 4.1 Germination performance of maize and sorghum seeds harvested from original (open bar), pre-flowering control and dehydrated (dark solid bar) and post-flowering control and dehydrated (grey bar) plants, respectively. Vertical bars denote standard errors of means (n=3). Letters followed by different letters are significantly different at $P<0.05$.

Original seeds of both species germinate similarly in the AA test. Sorghum seeds from both pre and post-flowering control plants and maize from post-flowering control plants were not affected by AA relative to the original seeds (Fig. 4.2a and b) indicating that their vigour was the same. In contrast, AA led to a significant ($P<0.05$) decline in vigour of control maize seeds from pre-flowering treatment, relative to the original seeds. There was no significant P<005) difference in vigour of sorghum seeds harvested from pre-flowering dehydrated plants AA as compared with the control and original seeds. By contrast, although it look like that germination of seeds harvested from post-flowering dehydrated plants was not affected (Fig. 4.1), it does affect vigour AA (Fig. 4.2) as compared to its control counter part and the original seeds.

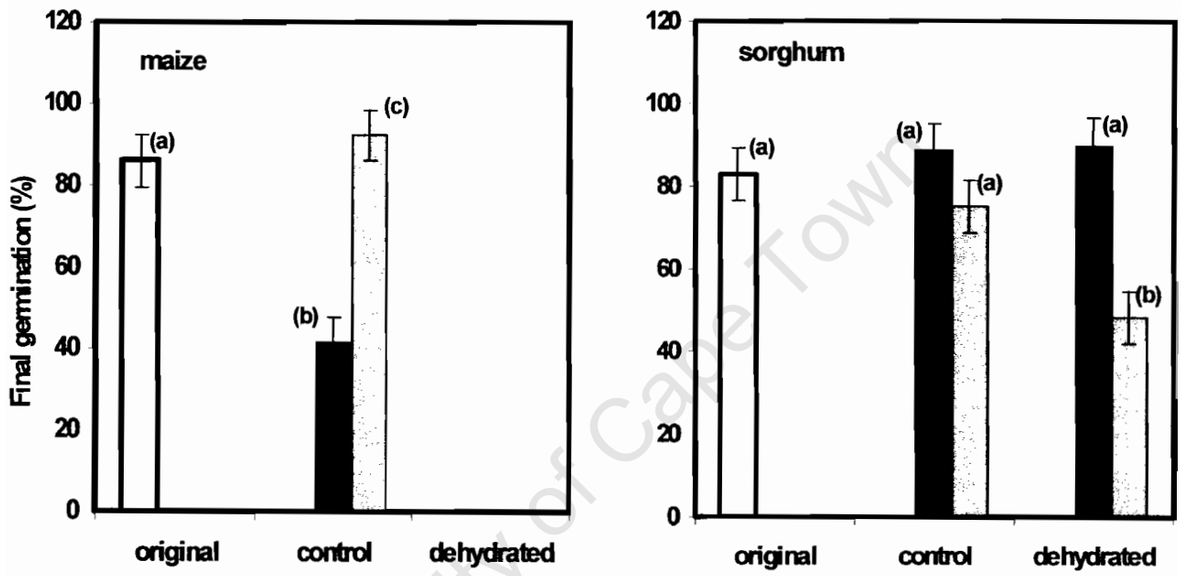

Figure 4.2 Germination performance after an accelerated ageing treatment of maize and sorghum seeds harvested from original (open bar), pre-flowering control and dehydrated (dark solid bar) and post-flowering control and dehydrated (grey bar) plants, respectively. Vertical bars denote standard errors of means (n=3). Letters followed by different letters are significantly different at $P<0.05$. Due to lack of seeds, the accelerated ageing test was not performed on maize pre and post-flowering dehydrated treatments.

4.3.2 Soluble and insoluble carbohydrates

Glucose contents from original seeds in maize (31.6 μmol g^{-1} DW) are markedly higher than in sorghum (26.4 μmol g^{-1} DW) (Fig. 4.3). In addition, glucose contents of control seeds of both species from pre-flowering treatment were generally lower than from their respective original seeds. In contrast, glucose contents of control seeds of both species from post-flowering treatment were significantly ($P<0.05$) higher than from their respective original seeds. Dehydration during pre and post-flowering stages in maize seeds and only post-flowering in sorghum seeds led to a significant ($P<0.05$) decline in glucose contents.

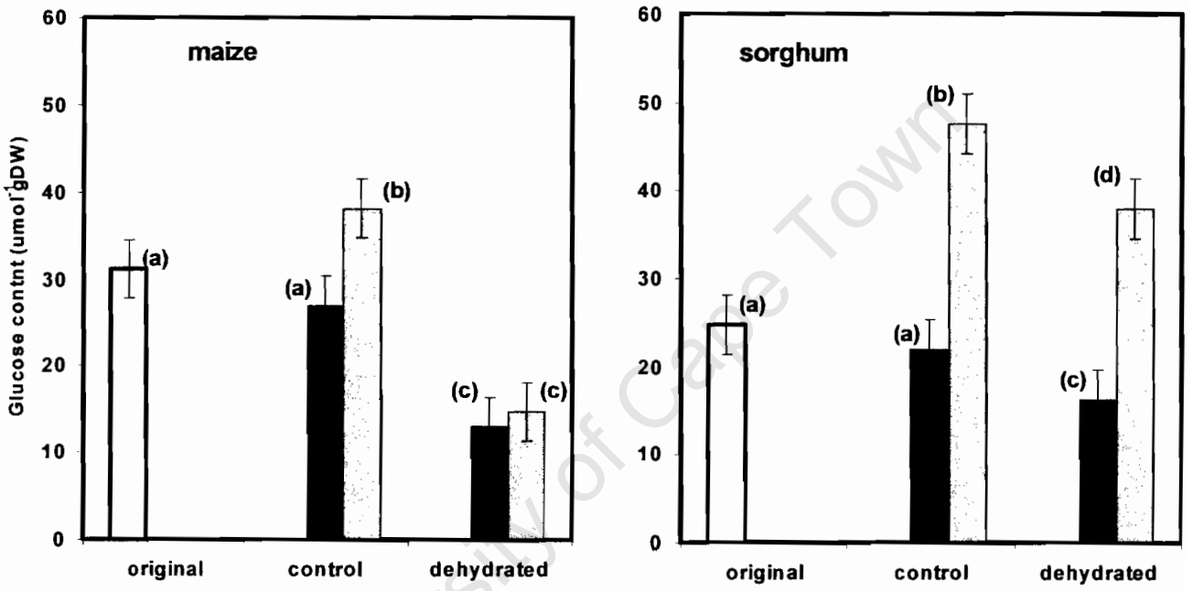

Figure 4.3 Glucose contents of maize and sorghum seeds harvested from original (open bar), pre-flowering control, dehydrated (dark solid bar) and post-flowering control, dehydrated (grey bar) plants, respectively. Vertical bars denote standard errors of means (n=3). Letters followed by different letters are significantly different at $P<0.05$.

Differences in glucose contents were observed between species in response to pre and post-flowering dehydration. Maize seeds harvested from pre and post-flowering dehydrated plants decreased by 52% and 61% in glucose levels as compared to their respective control and 58% and 52% as compared to their original seeds, respectively. Sorghum on the other hand exhibited only 27% and 21% decrease as compared to pre and post-flowering control seeds, respectively. When compared with the original seeds, glucose levels of sorghum seeds from pre and post-flowering dehydration declined by 36%, and increased by 52%, respectively.

Similar to those of glucose levels, fructose contents of original maize seeds were higher than in sorghum (Fig. 4.4). On the other hand, sorghum seeds from both pre and post-flowering control plants appears to have slightly higher fructose contents than in maize.

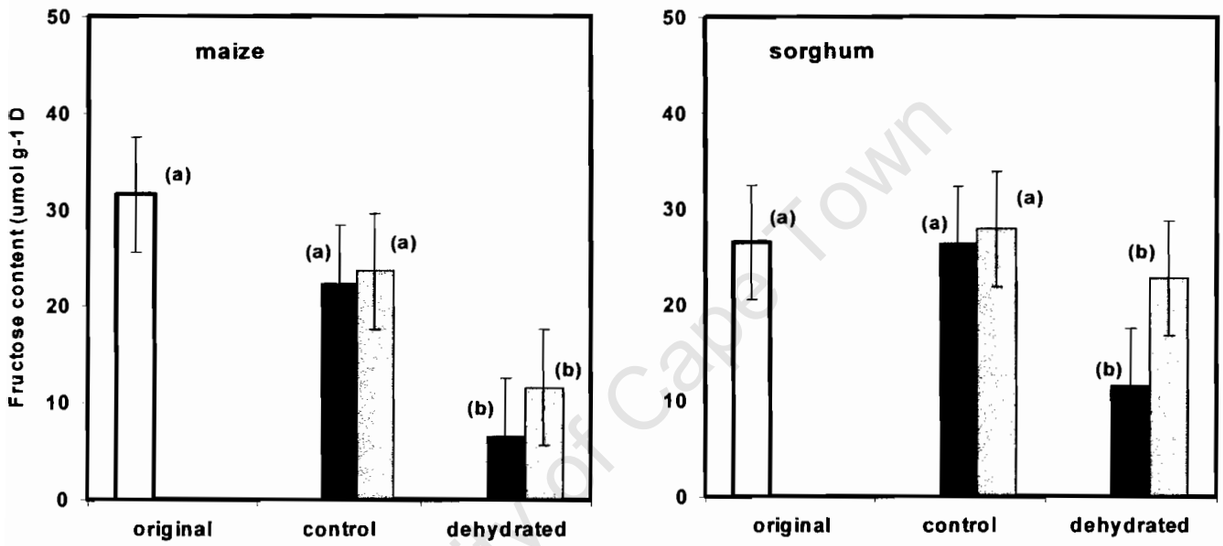

Figure 4.4 Fructose contents of maize and sorghum seeds harvested from original (open bar), pre-flowering control, dehydrated (dark solid bar) and post-flowering control, dehydrated (grey bar) plants, respectively. Vertical bars denote standard errors of means (n=3). Letters followed by different letters are significantly different at P<0.05.

In general, dehydration decreased fructose contents of both species, but differences between species was not significant ($P<0.05$). In maize, fructose contents of seeds from pre-flowering dehydration plants decreased by 70% and 79% when compared to seeds harvested from its respective control and original seeds, respectively. Fructose contents of seeds from post-flowering dehydrated plants decreased by 51% and 63% relative to seeds harvested from its control counter part and original seeds, respectively. The decline in fructose contents of sorghum seeds harvested from pre and post-flowering dehydrated plants were in the order of 56% and 19% as compared to the controls, and 56% and 14% as compared to the original seeds, respectively.

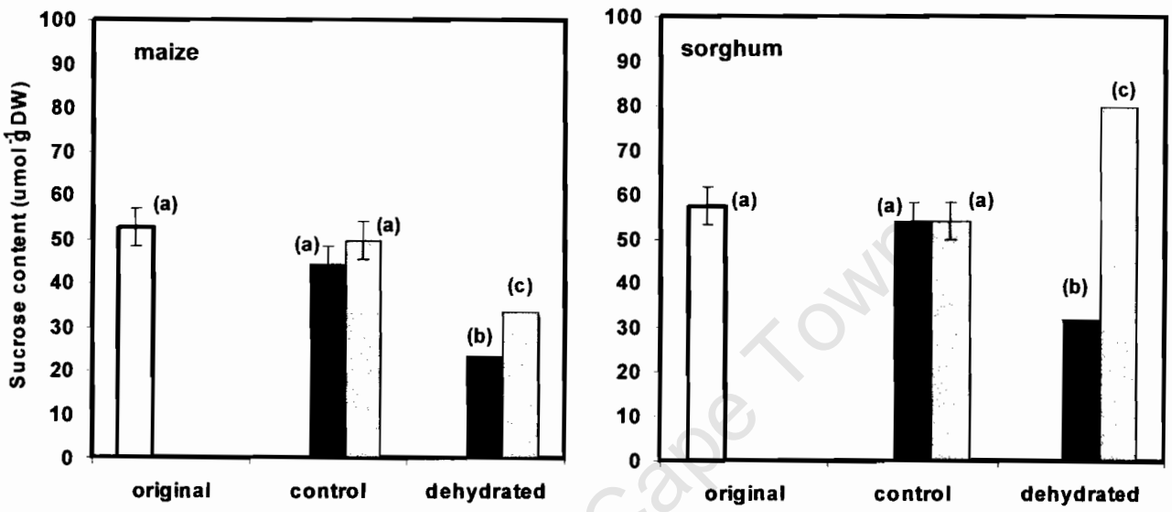

Figure 4.5 Sucrose contents of maize and sorghum seeds harvested from original (open bar), pre-flowering control, dehydrated (dark solid bar) and post-flowering control, dehydrated (grey bar) plants, respectively. Vertical bars denote standard errors of means (n=3). Letters followed by different letters are significantly different at $P<0.05$.

Sorghum seeds harvested from original plants appear to have higher sucrose contents than maize seeds (Fig. 4.5). Significant ($P<0.05$) differences in sucrose contents were observed between species in seeds harvested from the control plants of pre and post-flowering stages.

Control maize seeds harvested from both pre and post-flowering treatment plants had significantly ($P<0.05$) lower sucrose contents than sorghum counter parts. In addition, control maize seeds harvested from pre-flowering plants had significantly ($P<0.05$) lower sucrose contents than the seeds harvested from post-flowering and original plants. Sorghum on the other hand, showed similar sucrose contents in original and seeds of pre and post-flowering control plants. Dehydration had a significant ($P<0.05$) effect on the sucrose contents of seeds of both species harvested from both pre and post-flowering dehydration. With the exception of sorghum seeds from post-flowering dehydrated plants, dehydration significantly ($P<0.05$) decreased sucrose contents of maize seeds harvested from both developmental stages and sorghum seeds harvested from pre-flowering dehydrated plants. The decrease in maize seeds harvested from pre-flowering dehydrated plants was by 47% and 56% relative to the control and original seeds, respectively. Sucrose contents of seeds of this species harvested from post-flowering dehydrated plants declined by 33% and 36% when compared to the control and original seeds, respectively. Therefore, it is apparent that the decrease was greater from seeds harvested from those dehydrated during pre than post-flowering plants. Sucrose levels of sorghum seeds harvested from pre-flowering dehydrated plants declined by 41% and 44% when compared to the control and original seeds, respectively. In contrast, sucrose levels of seeds harvested from post-flowering dehydrated plants showed an increase of 47% and 39% relative to the control and original seeds, respectively.

Original sorghum seeds showed higher starch contents than maize seeds (Fig. 4.6). It can be seen that no significant ($P<0.05$) differences were observed between species in their starch contents of seeds harvested from control plants. In addition, dehydration during pre and post-flowering stages had no significant ($P<0.05$) effects on grain starch contents of both species. The only statistically significant ($P<0.05$) difference was that starch content

of control maize seeds harvested from post-flowering control plants was higher than those pre-flowering control plants. This difference also holds true between seeds harvested from post and pre-flowering dehydrated maize plants.

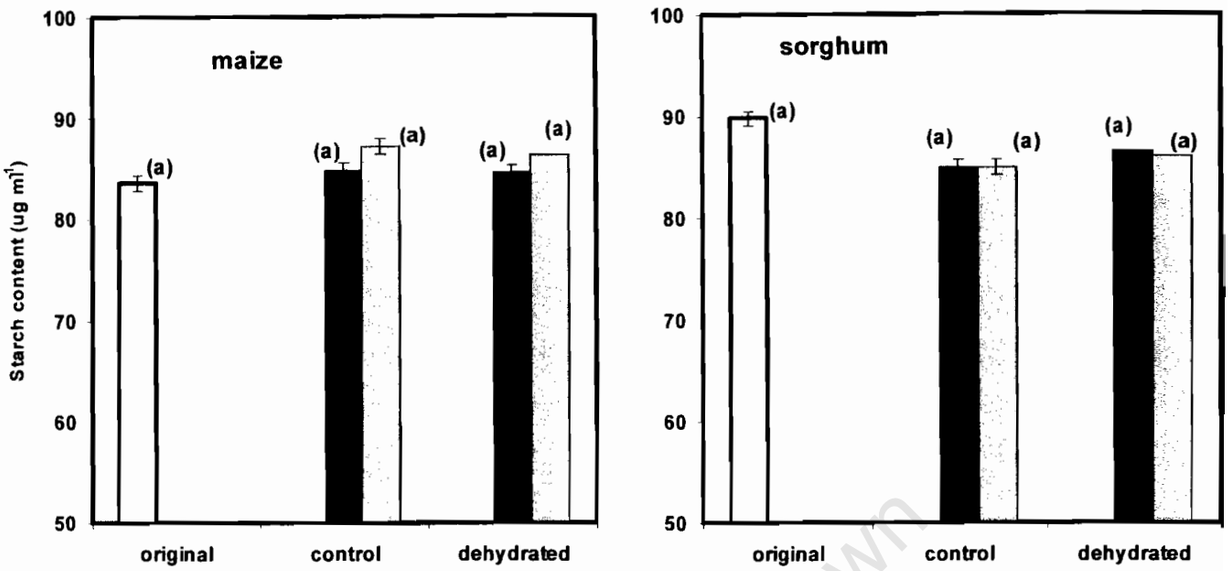

Figure 4.6 Starch contents of maize and sorghum seeds harvested from original (open bar), pre-flowering control, dehydrated (dark solid bar) and post-flowering control, dehydrated (grey bar) plants, respectively. Vertical bars denote standard errors of means (n=3). Letters followed by different letters are significantly different at P<0.05.

4.3.3 Lipid

In general, lipid content of maize seeds from original and pre and post-flowering control plants was higher than sorghum seeds (Fig. 4.7). Dehydration significantly ($P<0.05$) decreased lipid contents of both maize and sorghum seeds harvested from pre and post-flowering dehydrated plants relative to the control and original seeds. There was no

significant (P<0.05) difference in lipid contents between species in response to pre and post-flowering dehydration. In both species seeds from pre-flowering dehydrated plants had significantly (P<0.05) lower lipid contents than those from post-flowering dehydrated plants.

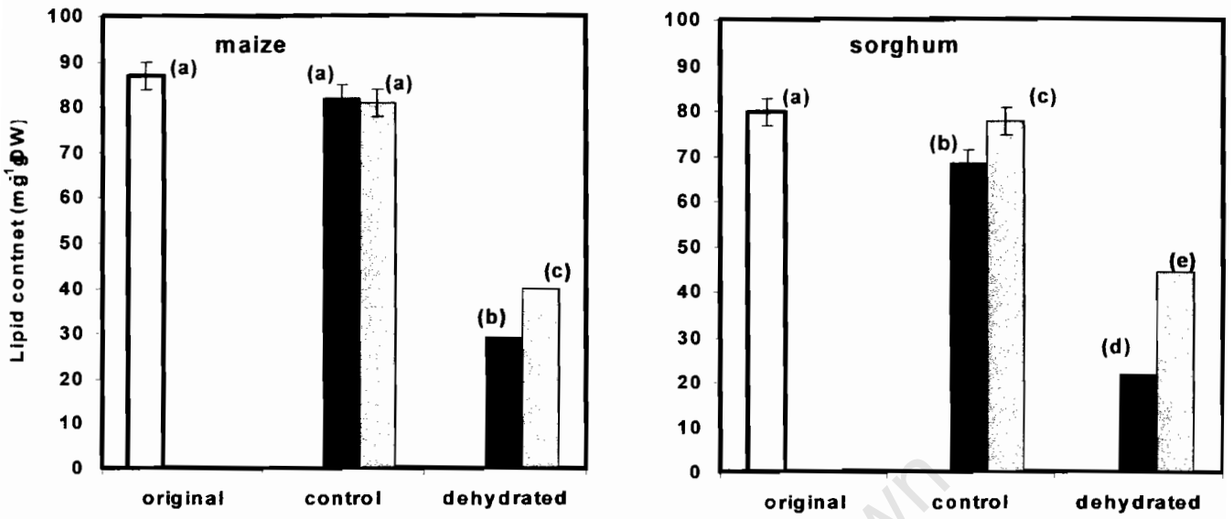

Figure 4.7 Lipid contents of maize and sorghum seeds harvested from original (open bar), pre-flowering control, dehydrated (dark solid bar) and post-flowering control, dehydrated (grey bar) plants, respectively. Vertical bars denote standard errors of means (n=3). Letters followed by different letters are significantly different at P<0.05.

4.3.4 Seed protein

There were considerable differences in the total protein contents of seeds between species harvested from original, control and dehydrated plants (Fig. 4.8a and b). Sorghum seeds showed remarkably higher protein contents than maize seeds from all seed sources. In general, dehydration had a significant (P<0.05) effect on the protein contents of both species harvested from pre and post-flowering dehydrated plants relative to the original and control seeds. However, the mode of the effect of dehydration at pre and post-flowering stages differed between species as compared to the control seeds. In maize,

protein contents of seeds harvested from pre-flowering dehydrated plants markedly decrease and those from post-flowering dehydrated plants increased relative to their respective control seeds.

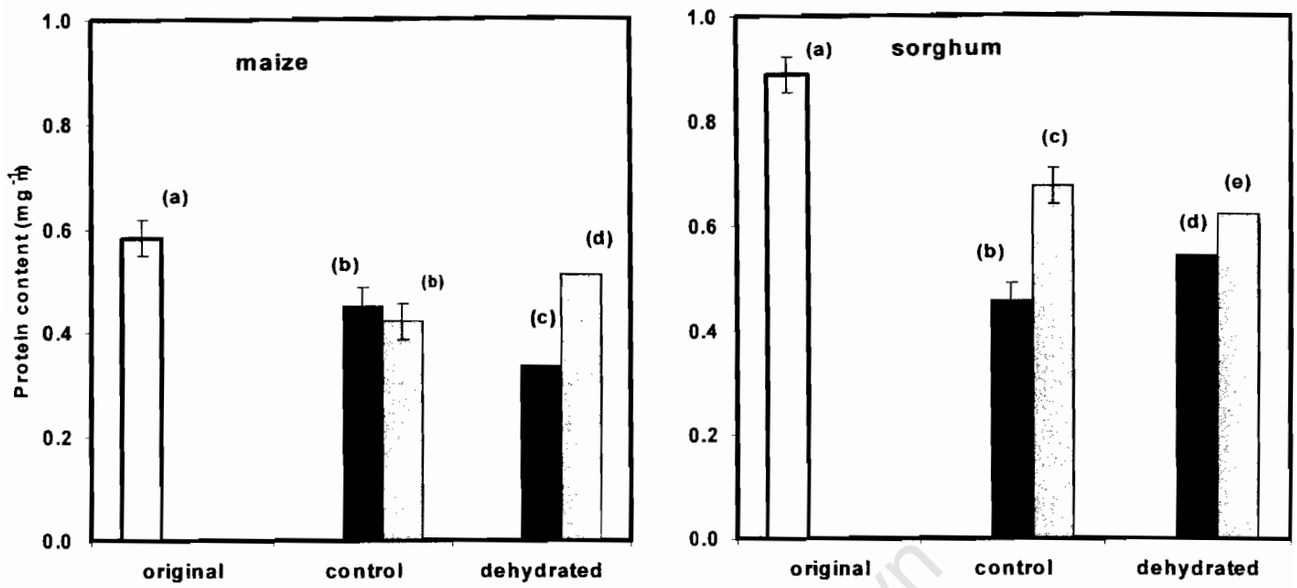

Figure 4.8 Protein contents of maize and sorghum seeds harvested from original (open bar), pre-flowering control, dehydrated (dark solid bar) and post-flowering control, dehydrated (grey bar) plants, respectively. Vertical bars denote standard errors of means (n=3). Letters followed by different letters are significantly different at P<0.05.

In contrast, sorghum protein contents harvested from pre-flowering dehydrated plants showed an increase, and seeds from post-flowering dehydrated plants decreased as compared with their respective control seeds. Nevertheless, in both species protein contents of seeds harvested from dehydrated pre and post-flowering plants declined as compared to the original seeds.

4.4 Discussion

This study examined the quality and composition of maize and sorghum seeds harvested from plants subjected to pre and post-flowering dehydration. Theses are developmental stages during which both rainfed maize and sorghum frequently experience intense drought stress under field conditions.

The greatest reduction in percentage germination observed in maize seeds harvested from both pre and post-flowering dehydrated plants (Fig. 4.1) suggests that development of maize grains, irrespective of developmental stages is more susceptible to both pre and post-flowering dehydration than sorghum seeds. According to Roberts (1972), the decrease of essential metabolites, including loss of precursors for storage reserves (such as free sugars), are important factors responsible for loss of seed viability. The present study showed that starch content is not correlated with loss of viability of maize seeds, since starch contents of both maize and sorghum seeds harvested from both pre and post-flowering dehydrated plants did not decrease relative to the control. It is, however, apparent that there are significant decreases in storage reserves of maize seeds such as soluble sugars (sucrose, fructose and glucose), lipids and proteins from both pre and post-flowering dehydrated plants. This decrease in the storage reserves could be responsible for loss of viability and vigor of maize seeds. The situation in sorghum is different from that of maize. Although a decline in storage reserves were also observed in sorghum seeds harvested from pre-flowering dehydrated plants, storage reserves do not appear to have a significant effect in the germinability of sorghum seeds, since percentage germination of sorghum seeds from pre-flowering dehydrated plants was not significantly decreased. On the other hand, with the exception of glucose that was markedly reduced; sorghum seeds harvested from post-flowering dehydrated plants maintained high contents of sucrose and fructose underwent little or no change as compared to the control (Fig. 4.3-Fig. 4.5). Despite high concentrations of sugars, percentage germination of sorghum seeds harvested from post-flowering dehydrated plants markedly decreased. It was proposed that species with high concentrations of sugars could have a more negative osmotic potential during seed hydration (Douglass et al, 1993) and when sorghum seeds were planted in paper towel, the steep osmotic gradient induced the rapid influx of water leading to membrane disruption and the leakage of the water soluble sugars from the

endosperm resulted in the reduction of the energy pool available to the embryo and ultimately reduced percentage germination.

Sorghum seeds showed age dependent difference in vigour after AA (Fig. 4.2b). The reduction in vigour of sorghum seeds harvested from post-flowering dehydrated plants after exposure to high temperature and high % RH suggest that the effect of poor storage conditions could lead to remarkable reductions in vigour and seedling establishment under field conditions if the plants had been subjected to drought during post-flowering stage. It was proposed that dehydration during post-flowering (grain filling stage) shortens the duration of grain filling by causing premature desiccation of the endosperm and by limiting embryo volume (Westgate, 1994; Tuinstra et al, 1997). This premature desiccation and limited embryo volume could be responsible for the observed decrease in vigour in aged sorghum seeds. This decrease in vigour of sorghum seeds after AA is in accordance with the results of a number of studies on other seed species (Lin and Pearce, 1990; Ray et al, 1990; Basavarajappa et al, 1991; Gurmu and Naylor, 1991 and Madhava Rao and Kalpana, 1994). The reduction in vigour could be attributed to changes in storage reserves that occur during ageing that will ultimately lead to poor germination, reduced seedling growth rates, decrease tolerance to adverse environmental conditions during germination and loss of germinability under field conditions (Gurmu and Naylor, 1991; Bingham et al, 1994). During AA, a decrease in starch and soluble sugar contents in pigeon pea seeds was observed (Madhava Rao and Kalpana, 1994). Basavarajappa et al, (1991) also reported a decline in starch, total carbohydrate and reducing sugar concentrations during AA of maize seeds. Although storage reserves were not quantified during AA of these species, exposure to further high temperature and high % RH conditions (ageing) could have led to depletion of storage reserves and resulted in reduced vigour. A loss in the integrity of the plasma membrane and changes in enzyme activities has been demonstrated in aged seeds of maize (Basavarajappa et al, 1991) and rice (Ghosh et al, 1981) by the extent of leakage of cytoplasmic components to the external medium. It is possible that similar changes in seeds of sorghum could have occurred during AA. The decline in vigour of maize seeds harvested from pre-flowering control plants may have been associated with the fact that maize plants have a larger leaf

area and greater overall plant size, and thus were exposed to higher light intensity and temperature conditions than sorghum plants (refer to chapter 2). These could possibly have led to abbreviated period of grain development which in turn resulted in poor seed quality and thus reduced vigour.

Dehydration during reproductive stages (anthesis and post-flowering) frequently reduces grain yield of maize (Ouattar, et al, 1987; Westgate, 1994) and sorghum (Rosenow et al; 1997, Tuinstra et al, 1997). Because dehydration during reproductive stages shortens the duration of grain development by causing premature desiccation of the endosperm and by limiting embryo volume, seed size is reduced (Westgate, 1994; Tuinstra et al, 1997). In the previous section (refer chapter 2), pre and post-flowering dehydration decreased final grain yield of maize and sorghum when compared to their respective control seeds. Visual observations indicated that seeds of both species harvested from pre and post-flowering dehydrated plants were smaller than their respective original and control seeds. This decrease in the size of seeds from pre and post-flowering dehydrated plants suggested that dehydration during pre and post-flowering stages of maize and sorghum resulted in premature ceasation of grain filling. The results found in this study is in agreement with the observation made by Westgate (1994) and Ouattar et al, (1987) in maize and Brooks et al, (1982) in wheat and barley. Reserve carbohydrate mobilization to the grain is affected by sink size, by the environment and by cultivar (Blum, 1996). Previous studies have shown that in maize subjected to water deficits during anthesis and grain filling stages, reserve carbohydrates were mobilized from the vegetative tissue and accumulated in the ovaries to levels similar to or higher than the control (Westgate and Thompson Grant, 1989). The results of the present study, however, indicated that the response of soluble sugar (glucose, fructose and sucrose) deposition in maize seeds was markedly decreased in both pre and post-flowering dehydrated plants (Fig. 4.3-Fig. 4.5). This would suggest that the soluble sugar deposition in maize grain development may have been limiting. Stem reserve mobilization has been reported to be affected by water deficits. Under dryland field conditions in spring wheat genotypes only half the amount of water soluble carbohydrates was available for remobilization during grain filling, as compared with irrigated conditions (Winzeler et al, 1989). Even the rate of development

of water deficit may affect mobilization (Blum, 1996). Palta et al, (1994) found that total grain carbon with rapid development of water deficit was reduced by 24% compared to slow development of stress. Since development of water deficit during pre and post-flowering stages in maize was relatively fast, availability of these sugars could have been limited as photosynthesis was rapidly inhibited with increase in intensity of dehydration (refer chapter 2; Fig. 2.1 and Fig. 2.7a and b). It is possible that dehydration during pre and post-flowering stages in maize plants could selectively increase the resistance to soluble sugar movement from the vascular bundle into the endosperm, thereby decreasing the concentration in the latter (Brooks et al, 1982). Stem reserve mobilization is a solid source of carbon for grain filling under any stress which would inhibit current photosynthesis (Blum, 1996). Since dehydration-induced senescence in maize was extensive (refer to chapter 2), it is possible that soluble sugars might have been available from stem reserves which was ultimately metabolized to starch during maturation, as the starch in these seeds was retained to the control level.

In sorghum seeds, dehydration during pre and post-flowering stages showed markedly different effects on soluble sugar deposition. As with maize, sugar deposition (glucose, fructose and sucrose) in sorghum seeds was affected by dehydration but a marked decrease was observed in only seeds harvested from plants dehydrated during pre-flowering compared to post-flowering stage (Fig. 4.3-Fig. 4.5). This is in agreement with the findings of Westgate and Thomson Grant (1989) who reported the level of carbohydrate concentration in maize was more severely affected by water deficits during anthesis than mid-grain filling stage. Since dehydration during pre-flowering stage coincides with zygote formation, endosperm cell division and early grain growth which clearly are sensitive to severe dehydration and developmental failure at low relative water contents has often been observed (Schoper et al, 1987; Westgate and Thomson Grant, 1989). Subsequently, the sink size of the grains might have been reduced by the effects of the dehydration because of the lower number of endosperm cells. It is possible; therefore, that the decrease in soluble sugars in pre-flowering dehydrated sorghum seeds was a consequence of low demand for assimilates by the main sink (developing grain). Previously, Nicolas et al, (1985) have shown that because of reduced number of endosperm cells and starch granules the sink size of the grains in wheat was reduced

under drought. On the other hand, in post-flowering dehydrated sorghum seeds, with the exception of glucose concentration, fructose was at a similar content to the original and control seeds, and sucrose markedly increased as compared to the original and control seeds. It has been suggested that, longevity of a leaf is intimately related to its carbon and nitrogen status (Thomas and Rogers, 1990; Van Oosterom, 1996), and the subsequent loss of photosynthetic capability are linked to the rate of assimilate exports (Thomas and Smart, 1993). In this study, sorghum leaves retained green leaf area for longer duration as compared to maize during post-flowering dehydration and this could be one factor for the deposition of higher concentration of soluble sugars in the former than in the later. Furthermore, during post-flowering dehydration sorghum leaves maintained turgor to a greater extent and for longer duration (chapter 2) and this may have been associated with osmotic adjustment. As osmotic adjustment is mainly due to sugars and K^+ (Morgan, 1984), it is possible that sorghum may have adjusted osmotically better than maize. Osmotic adjustment allows slower decrease of photosynthesis and for it to continue at reduced level under dehydration. The continued rate of photosynthesis allows remobilization of current assimilates to the growing grains by reducing leaf senescence (Ludlow and Muchow, 1990). Since sorghum had higher rates of photosynthesis as compared to maize during post-flowering dehydration (chapter 2), this could explain why sorghum seeds exhibited relatively higher soluble sugars.

Sucrose is considered to be the primary carbon source for starch synthesis (Duffus and Duffus, 1984). In the present study starch deposition in both maize and sorghum seeds were not affected by dehydration during pre and post-flowering stages (Fig. 4.6). This indicated that the decrease in sucrose deposition in maize seeds harvested from pre and post-flowering dehydrated plants and sorghum seeds harvested from pre-flowering dehydrated plants did not result in reduction of starch synthesis. It is probable that a part of accumulated sucrose in seeds of maize and sorghum was metabolized to starch. It is well known that starch content increases under the conditions where sucrose content is increased (Nakamura et al, 1991). If this is also the case with sorghum seeds harvested from post-flowering dehydrated plants, that the stress causes an increase in sucrose concentration and did not affect starch deposition may be explainable.

The ultimate carbon source for the formation of storage lipids by developing seeds is usually sucrose (Murray, 1984). The observed decrease in storage lipid in maize seeds harvested from pre and post-flowering dehydrated plants and sorghum seeds harvested from pre-flowering dehydrated plants (Fig. 4.7) may suggest that sucrose might have been unavailable in sufficient amount for the biosynthesis of lipids, since sucrose concentrations in these seeds were low. Sorghum seeds harvested from post-flowering dehydrated plants might be expected to maintain relatively high concentrations of storage lipid due to their increased concentration of sucrose. It appears, however, that storage lipids deposition might have ceased as a result of the effect of dehydration during the final stage of grain filling and the enzymes involved might have been destroyed and their synthesis stop (Bewley and Black, 1994).

It has been reported frequently that the percentage of protein or of nitrogen in grain is increased by plant water deficits (Brooks et al, 1982; Barber and Jessop 1987; Garrot et al, 1994). In severe cases, however, translocation of nitrogen may be inhibited to the extent that mobilized amino acids become trapped in senescing leaf tissues (Tully et al, 1979). In the present study, the decrease in protein content of maize seeds harvested from pre-flowering dehydrated plants (Fig. 4.8a) may have been due to inhibition of nitrogen remobilization as a result of development of rapid water deficit, although this was not measured. Jenner et al, (1991) suggested that rapid onset of water stress severely curtailed nitrogen remobilization through translocation effects. However, the increase in protein concentration in seeds harvested from post-flowering dehydrated maize plants as compared to the control seeds may be due to the lower water content of stressed grains at maturity. This result is in agreement with the findings of Brooks et al, (1982) who reported an increase in the amino acid concentration of barley seeds by water deficits at grain filling stage. It is well known that higher specific leaf nitrogen is closely associated with 'stay-green' genotypes compared with 'intermediate' and 'senescent' genotypes at anthesis, mid-grain filling and maturity (Thomas and Rogers, 1990). In crops adapted to semi-arid areas such as maize, sorghum and wheat, genotypes with delayed senescence that retain their leaves in an active photosynthetic state during the grain filling period are proposed to have enhanced the stress tolerance due to increase the assimilate supply for

grain filling and maintaining the root function and water and nutrient uptake, or both (Van Oosterom et al, 1996). We propose that as a result of delayed senescence, sorghum seeds in the present study harvested from pre-flowering dehydrated plants were able to increase protein concentration as compared to the control seeds (Fig. 4.8b), presumably due mainly to high rate of export of nitrogen from the leaf tissue. On the other hand, protein concentration in sorghum seeds harvested from post-flowering dehydrated plants was markedly reduced. This may have been due to reduced uptake of soil nitrogen under dehydration conditions, as soil nitrogen could be less available to the dehydrated plants because smaller soil water was available for diffusion and the soil solution eventually became discontinuous when the soil moisture decreased (Nicolas et al, 1985).

4.5 Conclusions

This study demonstrated that dehydration at pre and post-flowering stages influenced seed quality (standard germination and vigour) of both maize and sorghum, but sorghum seeds were more tolerant to dehydration irrespective of developmental stages than maize seeds. Sorghum appeared to lose vigour only when dehydration occurred during post-flowering stage.

Remobilization of reserve assimilates is an important strategy of increasing harvest index under dehydration that occurred during pre and post-flowering stages and consequently drought resistance. It is proposed that genotypes which have the capacity to remobilize reserve assimilates under drought stress during reproductive stages would tend to improve yield stability (Ludlow and Muchow, 1990).

Therefore, it is concluded that under the conditions of this study sorghum appeared to be superior than maize. However, further studies under more realistic conditions are necessary to generate additional and conclusive information.

Chapter 5.

5. Summary, Conclusions and Recommendations

5.1 Summary

Climate influences plant life in many ways and can inhibit, stimulate, alter or modify crop performance. Maize and sorghum are important cereal food crops in many parts of the semi-arid regions of the world. Since both species are cultivated almost entirely under rain dependent conditions by subsistence farmers, water deficit stress is one of the major environmental stresses encountered by these species in the semi-arid areas. In both species, reduction in yield due to drought depend on the developmental stages at which drought occurs. Although several studies have investigated physiological and morphological traits in various crop plants to develop effective screening methods for drought tolerance, consistent relationships between variations of physiological, morphological and biochemical traits have not been observed in maize and sorghum for drought tolerance. Attempts to incorporate biochemical protection mechanisms as selection criteria in these species for drought tolerance are very scant. A possible avenue to use physiological, morphological and biochemical traits as selection criteria during recovery after exposure to dehydration have not been addressed. The aim of the present study was to examine the physiological (plant water relations, gas exchange characteristics, membrane leakage and photosynthetic pigment compositions) and biochemical (antioxidant protection mechanisms) responses and resultant seed quality response of maize and sorghum after exposure to and recovery from pre and post-flowering dehydration. Pre and post-flowering developmental stages were chosen because water deficit at these developmental stages are known to cause the greatest reduction in grain yield of both species.

The identification of underlying physiological, morphological and biochemical traits that can enable superior performance (yield stability) of crop plants under water deficit conditions is important for development of selection criteria for drought resistance to complement conventional plant breeding programs.

In chapter 1 a wide variety of adaptation mechanisms to drought stress and their relative contribution to crop yield was reviewed. This included a synopsis of drought induced injuries and associated protection mechanisms, synthesis of abscisic acid, expression of dehydration-induced proteins and genes as related to crop plants.

Chapters 2-4 present data from the physiological, biochemical and seed studies respectively. The data were interpreted and discussed in relation to current data on only crop plants.

The major conclusions from this study are summarized as follows:

5.2 Conclusions

5.2.1 Dehydration

The physiological and morphological responses of maize and sorghum to pre and post-flowering dehydration and subsequent rehydration (chapter 2) revealed that both species employed dehydration avoidance strategies. There were differences between the two species when exposed to moderate dehydration (approximately between RWCs of 95% and 65%; 0 to 10 days after dehydration began), but as RWCs declined further the differences between them disappeared.

Maize was found to lose RWCs faster than sorghum during the early phase of dehydration. During the course of dehydration period, no differences were observed in the mean RWCs between pre and post-flowering stages for both species. The most obvious visual morphological differences was that sorghum delayed leaf rolling for an extended period during both pre and post-flowering dehydration whereas during the early phase of pre-flowering dehydration maize displayed leaf rolling, a typical symptom of turgor loss. As a result, leaf rolling appeared to be the earliest dehydration avoidance mechanism in maize, and this trait appears to be an important tool for identification of cultivars for drought resistance in these C_4 species.

While stomata remain partially open in sorghum, early and complete stomatal closure was a typical response of maize during both pre and post-flowering dehydration. The rapid decline in all gas exchange characteristics (P_n, transpiration and respiration rates),

WUE and increase in Ci in maize during the early phase of pre and post-flowering dehydration (moderate dehydration) is a consequence of early stomatal closure. Sorghum displayed other characteristics which are often associated to drought resistance: delayed chlorophyll (commonly known as stay-green in cereal crops) and carotenoid loss and P_n continued at a reduced rate until RWCs reached to approximately 55% during both pre and post-flowering dehydration. Maize on the contrary displayed extensive early chlorophyll and carotenoid degradation and exhibited a rapid decline of P_n during the early phase of dehydration. On the basis of the strategies employed by both species, it may be concluded that sorghum by delayed stomatal closure and delayed chlorophyll and carotenoid breakdown could all together be factors that enabled to continue P_n at reduced rate and maintain higher WUE compared to maize during both pre and post-flowering dehydration. On the other hand stomatal regulation is a principal dehydration avoidance mechanism in maize.

A common characteristic in response to pre and post-flowering dehydration was that an increase in the rate of electrolyte leakage and a decrease in Fv/Fm ratio were noticed at RWCs below 60%, with the effect being greater in both species during post than pre-flowering dehydration. This suggests that in both species post-flowering stage is susceptible to severe dehydration than pre-flowering stages.

The present study also revealed that exposure of maize and sorghum to pre and post-flowering dehydration resulted in an increase in the activities of several enzymatic and contents of non-enzymatic antioxidants which are involved in the detoxification of reactive oxygen species (chapter 3). Under both pre and post-flowering dehydration conditions, sorghum exhibited a consistent and comparatively higher increases in all enzymatic and non-enzymatic antioxidant activities than maize. This resulted in minimizing the extent of lipid peroxidation (as determined by Malondialdehyde content) but not in maize. It is proposed that a capacity to minimize membrane damage to a reparable level by controlling lipid peroxidation may be an important mechanism of drought resistance in plants. Whereas in maize with the exception of superoxide dismutase and ascorbate peroxidase, the activities of other enzymatic (glutathione

reductase and catalase) and contents of non-enzymatic antioxidants declined with increase in the intensity of pre and post-flowering dehydration. This might be the reason why maize had high amount of malondialdehyde and extensive chlorophyll loss. Since sorghum exhibited comparatively over all higher activities of enzymatic and non-enzymatic antioxidants during both pre and post-flowering dehydration, selection based on these criteria may help in the development of genotypes tolerant to dehydration. However, several other antioxidants which were not measured in the present study and could eliminate reactive oxygen species or limit dehydration induced damage in both species need in depth study in this direction.

Percentage germination of seeds harvested from pre and post-flowering dehydrated sorghum plants were not affected whereas maize seeds were more adversely affected, suggesting that development of maize seeds irrespective of developmental stage is more susceptible to pre and post-flowering dehydration than sorghum seeds. In addition vigour of sorghum seeds harvested from pre-flowering dehydrated plants was not affected when subjected to accelerated ageing, whereas seeds harvested from post-flowering dehydrated plants was markedly reduced by this measurement suggesting that vigour of seeds harvested from post-flowering dehydrated plants is more susceptible than seeds from pre-flowering dehydrated plants.

With the exception of starch, dehydration significantly affected the quantity of sugars (sucrose, glucose and fructose) lipid and protein in both species as compared to the control seeds. Dehydration consistently reduced sugars (glucose, fructose and sucrose) and lipid contents of maize seeds harvested from both pre and post-flowering dehydrated plants. This could have been a result of reduced stem reserve mobilization to the developing grains. Sugar deposition in sorghum seeds was affected by dehydration but a marked decrease was noted in only seeds harvested from plants dehydrated during pre-flowering stage. This could have been a consequence of low demand for assimilates by the main sink (developing grains). Fructose was not affected and sucrose markedly increased in sorghum seeds harvested from post-flowering dehydrated plants as compared to the original and control seeds. This was probably a result of effective mobilization of

assimilates to the growing grains from photosynthetically active leaves. On the other hand, as a result of delayed senescence, sorghum seeds harvested from pre-flowering dehydrated plants were able to increase protein concentrations as compared to the control seeds. But the decrease in seeds harvested from post-flowering dehydrated plants may have been a consequence of reduced uptake of soil nitrogen. The decrease in protein concentration of maize seeds harvested from pre-flowering dehydrated plants may have been due to inhibition of nitrogen remobilization as a result of development of rapid water deficits. However, the increase in protein concentration in seeds harvested from post-flowering dehydrated plants as compared to the control seeds may be due to the lower water content of stressed grains at maturity. The capacity to remobilize reserve and assimilates to the growing grains under drought stress is a dehydration tolerant processes in crop plants (Blum, 1988).

5.2.2 Rehydration

g_s of maize and sorghum plants undergoing pre-flowering dehydration were able to fully recover to the control level upon rehydration. On the other hand, g_s of both species did not recover to the control level following post-flowering rehydration. It is possible that damage on drying was more deleterious at post-flowering than pre-flowering or the repair on rehydration was less effective on post-flowering rehydration. The lack of recovery of other gas exchange characteristics (P_n, E, R_d) and WUE during post-flowering stage upon rehydration is probably a consequence of g_s.

Fv/Fm ratios and rate of electrolyte leakage of both pre and post-flowering dehydrated maize plants and pre-flowering dehydrated sorghum plants were able to recover to the control level, suggesting a complete repair of photosynthetic apparatus and cell membranes upon rehydration. In sorghum, the damaging effects of dehydration were worsened by post-flowering rehydration. The ability of maize and sorghum plants to repair the photosynthetic apparatus and cell membranes upon rehydration is a desirable trait and merits further detailed investigations under more realistic conditions for use as a tool in the development of drought resistant cultivars.

Photosynthetic pigment compositions (chlorophyll and carotenoid) of both maize and sorghum were higher during pre-flowering than post-flowering rehydration. Recovery of chlorophyll and carotenoid contents to the control level was noticed in sorghum during pre and post-flowering rehydration. This may be due to photosynthetic pigment synthesis upon rehydration. On the other hand, there was no change in the pigment composition of maize plants undergoing pre and post-flowering rehydration. However, because there was a marked decrease in the control plants both chlorophyll and carotenoid contents were at the control level during both pre and post-flowering rehydration. We therefore suggest that selection for drought resistance on the basis of pigment compositions in sorghum plants during pre and post-flowering rehydration could contribute in the development of drought resistant cultivars.

An increase in enzymatic antioxidant (Superoxide dismutase, Glutathione reductase, Catalase and Ascorbate peroxidase) activities during the late phase of rehydration following pre-flowering dehydration was noticed in sorghum. In addition, these enzymes were retained elevated during post-flowering rehydration in sorghum as compared with their respective control suggesting that these enzymes are actively involved in detoxification of reactive oxygen species during the process of both pre and post-flowering rehydration. The increase in glutathione reductase and catalase activities during the late phase of pre-flowering rehydration in maize may indicate that the operation of these enzymes appeared to be more efficient when maize regains relatively higher relative water contents. On the other hand, the retention of superoxide dismutase and ascorbate peroxidase activities at high levels during post-flowering rehydration may be viewed as a key factor in controlling the oxidative stress that might have occurred during the rehydration process and providing additional protection against reactive oxygen species.

While α-tocopherol content decreased to the control level in maize during pre and post-flowering and sorghum during pre-flowering rehydration, the retention of α-tocopherol contents up-regulated in sorghum during post-flowering rehydration may help sorghum plants to eliminate $^1O_2$ which are formed during rehydration as a result of excited triplet

chlorophyll with oxygen in the pigment bed and minimize membrane damage. Reduced glutathione, oxidized glutathione contents and GSH:GSSG ratio were retained elevated in both species during post-flowering rehydration and could play an important role in protecting against reactive oxygen species that might have been formed during post-flowering rehydration.

Since an enhancement in the antioxidant defence system is considered as an adaptive mechanism of environmental stress, the activities of these antioxidants in the rehydration cycle during pre and post-flowering stage may be a good indicator for selecting drought resistant crop plants. We, therefore, suggest that breeders should opt for screening for cultivars which possess a capacity to enhance enzymatic and non-enzymatic antioxidants. However, further investigations are required with respect to the protective advantages of enzymatic and non-enzymatic antioxidants in response to rehydration during both developmental stages in both species.

Variability between species was noticed in the amount of malondialdehyde contents in response to pre and post-flowering rehydration. During pre-flowering rehydration malondialdehyde levels were reduced to 85% of the control while in sorghum the tissues damaged by ROS were repaired as with the control level. During post-flowering rehydration maize had 85% and sorghum had 95% less malondialdehyde contents as compared with the control. Under the condition of our experiment malondialdehyde contents provides a good indication of tissue damage due to oxidative stress and may be used as a tool in screening cultivars for drought resistance at various developmental stages.

5.3 Recommendations

Although we recognized that a remarkable work has been done towards the effects of dehydration on the physiological, morphological and biochemical responses in wide varieties of cultivated crops, much of the works has been focused at early seedling and vegetative stages. Very little work has been directed towards the effects of rehydration following dehydration during pre and post-flowering stages in crop plants. Moreover, most of the studies were undertaken under controlled environments using small

containers. This study represents an initial attempt to examine the response of these C_4 crop species after exposure to and recovery from pre and post-flowering dehydration. The results indicated that there are indicators to be used for improving the drought resistance, and improving the potential of the current low and variable production of these species in the semi-arid areas. This can be achieved by integrating all possible ways, including utilization of morphological, physiological, biochemical indirect selection traits in conjunction with yield testing across different target environments. However, since the study was carried out under controlled environment excluding other interacting factors which occurred under natural environmental conditions, extrapolating the results would be difficult. Future investigations on the physiological and biochemical traits of these species should, therefore, focus on:

1. Physiological, morphological and biochemical responses of maize and sorghum after exposure to and recovery from pre and post-flowering dehydration under field conditions where other interacting factors are involved.
2. Future studies should include long maturing genotypes, since subsistence farmers in the semi-arid areas largely depend on long maturing genotypes.
3. In depth studies on the response of antioxidant defence mechanisms to dehydration and rehydration of these species should also include other enzymatic antioxidants.
4. Sorghum and maize are major important staple crops in the semi-arid areas and plays a significant role in the nutrition of poor farmers in the regions. Therefore, to insure the nutritional requirements of subsistence farmers, it is important that attention needs to be emphasised towards the effects of dehydration and rehydration during pre and post-flowering stages on seed qualities of diverse genotypes.

Chapter 6

6.1 A synthesis

It is well documented that dehydration is one of the most common environmental stresses to which plants are exposed, and in many regions it is the bottleneck of agricultural development. It appears that the intrinsic ability of a plant to tolerate dehydration stress is a result of different physiological, morphological and biochemical mechanisms, and the elucidation of the nature of these mechanisms and then the use of some of the mechanisms in the breeding for drought tolerance is a major issue. The purpose of this chapter is to try to integrate the physiological, morphological and biochemical mechanisms of maize and sorghum after exposure to and recovery from pre and post-flowering dehydration and to make clear how these mechanisms could be exploited with implications for plant breeding to increase food production in Africa.

One approach to improve crop performance in drought stressed environments is to select for genotypes that have improved yield in this environments. This approach has proved partially successful, but difficult due to the variability of rainfall and the polygenic nature of drought avoidance and tolerance (Mullet and Whitsitt, 1996). A complementary approach to improve plant performance in drought stressed environments involves the identification of secondary traits that contribute to drought avoidance, tolerance or water use efficiency (Blum, 1988; Ludlow and Muchow, 1990; Turner, 1997).

g_s has been shown to be associated with performance of various cereal crops under drought stress conditions (Reynolds et al, 1994; 1999; Fischer et al, 1998). Evaluation of g_s is highlighted by studies suggesting that g_s, is a better indicator of the plant water status than, for example, the water potential or the relative water content, with changes in photosynthetic metabolism during progressive drought being tracked by g_s (Flexas et al, 2000). In our study, g_s appeared to be a good indicator of plant water stress, since differences in g_s was observed between maize and sorghum during pre and post-flowering dehydration. The ability of sorghum to have partially opened its g_s during both pre and post-flowering dehydration was partially implicated in the maintenance of relatively high rate of photosynthesis, E and water use efficiency. Relatively high rate of photosynthesis observed in sorghum than maize during both pre and post-flowering

dehydration also enabled seeds of the former to efficiently accumulate soluble sugars, protein and lipid reserves better than the latter. Moreover, leaf cooling due to relatively high rate of E as a result of partially opened g_s in sorghum than maize contributed to improvement of the photosynthetic activity of leaves and prevents premature ageing (retain green leaf area). g_s is therefore a good indicator of a genotype's physiological fitness, since a relatively high value is indicative of good expression of all those traits under a given set of environmental conditions.

Where there is decrease in water availability, increased water use efficiency appears to be an alternative strategy for improving crop performance. Therefore, genotypes producing greater biomass and yield due to superior water use efficiency are selected in the breeding program. Techniques that may have applications in screening for physiologically superior genotype are leaf chlorophyll content and leaf area. These parameters are related to the photosynthetic size of a canopy and include green biomass and leaf area. As was observed in this study, genetic variation exists for stay green character in cereals. Our result indicated that sorghum by retaining its green leaf area better than maize during post-flowering dehydration enhanced its drought stress tolerance by increasing the assimilate supply during grain filling. Since maintaining green leaf area during post-anthesis drought increase grain yield, compared with senescent genotypes, we suggest that future research in crop improvement should aim at delaying the onset of leaf senescence or to reduce its rate.

It is generally accepted that the maintenance of membrane integrity and stability and development of highly efficient antioxidant defense mechanisms against reactive oxygen species under dehydration conditions is a major component of drought tolerance in plants. Rate of electrolyte leakage as a measure of cell membrane stability have been found highly correlated to yield stability in several cereal crops under stress conditions that have a dehydrative component and suitable to be used in a breeding program (Blum and Ebercon, 1981). The metabolic indicators of drought tolerance are antioxidant defense mechanisms composed of both enzymatic and non-enzymatic antioxidant constituents. In the present study we have identified different physiological and metabolic indicators of drought tolerance in both maize and sorghum. Particularly sorghum appeared to be characterized by an efficient antioxidant defense system constituted by

both enzymatic and non-enzymatic components. Although metabolic traits particularly antioxidant defense systems have seldom been used successfully as selection criteria for crop plants in breeding programs, we suggest that these traits can contribute greatly to enhance the survival of crop plants under sever dehydration conditions and increase yield stability, if they are used as selection criteria in the breeding programs.

In conclusion, physiological and biochemical mechanisms identified as drought resistance traits in response to pre and post-flowering dehydration and rehydration in both sorghum and maize could be used in two ways.

1. Traits of interest can be used as selection criteria to complement conventional breeding programs.
2. By using molecular marker assisted selection, useful traits most likely to be important in determining yield under dehydration conditions can be identified and introduced in to improved varieties.

Chapter 7

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