DROUGHT TOLERANCE STUDIES IN SPRING WHEAT CULTIVARS PRODUCED IN SOUTH AFRICA

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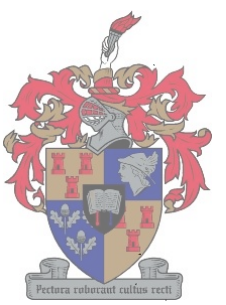

Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entire entity or in part submitted it at any university for a degree.

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Abstract

Drought is considered, worldwide, to be the most important factor limiting crop yields. Spring wheat produced in the Western Cape region of South Africa is also affected by water stress. The onset of global warming may cause periods of water stress in the winter rainfall regions of South Africa more frequently, which makes it important to identify spring wheat cultivars that can withstand these conditions. The aim of this study was to determine whether cultivar differences in drought tolerance could be detected through the use of a series of physiological tests on spring wheat plants subjected to water stress at different growth stages. Furthermore to determine whether differences in physiological responses would be of any commercial value. To evaluate this, plants were analyzed to compare biomass production, yield and quality of stressed and control (unstressed) plants.

A preliminary trial showed that the withholding of water might be more appropriate in the induction of water stress than the use of polyethylene glycol. The trial also showed that the reduction of 2,3,5-triphenyltetrazoliumchloride (modified method) was not sensitive enough to detect water stress in spring wheat.

The evaluation of the physiological parameters showed that differences in drought tolerance do exist in spring wheat cultivars produced in the Western Cape region. The accumulation of proline and the water content of leaves proved to be the most sensitive parameters tested. A combination of these parameters may provide valuable information in newly bred spring wheat cultivars.

In a study on biomass production it was shown that the above ground biomass was reduced by applied water stress. No clear distinctions in drought tolerance could, however, be made between cultivars. The use of leaf area (rate of leaf abscission) shows promise as a method to distinguish between drought tolerant cultivars.

Yield and the yield components of all cultivars tested were severely reduced by water stress at both the flag leaf -, milky kernel growth stage. Although reductions in yield and yield components was shown, no single cultivar proved more tolerant than the other.

The application of water stress resulted in a general increase in kernel protein content. Flour yield was lower, due to a relatively smaller production of non-protein components in the kernel. Although protein content increased with increased water stress, no significant differences were noted in micro-loaf volumes. The results of the mixograph parameters tested were also similar.

Uittreksel

Droogte word wêreldwyd beskou as een van die belangrikste faktore wat gewasopbrengste beperk. Lentekoringkultivars wat in die Wes-Kaap streek in Suid-Afrika verbou word, word ook deur waterstremming beïnvloed. Die koms van die kweekhuis-effek mag veroorsaak dat die winterreënvalgebiede van Suid-Afrika meer en meer bloogestel word aan periodes van waterstremming. Dit is dus belangrik om kultivars te identifiseer wat heirdie toestande kan weerstaan. Die doel van die studie was om te bepaal of kultivarverskille ten opsigte van droogteverdraagsaamheid in lentekoring kultivars, wat aan waterstremming tydens verskillende groeistadiums, blootgestel is, geïdentifiseer kan word. 'n Reeks fisiologiese toetse is gebruik om die bepalings te doen. Verder is die biomassa produksie, opbrengs en kwaliteit van gestremde en kontrole (geen stremming) plante ontleed om te sien of verskille in fisiologiese reaksies van enige kommersiële waarde is.

Tydens 'n loodsproef is aangetoon dat die onthouding van water dalk meer geskik is as die gebruik van poli-etileen glikol om waterstremming te induseer. Verder is ook aangetoon dat die reduksie van 2,3,5-trifenieltetrazoliumchloried (aangepaste metode) nie sensitief genoeg was om waterstremming in lentekoringkultivars uit te wys nie.

Die evaluering van die fisiologiese parameters het daarop gewys dat daar wel verskille in droogteverdraagsaamheid bestaan tussen lentekoring kultivars wat in die Wes-Kaap verbou word. Die ophoping van vry prolien en die waterinhoud van blare het geblyk die sensitiefste parameters te wees. 'n

Kombinasie van al die getoetste parameters kan waardevolle inligting verskaf in die teel van nuwe lentekoringkultivars.

In 'n studie van biomassaproduksie is aangetoon dat biomassa van lentekoring verlaag word deur waterstremming tydens die vlagblaar- en melkdeegstadium individueel en tydens beide groeistadiums gekombineerd. Geen duidelike verskille in droogteverdraagsaamheid tussen verskillende kultivars kon egter verkry word nie. Die gebruik van blaaroppervlaktebepalings toon egter belofte as 'n metode om tussen kultivars vir droogteverdraagsaamheid te onderskei.

Die opbrengs en opbrengskomponente van lentekoringkultivars is erg verlaag deur waterstremming tydens die vlagblaar-, melkdeeg- en beide groeistadiums gekombineerd. Alhoewel verlagings in die opbrengs van lentekoring gevind is, het geen kultivar beter gevaar as 'n ander opbrengs as parameter gebruik word nie.

Die indusering van waterstremming het 'n algemene verhoging van korrelproteïen veroorsaak. Meel opbrengs is verlaag deurdat 'n verlaging in die produksie van die nie-proteïen komponent van die korrels veroorsaak is. Alhoewel korrelproteïen verhoog is, is geen betekenisvolle verskille in die mikro-broodvolumes of die mixogram-parameters vir die getoetste kultivars waargeneem nie.

THIS THESIS IS DEDICATED TO MY MOTHER; THANK YOU FOR ALL THE OPPORTUNITIES AND YOUR ENLESS LOVE

“Facts are stubborn, but statistics are more pliable.”
- Mark Twain -

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List of Abbreviations

ABA	Abscisic Acid
C X T	Cultivar by Treatment
CUL	Cultivar
CV	Coefficient of variation
HI	Harvest Index
kPa	kilopascal
LDR	Leaf diffusive resistance
MS	Mean square
PEG	Polyethylene glycol
RWC	Relative water content
ST	Stress treatment
TKM	Thousand kernel mass
TRANS	Transpiration rate
TRT	Treatment
TTC	2,3,5-Triphenyltetrazoliumchloride
WOW	Withholding of water
WUE	Water use efficiency

Contents

Language and style used in this thesis are in accordance with the requirements of the South African Journal of Plant and Soil. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some redundancy between chapters has, therefore, been unavoidable.

Chapter 1

Introduction

The population of the world continues to increase at an incredible rate. Approximately 100 million people are added to the global population of ± 6 billion every year, adding up to an estimated 8.5 billion by the year 2025 (Turner, 1996). Along with this, the area of cropable land per person will decrease because the area of arable land is stable or declining. The potential for increased irrigation is limited and the increasing future populations will need to be fed from higher food production per unit land area and without the aid of increased irrigation resources.

A large percentage of the world's crops are exposed to chronic or sporadic periods of drought (Boyer, 1982). Even in the absence of drought, the timing and amount of rainfall are primary determinants of crop selection and yield (Boyer 1982; Ludlow & Muchow, 1990). Thus, for improved food production the use of rainfall and irrigation water will need to be utilized more efficiently and the importance of understanding and managing crop water deficits is a necessity.

A number of methods exist for the improvement of the efficiency of water use and have been summarized by Taylor, Jordan & Sinclair (1983) and by Stewart and Nielsen (1990). These methods can be grouped into three broad classes: (1) increasing the efficiency of water delivery and the timing of water application, (2) increasing the efficiency of water use by the plants, and

(3) increasing the drought tolerance of plants. The first method is practiced most because it depends on the basis of engineering and only minimally on the crop. The transportation of water in such a way that evaporation is cut to a minimum, the prevention of runoff, catchments for water storage, delivery of water to the root zone and the timing of irrigation to meet the needs of the plant have been successful when it comes to the improvement of productivity per unit of water delivered to the farm (Boyer, 1996). The second and third methods depend on the understanding of crop biology and whether it can be manipulated to achieve the same level of productivity with a smaller amount of water.

Water use efficiency

Water use efficiency (WUE) is usually defined as the total dry matter produced by plants per unit of water used,

$$WUE = D / W,$$

where D is the mass of dry matter produced and W is the mass of water used. The mass of dry matter produced usually refers to that aboveground and the mass of water used usually includes direct evaporation from the soil (Boyer, 1996). As pointed out by Turner (1996) WUE can be observed at several levels. For an experiment conducted in the field, D and W would be expressed on the basis of land area. For a pot experiment, D and W would be used for single plants and expressed on the basis of the whole plant. Sometimes D is

expressed as the economically valuable part of the crop and WUE refers to the yield. One may also consider the water use efficiency of a single leaf and so on. The higher the production per unit of water, the higher the efficiency.

Much of the improvement in crop yield in the past decade has been as a result of farmers managing their crops in order to achieve their potential WUE (Edwards, 1992). Improvement in WUE has been aided by practices such as earlier planting, use of increased levels of fertilizer (especially nitrogen and phosphorus), stubble retention, minimum tillage and use of rotations to improve the nutrition and root penetration of cereal crops (Cooper, Gregory, Tully & Harris, 1987; French & Schultz, 1994 a and b; Anderson & Smith, 1990 a and b).

WUE - measurements

The most accurate way of measuring WUE is to monitor the evapotranspiration and harvesting the crop at the end of the growing season in order to do biomass measurements. The WUE can be determined for the total biomass or for any part of the biomass. These are labour intensive and costly measurements. Methods that are less expensive have been identified.

One of these methods directly measures the CO_2 and H_2O exchange of individual leaves (Bierhuizen & Slatyer, 1965; Brown & Simmons, 1979; Robichaux & Pearcy, 1984). The gas exchange efficiency can be defined as the ratio of the mass of CO_2 gained to the mass of H_2O lost, because the CO molecule is the main contributor to the dry mass. Another method is based on the relative abundance of natural isotopes in plant tissue. The atmospheric

CO_2 consists of $^{12}CO_2$ and a small amount of $^{13}CO_2$. Because $^{12}CO_2$ molecules are lighter, it diffuses more rapidly than $^{13}CO_2$. The fixation of $^{12}CO_2$ by ribulose-1,5-biphosphate is more rapid than $^{13}CO_2$. The cells thus accumulate relatively more ^{12}C than ^{13}C. The unused C diffuses out according to the extent of stomatal opening and this outward diffusion is correlated with transpiration. Because inward diffusion and the use of $^{12}CO_2$ correlates with photosynthesis and dry mass while the outward diffusion of $^{13}CO_2$ correlates with transpiration, the relative uptake of ^{12}C and ^{13}C correlates with the WUE. Generally, higher WUE correlates with lower tissue ^{12}C relative to ^{13}C for wheat, peanut, barley and wheatgrass (Hubick, Shorter & Farquhar, 1988; Johnson, Asay, Tieszen, Ehlleringer & Jefferson, 1990). The measurements thus detect differences in WUE among individual plants within a species and they only require the ratio of the isotopes in tissue samples to be compared to a standard (Bowman, Hubick, Von Caemmerer & Farquhar, 1989; Brugnoli, Hubick, Von Caemmerer, Wong & Farquhar, 1988; Condon *et al.*, 1987; Condon, Farquhar & Richards, 1990). The ratio technique makes it possible to survey a large number of plants at moderate cost. Genetic variation clearly exists but in crop canopies the variation becomes less clear. Condon and Richards (1993) estimated that the leaf diffusive conductance difference between two wheat genotypes in the order of 40-50% only differed by 15% in canopy transpiration efficiency. This difference was not reflected in improved WUE for the crop in the field because soil evaporation differed in opposition,

thus canceling the 15% effect. Therefore differences in WUE may need to be combined with other crop traits to be realized as water savings.

Drought tolerance - responses and interpretations of adaptation

Drought tolerance is a nebulous term that becomes more nebulous the more closely we look at it (Passioura, 1996). There are no prizes for pointing out that a cactus is more drought tolerant than a carnation. It is when we shift our focus to crop plants that the features that confer drought tolerance are far from clear. Drought resistance is a term that conveys the ability of a crop to produce satisfactorily in areas subjected to water deficits (Fischer, 1981). The mechanisms of drought resistance have been categorized in different ways. All the different ways can be classified into three major categories: (1) drought avoiders, (2) dehydration postponement and (3) dehydration tolerance. Drought avoiders depend on timing of development, which is under internal control. Their tendency is to reproduce after a minimal accumulation of dry matter. Their success ensures that they are represented in the next generation (Alvim, 1960; Alvim, 1965).

Dehydration postponers have deep roots or seal themselves against transpiration or they accumulate water in fleshy tissues. Their adaptations are therefor mostly structural and take time to build up, requiring the expenditure of photosynthetic products. Dehydration tolerators might have the same water use efficiency as dehydration-sensitive species when there is sufficient water available but can grow at levels of tissue dehydration that the other species cannot (Boyer, 1996).

Drought is a multi-dimensional stress factor affecting plants at various levels of their organization (Monneveux & Belhassen, 1996). The effect of and plant response to drought at the whole plant and crop level is most complex because it reflects the integration of stress effects and responses at all underlying levels of organization over space and time (Blum, 1996).

Metabolism diversity of drought tolerance

Stomatal transpiration is the main way of water loss in the plant. When CO_2 enters the stomatal cavity, the result is a water loss, depending on the temperature and hygrometry of outside air. The reduction of water loss can be an important mechanism of drought tolerance. There are three metabolic pathways through which CO_2 assimilation can occur: C3, C4 and CAM species.

In the CAM (Crassulacean Acid Metabolism) species, such as the pineapple, CO_2 assimilation occurs during the night and uses PEP carboxylase, which produces oxaloacetate acid (OAA). The stomata are opened at night and closed during the day in order to avoid stomatal transpiration during the hot period of the day. In C3 plants, CO_2 is assimilated during the day by ribulose bisphosphate carboxylase (Rubisco) and enters the Calvin cycle through 3-phosphoglyceric acid. The difference in water consumption between these two groups of plants is very important to note as the transpiration is 10 to 20 times higher in C_3 species. The third metabolic pathway is found in C_4 plant species. The CO_2 is assimilated in the same way as in CAM plants by carboxylation of PEP to oxaloacetate but this occurs in

the mesophyll cells. Subsequently, OAA is converted to malate which is then transferred to the bundle-sheath cells. Here it is assimilated, after decarboxylation of the C_4 molecules (malate). CO_2 is then fixed by Rubisco and enters the Calvin cycle. Photosynthesis efficiency is also higher in C_4 species than in C_3 species (Monneveux & Belhassen, 1996).

Morphological and physiological diversity and adaptation

Seedling emergence and establishment

Seedling mortality in a drying seedbed is a common problem in drought prone areas (Johnson & Asay, 1993). The problem is aggravated under some conditions by excessively high soil temperatures during the periods of seedling emergence and establishment (Peacock, Miller, Matsuda & Robinson, 1990).

Seedling establishment is a critical stage, when stable rooting and initial green leaf area are attained. Drought-affected stand losses may occur after full emergence but before establishment. Sorghum seedling establishment depends mainly on the development of crown or adventitious roots to support the plant before the seminal root looses its functionality (Blum, Arkin & Jordan, 1977; Blum, 1988). This is not the case in wheat where seminal roots function for most of the plant's life (Blum *et al.*, 1977).

We still lack understanding of the interactions between processes such as germination, emergence and establishment with seed and plant water status. Developmental factors are no less important here than the biochemical and physiological ones (Blum, 1996).

Phenology

Phenology, the developmental stages of the plant, has a powerful effect on plant growth, response to and productivity under drought stress. Drought often delays developmental events because of the inhibition of growth by a water deficit (Bidinger, Mahalakshmi & Roa, 1987; Craufurd, Flower & Peacock, 1993; Donatelli, Hammer & Vanderlip, 1992).

The delaying effect of drought on flowering could perhaps be a result of abscisic acid (ABA) accumulation under the influence of a water deficit. ABA was implicated in the past as causative of flowering delay by drought stress in Lolium (King & Evans, 1977). A review of documented evidence of the effect of ABA on flowering indicated that ABA might delay or advance flowering time, depending on the time when ABA is in effect and the species (Trewavas & Jones, 1991) and possibly the rate of stress. Advanced flowering in wheat was caused by mild stress (Angus & Moncur, 1977) while delayed flowering in wheat was caused by severe stress in wheat (Angus & Moncur, 1977) and barley (Dwyer & Stewart, 1987). Advanced flowering due to mild stress may also stem from plant heating due to reduced transpiration. Under severe stress this effect may be overridden by factors causing delayed flowering (Blum, 1996).

Phenology, and its modifications by drought stress, affects plant production under drought stress in different ways. Water requirement and the probability of exposure to stress, both of which decrease in early flowering genotypes, are determined by growth duration. Longer growth duration is

associated with greater biomass, both above and below ground, leading to greater root length density in soil. Later flowering genotypes thus posses a greater productivity potential and the developmental attributes to achieve that potential in terms of their capacity for resource capture. However, later flowering genotypes of cereals tend to lack in their harvest index compared to earlier flowering genotypes (Blum, Golan, Mayer, Sinmena & Obilana, 1992).

The root and water uptake

A common observation concerning roots under drought stress is the increase in root/shoot dry matter weight ratio. The increase in ratio results from the relatively greater decrease in shoot growth than in root growth under drought stress. This increase often implies that the development of a larger ratio of root length density to leaf area, which translates into a better capacity for sustaining plant water status under a given evapotranspirational demand (Blum & Arkin, 1984). In some rare cases root weight increased in absolute terms under drought stress (Malik, Dhankar & Turner, 1979). Westgate and Boyer (1985) and Sharp (1990) found that root growth continued at substrate water potentials that cause complete inhibition of shoot growth. The indication is there that ABA accumulation in roots under the effect of substrate water deficit was responsible for reducing shoot growth on the one hand and sustaining root growth on the other (Sharp, 1990).

Plants adapted to drought are characterized by deep and vigorous root systems (Taylor & Klepper, 1978). However, the importance and shape of root systems differ considerably between plants with even drought-adapted plants

having superficial root systems (Cactaceae) or very deep systems. The characteristics of a desirable root system may also vary with edaphic and climatic conditions. A deep root system will be a necessity if the water is stored deeply in the soil profile (Hurd, 1974). If the amount of rainfall is uncertain and the crop is forced to survive on water stored in the soil, a sparse root system extracting water at a slow rate during a prolonged period may be advantageous (Passioura, 1972). In situations where evapotranspiration from the soil surface is a major component of the total water use, a dense root system, ensuring that a great proportion of the water is transpired, may be favourable (Brown, Gregory & Wahbi, 1987).

Leaf area

Plasticity in leaf area is an important means by which a drought-stressed crop maintains control over water-use. In sorghum, leaf area was significantly reduced under drought stress before stomatal conductance in the remaining viable leaf area was seriously reduced (Blum & Arkin, 1984). A reduction in canopy photosynthesis of 14-26% under drought stress, was accounted for by leaf area reduction rather than by stomatal response as observed by Garrity, Watts, Sullivan and Gilley (1982). The degeneration of existing tillers (Elalaoui, Simmons & Crookston, 1992) and the total cessation of the appearance of new tillers (Blum, Ramaiah, Kanemasu & Paulsen, 1990) in small grains are also important factors limiting leaf area under drought stress. Compared to growth cessation of single leaves on a stem, the control of leaf area by tillers

allows an impressive recovery of leaf area as tillers appear after very high rates of dehydration (Blum *et al.*, 1990).

The effective alive and light-intercepting leaf area on a single stem is reduced by drought by way of reduced cell expansion, reduced cell division, leaf rolling, para-heliotropism, death of apical parts of leaves and death of whole leaves (first basal and then apical leaves). A severe reduction in stomatal conductance is generally observed when leaves approach wilting, typically when cereal leaves roll. Before flowering, the reduction in leaf area index and intercepted radiation under stress are largely a result of impaired leaf expansion and changes in leaf display. After flowering, this reduction is mainly a result of progressive leaf senescence. Evidently, the control over leaf viability under drought stress is different before and after flowering (Blum, 1996).

Transpiration and morphological leaf adaptation

Water losses due to transpiration present tremendous variation among plant species with maximal transpiration rates of 10, 1.0 and 0.1g H_2O dm^2h^1 in hygrophytes, mesophytes and xerophytes respectively (Monneveux & Belhassen, 1996). Water loss at the plant level largely depends upon the size of the evaporating areas. It is also related to the absolute and relative quantities of water lost by the different existing ways of gas exchange: stomatal, cuticle and lenticels.

Levitt (1980) distinquished between two types of plants among species from natural habitats, with respect to transpiration mechanisms. The first type

is plants that avoid dehydration by reducing transpiration through closure of stomata and low residual transpiration. These 'water-saving' plants are mainly encountered beyond xerophytes subjected to severe and prolonged water stress and have been selected for survival. Their level of productivity, regarding biomass and reproductive organs, is low. On the other hand there is the second type, referred to as the 'water-spender' plants that use other means for water conservation, such as osmotic adjustment rather than reduced transpiration, to avoid dehydration. It appears that domestication lent to the second strategy. A compromise between both strategies still persists in many of the cultivated species.

Water status components and osmotic adjustment

Wide genetic variability exists in the water potential of plant species, even under optimal conditions. As a consequence of osmotic adjustment and other traits of adaptation to drought, such as root development and reduced transpiration, water content can be maintained in cells and tissues to allow metabolic activity, so that the genetic variability of this component is rather limited. Water content, expressed as a percentage of the fresh weight, generally comprises between 70% and 85% of the plant green tissue (Monneveux & Belhassen, 1996).

Osmotic potential in plants generally varies between -0.3 and 3 MPa. The lowest osmotic potential can be found in species subjected to water (xerophytes) and salt stress (halophytes). The reduction in osmotic potential is due to the accumulation of osmolytes. These solutes can be direct products of

photosynthesis (soluble sugars) or can have their origin in the hydrolysis of storage compounds. The nature of osmolytes can differ considerably among species. Osmotic adjustment is a typically inducible trait, which could constitute a partial explanation of the association between osmotic adjustment capacity and yield stability, as noted by several authors including Morgan (1983) and Morgan & Condon (1986).

Solute accumulation or osmotic adjustment under stress is probably the most distinctive feature of an adaptive response to stress, which involves a component of water deficit, such as drought, freezing and salinity. The association between osmotic adjustment and yield, and its stability under drought stress, has been well demonstrated for a number of species (Martin, 1930; Blum, Mayer & Golan, 1983; Morgan, Rodriquez-Maribona & Knights, 1991; Rodriquez-Maribona, Tenores, Conde & Ayerbe, 1992).

Formation of yield

Most of the research dealing with yield reduction under drought stress is largely of a descriptive nature. This is simply because the disciplines concerned with crops and yield are not equipped to investigate lower levels of plant organization where explanations reside, while disciplines involved with lower levels of plant organization are generally not interested in yield (Blum, 1996).

Yield is established by the creation of a sink during inflorescence and its subsequent filling by photosynthate from the source. Drought stress affects yield by depressing both sink and source, depending on the timing and the

severity of stress with respect to plant phenology. As some yield components are developmentally correlated, such as kernel mass and the number of kernels per inflorescence, component compensation is an important developmental mechanism for reconstituting yield under or upon recovery from stress, to some extent. It is not uncommon to observe an increase in sorghum kernel weight under drought stress, due to a decrease in kernel number per panicle in compensation for a decrease in panicle number.

The plant has a large potential for the creation of yield sinks, beyond what is realized even under non-stress conditions. Cotton produces more flower buds and wheat produces more florets than will ever bear fruit to maturity. Despite constant breeding for a more efficient small grain plant, excessive tillering and the natural degeneration of a proportion of the tillers have been apparently retained in present cultivars. This may also have been the result of the selection pressure for stability of yield across different environments (Blum, 1996).

When cereals are subjected to conditions which depress photosynthesis during grain filling, as in the case of severe drought or heat stress, grain filling depends largely or even exclusively on remobilization of stem reserves to the grain (Schnyder, 1993). Various factors can affect the constitutive capacity of cereals to support grain filling from stem reserves, but the amount of storage material in stems is a major one. This is expected to be affected by conditions influencing assimilation during pre-anthesis stages of development as well as by the genotype (Blum, Sinmena, Mayer, Golan &

Shpiler, 1994). In many plants, including the small grains, fructans are the main constituent of the long-term storage pool and there seems to be little or no competition between fructan accumulation in storage and the development of yield sinks (Schnyder, 1993).

Drought - challenge to the plant breeder

Drought continues to be a challenge to agricultural scientists in general and plant breeders in particular, despite many decades of research. The development, through breeding, of cultivars with higher harvestable yield under drought conditions would be a major breakthrough and this is one of the most important challenges for plant breeders. However, the ability of some plants to give a higher economic yield under drought conditions than others, is a very elusive trait from a genetic point of view. This is because severity, timing and duration of drought will vary from year to year and cultivars successful in one dry year may fail in another. To make matters worse, drought seldom occurs in isolation, it often interacts with other abiotic stresses (particularly high temperature extremes) and with biotic stress. Also, areas with a high risk of drought generally have low-input agriculture. Thus, breeding for drought resistance is made more complex by the interactions of drought with other stresses (Ceccarelli & Grando, 1996).

In areas affected by drought, progress with empirical breeding has been negligible. As a result the yield of some important staple crops has shown only modest increase. This has been attributed to the difficult nature of the target environment and has been accepted as inevitable (Blum, 1988; Passioura,

1986). Therefore, most of the selection work in breeding programs is done under favourable conditions and much research has been done to seek alternatives to empirical breeding for unfavorable conditions. Much less has been done to question whether the slow progress of empirical breeding for stress environments has been due to a wrong breeding approach.

Breeding for stress environments

The key aspects of these strategies and methodologies are (1) direct selection for specific adaptation in the target environment, (2) use of locally adapted germplasm, and (3) use of plot techniques and experimental design to control environmental variation.

The environment of selection and specific adaptation has been one of the hottest topics of debate among breeders during the last 30 years. The debate is between those who advocate selection in favorable environments, where genetic differences are maximized and environmental noises minimized, and those who believe that selection has to be done in the target environment or under conditions closely resembling it (Ceccarelli & Grando, 1996).

The latter are a minority among breeders but enjoy support from geneticists and physiologists. The theoretical framework to this issue, which in essence is a problem of genotype by environment (G x E) interaction, has been provided by Falconer (1952), who wrote: "If a breeder wants to improve performance in environment A he should select in environment A".

Defining selection criteria to improve yield under drought

The many selection criteria that have been proposed to increase the drought resistance of crops have had little, if any, impact on improving crop yields in dry environments. There are several likely reasons for this lack of success. Some of these are: (i) proposed criteria have been related more to survival mechanisms under drought than to productivity, (ii) criteria were inappropriate for the target environment, and (iii) criteria were temporal and therefore likely to have minimal impact on growth and yield over the entire lifecycle.

Another important reason is that breeders were convinced that the proposed criteria would be unsuccessful, as they were too difficult to measure. On the other hand, empirical breeding programs to improve yield under drought, have been successful. Surprisingly some of the greatest successes have been achieved by breeding in environments where water was non-limiting (Richards, 1996).

Crop Quality

Wheat produced in the Western and Southern Cape wheat producing regions annually accounts for up to one third of the total wheat crop in South Africa. These regions have been very consistent in yield production in the past, but experience problems with the fluctuation in wheat quality from season to season (Traut, 1993). The instability noted in crop quality in these regions in turn created problems for the milling and baking industries. These industries were constantly challenged by their respective clients to produce consistent products. Wheat quality factors may subsequently be divided into those largely

inherited (Baenziger *et al.*, 1985) and those predominantly influenced by growing or environmental conditions (Mailhot & Patton, 1988).

Growth conditions that affect crop quality the most, include such factors as residual nitrogen in the soil, climatic conditions, husbandry as well as the rate and timing of fertilization (Benzian & Lane, 1986; Van Lill, Purchase, De Villiers & Smith, 1993). Soil cultivation and production techniques vary very little between seasons in specific production areas, thus the dominant variables seem to be weather conditions (Nel, Agenbag & Purchase, 1998). The two most critical factors affecting crop yield and grain quality in the Western and Southern Cape regions are those of temperature and rainfall (Beyers, 1992). Randall and Moss (1990) reported that a short period of very high temperature stress can have a serious effect on the crop yield and quality, whereas water stress during kernel set resulted in an acceleration of leaf senescence which shortened the period of kernel set and reduced individual kernel mass (Austin, 1989).

Laubscher (1980) reported that the environment in which spring wheat cultivars are grown in the Western and Southern Cape had the biggest effect on protein content and loaf volume, rather than the different cultivars. He, however, based his conclusions on locality groupings and differences between localities and cultivars, but did not calculate the contribution made by each factor to the total variance. The result is that very little is thus known about the effect of the environment on the stability of cultivars in this area.

Agenbag and De Villiers (1995b) have shown that a high intensity of water stress can enhance leaf senescence and reduce grain yield, the result of smaller kernels. This can have a serious effect on the breadmaking quality of wheat (Bushuk, 1988). Johnson and Mattern (1980) have shown that protein content is mainly determined by the growing conditions of the crop, while protein quality is primarily determined genetically (Finney, Yamazaku, Young & Rubenthaler, 1987). Protein quality can also be affected by growth conditions after anthesis (Evans, Wardlaw & Fischer, 1975; Huebner, Kaczkovski & Bietz, 1990). The effect of water stress on different protein fractions will therefore depend on the growth stage during which the stress develops (Agenbag & De Villiers, 1995a). Ohm, Chung and Deyoe (1998) reported that kernel weight and size are important factors in the evaluation of milling characteristics.

The induction of water stress

Control of the environment is important when studies of plant responses to water stress are undertaken. Growth chambers are equiped for light, temperature and humidity control, but precise control of soil and plant water potential is seldom achieved (Kidder & Behrens, 1991). Some of these problems were overcome when investigators began growing plants hydroponically in nutrient solutions containing polyethylene glycol (PEG). Polyethylene glycols are neutral polymers available in a range of molecular weights, highly soluble in water and with low toxicity to mammals. Because of these properties they have been used in the past to exert water stress on plants by decreasing the water potential of the rooting medium and

subsequently the water potential of the plant. Lawlor (1970), however, demonstrated that PEG molecules from the nutrient solution entered and damaged water stressed roots and exerted phytotoxic effects. Other problems encountered with this technique include inhibition of phosphorus uptake (Emmert, 1974) and reduced oxygen availability to roots (Mexal, Fischer, Osteryoung & Patric-Reid, 1975). Another problem with the use of PEG is that the stress is applied rapidly, which does not represent the true situation under natural conditions where water deficits develop slowly over considerable timespans. Withholding of water also pose problems because of the difficulty to maintain a constant soil and plant water potential (Kidder & Behrens, 1991).

Parameters to be used as screening methods for drought tolerance

As different traits play an important role during drought stress, it is advisable to investigate the value of different drought tolerance mechanisms to be used as possible screening methods. In earlier studies both proline accumulation and the reduction of 2,3,5-triphenyltetrazoliumchloride showed promise as indicators of drought tolerance in a wide range of crops.

Free proline

Severe water stress induces numerous metabolic irregularities in plants. Free proline accumulates in response to water stress in attached and detached leaves of many crop plants (up to 100 times the normal level) under laboratory (Barnett & Naylor, 1966; Routley, 1966; Waldren, Teare & Ehler, 1974) and field conditions (Pálfi & Juház, 1971; Waldren, Teare & Ehler, 1974).

Studies with detached leaves indicated that net synthesis of proline from carbohydrates via α-ketoglutarate and glutamate is the main source of free proline accumulated during drought stress (Morris, Thompson & Johnson, 1969; Boggess, Aspinall & Paleg, 1976; Boggess & Stewart, 1976; Boggess, Stewart, Aspinall & Paleg, 1976). Upon relief of stress, free proline levels decline rapidly in viable tissue, probably due both to proline oxidation and to incorporation into protein (Stewart, 1972; Singh, Paleg & Aspinall, 1973).

Whether proline accumulation is of adaptive value, as part of a plant's strategy for withstanding drought, is not known. The suggested roles for proline accumulation during drought stress include the maintenance of osmotic potential in response to stress (Rajagopal, Balasubramanian & Sinha, 1977), a nitrogen reserve in those leaves able to recover fully after stress (Tully, Hanson & Nelsen, 1979) and as a storage compound for reduced nitrogen and carbon under stress situations.

The method

Chinard (1952) described an acid-ninhydrin method for detecting proline determinations which was used in the past for studying the effects of various interference's (Troll & Lindsley, 1955; Messer, 1961). Although several free amino acids can interfere with proline determinations, the free amino acid levels reported in stressed plants (Barnett & Naylor, 1966; Thompson, Stewart & Morris, 1966) were low compared to proline. The techniques described by Chinard (1952) and Troll and Lindsley (1955) worked well with purified or semi-purified proline samples, but did not work with the simple fractionation

and filtration techniques needed for rapid field analysis. Bates, Waldren and Teare (1973) developed a simple, rapid colorimetric method for determining proline. This method detected proline in the 0.1 to 36.0 mol g^{-1} range of fresh weight leaf material.

Samples. Fully expanded 'sun' leaves.

Reagents. Acid-ninhydrin is prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6M phosphoric acid, with agitation, until dissolved.

Procedure. Approximately 0.5 g of plant material is homogenized in 10 ml 3% (v/v) aqueous sulfosalicyclic acid and the homogenate filtered through Whatmann #2 filter paper. Two milliliter of filtrate is allowed to react with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100ºC, after which the reaction is terminated in an ice bath. The reaction mixture is subsequently extracted with 4 ml toluene and mixed vigorously with a test tube stirrer for 15-20 sec. The chromophore containing toluene is then aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene as a blank. The proline concentration is determined from a standard curve and calculated on a fresh weight basis as follows:

[(µg proline / ml x ml toluene) / 115.5 µg / µmole] / [(g sample) / 5] =

µmoles proline / g of fresh weight material.

Research on the accumulation of free proline

Hanson, Nelsen and Everson (1977) evaluated free proline accumulation as an index of drought resistance in barley. Two barley cultivars of contrasting drought resistance were used. The varietal difference in drought resistance was clearly expressed at the three to four-leaf stage, both in soil grown plants in greenhouse tests and in perlite-grown plants in growth chamber experiments.

Stewart, Boggess, Aspinall and Paleg (1977) investigated the inhibition of proline by water stress in barley. They found that the conversion of proline to glutamic acid and hence other soluble compounds (proline oxidation) proceeds rapidly in turgid barley leaves and is stimulated by higher concentrations of proline. This suggests that proline oxidation could function as a control mechanism for maintaining low cellular levels of proline in turgid tissue. In water stressed tissue, however, proline oxidation is reduced to negligible rates. These results are consistent with the idea that proline accumulation results from inactivation by water stress of normal control mechanisms.

Huang and Cavalieri (1979) reported on the accumulation of free proline in leaf discs of spinach (*Spinacia oleracea* L.). The leaf discs were exposed to solutions of polyethylene glycol with water potentials of less than –1000 kPa. At –2000 kPa the proline accumulation was 11 µmoles per gram of original fresh weight in a 24-hour period.

Handa, Handa, Hasegawa and Bressan (1986) reported on the accumulation of proline and the adaptation of cultured plant cells to water stress. When they transferred cultured tomato cells to a low water potential environment an increase in the dry weight to fresh weight ratio, accompanied by a rapid accumulation of proline, was the result. Proline levels remained high after more than 100 cell generations in low water potential media, but declined rapidly after transfer to media with less negative water potential.

Argandowa and Pahlich (1991) studied the effect of water stress on the proline content and enzyme activities in barley seedlings. They found that proline increased three-fold in lower epidermal tissue and 2.3-fold in primary leaves. The relative water content decreased to 86% and 81%, respectively.

Van Rensburg and Krüger (1994) tested the efficiency of various aspects of abscisic acid and proline accumulation as potential selection parameters for drought tolerance in tobacco under controlled conditions. The results indicated that both abscisic acid (though being less pronounced) and proline accumulated rapidly after a distinct threshold water potential value. Proline concentrations increased sharply at a leaf water potential of -1.27 MPa in the drought-tolerant cultivars and at a leaf water potential of -1.50 MPa in the drought-sensitive cultivars.

Ali Dib *et al.* (1994) investigated the possibility of using proline accumulation and fluorescence inhibition as predictive tests for drought tolerance in durum wheat. A significant effect due to water stress could be noted on the two physiological traits measured in controlled conditions. Proline

accumulation in stressed plants increased by a factor of 15.7 in relation to the control. The results of analysis of variance for proline accumulation, was highly significant for the effects of variety, treatment and the variety x treatment interaction.

Van Heerden and De Villiers (1996a) evaluated the accumulation of proline as an indicator of drought tolerance in spring wheat. They found that proline accumulation in whole plants showed clear genotypical differences in the time of onset of proline accumulation as well as in the amount of proline accumulated as a result of drought stress.

2,3,5-Triphenyltetrazoliumchloride (TTC)

Photosynthesis can drastically be inhibited by extreme environmental conditions that disrupt the electron transport system. To quantify the plant injury response and to be able to express differences between cultivars, much research has been devoted to find absolute measures of plant injury. These methods include electrolyte leakage for drought, heat and freezing resistance and accumulation of formazan, the reduced form of TTC, for heat and cold resistance (De Ronde & Van der Mescht, 1997).

Electrolyte leakage is a measure for membrane injury (Ruter, 1993) but has been found to be unreliable in some cases (Zhang, Willison & Hall, 1993). The ability of cells to reduce TTC in vitro has been shown in a variety of organisms (Towill & Mazur, 1974). The reduction of TTC probably takes place in the mitochondria. Electrons from the electron transport system reduce TTC to formazan (a colored complex) (Towill & Mazur, 1974). The degree of TTC

reduction gives an indication of the viability of cells (Towill & Mazur, 1974; Chen, Shen & Li, 1982). Chen *et al.* (1982) showed that TTC reduction could be used as a reliable indicator of heat tolerance in various crops.

Plants with a tolerance to environmental stress produce a higher activity of succinic dehydrogenase and have the ability to reduce more of the TTC into formazan. Formazan has a red color which can be monitored spectrophotometrically (Chen *et al.*, 1982). De Ronde and Van der Mescht (1997) showed that it can be used as a reliable indicator of drought tolerance in cotton cultivars.

The method

The determination of TTC reduction can be done by a modified method of Chen et al. (1982), as described by Van Heerden and De Villiers (1996b). Two leaf discs (10 mm in diameter) are collected from each of the control and stressed leaves and immediately transferred to 3 ml TTC solution [0.8% (w/v) in 0.2M phosphate buffer, pH 6.0] in small glass bottles, covered with tin foil, with screw caps. Each of the bottles is vacuum infiltrated in the dark for 5 min. and then incubated in the dark for 20 h at 29°C while being stirred continuously.

Following incubation, the samples are rinsed twice with distilled water, blotted dry on filter paper and transferred to 3 ml ethanol (95%) in a test tube and boiled in a water bath until the ethanol has evaporated. After the samples are cooled to room temperature, 3 ml ethanol is added to each tube and

vortexed for 20-30 sec. After this is done the reduced TTC is determined at 485 nm, using ethanol as a blank.

Research on the use of the TTC test

De Ronde and Van der Mescht (1997) used the TTC test to predict drought tolerance in six cotton cultivars. A lower absorbance value for the control sample compared to the stressed material indicated that the cultivar reacted as a drought-tolerant cultivar. Water stress was affected in the laboratory but this can be different from field conditions since heat stress cannot be eliminated in the latter. They also did a study where heat stress was combined with water stress. There was a slight difference in the ranking of the cultivars.

Van Heerden and De Villiers (1996b) evaluated the TTC test as an indicator for drought tolerance in spring wheat cultivars. They used the modified test. The results obtained with the reduction of the TTC during water stress showed only minor genotypical differences and no correlation could be shown between the degree of reduction and drought tolerance.

Physiological responses to water stress

Relative water content (RWC)

Changes in RWC of leaves are considered to be a sensitive indicator of water stress (Henson, Mahalakshmi, Bidinger & Alagarswamy, 1981; Van der Mescht, 1989). Research with RWC of leaves is well-documented (Ferreira, De Souza & Prisco, 1979; O'Reagan, Cress & Van Staden, 1993) and differences in the decrease in RWC during water stress are common for a number of crops. It was found that in most cases this decrease was smaller

and occurred later during the stress period in drought tolerant cultivars of potato (Van der Mescht, 1989), maize (O'Reagan *et al.*, 1993), cotton (Ferreira *et al.*, 1979), wheat (Dedio, 1975; Schonfeld, Johnson, Carver & Mornhinweg, 1988; Van Heerden & De Villiers, 1996b) and tobacco (Van Rensburg & Krüger, 1994).

The determination of RWC is conducted according to the method of Henson et al. (1981). Harvested leaves are weighed immediately to determine fresh weight (FW), after which they are placed in water in test tubes in the dark for 24 hours. They are then blotted dry with filter paper and weighed to determine their turgid weight (TW). The dry weight (DW) is determined by drying the leaves in an oven for 48 hours at 70°C. the RWC (%) of each harvested leaf is then calculated using the following formula:

RWC = [(FW - DW) / (TW - DW)] x 100.

Leaf diffusive resistance (LDR)

Resistance created by stomatal pores is of significance in determining the total water loss from plants. The value increases or decreases with closing and opening of stomata. Factors such as light, CO_2 concentration, temperature and leaf water potential influence the resistance on stomatal pores significantly, and they regulate water loss from plants (Shrivastava & Kumar, 1995).

The leaf diffusive resistance can be determined by using a LICOR 1600 steady state porometer. Oosterhuis and Walker (1987) showed that LDR is a useful and reliable indicator of crop water status as well as the onset of water stress. Agenbag and De Villiers (1995a) also demonstrated that LDR is a

sensitive parameter of stress conditions in wheat plants. Along with the LDR, the transpiration rate of the leaves can also be determined using the porometer.

Water potential

Many researchers, including Sullivan and Eastin (1974) and Levitt (1980), have suggested that total water potential of plant tissue may differentiate between drought-resistant and drought-susceptible cultivars. Genotypic total water potential variation in large populations of plants has been obtained by Sammons, Peters and Hymowitz (1978), Blum (1974) and Quarrie and Jones (1979) for soybean, sorghum and spring wheat, respectively. These studies concluded that higher total leaf water potentials in specific cultivars within the populations indicated increased drought resistance.

Jarvis and Jarvis (1963), Sanchez-Diaz and Kramer (1971) and Levitt (1980) have shown that drought-resistant plants have smaller water deficit per unit decrease in leaf waterpotential than more drought-susceptible plants when subjected to water stress. The effectiveness of leaf water potential for drought sensitivity tests is well documented (Sanchez-Diaz & Kramer, 1971, Levitt, 1972; Bates & Hall, 1981). However, there is a lack of research on the use of the ear to measure the water potential of the plant.

Plant components

Austin (1989) found that water stress treatments hastened leaf senescence, which in turn can cause a reduction in the duration of grain filling and eventually kernel mass. To determine this, plants are sampled before and after

stress induction. The area of green leaves can be determined as well as the dry mass of the plant components (leaf mass, stem mass and ear mass) as suggested by Agenbag and De Villiers (1995b). Yield and yield components can be determined during the final harvest to determine the effect of the water stress on different cultivars.

Quality Parameters

Protein

Kernel protein is determined by the use of a Technikon Infralyzer 400, previously calibrated against Kjeldahl nitrogen content (AACC, 1983).

Mixograph

The mixograph is an instrument that performs measurements on the dough during the mixing action (Wikström & Bohlin, 1996). The mixograph was developed by Swanson and Working (1933) and is still one of the most widely used instruments for physical dough testing. Parameters from the mixograph are used to classify wheat and predict properties on the finished product.

The mixograph characteristics are dependent on the changes of the plastic, elastic and viscolastic properties of the dough during the mixing (Kunerth & D'Appolonia, 1985). In the initial phase, water is brought into contact with the flour particles. The mixing action helps to break down the particles by rubbing them against the pins and the walls of the mixer bowl and facilitates water absorption by the starch and the protein. In the next phase, the gluten proteins are orientated in the dough by the folding and stretching action of the mixing pins, and the dough begins to develop. Further mixing will

lead to a breakdown of the dough (Hoseney, 1994). Shogren & Finney (1984) described a 35-gram mixograph for determining and predicting the functional properties of wheat flours.

Micro-loaf baking test

Test baking is still the only reliable method for determining the breadmaking performance of wheat flour (Wikström & Bohlin, 1996).

The loaf volume is the most inclusive parameter of bread-baking quality of wheat (Blackman & Payne, 1987). The test baking is done by way of micro-loaves that are baked from 10 grams of flour, using the optimized straight dough method as described by Shogren and Finney (1984). Loaf volumes are determined by rapeseed displacement.

Objectives of this study

From the literature it was evident that water stress plays a significant role in the production and quality of wheat. With the possibility of more frequent periods of water stress in the future, due to the effect of global warming, this study was undertaken to determine the effect of water stress on the physiology and quality of spring wheat cultivars grown in the Western Cape region of South Africa with the following objectives:

- To determine the most suitable method for inducing water stress in the wheat plants grown in a greenhouse (Chapter 2).

- To determine whether total ear water potential, relative water content, leaf diffusive resistance, transpiration rate and the accumulation of free proline can be used to distinguish between drought tolerant and drought sensitive spring wheat cultivars (Chapter 3).

- To determine whether the effect of water stress on the different plant components of wheat can be used to distinguish between drought tolerant and drought sensitive spring wheat cultivars (Chapter 4).

- To determine the effect of water stress during different growth stages on the yield and yield components of different spring wheat cultivars (Chapter 5).

- To determine the effect of water stress at different growth stages on protein production and baking quality of spring wheat cultivars (Chapter 6).

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Chapter 2

A comparison of two methods of inducing water stress in wheat (*Triticum aestivum* L.)

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Abstract

The aim of this study was to compare the withholding of water (WOW) and the use of polyethylene glycol (PEG) as methods of inducing water stress in spring wheat cultivars (*Triticum aestivum* L.) grown in the Western Cape. Water stress was induced in two cultivars that had previously shown differences in drought tolerance. Proline accumulation in plants and the reduction of 2,3,5-triphenyltetrazoliumchloride (TTC) were used as indicators of water stress in the test plants. Proline accumulation proved to be a more sensitive indicator of water stress compared to TTC, showing significant increases with both the withholding of water and PEG as methods to induce water stress. Although both methods of stress induction proved to be effective, the withholding of water may be more appropriate due to the possibility of root injuries with PEG. Good control of climatic conditions and measurements of plant water potential will, however, be essential if the withholding of water is used to induce stress.

Drought is considered, worldwide, to be the most important factor limiting crop yields (Jones & Corlett, 1992). Quick, reliable and consistent tests to provide information on drought tolerance of newly bred wheat cultivars could therefore be of great help in breeding programmes. Both most commonly used techniques to induce water stress, namely the withholding of water and the use of polyethylene glycol (PEG), create some problems (Kidder & Behrens, 1991). PEG may enter and damage the roots (Lawlor, 1970), while it is not possible to maintain a constant soil and plant water potential where water is withheld. In earlier studies (Van Heerden & De Villiers, 1996a, 1996b), both proline accumulation and to a lesser extent the reduction of 2,3,5-triphenyltetrazoliumchloride showed promise as indicators of drought tolerance in spring wheat cultivars. In their experiments water was withheld to induce water stress in whole plants, but this method may create some problems as a routine technique to screen cultivars due to the mentioned difficulty to maintain a constant level of stress. This preliminary study was undertaken to compare the withholding of water (WOW) and the use of polyethylene glycol (PEG) as methods to induce water stress in spring wheat cultivars grown in the Western Cape.

The study was conducted in a temperature-controlled glasshouse (16°/10°C day/night temperature). Two cultivars (Gamtoos and SST66) which showed differences in drought tolerance in studies done by Van Heerden and De Villiers (1996a, 1996b) were grown in 2 litre pots filled with sand. Three treatments, namely, control; WOW and PEG were applied between the flag leaf

stage and pre-anthesis for a period of 21 days. The control plants were not subjected to any water stress and water deficits were imposed by the withholding of water from the soil or by flooding the soil with polyethylene glycol 6000 with an osmotic potential of –1900kPa (Hanson, Nelson & Everson, 1977). To compare the effect of the two methods of stress, proline accumulation in whole plants and the reduction of 2,3,5-triphenyltetrazoliumchloride was used as indicators of water stress. Proline content of leaves was determined by homogenizing the samples in a mortar and pestle in 3% sulfosalicylic acid, whereafter the absorbance of the solutions was determined at 520 nm by a ninhydrin colorimetric method (Bates, Waldren & Teare, 1973). The determination of TTC reduction was performed using a modified method of Chen, Shen & Li (1982) which was also used by Van Heerden & De Villiers (1996b). The reduced TTC was determined at 485 nm, using ethanol as a blank. Both proline and TTC were determined on day 0, 7, 14 and 21 of the stress period. Treatments were replicated five times, while proline and TTC measurements were done in quadruplicate. Results of day 0 were not analyzed because a combined sample of all treatments was used to determine proline and TTC. Least-significant difference (LSD) was used to compare treatment means.

In general, results obtained with the proline-test confirmed findings of earlier studies (O'Regan, Cress & Van Staden, 1993; Van Rensburg, Krüger & Krüger, 1993; Van Heerden & De Villiers, 1996a). In spite of large coefficients of variation (CV), significant differences due to treatments at days 7, 14 and 21 and between cultivars at days 7 and 21 were obtained (Table 1).

Table 1 Results of the ANOVA done on the proline-test

Sources of Variation	Degrees of Freedom	Significancy (Pr > F) Days after onset of stress		
		7	14	21
Cultivar (C)	1	0.0007	0.8381	0.0001
Treatment (T)	2	0.0034	0.0001	0.0001
C x T	2	0.6362	0.0007	0.0001
Repetition	5	0.4736	0.6648	0.2725
Error	20			
CV (%)		39.03	21.97	16.78

The differences for proline accumulation between the three treatments used in this study are shown in Figure 1. Both the withholding of water and the use of PEG resulted in a significant increase in proline accumulation compared to that of the control treatment.

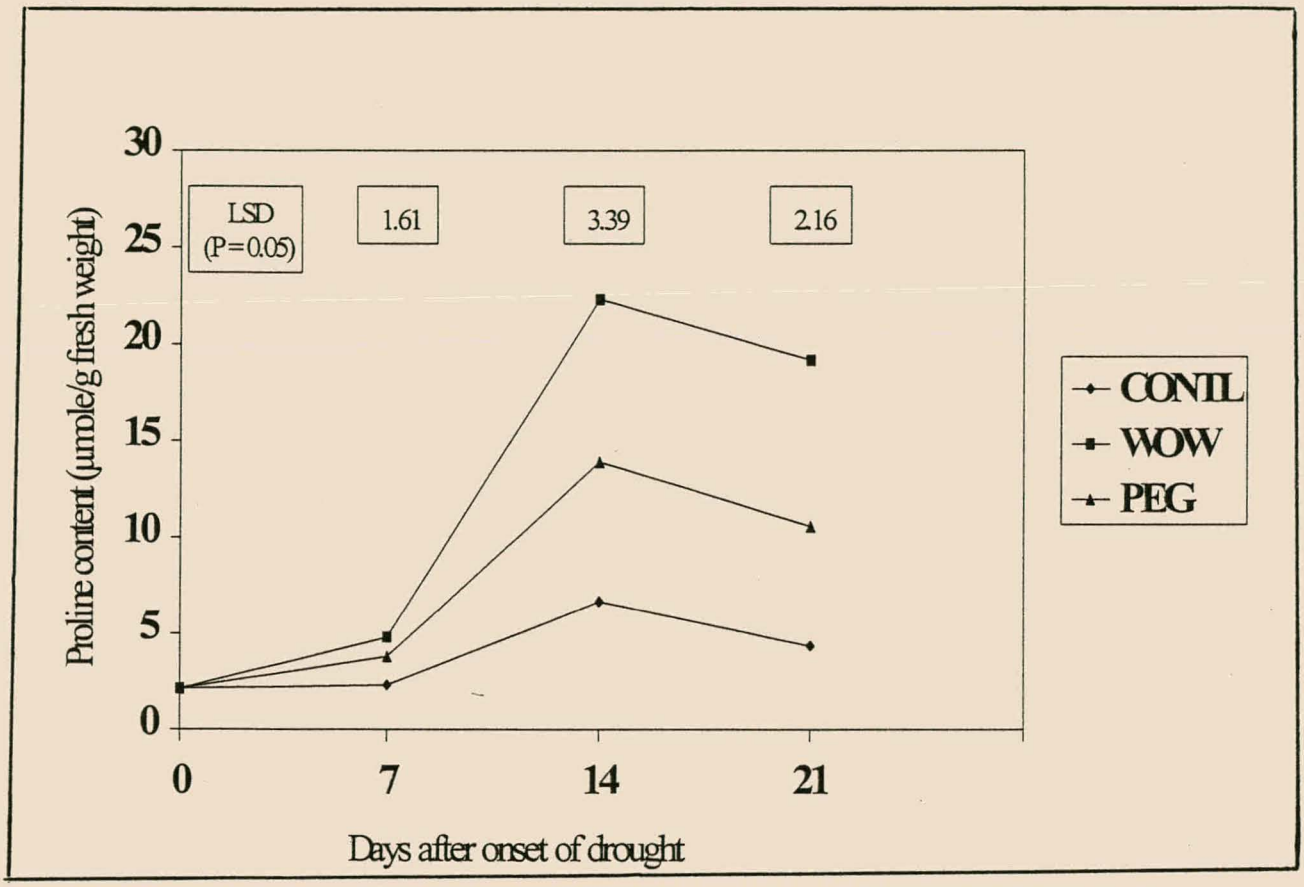

Figure 1 Proline accumulation in two Western Cape spring wheat cultivars subjected to three different water stress treatments. LSD-values given for the 5% confidence level for each of the stress days.

Higher proline values for the WOW-treatment at both 14 and 21 days after the stress was induced indicated that it would be easier to show differences

between cultivars where this method of stress induction was used compared to the PEG-method. These results also confirm that proline accumulation can be used as an indicator of water stress, but plant sampling and chemical measurements should be done very carefully to minimize variation in the data.

Table 2 Results of the ANOVA done on the TTC-test

Sources of Variation	Degrees of Freedom	Significancy (Pr > F) Days after onset of stress		
		7	14	21
Cultivar (C)	1	0.2882	0.4603	0.9437
Treatment (T)	2	0.5404	0.2401	0.0165
C x T	2	0.8328	0.4091	0.3764
Repetition	5	0.5890	0.7385	0.5179
Error	20			
CV (%)		10.69	12.01	13.16

Results of the TTC-test given in Table 2 showed less variation (CV < 15%) compared to proline data. Significant differences between treatments were however, only found after 21 days of stress, while no significant differences were found between cultivars. Differences between the two methods of stress induction could not be detected by the TTC test at an early stage. The adapted test of TTC reduction as an indicator of drought tolerance appears to be less sensitive than the test for the accumulation of proline. In general, it can be concluded that although both methods of stress induction proved to be effective, the withholding of water may be more appropriate due to the possibility of root injuries with PEG as found by Lawlor (1970). Another problem with PEG is that

the stress is applied rapidly, which does not represent the true situation since water deficits normally develop slowly over considerable timespans. Good control of climatic conditions and measurements of plant water potential will, however, be essential if the withholding of water is used to induce stress.

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Chapter 3

THE USE OF PHYSIOLOGICAL PARAMETERS TO IDENTIFY DROUGHT TOLERANCE IN SPRING WHEAT CULTIVARS

(In Press: South African Journal of Plant and Soil)

Abstract

Wheat crops produced under dryland conditions in the winter rainfall region of South Africa often experience periods of water stress. It is therefore important to identify spring wheat cultivars that can withstand these conditions. The multitude of factors potentially involved in drought resistance make it unlikely that a single measurement will provide an all-encompassing test for drought tolerance. The aim of this study was to determine whether total ear water potential, relative water content (RWC), leaf diffusive resistance (LDR), transpiration rate and the accumulation of free proline could be used to distinguish between drought tolerant and drought sensitive spring wheat cultivars. The withholding of water (WOW) and the use of polyethylene glycol (PEG) imposed stress at different physiological growth stages. It was shown in this study that these physiological parameters could be used to identify drought tolerance in spring wheat cultivars. Although genetic differences, as measured by the abovementioned parameters, do exist between cultivars, the effect of water stress on plant growth and harvest data should be taken into account to decide whether these differences are of any commercial benefit.

Introduction

According to Du Pisani and Partridge (1990), global warming may result in increased winter temperatures and a decrease in rainfall in the Western Cape region of South Africa. It is therefore important to identify spring wheat cultivars that can better tolerate these conditions. The multitude of factors potentially involved in drought resistance make it unlikely that a single measurement will provide an all-encompassing test for drought tolerance (Martin, Brown & Ferguson, 1989).

Stomatal response, a major factor controlling plant water loss, has long been considered a promising method to screen for drought tolerance. It can easily be measured by determining the leaf diffusive resistance (LDR). Differences in stomatal sensitivity and diffusive resistance of different crops have been reported by several researchers (Boyer, 1970; Teare & Kanemasu, 1972; Quarrie & Jones, 1979). The importance of plant water for the maintenance of turgidity needed for the growth and/or the survival of plants is widely recognized. Work done by Clarke and McCaig (1982) and Schonfeld, Johnson Carver & Mornhigweg (1988) indicated that cultivars with higher relative water content (RWC) during stress treatments are more drought resistant.

Several authors (Slayter, 1960; Sanchez-Diaz & Kramer, 1971; Levitt, 1972) have reported that drought resistant plants have a smaller water deficit-per-unit decrease in leaf water potential than drought susceptible plants. Researchers therefore have speculated that total leaf water potential might be used to differentiate between drought resistant and drought sensitive cultivars

(Sullivan & Eastin, 1974; Levitt, 1972). Studies done by Blum (1974) and Quarrie and Jones (1979) on the total water potential variation in large populations of sorghum and spring wheat concluded that higher total leaf water potentials within the populations of specific cultivars indicated increased drought tolerance.

Free proline has been reported to accumulate in a range of plants in response to a wide range of environmental stress. Proline accumulation in leaves of plants during water stress is well-documented (Barnett & Naylor, 1966; Tan & Halloran, 1982; Van Rensburg, 1991; O'Reagan, Cress & Van Staden, 1993). Usually, positive correlations are obtained between the rate of proline accumulation and the degree of tolerance to stress (O'Reagan *et al.*, 1993, Van Rensburg, Krüger & Krüger, 1993; Van Heerden & De Villiers, 1996a), although negative correlations have also been shown (Nath & Ghoshal, 1978; Hanson, 1980).

The objectives of this study were to determine whether total ear water potential (instead of leaf water potential), relative water content, leaf diffusive resistance, transpiration rate and the accumulation of free proline could be used to distinguish between drought tolerant and drought sensitive spring wheat cultivars.

Material and Methods

Plant material

Four spring wheat cultivars were used in this study. Two of these, Gamtoos (drought tolerant) and SST 66 (slightly drought sensitive), had showed differences in drought tolerance in studies done by Van Heerden and De Villiers

(1996a). The other two, Kariega and SST 57, were of unknown drought tolerance.

Growth conditions

Six seeds of the respective cultivars were planted (1997 and 1998) in 2-litre plastic pots filled with coarse sand. Eight days after emergence, seedlings were thinned to 3 plants per pot. Experiments were conducted in a temperature-controlled (16°/10°C day/night) glasshouse at the Welgevallen Experimental Farm, Stellenbosch. Irrigation was done by a computerized system, according to the daily solar radiation received. More pulses were thus given on sunny days than on cloudy days. For the first two weeks seedlings were irrigated with water only and for the rest of the growth period with a balanced nutrient solution (75%).

Induction of water stress

Water stress was induced by the withholding of water (WOW) at different stages of plant development in both experimental years (1997 & 1998). In 1998 polyethylene glycol (PEG) 6000, with an osmotic potential of -1900kPa (Hanson, Nelson & Everson, 1977), was used as an additional treatment to induce water stress. Three stress treatments, each lasting seven days, were applied, with the first treatment commencing at the flag leaf stage, the second at the milky kernel stage and the third a combination of the first two. At the end of each stress period, pots of these treatments were flushed with the Hoagland nutrient solution to ensure that stress conditions were terminated. Plants which were kept well-watered throughout their development, were used as a control treatment.

Measurements

Leaf diffusive resistance (LDR) is a useful indicator of crop water status and the onset of water stress (Oosterhuis & Walker, 1987). LDR and transpiration rate were determined at 12h00 on cloudless days using a LICOR 1600 steady state porometer. The relative water content (RWC) (Martin *et al.*, 1989) of the flag leaf was determined on day 2, 4 and 6 of each stress treatment. Proline content of leaves was determined on day 2, 4 and 6 of each stress treatment, by homogenizing the flag leaf in a mortar and pestle in 3% sulfo-salicyclic acid, whereafter the absorbance of the solutions were determined at 520 nm by a ninhydrin colorimetric method (Bates, Waldren & Teare, 1973). To determine total ear water potential, the ear of the main shoot was used. Water potential was determined before and after the stress. This was done by cutting the ear, sealing it in a plastic bag and transferring it to the PMS pressure chamber to determine the water potential (Scholander, Hammel, Hemingstein & Bradstreet, 1964).

All treatments were done in triplicate and an analysis of variance was done on all parameters. The Studentized T test (P=5%) was used to compare treatment means.

Results and Discussion

Leaf diffusive resistance and transpiration rate

LDR and transpiration rate proved to be useful parameters of stress conditions in wheat, since significant differences due to treatments were obtained in both trial years (Table 1). Results obtained in this study showed the same tendencies as found by Agenbag and De Villiers (1995). Cultivar differences were also

Table 1 Results of the ANOVA done on leaf diffusive resistance (LDR) and transpiration rate (TRANS) on two sample days of the three stress treatments in both trial years

SOURCES OF VARIATION	MS CULTIVAR (CUL)	MS TREATMENT (TRT)	MS CUL X TRT	CV(%)
Df	3	1	3	
		1997		
		LDR (scm^{-1})		
ST 1				
Day 2	0.21**	2.30**	0.36**	8.87
Day 6	0.78**	5.82**	0.15	10.49
ST 2				
Day 2	0.19	26.95**	0.46**	6.63
Day 6	44.78**	1164.69**	22.37**	8.54
ST3				
Day 2	0.40**	0.28*	0.43**	8.09
Day 6	35.18**	769.19**	17.65**	10.45
		TRANS ($\mu g\ cm^2s^{-1}$)		
ST 1				
Day 2	3.84**	19.75**	2.21**	5.67
Day 6	10.52**	38.00**	3.53**	8.65
ST 2				
Day 2	0.33	15.46**	4.53**	9.64
Day 6	4.20**	108.34**	3.96**	17.72
ST 3				
Day 2	3.35**	2.05**	0.31	10.30
Day 6	3.81**	101.61**	4.05**	17.29
SOURCES OF VARIATION	MS CULTIVAR (CUL)	MS TREATMENT (TRT)	MS CUL X TRT	CV(%)
Df	3	2	6	
		1998		
		LDR (scm^{-1})		
ST 1				
Day 2	4.03**	353.34**	29.97**	9.48
Day 6	37.06**	911.80**	54.11**	9.90
ST 2				
Day 2	86.09**	175.01**	43.34**	10.22
Day 6	97.33**	1019.54**	48.33**	24.51
ST3				
Day 2	79.02**	187.41**	44.43**	9.98
Day 6	185.31**	909.59**	77.13**	17.63
		TRANS ($\mu g\ cm^2s^{-1}$)		
ST 1				
Day 2	0.76**	13.65**	1.28**	11.67
Day 6	0.29**	11.13**	0.27**	14.36
ST 2				
Day 2	1.42**	57.00**	7.66**	11.03
Day 6	7.39**	131.40**	16.73**	11.47
ST3				
Day 2	2.32**	61.14**	6.76**	6.71
Day 6	6.44**	127.95**	17.96**	10.62

* significant at 5% confidence level, ** significant at 1% confidence level,

ST – stress treatments, MS – mean square values, CV(%) – coefficient of variation

significant in both years and in general the cultivar x treatment interactions also proved to be significant.

LDR of control plants varied from day to day due to the differences in daily solar radiation received. To reduce this variation, treatment means for both years are presented as percentages of their respective control values (Table 2).

Table 2 Leaf diffusive resistance (LDR) and transpiration rate (TRANS) values expressed as percentages of control values for the respective cultivars in both trial years represented as a percentage of the respective control values for both trial years

	1997 W				1998 W				1998 P			
	GAM	KAR	57	66	GAM	KAR	57	66	GAM	KAR	57	66
LDR												
ST 1												
Day 2	145.34	93.59	166.18	155.80	157.75	129.38	169.84	162.10	263.90	246.88	1265.08	1248.39
Day 6	179.85	170.87	173.51	131.47	160.76	94.37	106.75	120.94	445.28	500.87	1497.55	964.10
ST 2												
Day 2	215.29	178.79	159.33	146.15	159.72	117.58	277.05	420.67	195.83	167.88	1225.41	857.54
Day 6	643.17	435.29	418.43	666.27	145.09	186.08	554.97	1136.47	564.73	84.52	1543.86	1504.73
ST 3												
Day 2	137.19	77.88	112.00	98.23	216.67	220.61	386.89	423.46	277.08	183.64	1286.07	869.83
Day 6	18.42	311.77	380.51	553.31	136.36	276.80	755.56	1355.41	674.55	467.01	147.78	1664.87
TRANS												
ST 1												
Day 2	37.37	97.66	29.90	46.88	69.27	74.06	35.31	44.83	49.72	42.45	12.63	17.24
Day 6	35.58	52.52	76.47	29.50	64.79	103.57	92.58	77.12	31.46	26.34	15.23	16.10
ST 2												
Day 2	45.37	124.12	47.55	70.20	51.26	81.85	32.76	11.56	36.33	84.52	10.67	6.35
Day 6	19.05	25.18	40.88	20.41	69.57	38.06	11.58	4.78	22.61	14.89	4.61	3.98
ST 3												
Day 2	79.44	99.71	89.06	87.65	44.25	55.36	24.95	11.82	44.78	79.76	11.05	6.48
Day 6	18.42	30.11	33.95	32.78	81.45	29.55	11.45	4.69	17.97	31.68	4.74	3.45

ST - stress treatment, W - WOW, P- PEG, GAM - Gamtoos, KAR - Kariega, 57 – SST 57, 66 – SST 66

During the 1997 experimental year, cultivars Gamtoos and Kariega showed increases in LDR and decreases in transpiration rate between days 2 and 6 for all stress treatments, when expressed as percentages of the control values (Table 2). With the exception of transpiration rate for the first stress treatment, a similar tendency was found for SST 57, while SST 66 showed a relative decrease in LDR for the first stress treatment.

In 1998 Gamtoos showed a relative decrease in LDR and an increase in transpiration rate for stress treatments 2 and 3. Kariega, SST 57 and SST 66 on the other hand, showed a relative decrease in LDR and an increase in transpiration rate for stress treatment 1. Although the effects of PEG treatments during 1998 were in general more drastic than the withholding of water, tendencies were more or less the same. During 1998, both SST 57 and SST 66 showed higher relative values for LDR and lower values for transpiration rate at stress treatments 2 and 3 in comparison to Gamtoos and Kariega.

These tendencies indicate that SST 57 and SST 66 might be more sensitive to drought conditions during the later growth stages in comparison to Gamtoos and Kariega. The differences found between cultivars reflect the same tendencies as reported by Gordon, Brown and Dixon (1997).

Relative water content

Changes in the RWC of leaves are considered to be a sensitive indicator of drought stress (Henson, Mahalashmi, Bidinger & Alagarswamy, 1981; Van der Mescht, 1989). The results from the two trial years support this view. A summary of the analysis of variance of the two trial years is given in Table 3. In both trial years significant differences due to stress treatments were shown for all three stress treatments. Although significant differences for cultivars did occur, this was not the case on all sampling days. As a consequence the interactions between cultivar x treatment were also only significant on some sampling days.

Table 3 Results of the ANOVA done on the relative water content (RWC) taken on three sample days of the three stress treatments in both trial years

SOURCES OF VARIATION	MS CULTIVAR (CUL)	MS TREATMENT (TRT)	MS CUL X TRT	CV(%)
Df	3	1	3	
		1997		
ST 1				
Day 2	48.64*	0.38	20.56	4.33
Day 4	17.91	3.53	43.51*	3.63
Day 6	16.71	1730.60**	152.73**	4.54
ST 2				
Day 2	300.28**	647.92**	248.86*	9.90
Day 4	63.07	5180.28**	105.53	22.33
Day 6	11.31	9028.76**	19.30	5.32
ST 3				
Day 2	151.08**	757.13**	170.12**	3.31
Day 4	22.35	1962.04**	11.99	10.71
Day 6	95.71**	4540.25**	71.06**	2.54
SOURCES OF VARIATION	**MS CULTIVAR (CUL)**	**MS TREATMENT (TRT)**	**MS CUL X TRT**	**CV(%)**
Df	3	2	6	
		1998		
ST 1				
Day 2	513.66**	51.25	12.89	5.43
Day 4	341.65**	1048.40**	100.80**	5.31
Day 6	375.03**	2369.42**	175.99*	9.46
ST 2				
Day 2	157.35	524.44**	262.65*	11.87
Day 4	53.88	3916.73**	23.56	15.67
Day 6	194.68**	8525.08**	61.88**	6.21
ST 3				
Day 2	38.71	528.68**	80.65	7.14
Day 4	15.89	1416.51**	87.85	10.55
Day 6	64.30	7633.74**	307.24**	12.60

* significant at 5% confidence level, ** significant at 1 % confidence level,
ST – stress treatment, MS – mean square values, CV (%) – coefficient of variation

In general stress treatments resulted in a decrease in relative water content (RWC) of all cultivars when compared to the control (unstressed) values (Table 4). Although the effect of stress induction by PEG was more drastic compared to the effect of the withholding of water, decreases in RWC during

earlier growth stages (stress treatment 1) were generally less than that of later growth stages (stress treatments 2 and 3).

Table 4 Relative water content (RWC) of stress treatments expressed as percentages of control values for the respective cultivars in both trial years

	1997 W				1998 W				1998 P			
	GAM	KAR	57	66	GAM	KAR	57	66	GAM	KAR	57	66
ST1												
Day 2	97.47	104.56	104.56	173.54	96.09	94.94	98.84	98.84	89.41	92.83	99.40	98.47
Day 4	92.40	101.61	101.61	171.91	94.44	98.30	99.77	99.77	82.77	65.18	79.55	94.40
Day 6	95.69	70.50	70.50	81.56	96.70	68.34	88.92	88.92	83.67	46.51	70.23	72.14
ST2												
Day 2	83.57	65.24	65.24	89.53	105.24	65.57	99.48	99.48	89.55	68.68	83.70	82.87
Day 4	73.81	51.79	51.79	68.53	74.41	65.60	66.68	66.68	53.27	59.72	63.38	65.73
Day 6	58.37	50.19	50.19	49.56	63.05	64.58	67.52	67.52	57.96	38.87	41.48	27.98
ST3												
Day 2	95.94	67.27	67.27	85.61	106.00	82.26	91.14	91.14	82.91	80.01	88.42	87.93
Day 4	79.72	75.39	75.39	81.49	89.64	83.14	82.01	82.01	66.47	80.98	82.97	73.12
Day 6	73.90	60.80	60.80	73.60	67.43	60.59	70.38	70.38	57.83	42.32	39.42	28.10

ST - stress treatment, W – WOW, P - PEG, GAM -Gamtoos, KAR - Kariega, 57 – SST 57, 66 – SST 66

This may indicate that plants become less drought tolerant during later growth stages since many researchers, including Van der Mescht (1989) and Van Heerden and De Villiers (1996b), are of the opinion that drought tolerance is obtained through the ability of cultivars to maintain a high RWC during drought conditions. This was especially true for Gamtoos, which showed very small decreases in RWC due to stress treatment 1. Larger decreases in RWC during stress treatment 1 for other cultivars tested (Kariega, SST 57 and SST 66)

indicated that these cultivars were less drought tolerant during earlier growth stages, compared to Gamtoos.

This tendency also applied for stress treatment 2 during the 1997 experimental year and the induction of water stress with PEG for this growth stage in 1998. During this growth stage (stress treatment 2) the largest decreases in RWC were found for SST 66, indicating higher drought sensitivity for this cultivar. Results obtained with Kariega and SST 57 were not clear, but slightly higher values compared to SST 66 and slightly lower values compared to Gamtoos found on sampling day 6 during stress treatment 2 may indicate that these cultivars are less drought tolerant than Gamtoos, but more tolerant than SST 66 during this growth stage.

These results correlate with those found by Van Heerden and De Villiers (1996b) and Joubert (1987) which classified Gamtoos as being drought tolerant and SST 66 as slightly drought sensitive.

Total ear water potential

Results obtained with total ear water potential corresponded with studies done by Cortes and Sinclair (1986) and El Hafid, Smith, Karrou and Samir (1998). Differences due to treatment, cultivar and the interactions of cultivar x treatment proved to be significant for all three stress treatments (Table 5).

Table 5 Results of the ANOVA done on total ear water potential for the data from before and after stress induction of the three stress treatments in the 1998 experiment

SOURCES OF VARIATION	MS CULTIVAR (CUL)	MS TREATMENT (TRT)	MS CUL X TRT	CV(%)
Df	3	2	6	
ST 1				
Before stress	5.22**	4.19**	1.59**	6.72
After stress	28.97**	701.51**	22.79**	8.57
ST 2				
Before stress	9.37**	7.34**	11.55**	6.22
After stress	50.39**	2762.85**	12.72**	3.11
ST 3				
Before stress	13.39**	3.34**	3.56**	6.00
After stress	268.24**	2065.30**	79.23**	4.89

* significant at the 5% confidence level, ** significant at the 1% confidence level

ST – stress treatment, MS – mean square values, CV (%) – coefficient of variation

The induction of water stress caused a general increase in the total ear water potential. This tendency was true for all three stress treatments of the respective cultivars (Table 6). Although all four cultivars showed increases in their total ear water potential, percentage increases differed between cultivars. The cultivars Gamtoos and Kariega showed smaller increases in total ear water potential in stress treatments 2 and 3 in comparison to SST 57 and SST 66. SST 66 differed slightly from SST 57 in the sense that a small decline in the difference of total ear waterpotential from before until after stress induction was shown in stress treatment 3. This difference, however, was still significantly larger than for stress treatment 1. Results obtained with the use of PEG as a method of stress induction showed the same tendencies, except for stress treatment 2 where

Gamtoos and Kariega showed a decline in the total ear water potential from the start until the end of the stress period.

Table 6 Total ear water potential values before and after stress induction of stress treatments expressed as percentages of control values for the respective cultivars in the 1998 experiment

	1998 W				1998 P			
	GAM	KAR	57	66	GAM	KAR	57	66
ST1								
Before	233.38	106.59	131.96	132.60	105.58	105.58	173.52	194.66
After	611.34	922.65	570.02	417.19	514.66	514.66	331.39	665.13
ST2								
Before	277.92	107.93	297.71	204.65	548.60	548.60	443.51	1031.86
After	412.84	423.84	458.35	688.97	315.75	315.75	1610.95	1648.20
ST3								
Before	449.75	324.24	136.64	176.05	249.26	249.26	1370.74	846.51
After	481.45	582.09	423.70	589.93	613.81	613.81	1994.08	1947.24

ST - stress treatment, W - WOW, P- PEG, GAM - Gamtoos, KAR – Kariega, 57 – SST 57,66 – SST 66

These tendencies indicate that SST 66 might be more sensitive to drought conditions during the later growth stages in comparison to Gamtoos. Results obtained with Kariega and SST 57 were not clear. Larger differences in proline accumulation, when compared to Gamtoos, and smaller differences in comparison to SST 66 may indicate that these cultivars are less drought tolerant than Gamtoos, but more drought tolerant than SST 66 during the later growth stages.

Accumulation of free proline

In general, results obtained with the proline-test confirmed findings of earlier studies done by O'Regan *et al.* (1993) and Van Rensburg *et al.* (1993). In spite of large coefficients of variation (CV) at certain sampling dates, significant differences due to stress treatments were obtained in both years (Table 7).

Table 7 Results of the ANOVA done on the accumulation of free proline (µmole g^{-1} fresh weight) on three sample days of the three stress treatments in both trial years

SOURCES OF VARIATION	MS CULTIVAR (CUL)	MS TREATMENT (TRT)	MS CUL X TRT	CV(%)
Df	3	1	3	
		1997		
ST 1				
Day 2	72.57**	41.87**	61.41**	8.87
Day 4	1692.73**	8317.93**	1657.89**	15.82
Day 6	4618.56**	15494.00**	3926.34**	13.64
ST 2				
Day 2	125.39**	234.38**	23.35**	16.62
Day 4	108.88**	6620.08**	178.50**	5.97
Day 6	2378.95**	13282.22**	2264.11**	12.00
ST 3				
Day 2	215.32**	976.65**	127.57**	17.87
Day 4	248.24**	3658.07**	323.01**	21.52
Day 6	1558.44**	7031.53**	1474.43**	9.08

TABLE 7
(CONTINUE)

SOURCES OF VARIATION	MS CULTIVAR (CUL)	MS TREATMENT (TRT)	MS CUL X TRT	CV(%)
Df	3	2	6	
		1998		
ST 1				
Day 2	27.37*	43.03**	32.11**	31.22
Day 4	2660.26**	5086.72**	601.98**	17.47
Day 6	5463.36**	6153.90**	1318.50**	19.61
ST 2				
Day 2	264.28*	1554.18**	213.59*	58.00
Day 4	464.14	6877.81**	1120.42**	52.76
Day 6	2589.04**	4109.12**	633.20**	25.21
ST 3				
Day 2	188.47	3738.70**	547.43	84.59
Day 4	203.03	12560.34**	1189.27**	25.14
Day 6	3179.60**	4949.54**	935.81**	25.75

*significant at the 5% confidence level, ** significant at the 1% confidence level, ST – stress treatment, MS – mean square values, CV (%) – coefficient of variation

Differences due to cultivar and the interactions of cultivar x treatment also proved to be significant for most sampling days in both years. Water stress induced by the withholding of water or the use of PEG resulted in increased proline contents in stressed plants when compared to control (unstressed) plants (Table 8). Percentage increases, as well as time for the onset and decline of proline accumulation, differed between cultivars, stress treatments and trial years.

Table 8 Proline accumulation taken on three sample days in the three stress treatments expressed as percentages of control values for the respective cultivars in both trial years

	1997 W				1998 W				1998 P			
	GAM	KAR	57	66	GAM	KAR	57	66	GAM	KAR	57	66
ST1												
Day 2	232.56	139.13	100.00	61.90	233.38	106.59	131.96	132.60	105.58	105.61	173.52	194.66
Day 4	481.81	1168.20	303.60	244.14	611.34	922.65	570.02	417.19	514.66	745.98	331.39	665.13
Day 6	1588.70	1281.29	175.88	612.77	1149.30	1034.89	269.01	506.27	631.41	866.25	232.55	435.54
ST2												
Day 2	276.38	138.98	206.55	200.29	277.92	107.93	297.71	204.65	548.60	220.41	443.51	1031.86
Day 4	552.35	520.13	264.22	443.07	412.84	423.84	458.35	688.97	315.75	376.39	1610.95	1648.20
Day 6	1504.76	2069.40	342.50	541.19	956.34	1848.28	393.65	363.83	815.07	1832.07	331.24	192.13
ST3												
Day 2	363.83	332.20	134.83	395.71	449.75	324.24	136.64	176.05	249.26	879.66	1370.74	846.51
Day 4	540.45	519.62	176.32	294.44	481.45	582.09	423.70	589.93	613.81	548.87	1994.08	1947.24
Day 6	1221.75	1580.13	277.39	268.33	962.96	1776.90	258.49	268.72	834.26	2631.03	455.56	305.75

ST - stress treatment, W - WOW, P- PEG, GAM - Gamtoos, KAR - Kariega, 57 – SST 57, 66 – SST 66

The results obtained for the accumulation of free proline in Gamtoos (drought tolerant) and SST 66 (slightly drought sensitive) correspond with those found by Van Heerden and De Villiers (1996a). Values for Gamtoos increased from sampling day 2 to day 6 for all three stress treatments in both years (Figure 1). Kariega showed the same tendencies as Gamtoos, with a more rapid increase in proline content from sampling day 2 to 4, but no significant difference when compared to Gamtoos at sampling day 6. Proline values for SST 66 also increased between sampling days 2 and 6 for stress treatment 1, but increases were considerably less than those found for Gamtoos (Table 8). For stress

treatment 2 (only 1998) and 3, proline content for SST 66 increased between sampling days 2 and 4 and declined between sampling days 4 and 6 (Figure 1). SST 57 showed similar tendencies for all three stress treatments in 1998. Although proline accumulation for SST 57, expressed as a percentage of the control, increased between sampling days 2 and 6 for stress treatments 2 and 3 during 1997, values were also much lower than those found for Gamtoos.

These results indicated that SST 57, although to a lesser extent than SST 66, might be classified as less drought tolerant than Gamtoos. The results obtained with Kariega on the other hand correspond closely to those of Gamtoos, indicating that Kariega may also be classified as drought tolerant. Although the data on the accumulation of free proline in Gamtoos and Kariega corresponds and the data of the two SST cultivars are similar, neither of these pairs are genetically related in such a way that it could explain the similarity of data (personal communications with Dr. B. Lombaard (Sensako) and Dr. H.A. van Niekerk (LNR –SGI)).

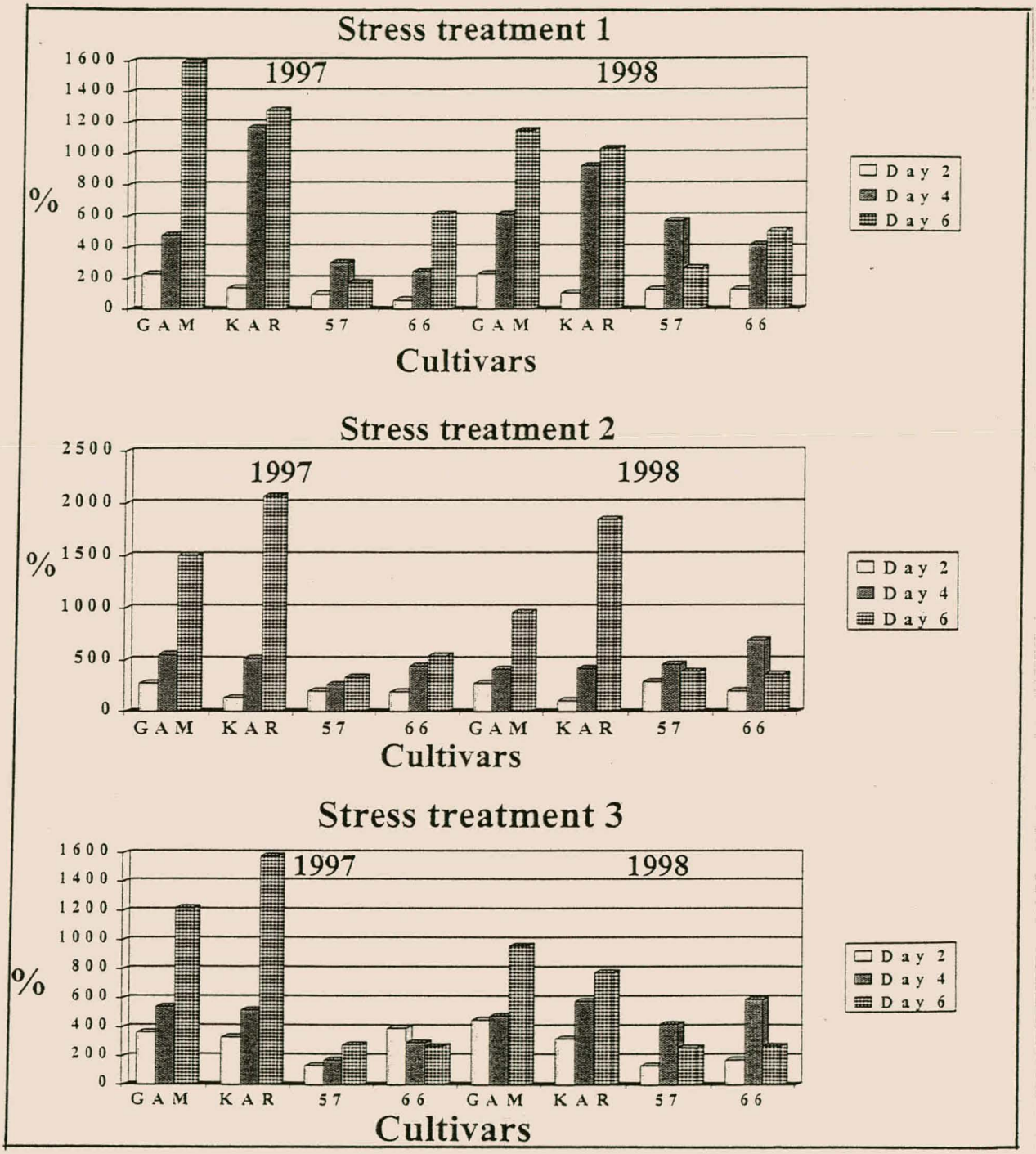

Figure 1 Proline accumulation in three stress treatments expressed as percentages of control values of the respective cultivars in both trial years (GAM – Gamtoos, KAR – Kariega, 57 – SST 57, 66 – SST 66).

Conclusion

This study showed that physiological parameters such as diffusive resistance, transpiration rate, leaf water content, ear water potential and proline content, can be used to identify drought tolerance in different spring wheat cultivars. Daily fluctuations in diffusive resistance and transpiration rate due to differences in solar radiation may however conceal genotypic differences. Leaf water content and proline content, on the other hand, proved to be the most sensitive parameters. Although this study showed that genotypic differences between South African spring wheat cultivars do exist in regard to the response of different physiological parameters to water stress, one should also look at the effect on plant growth and harvest data to decide whether these differences are of any commercial benefit. This was addressed in chapters 4 (growth) and 5 (yield).

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Chapter 4

DROUGHT TOLERANCE DETERMINATIONS USING THE EFFECT OF WATER STRESS ON THE BIOMASS PRODUCTION OF SPRING WHEAT CULTIVARS

Abstract

Water stress affects the anatomy, physiology and biochemistry of plants at every growth stage. Wheat crops produced under dryland conditions often experience periods of water stress. It is therefore important to identify spring wheat cultivars that can withstand these conditions. The aim of this study was to determine whether the effect of water stress on different plant components could be used to distinguish between drought tolerant and drought sensitive spring wheat cultivars grown in the Western Cape region of South Africa. The withholding of water (WOW) and the use of polyethylene glycol (PEG) at the flag leaf - and milky kernel stages individually, and at both growth stages combined, induced water stress. It was shown that the use of the plant components were unable to distinguish between cultivars for drought tolerance when stress was applied at the flag leaf – and kernel filling stages. The use of leaf area determinations, however, shows promise as a method to distinguish between drought tolerant and drought sensitive cultivars.

Introduction

Water stress affects the anatomy, morphology, physiology and biochemistry of plants at every growth stage (Kramer, 1969). Earlier work has indicated that each organ and physiological process may respond differently to increasing water deficits (Navikov, 1952). The effect of water stress is also related to both the growth stage and intensity of the stress period (Campbell & Davidson, 1979).

A significant reduction in leaf area due to the effects of water stress was observed by several researchers (Quarrie & Jones, 1977; Agenbag & De Villiers, 1995). Wardlaw (1971) noted depression of the accumulation of dry weight in both stems and roots by water stress, while Keim and Kronstad (1981) reported a 55% reduction in tiller number in water stressed plants compared to well-watered plants. Blum, Mayer and Gozland (1983) demonstrated, through the use of mild and severe stress treatments, that water stress always reduces vegetative growth.

Du Pisani and Partridge (1990) warned that global warming might result in an increase in winter temperatures and a decrease in the rainfall of the Western Cape region. This means that spring wheat produced in these regions may in future, be more often subjected to periods of water stress, which is considered to be the most important factor limiting crop yields (Jones & Corlett, 1992).

The aim of this study was to determine whether the effect of water stress on the different plant components could be used to distinguish between drought tolerant and drought sensitive spring wheat cultivars grown in the Western Cape region of South Africa.

Material and Methods

Plant Material

Four spring wheat cultivars were used in this study. Two of these, Gamtoos (drought tolerant) and SST 66 (slightly drought sensitive) had showed differences in drought tolerance in studies done by Van Heerden and De Villiers (1996). The other two, Kariega and SST 57, were of unknown drought tolerance.

Growth Conditions

Six seeds of the respective cultivars were planted (1997 and 1998) in 2-litre plastic pots filled with coarse sand. Eight days after emergence, seedlings were thinned to 3 plants per pot. Experiments were conducted in a temperature-controlled (16°/10°C day/night) glasshouse at the Welgevallen Experimental Farm, Stellenbosch. Irrigation was done by a computerized system according to the daily solar radiation received. More pulses were thus given on sunny days than on cloudy days. For the first two weeks seedlings were irrigated with water and for the rest of the growth period with a balanced nutrient solution.

Induction of water stress

Water stress was induced by the withholding of water at different stages of plant development in both experimental years (1997 and 1998). In 1998 polyethylene glycol 6000, with an osmotic potential of –1900kPa (Hanson, Nelson & Everson, 1977), was used as an additional treatment to induce water stress. Three stress treatments, each lasting seven days, were applied. The first treatment commenced at the flag leaf stage, the second at the milky kernel stage and the third a combination of the two stages. At the end of each stress period, pots of

these treatments were flushed with the Hoagland nutrient solution to ensure that stress conditions were terminated. Plants, which were kept well watered throughout their development, were used as a control treatment.

Measurements

Three plants per treatment were harvested before each stress treatment started and again when the stress was relieved. Control plants were sampled at the same growth stages. The green leaf area was determined by the use of a leaf area meter. Plants were divided into ears, leaves and stems. Each component was allowed to dry for 48 hours in an oven at 70°C, before their dry mass was determined.

An analysis of variance was done an all parameters. The T-test (P = 5%) was used to compare treatment means.

Results and Discussion

Plant components

Number of stems and ears

In both experimental years (1997 & 1998) no significant differences in the number of stems and ears, due to stress treatments, were observed at the start of the stress treatments. Similar results were obtained after seven days of stress (when stress was relieved) (Table 1 & 2). Differences due to cultivars were found before and at the end of stress treatment 1 (flag leaf), but not for stress treatments 2 (milky kernel) and 3 (flag leaf and milky kernel stage) during 1997. These observed differences were therefore most probably due to differences in the rate of development of the different cultivars. During the 1998 experiment

significant differences, probably due to cultivar differences, for number of ears were found at the beginning of stress treatment 1, as well as at the start of stress treatment 3. No differences were noted at the end of these stress treatments.

Table 1 Results of the ANOVA done on plant components of the 1997 experiment

	Sources of variation	MS Cultivar (CUL)	MS Treatment (TRT)	MS CUL x TRT
	Df	3	1	3
ST1	Tillers - B	1.95*	0.17	1.06
	- A	3.67*	1.50	1.61
	Ears – B	1.39**	0.17	0.06
	- A	2.82*	2.04	1.15
	Leaf area - B	16993.71	2093.65	21869.66
	- A	4427.67	45004.21**	16169.79*
	Leaf dry mass - B	0.49	0.09	0.02
	- A	0.99	0.16	0.98
	Stem dry mass - B	1.21**	0.03	0.04
	- A	1.19	0.35	0.48
	Ear dry mass – B	0.06**	0.02	0.01
	- A	0.27*	0.64	0.06
ST2	Tillers - B	2.82	0.04	0.82
	- A	3.38	2.04	5.26
	Ears – B	1.93	1.04	0.82
	- A	2.50	0.67	5.17
	Leaf area - B	91697.13**	3365.64	9909.84
	- A	5441.63	353461.00**	56313.53**
	Leaf dry mass - B	5.79**	0.14	1.10
	- A	1.31*	0.24	3.53**
	Stem dry mass - B	9.57**	0.35	3.73**
	- A	0.56	2.15	6.34*
	Ear dry mass – B	8.12	0.02	8.39
	- A	23.21*	1.19	9.10

Table 1 (continue)

	Sources of variation Df	MS Cultivar (CUL) 3	MS Treatment (TRT) 1	MS CUL x TRT 3
	Tillers - B	0.56	1.50	3.61
	- A	5.61	1.56	2.50
	Ears – B	0.49	1.04	1.60
	- A	2.39	0.17	2.83
	Leaf area - B	33761.87	7053.11	31799.74
ST3	- A	11053.52	582040.88**	8251.52
	Leaf dry mass - B	4.07**	0.23	1.56
	- A	0.51	0.56	0.11
	Stem dry mass - B	6.96**	0.55	5.43**
	- A	0.85	0.12	2.99
	Ear dry mass – B	4.95	3.74	2.58
	- A	26.42*	46.79	12.75

* significant at 5% confidence level, ** significant at the 1% confidence level, ST1 – stress treatment at flag leaf, ST2 – stress at milky kernel stage, ST3 – combination of ST1 and ST2, MS – mean square values, B – before stress induction, A – seven days after stress induction

Table 2 Results of the ANOVA done on plant components of the 1998 experiment

	Sources of variation Df	MS Cultivar (CUL) 3	MS Treatment (TRT) 2	MS CUL x TRT 6
	Tillers - B	1.00	0.53	3.09
	- A	2.69	3.11	3.44
	Ears – B	1.29**	0.44	0.15
	- A	1.22	0.86	0.64
	Leaf area - B	74992.78**	961.33	75026.35**
ST1	- A	23826.26*	33485.07**	27371.09**
	Leaf dry mass - B	1.78*	0.12	0.40
	- A	2.36*	0.43	0.97
	Stem dry mass - B	1.21**	0.07	0.43
	- A	4.39**	0.21	0.61
	Ear dry mass – B	0.12*	0.01	0.01
	- A	0.20*	0.17	0.02

Table 2 (continue)

	Sources of variation Df	MS Cultivar (CUL) 3	MS Treatment (TRT) 2	MS CUL x TRT 6
ST2	Tillers - B	0.67	1.03	2.81
	- A	1.52	2.53	0.71
	Ears – B	1.51	2.11	1.37
	- A	0.77	2.53	0.71
	Leaf area - B	82436.86**	29892.30	10296.64
	- A	NA	NA	NA
	Leaf dry mass - B	2.13**	0.90	0.58
	- A	3.32**	0.30	1.22
	Stem dry mass - B	13.20*	10.18	3.45
	- A	23.29**	1.59	3.14
	Ear dry mass – B	7.56*	17.28	2.32
	- A	34.67**	0.12	2.02
ST3	Tillers - B	1.73	3.86	0.79
	- A	5.66	3.11	3.19
	Ears – B	5.52**	5.25	0.88
	- A	6.55*	2.11	1.30
	Leaf area - B	118501.62**	164967.14**	11170.32
	- A	4015.43	191090.98**	4956.79
	Leaf dry mass - B	6.26**	1.81**	0.60
	- A	2.64*	3.15*	1.79
	Stem dry mass - B	27.50**	34.44**	3.37
	- A	14.26**	6.92	1.09
	Ear dry mass – B	9.57**	43.71**	2.12
	- A	15.47*	32.01**	3.63

* significant at 5% confidence level ** significant at the 1% confidence level
NA – not available due to equipment error, ST1 – stress treatment at flag leaf, ST2 – stress treatment at milky kernel stage, ST3 – combination of both ST1 and ST2, MS – mean square values, B – before stress induction, A – seven days after stress induction.

No significant differences due to cultivar x treatment interactions were obtained in both experimental years. Because no differences between stress and control (unstressed) treatments were obtained, it could therefore be concluded that

water stress during the flag leaf and kernel filling stages did not result in the abscission of secondary tillers in these experiments.

Leaf area

With the exception of stress treatment 3 in 1998 (Table 2), no significant differences due to stress treatments were found at the beginning of the stress treatments, indicating that all plants experienced similar growth conditions before the onset of the stress treatments. However, significant differences were observed at the end of the stress treatments. Unfortunately no leaf area determinations could be done at the end of stress treatment 2 in 1998, due to a faulty leaf area meter. Although no significant differences due to the stress treatment at the beginning of stress treatment 3 were found in 1997 (Table 1), differences found in 1998 (Table 2) were probably the effect of the earlier stress treatment (treatment 1) as these plants were stressed at both the flag leaf and milky dough stage of development. Significant differences due to cultivar were only found at the beginning of stress treatment 2 in 1997. In the 1998 experiment significant differences were noted at the start of all three stress treatments, as well as at the end of stress treatment 1.

Differences due to cultivar are to be expected because of the different rates of development of different cultivars. The significant differences in stress treatment 3 of the 1998 experiment could also be attributed to the fact that this treatment received two periods of stress. Statistically significant differences due to cultivar x treatment interactions were also noted at the end of stress treatments 1 and 2 in 1997. Only stress treatment 1 of the 1998 experiment

showed significant cultivar x treatment interactions before and at the end of the stress treatment. Interactions at the start of the stress treatment might be attributed to the different rates of development of the different cultivars.

When expressed as percentages of the respective control values, the general tendency for plants in all three stress treatments was a reduction in total leaf area per plant in both experimental years (Table 3). This corresponds with the conclusion of Begg and Turner (1976) that one of the most important consequences of water deficits is a significant reduction in leaf area. The use of polyethylene glycol as method of stress induction in the 1998 experiment showed similar results.

Table 3 Treatment means of leaf area (cm^2) per plant expressed as percentages of their respective control (unstressed) values in both trial years

	Gamtoos	Kariega	SST 57	SST 66
1997				
ST1				
W – B	90.04	76.28	97.09	124.68
W – A	56.60	41.71	60.72	81.73
ST2				
W – B	96.44	82.84	113.94	100.00
W – A	101.44	47.66	34.43	50.57
ST3				
W – B	72.57	105.91	126.36	173.67
W – A	28.96	29.37	30.19	17.08

Table 3
(continue)

	Gamtoos	Kariega	SST 57	SST 66
1998				
ST1				
W – B	98.51	76.45	100.69	137.97
W – A	64.61	40.43	47.86	62.88
P – B	90.25	50.99	97.25	103.66
P – A	60.13	23.79	56.81	53.68
ST2	NA	NA	NA	NA
ST3				
W – B	93.89	68.22	63.10	34.29
W – A	31.34	27.97	21.33	34.69
P – B	49.87	34.88	41.77	36.86
P – A	28.77	24.33	27.78	11.33

NA – not available, ST1 – stress treatment at flag leaf, ST2 – stress treatment at milky kernel stage, ST3 – a combination of ST1 and ST2, W – WOW, P – PEG, B – before stress induction, A – after seven days of stress

Two exceptions to the general tendency were noted. In 1997 Gamtoos showed a 5.2% increase in total leaf area per plant from the start to the end of stress treatment 2 when leaf area of stressed plants were expressed as a percentage of control plants (Table 4).

Table 4 Percentage reduction in leaf area per plant over each stress treatment of both trial years

	Gamtoos	Kariega	SST 57	SST 66
1997				
ST1 – W	37.14	45.32	37.46	34.45
ST2 – W	5.18(+)	42.47	69.78	49.43
ST3 – W	60.09	72.27	76.11	90.16
1998				
ST1 – W	34.41	47.12	52.47	54.42
ST2 – W	NA	NA	NA	NA
ST3 – W	66.62	59.00	66.20	1.17(+)
ST1 – P	33.37	53.34	41.58	48.21
ST2 – P	NA	NA	NA	NA
ST3 – P	42.31	30.25	33.49	69.26

NA – not available, ST1 – stress treatment at flag leaf, ST2 – stress treatment at milky kernel stage, ST3 – combination of ST1 and ST2, W – WOW, P – PEG, + - increase in leaf area per plant

Similarly SST 66 showed a 1.2% increase in leaf area in stress treatment 3. On the other hand, stress treatment 1 of the 1997 experiment, Kariega showed the highest reduction in leaf area of 45.3%, while SST 66 had the lowest reduction (34.5%) (Table 4). In stress treatment 2, SST 57 showed the highest reduction of nearly 70%. From Table 4 it is clear that, when expressed as percentages of control values, stress treatment 3 caused the largest reductions in leaf area. During 1997 Gamtoos was the least affected by stress treatment 3 (60.1% reduction) and SST 66 the most affected (90.2% reduction). Reductions for Kariega and SST 57 were 72.3% and 76.1% respectively. In the 1998 experiment

Gamtoos, again, showed the smallest reduction in leaf area per plant as a result of stress treatment 1 (both methods of stress induction) and SST 66 the largest (Table 4). Although SST 66 showed an increase due to stress treatment 3, when stress was induced by the withholding of water, stress due to polyethylene glycol again resulted in the largest decrease in this cultivar.

As maximum leaf area is attained before heading when the flag leaf has fully emerged (Fischer & Kohn, 1966), it could be argued that reduction in leaf area was due to a natural genetically controlled decline as leaf senescence progressed (Simmons, 1987). Significant reductions in leaf area of plants under stress, when expressed as percentages of control plants, clearly indicated that this was not the case. Differences in reductions found for different cultivars could therefore be an indication of differences in drought tolerance and not differences in growth patterns.

Dry mass of plant components

Leaf mass

In the 1997 experiment no significant differences in leaf dry mass due to stress treatments, were observed (Table 1). Significant cultivar differences were noted for stress treatment 2 and 3 at the beginning of each treatment, while only stress treatment 2 showed significant differences at the end of the stress treatment. Significant differences due to cultivar x treatment interactions were only obtained at the end of stress treatment 2. The 1998 experiment showed the same tendencies in the sense that no significant differences due to stress treatments were noted in stress treatments 1 and 2 (Table 2). Stress treatment 3 however

showed significant differences at the start and the end of the stress treatment. Differences due to cultivar were significant at both the start and end of all stress treatments. No significant differences due to cultivar x treatment interactions were observed. The significant cultivar differences showed that cultivars do differ in regard to their potential to produce leaves under similar growth conditions.

Although no significant differences were noted due stress in treatment 2 of the 1997 experiment, cultivars responded differently. When leaf dry mass per plant of the stressed plants were expressed as a percentage of their respective controls, Gamtoos and Kariega showed increases, while the two SST cultivars showed a decrease (Table 5).

Table 5 Increases (+) or decreases (-) in leaf dry mass per plant shown in the milky kernel stage, expressed as a percentage of the respective control (unstressed) values

	Gamtoos	**Kariega**	**SST 57**	**SST 66**
ST2				
W	1.82 (+)	56.40 (+)	23.21 (-)	8.70 (-)
P	31.37 (-)	29.03 (-)	57.40 (-)	14.03 (-)

ST2 – stress treatment at milky kernel stage, W – WOW, P – PEG

In the 1998 experiment, significant differences in leaf dry mass were found for stress treatment 3 as a result of significant differences between control and stress values for the two SST cultivars (Table 6).

Table 6 Leaf and ear dry mass per plant of all four cultivars as affected by stress treatment 3 of the 1998 experiment

	Gamtoos	Kariega	SST 57	SST 66	LSD (P=0.5)
Leaf dry mass (g/plant)					
Before					
W	4.40	2.27	2.70	2.07	
P	4.63	2.83	3.07	2.63	0.95
C	4.30	2.60	3.90	3.73	
After					
W	5.51	1.42	2.80	2.63	
P	2.92	2.06	2.43	1.83	0.56
C	2.38	1.81	3.17	2.70	
Ear dry mass (g/plant)					
Before					
W	5.30	3.67	5.60	2.93	
P	3.07	2.60	2.80	1.27	4.22
C	5.50	5.47	8.20	5.50	
After					
W	2.72	3.52	4.00	1.70	
P	2.51	1.80	2.60	1.03	2.18
C	3.52	6.25	8.10	5.70	

W – WOW, P – PEG, C – control plants, B – before stress induction, A – after seven days of stress, LSD – least significant difference values

These significantly lower stress values when compared to their control (unstressed) values might be a remnant of the first stress treatment (ST1 at flag leaf). Although these two cultivars showed differences at the start of the stress treatment, no differences due to stress were obtained for these two cultivars at

the end of the stress treatment. The treatment differences obtained at the end of this stress treatment were the result of lower overall values obtained by Kariega. Although there were no significant difference between the stress and control value of Kariega, these values were significantly lower in comparison to that of the other three cultivars (Table 6). In general no significant differences were obtained between the withholding of water and the use of polyethylene glycol as methods of stress induction. These results clearly indicated that leaf mass, as affected by water stress, can not be used as an indicator to identify tolerant cultivars. These results may, however, be due to the fact that stress treatments were only applied at or after the flag leaf stage.

Stem mass

No significant differences due to stress treatments were shown in the 1997 experiment (Table 1). Cultivar differences were only noted at the beginning of each stress treatment, with no differences at the end of these stress treatments. Significant differences due to cultivar x treatment interactions were obtained with stress treatment 2 at both the start and the end of the stress treatment, while stress treatment 3 only showed differences at the beginning of the stress treatment. Cultivar differences found at the beginning of each stress treatment, as with leaf dry mass, showed that cultivars do differ in regard to their potential to produce stems under similar growth conditions. The 1998 experiment also showed no significant differences due stress treatments 1 and 2, while stress treatment 3 showed the same tendency as with leaf dry mass (Table 2). The

same tendency with differences due to cultivar was shown as in the 1997 experiment. No significant cultivar x treatment interactions were obtained.

Although significant interactions were shown for the 1997 experiment, these interactions were due to differences between control or stress values between cultivars and not due to differences in the response to various stress treatments. No significant interactions were shown in the 1998 experiment. It could therefore be concluded that stem mass can not be used to identify tolerant cultivars. Stress treatments at early growth stages, such as tillering, may however produce different results.

Ear mass

The 1997 experiment showed no significant differences in ear mass due to stress treatments (Table 1). Differences due to cultivar were only noted at the start of stress treatment 1, while all three stress treatments showed differences at the end of the treatments. No significant cultivar x treatment interactions were noted. The 1998 experiment showed similar tendencies. The only significant differences due to stress treatments noted were in stress treatment 3. Differences due to cultivar were shown at the start and end of each stress treatment. Again no significant differences due to cultivar x treatment interactions were noted (Table 2).

The significant differences obtained due to cultivar might be due to the different growth potentials of the different cultivars. In 1998 stress treatment 3 caused significant decreases in the ear masses of the two SST cultivars, while it was not the case for Gamtoos and Kariega. Although this tendency was not noted in the

1997 experiment it could be an indication that SST 57 and SST 66 are more susceptible to water stress than Gamtoos and Kariega.

Conclusion

It was evident from the dry mass data of plant components that the leaf mass, stem mass and ear mass were unable to clearly distinguish between cultivars for drought tolerance when stress was applied at either the flag leaf or kernel filling stages. This was also the case for the number of stems and ears. The use of leaf area determinations, however, showed promise as a method to distinguish between drought tolerant and drought sensitive cultivars. There are also definite differences between cultivars in the rate at which leaves die when water stress is applied. Gamtoos seemed the least affected by the water stress, while SST 66 showed the highest decrease in leaf area. Kariega and SST 57 did better than SST 66 but worse than Gamtoos. For ear mass, SST 57 and SST 66 also showed indications of being more susceptible to drought than did Gamtoos and Kariega.

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Chapter 5

DROUGHT TOLERANCE DETERMINATIONS USING THE EFFECT OF WATER STRESS ON YIELD AND YIELD COMPONENTS OF SPRING WHEAT CULTIVARS

Abstract

The effect of water stress on wheat yield depends on both the growth stage of the crop and the intensity of the stress. Wheat crops produced under dryland conditions often experience periods of water stress. It is therefore important to identify spring wheat cultivars that can withstand these conditions. The aim of this study was to determine whether the effect of water stress on the yield and yield components of wheat could be used to distinguish between drought tolerant and drought sensitive spring wheat cultivars grown in the Western Cape region of South Africa. Water stress was applied at the flag leaf and milky kernel stages individually, as well as at both growth stages combined, by the withholding of water. It was shown that water stress at these growth stages did reduce the yield and yield components of the four cultivars tested, but no clear distinction for drought tolerance could be made between cultivars on this basis.

Introduction

The Western and Southern Cape wheat producing areas annually produce up to one third of the South African wheat crop. Du Pisani and Partridge (1990) predicted that global warming might result in an increase in winter temperatures and a decrease in the rainfall of these regions. This means that spring wheat production in these regions may more often be subjected to periods of water stress, which is considered to be the most important factor limiting crop yields (Jones & Corlett, 1992).

The effect of water stress on wheat yield depends on both the growth stage of the crop (Entz & Fowler, 1984) and the intensity of the stress (French & Schultz, 1984). The effect of water stress on the components of yield is also determined by these factors (Campbell & Davidson, 1979). Research has also shown that water stress during seed development is more detrimental to grain yield than stress during the vegetative period (Campbell, 1968). Yield losses might also occur if stress conditions prior to anthesis cause a reduction in kernels per plant (Aspinall, 1984). Water stress during the grain filling period, on the other hand, could shorten the period of grain filling and reduce kernel mass (Austin, 1989).

This study was undertaken to determine the effect of water stress, during the flag leaf and milky kernel stages, on yield and yield components of different spring wheat cultivars.

Material and Methods

Plant Material

Four spring wheat cultivars were used in this study. Two of these, Gamtoos (drought tolerant) and SST 66 (slightly drought sensitive), had showed differences in drought tolerance in studies done by Van Heerden and De Villiers (1996). The other two, Kariega and SST 57, were of unknown drought tolerance.

Growth Conditions

Six seeds of the respective cultivars were planted (1997 and 1998) in 2-litre plastic pots filled with coarse sand. Eight days after emergence, seedlings were thinned to 3 plants per pot. Experiments were conducted in a temperature-controlled (16/10°C day/night) glasshouse at the Welgevallen Experimental Farm, Stellenbosch. Irrigation was done by a computerized system according to the daily solar radiation received. More pulses were thus given on sunny days than on cloudy days. For the first two weeks seedlings were irrigated with water and for the rest of the growth period with a balanced nutrient solution.

Induction of water stress

Water stress was induced by the withholding of water at different stages of plant development in both experimental years (1997 and 1998). Three stress treatments, each lasting seven days, were applied. The first treatment commenced at the flag leaf stage, the second at the milky kernel stage and the third at both stages. At the end of each stress period, pots of these treatments were flushed with the Hoagland nutrient solution to ensure that stress conditions were

terminated. Plants, which were kept well watered throughout their development, were used as a control treatment.

Measurements

Three pots per treatment were used to determine the final yield, each of the three pots served as a replication. Each component was allowed to dry for 48 hours in an oven at 70°C before their dry mass was determined. Subsequently the spikelets per ear were counted, ears were threshed and the kernel mass and number were determined. To determine the kernel number per spikelet, the kernel number per plant was firstly divided by the number of ears followed by the number of spikelets per ear. The harvest index (HI) was calculated by expressing the yield as a factor of the aboveground biomass. Data gathered from the three plants per replication were then recalculated on a single plant basis.

An analysis of variance was done an all parameters. The T-test (P = 0.05) was used compare treatment means.

Results and Discussion

Ears

Ears per plant for different cultivars differed significantly in 1997, irrespective of the induced stress treatment (Table 1). Cultivars used in these experiments therefore differed in their potential to produce ears under similar growth conditions, which corresponds with findings of Stern and Kirby (1979). The induction of water stress at the flag leaf, milky kernel or at both growth stages combined, did not affect ears per plant in 1997.

Table 1 Results of the ANOVA done on the yield and yield components of the1997 experiment

	Sources of variation	MS Cultivar (CUL)	MS Treatment (TRT)	MS CUL X TRT
	Df	3	1	3
	Ear number	18.01**	0.63	3.89*
	Ear mass	9.18	75.78**	9.27
	Number of spikelets	69.56**	13.50	2.39
ST1	Kernel number	531.87	29005.14*	3002.75
	Thousand kernel mass	66.32*	60.67*	95.43*
	Kernel mass	3.94	67.44**	4.63
	Harvest index	0.00	0.03**	0.00
	Ear number	14.46**	3.01	0.63
	Ear mass	11.65	197.46**	20.23*
	Number of spikelets	39.22**	0.17	1.61
ST2	Kernel number	5196.97	471.09	581.08
	Thousand kernel mass	103.03**	2376.66**	248.43
	Kernel mass	6.25	123.85**	10.29
	Harvest index	0.02	0.08**	0.03
	Ear number	5.90*	6.62	1.28
	Ear mass	3.72	298.15**	9.54
	Number of spikelets	36.61**	2.67	4.00
ST3	Kernel number	1597.90	50264.62**	1146.63
	Thousand kernel mass	87.88**	1519.56**	264.49
	Kernel mass	3.00	209.45**	6.36
	Harvest index	0.01	0.07**	0.01

* significant at 5% confidence level, ** significant at the 1% confidence level, ST1 – stress treatment at flag leaf stage, ST2 – stress treatment at the milky kernel stage, ST3 – combination of ST1 and ST2, MS – mean square values

Table 2 Results of the ANOVA done on the yield and yield components of the 1998 experiment

	Sources of variation	MS Cultivar (CUL)	MS Treatment (TRT)	MS CUL X TRT
	Df	3	1	3
ST1	Ear number	3.49	10.80**	2.61
	Ear mass	17.68	248.52**	2.14
	Number of spikelets	59.72**	2.67	0.89
	Kernel number	6022.42	72233.07**	818.71
	Thousand kernel mass	32.63**	213.85**	213.60**
	Kernel mass	8.36	168.70**	1.50
	Harvest index	0.01	0.07**	0.01
ST2	Ear number	0.95	0.00	1.34
	Ear mass	14.74	52.13*	2.32
	Number of spikelets	53.38**	0.04	2.60
	Kernel number	7412.40	136.85	1704.88
	Thousand kernel mass	95.41**	882.94**	158.56
	Kernel mass	8.12	41.11**	1.31
	Harvest index	0.00	0.04**	0.00
ST3	Ear number	2.02	15.52**	0.42
	Ear mass	8.80	232.50**	1.45
	Number of spikelets	74.04**	1.04	2.15
	Kernel number	2929.43	56584.05**	1013.10
	Thousand kernel mass	170.30**	233.19**	32.18
	Kernel mass	5.27	143.33**	1.15
	Harvest index	0.00	0.01	0.00

* significant at 5% confidence level, ** significant at the 1% confidence level, ST1 – stress treatment at flag leaf stage, ST2 – stress treatment at the milky kernel stage, ST3 – combination of ST1 and ST2, MS – mean square values

In the 1998 experiment (Table 2), ears per plant were significantly reduced when water stress was applied at the flag leaf stage (stress treatments 1 and 3) (Table 3). No effect was obtained when stress was applied at the milky kernel stage only.

The fact that the 1998 experiment experienced slightly higher temperatures, might explain why less ears per plant were formed because less tillers were produced.

Table 3 Means of stress and control values of number of ears per plant in stress treatments 1 and 3 of the 1998 experiment

Treatment and Cultivar	Stress	Control	LSD (P=0.05) Treatment	Percentage Increase or decrease
		1998		
ST1				
Gamtoos	4.57	4.00	0.89	+39.2
Kariega	2.87	5.33		-46.2
SST 57	3.10	4.87		-36.3
SST 66	1.77	3.47		-49.0
ST3				
Gamtoos	2.67	4.00	1.04	-33.3
Kariega	3.23	5.33		-39.4
SST 57	2.87	4.87		-41.1
SST 66	2.47	3.47		-28.8

(+) - % increase, (-) - % decrease, ST1 – stress treatment at flag leaf, ST3 – combination of stress at the flag leaf and milky kernel stage, LSD – least significant difference values

In contrast to the flag leaf stage, these results clearly showed that ears per plant of spring wheat cultivars could not be affected if water stress occurs after the primary tiller had reached the milky kernel stage. With the exception of stress treatment 1 (water stress applied at the flag leaf stage) in the 1997 experiment, no significant cultivar x treatment interaction was shown (Table 1 and 2). Although these results mean that cultivars did not differ in their general response to water stress at

different growth stages, differences may surface when cultivar responses to water stress are expressed as percentage increases or decreases. If main effects are highly significant these responses of some cultivars may even be significant, although the ANOVA did not indicate significant interactions (Snedecor & Cochran, 1967).

In this study ears per plant were, as already mentioned, only significantly affected in 1998 when water stress was applied during the flag leaf stage (treatments 1 and 3) of the wheat. In these treatments, ears per plant of Gamtoos showed an increase of 39.2% and a decrease of 33.3% due to water stress (Table 3). On average it could thus be said that Gamtoos showed little reaction, but such a statement may be doubtful due to the large difference in response between treatments 1 and 3. Kariega, SST 57 and SST 66 showed large decreases in ears per plant for both stress treatments. Although decreases for these cultivars varied on average between 42.8% for Kariega and 38.1% and 38.2% for SST 57 and SST 66 respectively, differences were too small to classify any of these cultivars as "more tolerant" than the other. There can be, however, little doubt that large decreases in number of ears per plant are to be expected for these cultivars if water stress occurs during the flag leaf stage.

Ear mass

In 1997 the ear mass for different cultivars did not differ significantly (Table 1). The induction of water stress at the flag leaf, milky kernel or at both growth stages combined, significantly reduced ear mass per plant (Table 4). Although the

number of ears per plant were not affected by the stress treatments in 1997 (discussed previously) the respective ear masses were reduced.

Table 4 Means of stress and control values of ear mass per plant (g / plant) in both experimental years, also expressed as the percentage reduction of the stress values in comparison to the respective control values

Treatment and Cultivar	Stress	Control	% Reduction
		1997	
ST1			
Gamtoos	8.23	12.80	36.7
Kariega	11.73	13.09	10.4
SST 57	8.97	10.68	16.0
SST 66	6.46	13.05	50.5
ST2			
Gamtoos	5.86	12.80	54.2
Kariega	2.74	13.09	79.1
SST 57	8.50	10.68	20.4
SST 66	9.56	13.05	26.7
ST3			
Gamtoos	2.96	12.80	76.9
Kariega	5.14	13.09	60.7
SST 57	6.83	10.68	36.1
SST 66	6.48	13.05	50.4
		1998	
ST1			
Gamtoos	6.97	12.35	43.6
Kariega	4.75	10.37	54.2
SST 57	7.27	14.03	48.2
SST 66	3.03	11.01	72.5

Table 4
(continue)

Treatment and Cultivar	Stress	Control	% Reduction
ST2			
Gamtoos	9.73	12.35	21.2
Kariega	6.39	10.37	38.4
SST 57	10.17	14.03	27.5
SST 66	9.69	11.01	12.0
ST3			
Gamtoos	6.32	12.35	48.8
Kariega	4.48	10.37	56.8
SST 57	6.40	14.03	54.4
SST 66	5.65	11.01	48.7

ST1 – stress treatment at flag leaf, ST2 – stress treatment at milky kernel stage, ST3 – combination of ST1 and ST2, LSD – least significant difference values

In the 1998 experiment the ear mass for different cultivars also did not differ significantly (Table 2). Similar reduction in ear mass per plant was noted in 1998 when water stress was applied (Table 4). With the exception of stress treatment 2 (water stress applied at the milky kernel stage) in the 1997 experiment, no significant cultivar x treatment interaction was shown (Table 1 and 2). Although cultivar x treatment interaction was shown in stress treatment 2 of the 1997 experiment (LSD {P=0.05} = 4.20), the interaction was due to differences between control or stress values between cultivars and not due to differences in the response of cultivars to the stress treatment applied (Table 4).

In this study ear mass per plant was, as already mentioned, significantly affected in both experimental years when water stress was applied during all three

stress treatments. In 1997 Gamtoos, on average over the three stress treatments, showed a reduction of 55.9%, followed by Kariega with a reduction of 50.1%. Lower reductions (SST 66 = 42.5%; SST 57 = 24.2%) were, however, obtained with the two SST cultivars. In the 1998 experiment Kariega (49.8%) and SST 66 (44.4%) showed reductions of nearly the same magnitude as in 1997, while the reductions for Gamtoos (37.9%) and SST 57 (43.4%) were respectively smaller and larger. When the average reduction over the two experimental years was calculated, Kariega obtained the highest reduction (50.0%). Gamtoos and SST 66 obtained reductions of 46.9% and 43.5% respectively, while SST 57 (33.8%) showed the lowest reduction of the four cultivars tested.

From the data it is clear that ear mass was seriously affected by water stress at the milky kernel stage (stress treatments 2 and 3) when kernel filling took place. The adverse effect on the ear mass when stress was applied at the flag leaf stage might be due to the fact that less ears per plant survived, as shown in the discussion of ears per plant.

Number of spikelets

The number of spikelets per ear for different cultivars differed significantly in both experimental years, irrespective of the stress treatment induced (Table 1 and 2). Cultivars used in these experiments therefore differ in their potential to form spikelets under similar growth conditions. The induction of water stress at the flag leaf and, milky kernel or at both growth stages combined, did not affect the number of spikelets per ear in both experimental years. No significant cultivar x treatment interactions were observed (Table 1 and 2).

The rate of spikelet initiation is faster than leaf initiation (Kirby & Perry, 1987) and ends at terminal spikelet formation (Loss & Siddique, 1994). The water stress applied at the flag leaf and milky kernel growth stages were therefore induced after spikelet formation had been completed, which could explain why the stress treatments did not affect the number of spikelets per ear.

Kernel number

Kernel number per plant for different cultivars did not differ significantly in both experimental years (Table 1 and 2), but kernel number per plant was significantly reduced when water stress was applied at the flag leaf stage (stress treatments 1 and 3) (Table 5). No effect was obtained when water stress was applied at the milky kernel stage only. No significant cultivar x treatment interaction was observed (Table 1 and 2).

Table 5 Means of stress and control values of kernel number per plant in both experimental years, also expressed as the percentage reduction of the stress values in comparison to the respective control values

Treatment and Cultivar	Stress	Control	LSD (P=0.05) Treatment	% Reduction
		1997		
ST1				
Gamtoos	189.44	214.33	54.38	11.6
Kariega	163.56	230.11		28.9
SST 57	152.55	208.00		26.7
SST 66	123.67	254.89		48.5
ST3				
Gamtoos	104.78	214.33	65.15	51.1
Kariega	154.67	230.11		32.8
SST 57	146.67	208.00		29.5
SST 66	135.11	254.89		47.0
		1998		
ST1				
Gamtoos	106.00	204.55	44.97	48.2
Kariega	68.11	151.55		55.1
SST 57	107.44	228.11		52.9
SST 66	38.00	173.22		78.1
ST3				
Gamtoos	103.22	204.55	38.68	49.5
Kariega	73.11	151.55		51.8
SST 57	95.89	228.11		58.0
SST 66	96.78	173.22		44.1

ST1 – stress treatment at flag leaf, ST3 – combination of stress at the flag leaf – and milky kernel stage, LSD – least significant difference values

Spikelet initiation starts when the main shoot has about three full leaves (Stern & Kirby, 1979). The terminal spikelet formation is synchronized with the spike emergence at the flag leaf stage. After the terminal spikelet is formed, environmental conditions should no longer influence spikelet number, unless long periods of severe drought stress prevails, but may affect the number of florets differentiated within each spikelet (Evans, Wardlaw & Fischer, 1975). The results obtained in this study, with the stress treatment of seven days, clearly show that kernel number per plant of spring wheat was not affected when water stress was induced after the primary tiller has reached the milky kernel stage. In the 1997 experiment Gamtoos showed reductions in kernel number per plant of 11.6% and 51.1% due to water stress in stress treatments 1 and 3 respectively (Table 5). On average Gamtoos showed a reduction of 31.4%. Kariega, SST 57 and SST 66 showed large decreases in kernel number per plant for both stress treatments. SST 66 showed a reduction on average of 47.8%, while Kariega and SST 57 showed reductions of 30.9% and 28.1% respectively. Although all four cultivars on average showed large decreases in kernel number per plant, SST 66 appears to be the most sensitive to water stress at the flag leaf stage. The same tendency was noted in the 1998 experiment. In these treatments larger reductions were shown than in the 1997 experiment. Gamtoos obtained on average a reduction in kernel number per plant of 49.9%, 53.5% for Kariega, 55.5% for SST 57 and 61.1% for SST 66. Although decreases for these cultivars were very large, differences between cultivars were too small to classify any of these cultivars as 'more tolerant' than the other. There can, however, be little doubt that large

decreases in kernel number per plant could be expected if water stress occurs during the flag leaf stage.

Thousand kernel mass (TKM)

The thousand kernel mass (TKM) of different cultivars differed significantly in both experimental years (Table 1 and 2). The induction of water stress at the flagleaf, milky kernel or at both these growth stages combined, caused a significant reduction in the TKM in both experimental years (Table 6).

Table 6 Means of stress and control values of thousand kernel mass (g) in both experimental years, also expressed as percentage reduction in the stress values compared to the respective control values

Treatment and Cultivar	Stress	Control	% Reduction
		1997	
ST1			
Gamtoos	32.98	47.01	29.8
Kariega	48.98	43.98	+11.4
SST 57	40.33	41.01	1.7
SST 66	37.67	40.68	7.4
ST2			
Gamtoos	15.38	47.01	67.3
Kariega	15.01	43.98	65.9
SST 57	37.01	41.01	9.8
SST 66	25.67	40.68	36.9
ST3			
Gamtoos	16.01	47.01	65.9
Kariega	21.02	43.98	52.2
SST 57	37.96	41.01	7.4
SST 66	34.04	40.68	16.3

Table 6 (continue)

Treatment and Cultivar	Stress	Control	% Reduction
		1998	
ST1			
Gamtoos	35.03	56.90	38.4
Kariega	45.31	50.46	10.2
SST 57	40.35	44.25	8.8
SST 66	49.42	42.37	+16.6
ST2			
Gamtoos	29.42	56.90	48.3
Kariega	44.89	50.46	11.0
SST 57	36.51	44.25	17.5
SST 66	34.63	42.37	18.3
ST3			
Gamtoos	44.54	56.90	21.7
Kariega	46.64	50.46	7.6
SST 57	42.53	44.25	3.9
SST 66	35.33	42.37	16.6

ST1 – stress treatment at flag leaf, ST2 – stress treatment at milky kernel stage, ST3 – combination of ST1 and ST2, (+) - % increase

With the exception of stress treatment 1 (water stress applied at the flag leaf stage) of both experimental years, no significant cultivar x treatment interactions were noted.

Expressed as percentage increases or decreases compared to control (unstressed) values, Gamtoos on average showed a reduction of 54.4% compared to 20.2% for SST 66 and 6.3% for SST 57 respectively (Table 6). Although Kariega showed a slight increase in TKM due to stress at the flag leaf stage (treatment 1) in 1997, TKM for this cultivar was on average reduced by 53.3%. The increase noted for Kariega in stress treatment 1 of the 1997 experiment was

not repeated in the 1998 experiment (Table 8). In the 1998 experiment Gamtoos on average showed a reduction of 36.1% compared to 9.6% for Kariega and 10.1% for SST 57 respectively. SST66 showed an increase of 16.6% in stress treatment 1, but on average a reduction of 17.5% for stress treatments 2 and 3. Gamtoos with reductions of 54.4% and 36.1% in 1997 and 1998 respectively, seems to be more susceptible to water stress than the other three cultivars tested, which is in contrast to the findings in Chapter 3. SST 57 seems to be the least affected by water stress with reductions in TKM of 6.3% and 9.6% respectively.

Kernel mass

Kernel mass per plant for different cultivars did not differ significantly in both experimental years, irrespective of the stress treatment received (Tables 1 and 2). The induction of water stress at the flag leaf, milky kernel and at both these growth stages combined, did reduce kernel mass per plant in both experimental years significantly (Table 7). No significant cultivar x treatment interaction was observed (Tables 1 and 2).

Table 7 Means of stress and control values of kernel mass per plant (g/plant) in both experimental years, also expressed as percentage reduction in the stress values compared to the respective control values

Treatment and Cultivar	Stress	Control	LSD (P=0.05) Treatment	% Reduction
		1997		
ST1				
Gamtoos	5.89	9.67	1.45	39.1
Kariega	7.97	9.99		20.2
SST 57	6.16	8.11		24.0
SST 66	4.60	10.25		55.1
ST2				
Gamtoos	3.29	9.69	1.45	66.1
Kariega	3.34	9.99		66.6
SST 57	6.63	8.11		18.3
SST 66	6.00	10.25		41.5
ST3				
Gamtoos	2.68	9.69	1.46	72.3
Kariega	3.21	9.99		32.1
SST 57	4.94	8.11		39.1
SST 66	4.58	10.25		55.3
		1998		
ST1				
Gamtoos	4.18	8.89	1.55	53.0
Kariega	3.52	7.76		54.6
SST 57	4.94	10.83		54.4
SST 66	2.03	8.40		75.8
ST2				
Gamtoos	6.31	8.89	1.83	71.0
Kariega	4.71	7.76		39.3
SST 57	7.33	10.83		32.3
SST 66	7.07	8.40		15.8

Table 7
(continue)

Treatment and Cultivar	Stress	Control	LSD (P=0.05) Treatment	% Reduction
			1998	
ST3				
Gamtoos	4.20	8.89	1.57	52.8
Kariega	3.21	7.76		58.6
SST 57	4.68	10.83		56.8
SST 66	4.25	8.40		50.6

ST1 – stress treatment at flag leaf, ST2 – stress treatment at milky kernel stage, ST3 – combination of ST1 and ST2, LSD – least significant difference values

In both experimental years kernel mass per plant was significantly affected when water stress was applied at either the flag leaf, milky kernel or at both growth stages combined. The growth of kernels progresses in several phases with dry weight accumulation the slowest just after anthesis when endosperm cell division occurs and cell number increase rapidly (Wardlaw, 1970). The number of endosperm cells formed by a kernel ultimately influences its growth rate and final weight (Singh & Jenner, 1982). The rates of endosperm cell division or final cell numbers are influenced by low intensity water stress (Brocklehurst, 1977). The reductions observed in kernel mass per plant under water stress at the milky kernel stage (stress treatment 2), might be explained by the above. The reduction in kernel mass per plant when water stress was applied at the flag leaf stage might be due to the fact that the final kernel weight is generally associated with the

weight of the ovary at anthesis (Singh & Jenner, 1982). The reductions in stress treatment 3 might be the result of a combination of the above. In the 1997 experiment SST 57 on average showed a reduction of 27.1% in kernel mass per plant, which was significantly less than the other three cultivars. Kariega on average showed a reduction of 39.6%, while SST 66 and Gamtoos showed reductions of 50.6% and 59.2% respectively. With the exception of SST 66 the four cultivars tested in 1998 showed the same ranking as in the 1997 experiment. On average SST 57 (47.8%) showed the least reaction to the applied stress, while Gamtoos (58.9%) showed the highest reduction. The differences noted were too small to classify any of these cultivars as 'more tolerant' than the other. There can be, however, little doubt that large decreases in kernel mass per plant could be expected for these cultivars if water stress occurs at either the flagleaf or milky kernel growth stages.

Harvest Index (HI)

The harvest index (HI) for different cultivars did not differ significantly in both experimental years (Tables 1 and 2). The induction of water stress at the flagleaf, milky kernel or at both growth stages combined, caused significant reductions in the HI in 1997 (Table 8). In the 1998 experiment reductions were also noted for stress treatments 1 and 2 (Table 8). No significant cultivar x treatment interactions were shown.

Table 8. Means of stress and control values of harvest index in both experimental years, also expressed as percentage reduction the stress values when compared to the respective control values

Treatment and Cultivar	Stress	Control	LSD (P=0.05) Treatment	% Reduction
		1997		
ST1				
Gamtoos	0.40	0.44	0.04	9.1
Kariega	0.39	0.47		17.0
SST 57	0.34	0.43		20.9
SST 66	0.35	0.42		16.7
ST2				
Gamtoos	0.19	0.44	0.04	56.8
Kariega	0.23	0.47		51.1
SST 57	0.40	0.43		7.0
SST 66	0.42	0.42		0.0
ST3				
Gamtoos	0.22	0.44	0.06	50.0
Kariega	0.35	0.47		25.5
SST 57	0.38	0.43		11.6
SST 66	0.37	0.42		11.9
		1998		
ST1				
Gamtoos	0.31	0.42	0.07	26.2
Kariega	0.40	0.46		13.0
SST 57	0.41	0.47		12.8
SST 66	0.25	0.45		44.5
ST2				
Gamtoos	0.36	0.42	0.04	14.3
Kariega	0.41	0.46		10.9
SST 57	0.37	0.47		21.3
SST 66	0.33	0.45		26.7
ST3				
Gamtoos	0.37	0.42	0.05	11.9
Kariega	0.42	0.46		8.7
SST 57	0.42	0.47		12.8
SST 66	0.43	0.45		4.5

ST1 – stress treatment at flag leaf, ST2 – stress treatment at milky kernel stage, ST3 – combination of ST1 and ST2, LSD – least significant difference values

Data presented by Perry and D'Antuono (1989) showed that the harvest index of modern cultivars has risen from 0.2 to more than 0.4. The harvest index of the control treatments of the four cultivars tested corresponded with these findings (Table 8). In the 1997 experiment the induction of water stress resulted, on average, in a reduction of 38.6% in the HI of Gamtoos compared to 31.2% for Kariega. The HI for SST 57 and SST 66 were, on average, reduced by 13.2% and 9.5% respectively. In the 1998 experiment SST 66 showed, on average, the highest reduction of 25.2% compared to the 10.9% for Kariega, 15.6% for SST 57 and 17.5% for Gamtoos respectively. Although all four cultivars showed reductions in the harvest index, variation between years made it impossible to draw any conclusions in regard to differences in drought tolerance.

Conclusion

In conclusion it can be said that the water stress treatments applied did reduce yield and yield components of all four cultivars tested. From the data it appears that water stress occuring during the flag leaf stage affects the number of ears, kernel mass and kernel number per plant of all four cultivars, while stress during the milky kernel stage only affected ear mass and kernel mass per plant. The reductions in these components were also reflected in the thousand kernel mass and the harvest index of these cultivars. Although the yield and yield components were reduced by the water stress treatments, no clear differences in drought tolerance could be made between the four cultivars tested on this basis.

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Chapter 6

EFFECT OF WATER STRESS ON WHEAT QUALITY OF SPRING WHEAT CULTIVARS

Abstract

The wheat producers regard high yield as important, while the quality characteristics of the wheat crop are of importance to the milling and baking industry. Protein content is mainly determined by the growth conditions, whereas protein quality is primarily determined genetically. The most critical climatic factors affecting plant yield and grain quality in the Western Cape region are those of temperature and rainfall. The aim of this study was thus to determine the effect of water stress at different growth stages on wheat quality parameters of spring wheat cultivars produced in the Western Cape region of South Africa. It was found that the water stress treatments applied at various stages of development of the wheat did affect the quality responses of the cultivars tested. Changes in bread-making quality were due to the increase in the relative protein content and a decrease in the relative non-protein components of the kernel and thus the flour.

Introduction

High yield is regarded as important by wheat producers, while the quality characteristics of the wheat crop are of commercial importance to the milling and baking industry (Van Lill, Purchase, Smith, Agenbag & De Villiers, 1995). The breadbaking quality of wheat might be affected by water stress because of enhanced leaf senescence and reduced grain yield as a result of smaller kernels (Agenbag & De Villiers, 1995b). This is due to the fact that wheat quality is primarily related to the content and composition of endosperm proteins (Bushuk, 1985).

Protein content is mainly determined by the growth conditions (Johnson & Mattern, 1980), whereas protein quality is primarily determined genetically (Finney, Yamazaki, Young & Rubenthaler, 1987). Protein quality may also be affected by growth conditions after anthesis (Evans, Wardlaw & Fischer, 1975). The most critical climatic factors affecting plant yield and grain quality in the Western Cape region are those of temperature and rainfall (Beyers, 1992). Although very consistent in yield, these areas regularly experience problems regarding the annual fluctuation in wheat quality (Traut, 1993).

The aim of this study was thus to determine the effect of water stress at different growth stages on protein production and baking quality of spring wheat cultivars produced in the Western Cape region.

Material and Methods

Plant material

Four spring wheat cultivars were used in this study. Two of these, Gamtoos (drought tolerant) and SST 66 (slightly drought sensitive), had showed differences in drought tolerance in studies done by Van Heerden and De Villiers (1996). The other two, Kariega and SST 57, were of unknown drought tolerance.

Growth conditions

Seeds of the cultivars were planted (1997 and 1998) in plastic pots (15x15cm) filled with coarse sand, on the Welgevallen experimental farm in Stellenbosch. Eight days after emergence, seedlings were reduced to 3 plants per pot. All experiments were conducted in a temperature-controlled (16°/10°C day/night) glasshouse. Automatic irrigation was done by way of a computerized system, according to the daily, solar radiation, so that more pulses were given on sunny days than on cloudy days. For the first two weeks seedlings were irrigated with water and for the rest of the growth period with a balanced nutrient solution (75%).

Induction of water stress

The induction of water stress in each of the two trial years was achieved by the withholding of water (WOW) at different stages of plant development. Three stress periods was adopted for each of the four wheat cultivars, with the first period commencing at the flag leaf stage, the second at the milky stage and the third a combination of the first two, each lasting seven days. Control plants for each cultivar were irrigated continuously throughout their development.

Measurements

Three pots per stress treatment were harvested for the determination of kernel protein, while the rest of the plants for each treatment were harvested in bulk to obtain enough kernels for the mixograph and baking test. Kernel protein was determined by the use of a Technikon Infralyzer 400, previously calibrated against Kjeldahl nitrogen content (AACC, 1983). Mixograph dough development time, peak height and bandwidth were determined using a 35 g mixograph as described by Finney and Shogren (1972). Micro-loaves were baked from 10 grams of flour, using an optimized straight dough method (Shogren & Finney, 1984) with a total fermentation period of 135 min. Following this, the loaves were baked for 13 min at 210°C in a carrousel-type oven. Loaf volume (LFV) was determined by rapeseed displacement directly after baking. Flour protein, flour yield and bran yield were determined as part of the baking test.

The data collected for kernel protein was subjected to an analysis of variance, but no statistical analysis could be done for the mixograph and baking test data due to bulking of the replications to ensure an acceptable sample size for evaluations. The T test (P = 5%) was used to compare treatment means.

Results and Discussion

Kernel protein

Kernel protein content for different cultivars differed significantly in 1997, irrespective of the stress treatment received (Table 1). The kernel protein content in the 1997 experiment was significantly increased when water stress was applied at the flagleaf stage (stress treatment 1 and 3) (Table 2). No effect was

obtained when stress was applied at the milky kernel stage. In the 1998 experiment, kernel protein content for different cultivars did not differ significantly. The induction of water stress at the flag leaf, milky kernel stages, as well as at both growth stages combined, generally caused a significant increase in the kernel protein content (Table 1). No significant cultivar x treatment interactions was shown in either experimental years (Table 1).

Table 1 The ANOVA done on the kernel protein content of four spring wheat cultivars subjected to water stress in 1997 and 1998

Sources of Variation	**Cultivar (CUL)**	**Treatment (TRT)**	**CUL X TRT**
Df	**3**	**1**	**3**
1997			
ST1	**	**	NS
ST2	**	NS	NS
ST3	**	*	NS
1998			
ST1	NS	**	NS
ST2	NS	**	NS
ST3	NS	**	NS

ST1 – stress at the flag leaf stage, ST2 – stress at the milky kernel stage, ST3 – combination of ST1 and ST2, NS – not significant, * significant at 5% confidence level, ** significant at 1% confidence level

In this study kernel protein content was only significantly affected in 1997 when water stress was applied during the flag leaf stage (stress treatments 1 and 3) of the wheat. In these treatments the kernel protein content of Gamtoos showed no increase due to water stress in stress treatment 1 but an increase of 8.2% in stress treatment 3 (Table 2). Although increases in kernel protein content varied

on average between 7.5% for Kariega and 6.9% and 9.0% for SST 57 and SST 66 respectively, differences were too small to classify any of these cultivars as 'more tolerant' than the other.

Table 2 Means of kernel protein content (%) of four spring wheat cultivars subjected to water stress at different growth stages in two experimental years (1997 and 1998).

Treatment and Cultivar	Stress	Control	LSD (P=0.05)		% Increase
			CUL	TRT	
1997					
ST1					
Gamtoos	20.40	20.40	1.19	0.84	0.0
Kariga	18.57	17.03			9.0
SST57	19.33	17.73			9.0
SST66	19.40	17.87			8.6
ST2					
Gamtoos	20.50	20.40	0.98	0.69	0.5
Kariga	18.43	17.03			8.2
SST57	17.77	17.73			0.2
SST66	18.27	17.87			2.2
ST3					
Gamtoos	22.07	20.40	1.36	0.96	8.2
Kariga	18.03	17.03			5.9
SST57	18.57	17.73			4.7
SST66	19.53	17.87			9.3
1998					
ST1					
Gamtoos	15.80	12.50	1.38	0.98	26.4
Kariga	14.47	12.73			13.7
SST57	15.97	12.47			28.1
SST66	16.17	13.43			20.4
ST2					
Gamtoos	15.60	12.50	1.42	1.01	24.8
Kariga	13.73	12.73			7.9
SST57	14.07	12.47			12.8
SST66	14.37	13.43			7.0

Table 2
(continue)

Treatment and Cultivar	Stress	Control	LSD (P=0.05)		% Increase
			CUL	TRT	
ST3					
Gamtoos	16.13	12.50	1.50	1.06	29.0
Kariga	14.73	12.73			15.7
SST57	16.23	12.47			30.2
SST66	15.07	13.43			12.2

ST1 – stress at flag leaf stage, ST2 – stress at milky kernel stage, ST3 – combination of ST1 and ST2, LSD – least significant difference values, CUL – cultivar, TRT – treatment, % Increase – percentage increase of stress value compared to the respective control (unstressed) values

The 1997 experiment showed higher kernel protein content than the 1998 experiment, but the abnormally high values for 1997 could not be readily explained by the thousand kernel mass (as described in Chapter 5), since the growing conditions of both experimental years were similar. The 1998 experiment, however, showed larger increases in kernel protein content when compared to their respective control (unstressed) values (Table 2). In the 1998 experiment Gamtoos showed the highest increase in kernel protein content with an average increase of 26,7% over the three stress treatments, followed by SST 57 with 23.7%, SST 66 with 15.2% and Kariega with 12.4%. These results may at most be used to speculate that the quality of Gamtoos and SST 57 will be less stable in comparison with Kariega and SST 66, during conditions of water stress.

Thousand kernel mass is a function of kernel size and density (Halverson & Zeleny, 1964). The protein fraction is thus higher compared to the non-protein fraction in less dense kernels. A general decrease in thousand kernel mass was noted in the stress treatments of both experimental years (Chapter 5). This accounts for the higher protein content of the stress treatments. The baking quality of the wheat might be affected by this rise in kernel protein content, since wheat quality is primarily related to the content and composition of endosperm proteins (Bushuk, 1985).

Milling, mixing and baking responses

The reaction of the mixograph and baking data are discussed in comparison to the kernel protein content, since the quality of wheat is primarily related to the protein content and composition of the endosperm proteins. Table 3 gives a generalized summary of the different quality responses of the stress treatments compared to their respective control treatments. These measurements were done on bulk sample without replications and not on the same plants as used to determine yield and kernel protein content.

Table 3 General reaction of quality parameters of both experimental years indicated as increases (+) and decreases (-) in the stress treatment values of the four cultivars tested, from their respective control (unstressed) values

	Flour protein	Flour	Bran	Mixing Time	Peak Height	Band Width	Volume
1997							
ST1	+	-	+	-	+	+	+
ST2	+	-	+	-	+	+	+
ST3	+	-	+	-	+	+	+
1998							
ST1	+	-	+	-	+	+	+
ST2	+	-	+	-	+	+	+
ST3	+	-	+	-	+	+	+

ST1 – stress treatment at flag leaf stage, ST2 – stress treatment at milky kernel stage, ST3 – combination of ST1 and ST2

Milling responses

Flour protein

The general response to the applied stress treatments was an increase in the flour protein when compared to the respective control values (Table 3). This tendency corresponded with the increase of kernel protein content as described. Protein quantity and quality are both considered primary factors in measuring the potential of a flour in relation to its end use (Mailhot & Patton, 1988).

In the 1997 experiment Gamtoos on average, over the three stress treatments, obtained an increase of 14.1%, with much smaller increases of 2.1%, 7.3% and 6.4% for Kariega, SST 57and SST 66 respectively (Table4). In the 1998 experiment Gamtoos and Kariega showed an average increase of 11.9%.

This was smaller than the increases of the two SST cultivars, with an increase of 22.9% for SST 57 and 18.0% for SST 66 respectively. Although it is difficult to explain why the cultivars tested differed in their ranking order in the two experimental years, there can be no doubt that the increase in kernel protein content by the applied water stress at both the flag leaf and milky kernel stage will cause an increase in the flour protein content of the wheat.

Table 4 Quality parameters of the four spring wheat cultivars tested in two experimental years indicated as percentage increases (+) or decreases (-) in the stress treatment values compared to their respective control values when water stress was applied

Treatment and Cultivar	Flour protein	Flour Yield	Bran Yield
1997			
ST1			
Gamtoos	+11.2	-3.6	+3.6
Kariga	+4.9	-4.1	+4.1
SST57	+10.8	-1.9	+1.9
SST66	+13.3	-7.1	+7.1
ST2			
Gamtoos	+16.4	-26.5	+26.5
Kariga	+1.8	-33.7	+33.2
SST57	+0.6	-6.1	+6.1
SST66	+0.7	-13.3	+13.3
ST3			
Gamtoos	+14.7	-1.4	+1.4
Kariga	+0.5	-6.1	+6.1
SST57	+10.5	-7.8	+7.8
SST66	+5.1	-2.9	+2.9
1998			
ST1			
Gamtoos	+10.2	-19.8	+19.8
Kariga	+11.9	-17.3	+17.3
SST57	+33.6	-6.7	+6.7
SST66	+26.1	-4.4	+4.4

Table 4
(continue)

Treatment and Cultivar	Flour protein	Flour Yield	Bran Yield
ST2			
Gamtoos	+17.2	-14.61	+14.61
Kariga	+10.8	-7.4	+7.4
SST57	+9.3	-7.9	+7.9
SST66	+8.5	-12.1	+12.1
ST3			
Gamtoos	+8.4	-11.3	+11.3
Kariga	+12.9	-20.4	+20.4
SST57	+25.8	-12.3	+12.3
SST66	+19.3	-14.1	+14.1

ST1 – stress treatment at flag leaf stage, ST2 – stress treatment at milky kernel stage, ST3 – combination of ST1 and ST2

Flour and Bran yields (%)

As also found by Halverson and Zeleny (1964), the decrease in kernel mass as described in Chapter 5 showed a good correlation to the percentage flour produced by the different cultivars. The induction of water stress at the flag leaf and milky kernel stages, as well as at both growth stages combined, caused a general decrease in the flour yield when compared to their respective control (unstressed) values (Table 3). The percentage bran therefore showed a general increase due to the different stress treatments. The decrease in the flour percentage is probably the result of a relative reduction in the formation of non-protein components in the kernel (Agenbag & De Villiers, 1995b).

In the 1997 experiment, Kariega on average, showed the highest reduction in flour yield (14.5%) due to the stress treatments, followed by Gamtoos (10.5%),

while SST 57 (5.3%) and SST 66 (7.8%) were slightly less affected. High values for Kariega and Gamtoos were due to large reductions found for treatment 2. These responses were not repeated in stress treatment 3, which also received the stress at the milky kernel stage. In the 1998 experiment the four cultivars tested were on average more affected. Gamtoos and Kariega showed the highest decreases of 15.2% and 15.0% respectively. SST 57 with a decrease of 9.0% and SST 66 with a decrease of 10.2% again were less affected.

Although the two SST cultivars in both experimental years showed the lowest decrease in the extracted flour percentage, the differences between the cultivars were small. It is, however, clear that water stress applied at the flag leaf- and milky kernel stage will result in a decrease in the extraction of flour from the kernel.

Mixing responses

Rheological properties of dough are important to the baker for two reasons. Firstly they determine the behavior of dough during mechanical handling and secondly they affect the quality of the finished loaf of bread (Bloksma & Bushuk, 1988). The mixograph characteristics are dependent on the changes of the plastic, elastic and viscoelastic properties of the dough during mixing (Kunerth & D'Appolonia, 1985).

Mixing time

A dough that has been mixed to a peak may be referred to as a mixed dough, a dough with minimum mobility, or optimal mixed dough (Hoseney, 1985). All of these infer that an end point has been reached and the inference is also that this

is the point to which a dough should be mixed for producing a loaf of bread (Hoseney & Finney, 1974). Variations from these levels will produce doughs that are difficult to handle and will not result in the best possible loaf of bread (Abdelrahman & Spies, 1986). Mixing time, in general, decreases as protein content increases to 12%, thereafter remaining more or less constant with flour protein. The baking industry has set the optimum mixing time for dough at 2.5 to 3 minutes. The average mixing time for the cultivars tested in this study was 2.3 min.

The induction of water stress at the flag leaf, milky kernel and at both growth stages combined, caused a general decrease in mixing time when the stressed wheat was compared to the respective control (unstressed) values (Table 3).

Table 5. Mixograph quality parameters of the four spring wheat cultivars tested in two experimental years indicated as percentage increases (+) or decreases (-) in the stress treatment values in comparison to their respective control values when water stress was applied

Treatment and Cultivar	Mixing time	Peak height	Bandwidth
1997			
ST1			
Gamtoos	-3.4	+0.9	+10.3
Kariega	-0.8	+7.1	+9.3
SST 57	-11.3	+9.3	+4.2
SST 66	-12.4	+8.9	+8.9
ST2			
Gamtoos	-3.4	+9.1	+0.9
Kariega	-11.4	+7.3	+3.2
SST 57	-5.1	+3.1	+16.9
SST 66	-0.5	+17.3	+41.6
ST3			
Gamtoos	-8.2	+7.1	+17.6
Kariega	-14.7	+6.9	+10.3
SST 57	-10.9	+6.3	+4.2
SST 66	-0.7	+13.8	+0.7
1998			
ST1			
Gamtoos	-7.4	+3.2	+6.3
Kariega	-2.2	+15.1	+9.9
SST 57	-4.1	+31.7	+15.8
SST 66	-14.0	+10.3	+51.7
ST2			
Gamtoos	-10.3	+15.0	+2.3
Kariega	-2.4	+18.3	+16.4
SST 57	-3.2	+2.7	+24.1
SST 66	-7.9	+21.3	+18.8
ST3			
Gamtoos	-3.7	+9.2	+24.3
Kariega	-3.3	+14.5	+7.4
SST 57	-14.1	+26.3	+23.9
SST 66	-2.1	+3.2	+4.4

ST1 – stress treatment at flag leaf stage, ST2 – stress treatment at milky kernel stage, ST3 – combination of ST1 and ST2

This is consistent with the increase of kernel protein content. In the 1997 experiment Kariega and SST 57 showed, on average, the highest decreases with 9.0% and 9.1% respectively. Gamtoos (5.0%) and SST 66 (4.5%) showed smaller decreases in mixing time (Table 5). In the 1998 experiment SST 66 on average showed the highest decrease in mixing time of the four cultivars tested with 14.2%, while Gamtoos (7.1%), Kariega (2.6%) and SST 57 (7.1%) were less affected compared to SST 66. The ranking order of the cultivars tested in the two experimental years were such that no clear distinction between cultivars could be made.

Peak height

The general tendency in both experimental years was an increase in peak height when water stress was applied at the flag leaf and milky kernel stages individually, as well as at both growth stages combined (Table 3). In the 1997 experiment SST 66 showed on average the highest increase in peak height of 13.3%, while the other three cultivars showed increases of less than 10% (Table 5). Although all four cultivars showed increases in the peak height of their respective mixographs, these differences were too small to be of any concern to the baker. In the 1998 experiment Gamtoos again showed a fairly small increase of 9.1%, followed by SST 66 with an increase of 11.6%. Kariega and SST 57 showed much larger increases of 16.0% and 20.2% respectively. As found for mixing time, results for peak height were also very inconsistent, which made it very difficult to draw any meaningful conclusions.

Bandwidth

The effect of flour water absorption on the mixograph mixing properties is of utmost importance. Stiff doughs produce mixograms having broad bandwidths, while slack doughs produce narrow bandwidths (Bloksma & Bushuk, 1988).

The general tendency in both experimental years was an increase in the bandwidth of the mixographs compared to their respective control (unstressed) values, when water stress was applied at the flag leaf, milky kernel and at both growth stages combined. In the 1997 experiment SST 66 on average showed the highest increase in bandwidth of 17.1%. Gamtoos, Kariega and SST 57 showed increases of less than 10% (Table 5). In the 1998 experiment SST 66 again showed on average the highest increase in bandwidth of 25.0%. SST 57 also showed an increase of more than 20%. Gamtoos and Kariega again showed low increases in bandwidth of 11%. It appears as if the bandwidth of the two SST cultivars might be more sensitive to water stress, but with the inconsistency of the data it was difficult to draw any conclusions.

Baking response

Loaf volume

Loaf volume is widely used as the most inclusive parameter of breadbaking quality of wheat (Blackman & Payne, 1987). Loaf volume is affected by both protein content and protein quality (Simonds, 1989).

Table 6. Micro-loaf volumes of the baking test for the four spring wheat cultivars tested in two experimental years indicated as percentage increase in the stress treatment values from their respective control values when water stress was applied

Treatment and Cultivar	Micro-loaf volume
1997	
ST1	
Gamtoos	+6.3
Kariga	+4.1
SST57	+8.2
SST66	+10.9
ST2	
Gamtoos	+6.1
Kariga	+4.2
SST57	+6.2
SST66	+13.8
ST3	
Gamtoos	+4.0
Kariega	+11.3
SST 57	+4.3
SST 66	+12.4
1998	
ST1	
Gamtoos	+8.7
Kariega	+11.3
SST 57	+4.2
SST 66	+5.9
ST2	
Gamtoos	+5.1
Kariega	+5.3
SST 57	+1.4
SST 66	+4.7
ST3	
Gamtoos	+9.1
Kariega	+7.3
SST 57	+4.9
SST 66	+16.3

ST1 – stress treatment at flag leaf stage, ST2 – stress treatment at milky kernel stage, ST3 – combination of ST1 and ST2

The micro-loaf volume increases with increasing protein content (Finney *et al.*, 1987). The average micro-loaf volume found in this study in 1997 was 83.07 ml for the stress treatments and 77.25 ml for the control (unstressed) treatments, while in 1997 it was 65.68 ml and 61.38 ml respectively. The general tendency in the baking of the micro-loaves of both experimental years was an increase in the loaf volume (Table 3). In the 1997 experiment, when taken as an average over the three stress treatments, SST 66 showed the highest increase in loaf volume of 12.4%. The other three cultivars tested showed lower increases with 5.5% for Gamtoos, 6.5% for Kariega and 6.2% for SST 57 respectively (Table 6). In the 1998 experiment all four cultivars tested showed increases of less than 10%. SST 57 showed the lowest increase of 3.5%. Agenbag and De Villiers (1995b) reported that low intensity water stress reduces loaf volume, whereas high intensity water stress had no significant effect on the loaf volumes of the micro-loaves. From this data it is clear that the water stress applied at the flag leaf and milky kernel stages did not significantly affect the loaf volume of the four cultivars tested.

Conclusion

It was found that the water stress treatments applied at the various stages of development of the wheat plant did affect the quality responses of the four cultivars tested. No clear differences could be detected between the different stages of development at which the stress was applied. Changes in breadbaking quality were due to the relative increase in the protein content and a relative

decrease in the non-protein components of the kernel and thus the flour. The general tendency was an increase in the loaf volumes of the micro-loaves, but not significantly different from the respective control values. The fact that results for the milling, mixing and baking response were obtained from bulk samples without replications made it difficult to clearly distinguish between the four spring wheat cultivars tested.

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Chapter 7

Summary

Drought is considered, worldwide, to be the most important factor limiting crop yields. This statement is also true for the production of spring wheat in the Western and Southern Cape regions. According to estimates, global warming will result in an increase in winter temperatures and a decrease in the rainfall of these regions. This means that spring wheat produced in these regions will more often be subjected to periods of water stress. It is therefore important to identify spring wheat cultivars that can withstand these conditions. The multitude of factors potentially involved in drought resistance makes it unlikely that a single measurement will provide an all-encompassing test of drought tolerance.

The primary aim of this study was to examine the effect of water stress, applied at different growth stages of the wheat, has on spring wheat cultivars. Furthermore to investigate whether quick, reliable and consistent physiological parameters could be found to provide information on drought tolerance of newly bred spring wheat cultivars.

In order to accomplish this, four spring wheat cultivars, namely Gamtoos, Kariega, SST 57 and SST 66, were subjected to water stress at the flag leaf and milky kernel stages and at both growth stages combined, of wheat plants. Two of these cultivars were of known drought tolerance. Gamtoos and SST 66 were classified by earlier research as being drought tolerant and slightly drought sensitive respectively. The other two cultivars, Kariega and SST 57, were of unknown drought tolerance.

The different cultivars were then tested for drought tolerance or sensitivity using a series of physiological tests. The methods used included the accumulation of free proline, the reduction of 2,3,5-triphenyltetrazolium chloride (TTC), relative water content (RWC), leaf diffusive resistance (LDR), transpiration rate and ear water potential. To determine whether differences in physiological responses would be of any commercial value, plants were analyzed at different growth stages to compare biomass production, yield and wheat quality of stressed to that of control (unstressed) plants.

In a preliminary trial the withholding of water (WOW) and the use of polyethylene glycol (PEG) to induce water stress were compared. To evaluate the efficiency of the methods the accumulation of free proline and the reduction of TTC were used as indicators of water stress in the test plants. Although both methods of stress induction proved to be effective, the WOW seem to be more appropriate due to the possibility of root injuries with PEG. Another problem with PEG is that the stress is induced very rapidly, which does not represent the true situation since water deficits in wheat fields normally develop slowly over considerable timespans. Good control of climatic conditions and measurements of plant water potential will, however, be essential if the withholding of water is used to induce water stress. Proline accumulation proved to be a more sensitive indicator of water stress compared to TTC. It was thus decided not to apply the TTC in the following experiments.

The experiments were conducted in a temperature-controlled (16°/10°C day/night) glasshouse in the months from April to November. Irrigation was done

by a computerized system, according to the daily solar radiation received. A problem with the use of the temperature-controlled glasshouse in wintertime was that it was difficult to completely control the temperature on very hot days. Another problem was encountered namely that no control over daily fluctuations in solar radiation was possible. The lack of space also severely restricted the number of replications that could be used per treatment. Data collection was based on three replications per treatment.

The evaluation of the physiological parameters showed that LDR, transpiration rate, RWC, ear water potential and proline accumulation could be used to identify drought tolerance in spring wheat cultivars. From the data it is clear that the RWC of the leaves and the accumulation of free proline were the most sensitive of the physiological parameters tested. The fact that LDR and transpiration rates were less sensitive might be due to differences in daily solar radiation, which might have concealed genotypical differences. Total ear water potential also showed promise as a possible indicator of drought tolerance, but because only data from a single trial year was available, more studies are needed to confirm this. Gamtoos and Kariega proved to be the most tolerant of the four cultivars tested. Both showed high proline accumulation in all three stress treatments which is an indication of drought tolerance. With LDR and transpiration rate it was also found that these two cultivars were less sensitive to drought in the later growth stages than the two SST cultivars. In the determination of the RWC of leaves Gamtoos also proved to be the most drought tolerant, with SST 66 showing the highest sensitivity to water stress. Kariega and

SST 57 proved to be more tolerant than SST 66 but more sensitive than Gamtoos. This tendency was also shown for total ear water potential. From the data it was clear that the differences in drought tolerance between the different cultivars became more evident in the later growth stages.

In the study of the effects of water stress on plant components it was evident that, although aboveground biomass was reduced by the applied water stress treatments, no clear distinctions between cultivars for drought tolerance could be made. The use of leaf area determinations (leaf abscission), however, shows promise as a method to distinguish drought tolerant and drought sensitive cultivars. Definite differences between cultivars were found in the rate at which leaves die off when water stress was applied. Gamtoos seemed to be the least affected by the water stress, while SST 66 showed the highest decrease in leaf area. Leaf area of Kariega and SST 57 were less affected than that of SST 66, but more in comparison with Gamtoos. Although no clear differences between cultivars with regard to the effect of water stress on ear mass could be detected, SST 57 and SST 66, generally, showed indications of being more susceptible to drought compared to Gamtoos and Kariega.

Water stress treatments applied at the flag leaf and milky kernel stages, as well as both growth stages combined, severely reduced yield and the yield components of all four cultivars tested. From the data it appears that water stress occuring during the flag leaf stage reduced the number of ears, stem mass, kernel mass and kernel number per plant, while water stress applied at the milky kernel stage only, also reduced ear mass and kernel mass. As a result of the

reduction in kernel mass per plant, the thousand kernel mass and the harvest index of all four cultivars tested were reduced when compared to their respective control (unstressed) plants. Although reductions in the yield and yield components were shown for all four cultivars, no single cultivar proved to be more tolerant than the other.

In the quality study it was shown that the application of water stress resulted in an increase in kernel protein levels. This was the result of a decrease in kernel size and density. In turn this resulted in an increase in flour protein and a decrease in the percentage of flour yield in all four cultivars. Although the protein content of the flour was increased no significant differences in micro-loaf volumes were found between the stress values and control (unstressed) values for all cultivars tested. The reactions of the four cultivars tested for the mixograph parameters were also similar.

Although cultivar differences in drought tolerance were obtained with the physiological parameters, these differences were not realized in the agronomic or quality data. This might be due to the morphological similarity and the multitude of factors involved with the adaptability of spring wheat cultivars planted in the Western and Southern Cape regions. This can be seen in the results of the micro-loaf baking test where no significant increase in loaf volume was detected. It appears that water stress does not have the same detrimental effect on the quality of spring wheat, as does temperature. Although no clear differences in the drought tolerance of cultivars based on the agronomic data could be made, the use of the physiological tests for drought tolerance holds promise for the future of

spring wheat breeding. The lack of significant differences between cultivars for both the agronomic and quality characteristics could be due to the fact that the data were collected on a single plant basis and that the number of repetitions were restricted due to the lack of sufficient space and which resulted in large coefficients of variation. The use of a combination of parameters namely the accumulation of proline, relative water content and leaf area determinations could provide valuable indications of drought tolerance potential for newly bred cultivars. Other parameters that could be used in a supportive role are diffusive resistance, transpiration rate and ear water potential. Diffusive resistance and transpiration rate studies must be done in a growth chamber to ensure constant daily solar radiation.

Based on the results of this study it is suggested that a wider range of cultivars should be tested to obtain data on drought tolerance for all spring wheat cultivars produced in the Republic of South Africa. A more in depth study on the quality parameters, with an added determination of the protein fractions, is also needed to clarify the differences between spring wheat cultivars. This is extremely important since very little research has previously been done in this regard.