Hydroponics as a tool in wheat breeding

By

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Table of Contents

1. Introduction p.1

2. Literature Review p.3

2.1. Drought tolerance in wheat p.3

2.2. Hydroponics p.12

2.3. Hydroponics and wheat p.15

2.4. SE-HPLC p.17

2.5. The composition of wheat proteins p.18

2.5.1. Classification of wheat proteins p.18

2.5.2. Wheat storage proteins p.20

2.5.3. Low Molecular Weight Storage Proteins (Gliadins) p.20

2.5.4. High Molecular Weight Storage proteins (Glutenins) p.20

2.5.5. Protein content p.21

2.5.6. Environmental effect on quality p.21

2.6. High performance liquid chromatography of wheat proteins p.21

2.6.1. SE-HPLC and wheat quality p.22

3. Hydroponics as a tool for drought tolerance breeding p.25

3.1. Introduction p.25

3.2. Material and methods p.27

3.2.1. Planting, maintenance and sampling procedure in hydroponic systems p.27

3.2.2. Proline extraction p.31

3.2.3. Total protein analysis p.32

3.2.4. Size exclusion high performance liquid chromatography (SE-HPLC) p.32

3.2.5. Statistical analysis p.34

3.3. Results and discussion p.34

3.3.1. Morphological characteristics of five cultivars under drought stress at seedling stage compared to the control p.34

3.3.2. The mean squares of the measured morphological characteristics for the five cultivars under drought stress during the seedling stage compared to the control p.36

3.3.3. The measured morphological characteristics of the five cultivars under drought stress during the seedling stage compared to the control p.36

3.3.4. The mean squares of the protein fractions of the five cultivars under drought treatment during the seedling stage compared to the control p.38
3.3.5. The mean squares of the protein fractions of the five cultivars under drought treatment during the seedling stage compared to the control p.39
3.3.6. The protein fractions of the five cultivars under drought treatment during the seedling stage compared to the control p.41
3.3.7. The correlation between all the protein fractions and morphological characteristics of the five cultivars during drought treatment together with the control at seedling stage. p.43
3.3.8. Morphological characteristics of five cultivars during drought treatment at anthesis p.47
3.3.9. The measured morphological characteristics of the five cultivars for drought treatment together with the control at anthesis p.48
3.3.10. The protein fractions of the five cultivars at anthesis under drought stress, compared to the control p.50
3.3.11. The mean squares of the protein fractions of the five cultivars under drought stress during anthesis compared to a control p.52
3.3.12. The protein fractions of the five cultivars under drought stress at anthesis compared to a control p.52
3.3.13. The correlation of all the protein fractions and morphological characteristics of the five cultivars under drought stress during anthesis with the control p.55
3.4. Discussion p.60
3.4.1. Morphological characteristics of five cultivars under drought stress at the seedling stage compared to a control treatment p.60
3.4.2. Morphological characteristics of five cultivars during drought treatment together with the control at anthesis p.63
3.5. Conclusion p.67
4. Comparison of the influence of nutrition in hydroponics and pots p.70
4.1. Introduction p.70
4.2. Material and methods p.71
4.2.1. Growth systems p.71
4.2.2. Emergence p.72

4.2.3. Statistical analysis p.73
4.3. Results and discussion p.73
4.3.1. The emergence percentages of wheat planted in three systems p.73
4.3.2. The mean squares of the measured morphological characteristics, for two cultivars planted in three different systems p.75
4.3.3. Means of the measured morphological characteristics of two cultivars planted in three different systems p.76
4.3.4. The correlation matrix of the measured morphological characteristics of two cultivars planted in three different systems p.79
4.4. Discussion p.81
4.5. Conclusions p.83
5. General conclusions and recommendations p.85
6. Summary p.88
Opsomming p.91
References p.94

Chapter 1

Introduction

Due to industrialization, erosion, urbanization, increased salinity, compaction, and the increase in acidity as a result of fertilization, there is a decrease in the available space for agriculture. Together with environmental conditions such as salt loading, drought, and freezing, this can cause adverse effects on the growth and productivity of cereal crops such as wheat (*Triticum aestivum* L.). Whether the cropping occurs in the temperate areas or the tropics, both types of environments are affected by global warming and the destabilizing effects that it causes, none more serious than the increased variability in rainfall and temperature that are occurring (Pellegrineschi *et al.*, 2002; Yamaguchi-Shinozaki *et al.***,** 2002).

Due to the limited insight into the physiological basis of drought tolerance in wheat, a better understanding of some of the mechanisms that enable the plants to adapt to stress and maintain growth during stress periods would help in breeding for drought tolerance (El Hafid *et al.*, 1998). If detection of drought tolerance can be done at an early stage of the wheat plant's development, the time for breeders for accurate selection can be shortened. Waterlogging, sodicity (alkalinity), and soil salinity can also play a major role in the growth of crops and their productivity in irrigation areas. Turner (2003) stated that for successful selection for drought tolerance in a breeding program, the breeder needs to first identify the type and timing of the stress that the crop might encounter. Understanding drought tolerance based on morpho-physiological traits, offers the potential to select germplasm based on key-traits linked with grain yield (Sapra *et al.*, 1991).

Experimental non-penetrating soluble components such as Sorbitol or Mannitol can be used to induce water stress (Santakumari and Berkowitz, 1990). By using a hydroponic cultivation technique one can simulate drought conditions in a controlled manner. Plants can also be provided with optimum conditions for development when drought conditions are not simulated.

Hydroponics is the art and science of growing plants without soil, by feeding it chemical solutions containing artificial forms of nutrients, which they usually draw from the earth. The basic principle of soil-less culture is not new. Over the centuries, scientists have been producing plants in this way for physiological experiments. Thus, hydroponics has developed from the findings of experiments carried out to determine what substances make plants grow, and the composition of plants (Deutschmann, 1998).

Today, hydroponics is an established branch of agronomical science. Progress has been extensive over the past 30 years. The two chief merits of the hydroponic cultivation of plants are, firstly much higher crop yields, and secondly, the fact that hydroponics can be used to cultivate crops where it is normally impossible (Douglas, 1972). For this reason hydroponics is often used for a number of applications in the study of plants. Space based applications is but one example (Steinberg *et al.*, 2000). It is yet unknown whether it is possible to improve the potential number of crosses that can be made in a breeding program, using a hydroponic growing method compared to the currently used potting method. If it is possible to increase the number of crosses that can be made, a breeder can make more combinations in the same time and same space available.

The first aim of this study was to evaluate the functionality and the practical application of hydroponics for wheat breeding, compared to conventional glasshouse cultivation. A further aim was to assess the screening capability of the hydroponic system for drought tolerance in wheat cultivars.

Chapter 2
Literature review

2.1. Drought tolerance in wheat

Wheat (*Triticum aestivum* L. and *T. turgidum* L.) is the world's leading cereal grain and most important food crop. Its importance derives from the properties of wheat gluten proteins that stretch with the expansion of fermenting dough, yet coagulate and hold together when heated to produce a 'risen' loaf of bread. Wheat is utilized for making bread, unleavened bread, flour for confectionary products and breakfast cereals. Its diversity of uses, nutritive content, and storage qualities has made wheat a staple food for more than one-third of the world's population (Poehlman and Sleper, 1995).

Due to industrialization, erosion, urbanization, increased salinity, compaction, and the increase in acidity as a result of fertilization, there is a decrease in the available space for agriculture. Together with environmental conditions such as salt loading, drought, and freezing, this can cause adverse effects on the growth and productivity of cereal crops such as wheat. Whether the cropping occurs in the temperate areas or the tropics, both types of environments are affected by global warming and the destabilizing effects that it causes, none more serious than the increased variability in rainfall and temperature that are occurring (Pellegrineschi *et al.*, 2002; Yamaguchi-Shinozaki *et al.***,** 2002).

Early season drought due to inadequate and erratic rainfall, together with uncertainty of rainfall immediately after plant emergence in rain fed farming systems of the Mediterranean, places major constraints on durum wheat production. To overcome this problem, several strategies have been devised. Due to the limited insight into the physiological basis of drought tolerance in durum wheat, a better understanding of some

of the mechanisms that enable the plant to adapt to stress and maintain growth during stress periods would help in breeding for drought tolerance (El Hafid *et al.*, 1998).

Pellegrineschi *et al.* (2002) reported that drought and declining fertilization is nowhere a bigger concern than in sub-Saharan Africa. For example, in 1997 15 kg/ha fertilizer was used compared to the 91 kg/ha in the global context. It is also essential to gather quantitative estimates of yield losses due to each abiotic stress.

The water potential of soil and the water availability is probably the most important factor for any crop to grow and develop. Many physiological and / or morphological adaptations that plants may have against water stress, have been studied and reported in the past. Reduction of leaf area, extensive root growth, closure of the stomata and the lowering of the leaf potential are some of the changes that plants go through in times of water stress (Sapra *et al.*, 1991).

If detection of drought tolerance can be done at an early stage of the wheat plant's development, the time for breeders for accurate selection can be shortened. Waterlogging, sodicity (alkalinity), and soil salinity can also play a major role in the growth of crops and their productivity in irrigation areas. Turner (2003) stated that for a breeder in any crop to do effective drought tolerance selection, he needs to firstly identify the type and timing of the stress that the crop might encounter.

Due to the unpredictable nature of drought, breeding for this tolerance is more challenging than for any other abiotic stress. The material selected must be outstanding under water-limited conditions, but more so, excel under normal or optimal conditions. For progress in cultivar development for dry regions, a number of factors must be considered. Firstly, identifying the prevailing stresses and understanding them better. Secondly, understanding of genetic control of drought tolerance should be improved. Thirdly, refinement of screening methods for drought tolerance is needed (Pellegrineschi *et al.*, 2002).

Sapra *et al.* (1991) stated that it is not possible for breeders to successfully select drought tolerant wheat using the limited information about the response of only a few wheat cultivars to drought. The screening techniques are inadequate due to the fact that it does not show a clear difference in the responses of plants to stress. Furthermore, because there is no concrete test method for early detection, grain yield as well as stability, remains the best selection methods used by breeders.

Wheat crops are affected by drought stress, not only in the plant development stages but also in the crop yield development. Furthermore, there is also a difference in the intensity of the stress that plays a role in both cases. For example, water stress during seed development affects the yield more than when the stress is experienced in the vegetative stage (Agenbag and De Villiers, 1995).

Modification of the physiological pathways during the growth and development of most cultivated crops are induced by abiotic stresses such as drought (Pellegrineschi *et al.*, 2002). According to Blum and Pnuel (1990) attempts have been made to compare the yield of different cultivars, iso–populations or isogenic lines for their physiological responses to water stress. Normally there is no problem with the physiological testing, but there is with the estimation of yield. Firstly, non-genetic variations in the yield between and within environments are very large, especially in drought stricken areas. Secondly, yield potential of the cultivar contributes to the yield, and not just the effects of the physiological responses. The yield potential cannot always be measured by physiological parameters.

The tolerance for dough mixing is an important quality characteristic in wheat. The composition and concentration of specific proteins in wheat has a big influence in bread making quality. For an increase in the loaf volume, one needs an increase in the protein concentration. The ratios of certain proteins also have an influence on baking quality. The baking and dough properties are influenced by both genotype and environmental factors. Firstly the protein concentration can be determined by the genetic background.

It is also true that environmental factors such as nitrogen availability, water access and temperature can have dramatic effects on protein concentrations (Johansson *et al.*, 2001).

Evaluation for drought tolerance requires a large number of testing sites and seasons. Understanding drought tolerance based on morpho-physiological traits offers the potential to select germplasm based on key-traits linked with grain yield under dryland conditions. Morpho-physiological traits can be used as indirect selection criteria for grain yield under dryland conditions. Their effectiveness depends on their correlations with grain yield under drought conditions and the degree to which each trait is genetically controlled. Durum drought tolerance studies have shown that some markers are associated with grain yield under dryland conditions and with morpho-physiological traits that can be used in selecting for drought tolerance (Nachit *et al.*, 2000).

Grasses were exposed to salinity of up to 600 m*M* NaCl. In solution culture, it was found that relative root length and relative root weight increased under saline conditions, compared to the control, in salt tolerant grasses. A negative correlation was found between leaf sap osmolality, Na1, Cl2, and proline concentrations and glycinebetaine was positively correlated with salinity tolerance (Marcum, 1999). Yet there is very little information available regarding the physiological bases of yield potential under water stress conditions. Wheat yield stability under variable drought conditions was associated with the plant's ability for osmotic adjustment (Blum and Pnuel, 1990). The characteristics of plant roots are thought to have an important role to play in the plant's drought and flooding tolerance. Thus, by development of drought tolerant wheat, one of the most important factors should be the roots. Because drought tolerance in wheat cultivars can be attributed to their difference in root development, it is possible that one of the contributing factors can be identified by the development of a more extensive root system that can penetrate deeper, thus obtaining more of the available moisture (Main *et al.*, 1993).

One of the biggest aspects of drought tolerance research in South Africa, is to establish easy and reliable methods for the assessment of water stress in plants. To develop such

methods, one should start to obtain a detailed characterization of the plant responses under drought conditions. Previous studies showed that the possible relationship between different stress parameters caused by water stress, namely changes in transpiration rate, leaf water potential, osmotic adjustment, leaf osmotic potential, rate of water loss during drying and leaf diffusive resistance is not always statistically satisfactory. Variations in the above relationships may vary due to the stages in the plant development, differences between species as well as the evaporative demand (Kumar and Tripathy, 1991; Moustafa *et al.*, 1996).

Kumar and Tripathy (1991) concluded that the transpiration rate was poorly related with the leaf diffusion resistance, water potential and the canopy temperature. These relationships became more conclusive when normalization was done when the normal watered plant measurements were taken into consideration. Furthermore, these studies indicated that above ground factors had the largest effect on plant water stress parameters. Thus, elimination of the soil induced components is essential in the accurate assessment of water stress parameters of plants.

Jat *et al.* (1991) reported that water potential and all its other facets is the most reliable component in the expression of water stress in plants. Although there are numerous documented cases of water potential studies, there is still a need for adequate information linking the crop performance of a plant to the water potential of the same plant under water stress conditions. A high correlation was found between the water potential of wheat and their performance under stress.

Exposing a plant to high salt concentrations or saline conditions to imitate drought conditions can be avoided in an experimental process when non-penetrating soluble components such as Sorbitol or Mannitol are used to induce water stress. Other studies also showed that when a chloroplast acclimatizes to low water potential, it can be beneficial to the photosynthesis potential of a water stressed plant (Santakumari and Berkowitz, 1990).

A plant responds to stress on molecular, cellular and physiological level. A variety of genes can be expressed due to stress. The products of these genes are not only functional in stress tolerance, but also in their gene expression and signal transduction in stress response as seen in Figure 1.

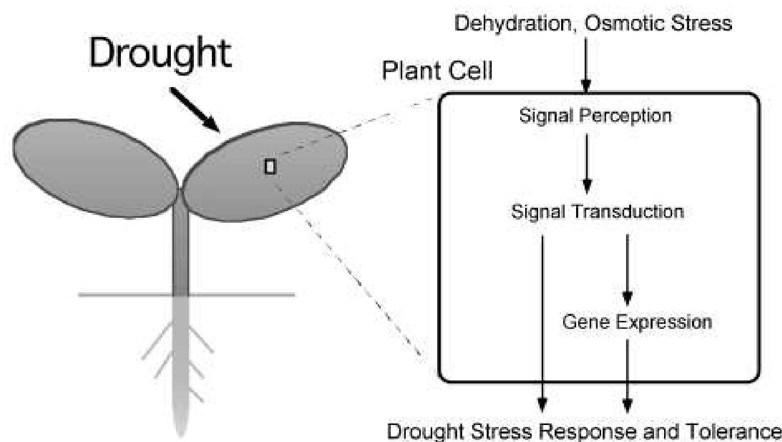


Figure. 2.1: A representation of a plant's molecular responses to drought stress. The perception of dehydration signal, signal transduction to cytoplasm and nucleus, gene expression, and responses and tolerance to drought stress are all part of the response of the plant cell (Yamaguchi-Shinozaki *et al.*, 2002).

When the internal organelles recalibrate their water potential to match the external drought environment, there is a signal process that lowers the photosynthetic rate of that cell (Santakumari and Berkowitz, 1990). Giunta *et al.* (1995) also stated the hypotheses that grain filling and the rate of grain filling can be coupled to the photosynthetic tempo of the plant, indicating the importance of the total leaf surface development of the wheat plant during times when stress is not experienced, making it possible for the plant to undergo effective grain filling during times of drought. Field studies have indicated that photosynthesis and several other related physiological traits do differ between drought tolerant and drought susceptible genotypes. The photosynthetic system's ability to resist dehydration is remarkable. This phenomenon may lead to the rapid recovery of the plant

after re-hydration. The ability of a plant to recover from drought has received little attention in the past (El Hafid *et al.*, 1998).

The spring wheat cultivars in Australia often experience an increase in temperature and evaporation together with a decrease in the rainfall during the crucial stages of grain filling. Grain filling depends on the availability of carbon from mostly three sources. They are; current assimilation, remobilization from pre-anthesis storage and also temporary stored carbon from post-anthesis assimilation. Water stress during grain filling affects the ratio of stored assimilates relative to current assimilates in the grain (Kobata *et al.*, 1992).

Differences in the response to pre-anthesis water stress of several winter wheat cultivars have been reported by Entz and Fowler (1990). The numbers of kernels per spike, and the ratio of spike dry mass are reported together with the total dry matter at anthesis, indicating that high water stress conditions pre-anthesis can dramatically reduce the yield due to the reduced dry matter accumulation and kernel production.

There is a multitude of factors involved in the response of plants to drought stress and Strauss and Agenbag (2000) reported that stomatal response is one of the major responses that plants use. Because it can easily be measured by means of Leaf Diffusive Resistance (LDR) it can be used as an easy screening method by breeders. Furthermore, drought tolerant plants have previously been shown to posses smaller water deficit-per-unit decrease in their leaf water potential compared to drought sensitive cultivars. The degree of drought tolerance has been linked to the accumulation of proline in leaves of plants that experience water stress, in fact, a positive correlation has been found between the accumulation of proline and the severity of water stress.

Salt stress and low temperatures also cause the expression of drought-inducible genes, suggesting the existence of similar mechanisms of stress responses, not only protecting cells from water deficit, but also regulating genes for signal transduction in the drought stress response. Gene products can be classified into two groups (Fig. 2). The first group

functions in stress tolerance; such as, osmotin, key enzymes for osmolytes, mRNA binding proteins, antifreeze proteins, water channel proteins, sugar and proline transporters, detoxification enzymes and various proteases. The second group contains protein factors involved in further regulation of signal transduction and gene expression.

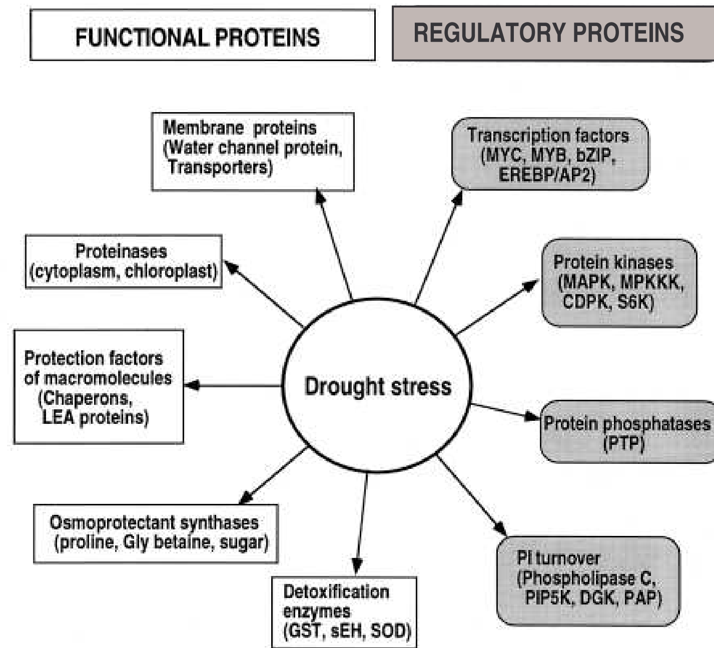


Figure 2.2: A schematic of the two protein groups thought to be active during the induction of stress in the plant cell (Yamaguchi-Shinozaki *et al.*, 2002).

The accumulation of proline in the leaves of wheat during drought stress is well documented. It was also found that in cases of severe water stress, there is a rapid accumulation of proline. Due to this fact, proline is not a good indicator for the onset of drought stress, but can be used as an indicator for drought tolerance in wheat plants. A direct correlation can be found between the degree of water stress and the amount of proline that is produced in the plant (Van Heerden and De Villiers, 1996). Sarker *et al.* (1999) reported that the accumulation of free proline is not the only possible indicator but the accumulation of free sugars can also be seen as an indicator of wheat under drought stress.

Tahara *et al.* (1991) reported that abscisic acid (ABA) plays an integral role in the control of plant responses to drought stress. This hypothesis is poorly supported by genetic investigation, due to technical difficulty in quantification of ABA. The results obtained by the enzyme immunoassay technique (EIA) did not differ much from those obtained from HPLC and GC analysis. The advantage of the EIA is that crude plant material can be used.

Cammue *et al.* (1989), reported that there is not only an ABA accumulation in wheat tissues, but there is also an accumulation of Wheat Germ Agglutinin (WGA) as a response to water stress. WGA was considered to be a seed or embryo–specific lectin, but recent studies have shown that it is also found in other tissue in the wheat plant. These tissues include the roots and coleoptiles of the wheat plant. Together with progress in the investigation of ABA, it was found that this plant growth regulator probably controls the synthesis of WGA in both embryos and growing plants. Also ABA was found to play a key role in the plant's defences to drought stress, not only in the leaves but more importantly in the roots. Ethylene production in wheat is also induced by numerous factors; water stress is one of the most commonly detected due to the evaporation of ethylene (Narayana *et al.*, 1991).

Changes in the cell walls of growing plants have been documented. There were differences found in the molecular mass of arabinoxylans between dwarf and normal cultivars of barley and rice. During water stress, plants such as chick-pea epicotyls, squash hypocotyls and cultured tobacco cells have been found to undergo changes in their cell wall composition. A marked decrease in the accumulation of polysaccharides in the cell walls of plants under water stress was found (Wakabayashi *et al.*, 1997). Many morphological and metabolic changes occur in a plant that is under drought stress. These changes are thought to be adaptive responses in the coping processes of the plant to its environment. Firstly there is an alteration in the cell wall structure and function, for example the formation of a gel-phase lipid layer in the liquid-crystalline bilayer of the cell wall. This increases the cell's ability of permeability and also the cell's micro viscosity (Navari-Izzo *et al.*, 1993).

Thus far, the data available on the effects of drought on the lipids in plant cell walls have been contradictory, probably because of the lack of information on the water status of the plants involved in the studies. Some studies indicate a decrease in the total polar lipid content and in other cases a degree of unsaturation together with an increase in free fatty acids and triacylglycerols (Navari-Izzo *et al.*, 1993).

2.2. Hydroponics

Hydroponics is the art and science of growing plants without soil by feeding it on chemical solutions by giving them artificial or manufactured forms of nutrients, which they usually draw from the earth. The basic principle of soil-less culture is not new. Over the centuries scientists have been producing plants for physiological experiments. Thus hydroponics has developed from the findings of experiments carried out to determine what substances make plants grow and the composition of plants (Deutschmann, 1998).

The birth of modern hydroponics was in 1929 by Dr. Gericke of the University of California when he succeeded to grow tomato vines twenty-five feet in height. Dr. Gericke named the new discovery “hydroponics” derived from the Greek meaning literally water works (Douglas, 1972).

Interest in hydroponic culture continued for several reasons. Firstly, no soil was needed, and a large plant population could be grown in a very small area. Secondly, when fed properly, optimum production could be attained. With most vegetables, growth was accelerated and, as a rule, the quality was better than that of soil grown crops. Produce grown hydroponically had a much longer shelf life or keeping quality (Deutschmann, 1998).

Hydroponic cultivation was used in the past for controlled environment life support systems (CELSS). This was done because of the optimal environment that could be supplied to the roots of the plant for optimal development, to ensure a high growth rate.

Nutrients, water and aeration, can be controlled to the highest degree. This platform of control is hard to match in solid media (Steinberg *et al.*, 2000).

Today, hydroponics is an established branch of agronomical science. Progress has been extensive over the past 30 years. The two chief merits of the hydroponic cultivation of plants are, firstly much higher crop yields, and secondly, the fact that hydroponics can be used to cultivate crops where it is normally impossible (Douglas, 1972). For this reason hydroponics are often used for a number of applications in the study of plants. Space based applications is but one example (Steinberg *et al.*, 2000).

In all hydroponic systems, the nutrition problem is solved in a similar fashion. Nutrient elements are placed in solution in the amounts and proportions required by various plants. The solution is brought into direct contact with the plant roots – hence the common name. According to Salisbury and Ross (1992) many plants invest 20-50 % of their total weight in roots, in some cases when plants are stressed by insufficient water or mineral nitrogen as much as 90 % of the plant biomass is in the roots. On the other hand, in plants grown hydroponically with adequate water and nitrogen, only 3-5 % of the plant biomass was in the roots.

To the question, can hydroponics be operated completely successfully against normal or conventional farming, Bentley (1959) answered by stating that many factors must be taken into account, but hydroponic cultivation of certain crops can give better yields and improved quality.

The advantages of hydroponic culture over soil may be summarized as follows:

1. Since the nutrient solution, unlike soil, is homogeneous, it is relatively easy to sample, test and readjust the nutrient supply periodically by replenishing those elements that are lacking. There is no manure contagion. Furthermore, less fertilizer and water is used, thus less waste.
2. Crops can be grown in localities where normal cultivation is difficult or impractical, e.g., in arid areas or areas of saline or shallows soils. This opens up new regions for

settlement and provides established settlements in such regions with new sources of income. No erosion, no drought and no monsoons could have an influence on the crop.

3. Both nutrient solutions and support medium are contained in beds. Beds and mediums can be sterilized to prevent root diseases or weeds, thus eliminating the need for crop rotation.
4. Seepage can be stopped and surface evaporation minimized so that less water is required for equal yields.
5. Watering can be automatically controlled, reducing labor cost. No big machinery is required
6. Since the nutrient solution can be adjusted and is constantly being replaced, relatively highly saline waters may be used.
7. Average yields are high and cultivation easy. Crops can grow faster due to the optimum environment. Individual plants can also be planted closer, for there are optimum nutrients available and competition should not occur. Fruits and flowers of excellent quality can be produced uniformly

The main disadvantages of hydroponic systems are:

1. The high initial investment and
2. The limited number of crops for which they are economically worthwhile.
3. One must stick to detail because the margin for error is greater than it is with soil.
4. The need for constant learning with progress and larger operations.

(Saffell, 1993)

The various hydroponic systems in use may be grouped according to the type of support medium:

1. Water (or tank) culture. The plant is supported above the roots on cardboard, plastic, wood, or wire; the root system hangs freely in a nutrient solution.
2. Sand culture. The plant is supported by its roots in fine-textured inert media such as sand, vermiculite, or fiberglass. The nutrient solution is mostly held between particles of the support medium. Similar in culture technique (though the medium is

not inert) is the use of peat (turf), or a mixture of peat and sand. Here, neither the peat nor the sand has any form of nutrients that is available to the plants.

3. Sub-irrigated gravel culture. The plant is supported by its roots in a relatively coarse-textured medium such as gravel or foam plastic. The nutrient solution, which is stored in an underground reservoir, is pumped into the beds. When the beds are filled to the required level, the pump is stopped, the solution drained back into the reservoir, and air fills the spaces between the gravel particles. The gravel is easily sterilized. Investment for this system is higher than for the others, but more growth factors can be controlled and better products ensured. As yet, no other hydroponic system has proved of greater economic value (Schwartz, 1968).

Schwartz (1968) also stated that for the most hydroponic units, inert gravel is the support medium. It is important to keep the cost of the system to a minimum. Rounded stones should be used to prevent injury to the developing roots of the plants in the system.

2.3. Hydroponics and wheat

The characteristics of wheat plant roots are thought to have an important role to play in the plant's ability to tolerate drought and flooding. When developing drought tolerant wheat, one of the important factors should be the roots. Because most wheat cultivars differ in their drought tolerance, it is possible that one of the contributing factors can be identified by the development of a more extensive root system that can penetrate deeper (Main *et al.*, 1993).

Knowing that the roots do have an influence on the drought tolerance of wheat, Main *et al.* (1993), decided to test the hypothesis. It was decided to use a hydroponic setup for determining the effects of water induced stress on wheat and to determine if there is a correspondence to wheat grown in soil. Hydroponic methods were conducted as follow: Seed weights were determined and germinated in the dark at 28 ºC on wetted paper towels after being soaked in 0.1mM $CaSO_4$. Plants were transferred to a static hydroponic system. Root weight was determined after drought was induced.

Oscarson *et al.* (1995), investigated the influence of nitrogen uptake and utilization in four different spring wheat cultivars in terms of protein yield content. The increase of N accumulation generally results in an increase of protein yield. Trials have shown that post-anthesis N uptake contributed significantly to the buildup of grain N. Grain-N content is regulated by the plant's ability to absorb the available N but also the remobilization and translocation of that absorbed N.

Oscarson *et al.* (1995), used the following planting and hydroponic methods in their trial. Seeds were germinated over four days in deionised water. Plants were transplanted to a hydroponic system and supplied with a N-free solution that was also used as a base solution. The nutrient solution contained the following:
0.83 mM K, 0.23 mM PO_4, 0.087 mM Ca, 0.175 mM Mg, 0.238 mM SO_4, 6.27 µM Fe(III), 3.64 µM Mn, 9.24 µM B, 0.24 µM Cu, 0.23 µM Zn, 0.037 µM Mo, 48.27 µM Na, 0.58 mM Cl and 24.1 µM EDTA. Nitrate was given as KNO_3. This was added once daily in exponentially increased doses according to the formulation $N_t = N_0$ x e RAxt. N_t and N_0 represents the N contents of the plants at days *t* and *0* respectively. RA is the relative NO_3- addition rate. Thus the daily addition is given by N_t - N_0. The nutrient solution was freshly prepared every seven days and the pH kept between 6.5 and 5. The NO_3- uptake measurements were done 24 hours after the previous NO_3- addition. Sets of plants were transferred to glass beakers with the N free basal solution. This solution was stirred to circulate through a quartz cuvette that was in an UV spectrophotometer. Absorption of NO_3- was measured at 202nm for four minutes.

Bugbee (1995) noted that to develop a refill solution, one must keep in mind that both water and nutrients should be replenished. Recipes, such as Hoagland solution, can be used as refill solution. Diluted to about a third strength, the electrical conductivity can be kept constant in the refill solution. However, the Hoagland solution was originally developed for tomatoes and is thus not always appropriate as refill solution for other types of plants.

Two things need to be considered in the developing of a refill solution, that is solution composition and solution concentration. The control of pH for plants are not so critical because plants grow equally well in a pH between 4 and 7 as long as there is no nutrient depletion. The recommended pH for hydroponics is between 5.5 and 5.8. There are three groups of essential plant nutrients based on the approximate uptake rates. Group one represents active uptake or fast removal nutrients, namely NO, NH_4, P, K, Mn. In group two, nutrients such as Mg, S, Fe, Zn, Cu, Mo, C are intermediately removed from the solution. The third group (Ca, B) is passively or slowly removed from the solution.

2.4. SE-HPLC

The world's leading cereal grain, wheat (*Triticum aestivum* L.) is known for its wheat gluten properties. A cohesive endosperm protein network that stretches with the expansion of fermenting dough and holding capabilities when heated to produce a 'risen' loaf of bread is what makes this product unique. The diversity of uses, storage qualities, and nutritive content ensured that wheat became and still is a staple food for more than one-third of the world's population (Poehlman and Sleper, 1995).

Carbohydrate compounds are the major storage compounds of wheat and play a role in the yield. The uniqueness of wheat comes in the second largest storing compound, namely proteins (Mamuya, 2000). These proteins are classified according to their solubility properties into gliadins and glutenins, and account for nearly 85 % of the endosperm proteins in wheat kernels (Osborne, 1907). The two groups of proteins differ in the role they perform in dough. Glutenin is primarily responsible for elasticity, whereas gliadin ensures viscosity and extensibility (Payne *et al.*, 1984). Glutenin can be subdivided into high molecular weight (HMW) and low molecular weight (LMW) subunits (Payne *et al.*, 1981). Gliadin forms roughly half of all storage proteins, while the two glutenin fractions, HMW and LMW, make up the other half with 10 % and 40 % respectively (Payne *et al.*, 1984).

2.5. The composition of wheat proteins

2.5.1. Classification of wheat proteins

The largest component of the grain is the endosperm, demanding the most attention with respect to the genetic analysis of quality traits. Starch makes up about 72 % of the endosperm and most of the remainder is protein (Worland and Snape, 2001). The protein percentage in common flour on a 14 % moisture basis is usually between 7-15 % (Table 2.1) (Atwell, 2001).

Up to 15 % of the flour proteins are made up of the water-soluble proteins or albumins (Table 2.1). The globulins have a small representation of only about 3 % of the total protein (Atwell, 2001). Between wheat varieties, composition of albumins and globulins does not vary much. There is also no apparent correlation between the amount of albumins and globulins and baking performance (MacRitchie, 1984).

The proteins soluble in 70 % aqueous ethanol are known as prolamins. Gliadin is a prolamin and represents about 33 % of all the proteins in flour. Glutelins are soluble in dilute acids or bases and 16 % of the flour protein is made up out of it. Some proteins do not totally dissolve in any of these solvents. The unclassified residue of 33 % accounts for these proteins (Atwell, 2001).

Table 2.1: Composition of flour and its primary components fractions (Atwell, 2001)

Property	Fraction	Compound
Moisture	14%	flour
Protein	7 – 15%	flour
Osborne classification		
Albumins	15%	protein
Globulins	3%	protein
Prolamin (gliadins)	33%	Protein
Glutenlin (glutenin)	16%	Protein
Residue	33%	protein
Gluten	6 – 13%	Flour
Gliadin	30 – 45%	Gluten
Glutenin	55 – 70%	Gluten
Starch	63 – 72%	Flour
No starchy polysaccharides	4.5 – 5.0%	Flour
Lipids	1%	Flour

2.5.2. Wheat storage proteins

Bietz and Wall (1973) indicated that gluten can be divided into two groups, namely the low-molecular-weight, alcohol-soluble subunits named gliadins and the high-molecular-weight, alcohol-insoluble subunits named glutenins.

2.5.3. Low Molecular Weight Storage Proteins (Gliadins)

Kaczkowski and Tkachuk (1980) defined gliadins as the following: "proteins of wheat endosperm soluble in alcohol such as 70 % ethanol at room temperature, and which migrate in polyacrylamide and starch gels without reduction as reasonable discrete bands, and which are not excluded during gel filtration on Sephadex-G-100". Gliadins can be fractionated by means of gel electrophoresis at low pH. The separation yields four groups, α-, β-, γ- and ω-gliadins, according to each group's mobility (Lafiandra *et al.*, 1994). Gliadins can also be characterized by a high proline content (Atwell, 2001) hence prolamins. The name prolamin is a combination of these amino acids (Gianibelli, 2001). According to Panozzo and Eagles (2000) the proportions of gliadin and glutenin in wheat flour protein are influenced by genotype as well as the environment. Gliadins are more sensitive to the environment.

2.5.4. High Molecular Weight Storage proteins (Glutenins)

Generally, when it comes to bread-making quality, glutenin is recognized as the wheat protein fraction that has the most influence. In its unreduced state it is a polymeric protein. Its molecular weight estimation varies from 100 000 up to 20 million (large polymers) (Huebner and Wall, 1976; Bietz and Huebner, 1980; Tatham *et al.*, 1985). Between 55 – 70 % of the gluten complex is made up of glutenin (Atwell, 2001). Two main characteristics are associated with glutenin subunits: they are insoluble in both salt and 70 % ethanol solution. Furthermore their disulfide bonds bind the macromolecule, composed of polypeptides (Lásztity, 1996).

2.5.5. Protein content

A basic amount of nitrogen (N) is required by a wheat plant from the soil to accumulate dry mass and nitrogen content in the vegetative tissue to ensure an acceptable yield with sufficient protein content (Dechard *et al.*, 1984). There are numerous limiting factors in protein production, namely the amount of available soil moisture and mineral nutrients availability (Pomeranz, 1988).

2.5.6. Environmental effect on quality

The genetic background of wheat determines the composition of proteins and protein subunits (Payne *et al.*, 1987; Johansson *et al.*, 1993; MacRitchie, 1999). The quantity of these groups, on the other hand, varies due to environmental conditions. Robert *et al.* (1996) found that the percentage and the concentration of the flour proteins present as gliadin and non-gluten proteins were severely affected by environmental fluctuations. Glutenin was the most genotype dependent (Graybosch *et al.*, 1996; Zhu and Khan, 2001).

2.6. High performance liquid chromatography of wheat proteins

Conventional chromatography is slow, column beds can become unstable, results difficult to accurately reproduce and quantification can be difficult (Bietz, 1985b). New developments in chromatographic methods provide superior separations. High performance liquid chromatography (HPLC), represents the improvement of instrumentation and columns. Chromatographic systems possessing reliable, small, uniform, and stable, silica-based columns that can withstand high pressures and flow rates, have become available. Improvements have been made in sensitivity, speed, resolution, reproducibility, and ease of use. Numerous bonded phases can be covalently attached to silica silanol groups, resulting in ion-exchange (IE-), reversed-phase (RP-),

and size-exclusion (SE-) HPLC columns. Separation has been done successfully using HPLC techniques for detection of differences in quality (Huebner *et al.*, 1990, Huebner and Bietz, 1985), and variety identification (Bietz, 1985a).

2.6.1. SE-HPLC and wheat quality

The first mode of liquid chromatography to be adapted to high performance methods for protein analysis was size-exclusion chromatography. The improvements of proper mobile phase, the adjustment of ionic strength and pH, and the use of detergents counteracting hydrophobic interactions, made high performance systems superior in resolution and analysis time. This allowed analyzing of any protein by SE-HPLC that could be separated on carbohydrate columns (Autran, 1994).

SE-HPLC has major advantages. It is very sensitive, reproducible, equipment is much simpler than, for example, the equipment of RP-HPLC and is easily automated. Data quantification can be done accurately (Bietz and Kruger, 1994, Autran, 1994). The most important advantage, however, is speed. Compared to an analysis of a day or more on a conventional column, a 20 – 30 minute analysis with SE-HPLC could give far better information (Bietz and Kruger, 1994).

Size distribution of protein polypeptides and protein aggregates can be examined by SE-HPLC. The size range of gluten proteins and the proportions of aggregating and monomeric proteins in flour or grain can be determined, because quality is normally associated with the presence of large protein aggregates. In other biochemical techniques (e.g., SDS-PAGE and RP-HPLC) the reduction of S-S bonds can lead to the loss of information concerning structure, interactive qualities and the stability of protein complexes. The potential to keep relatively large aggregates in an undisturbed state, to retain information is a major advantage of SE-HPLC (Autran, 1994).

Glutenin, gliadin, and albumins-globulins can be accurately separated by SE-HPLC (Larroque *et al.*, 1997). The results obtained correlate well with bread-making quality, especially when focusing on the first peak of the chromatogram (polymeric protein) (Batey *et al.*, 1991). The 210 nm wavelength is the preferred detection wavelength for protein. It is a good compromise between potential detection interference and detection sensitivity (Burke *et al.*, 1991).

By using SE-HPLC, proteins are sorted by size. Accurate molecular weight (MW) estimations can be obtained. Separations occur when larger proteins are rapidly eluted from the column and smaller proteins are retarded due to the inverse relation to their molecular size (Bietz, 1985b). By calibrating the column, using known protein standards, the molecular size of proteins separated can be calculated. Computer programs can be used to calculate each variable of each peak to determine the area percentage of each separation (Autran, 1994).

SE-HPLC methods are still improving in resolution and pore size of columns. This could improve the ability to analyze larger protein aggregates. Better ways to use these methods are being formulated. It is unacceptable to work on only partially solubilized material. It is essential that measurements are done on the total protein extract (Singh *et al.*, 1990a). It is difficult to completely dissolve the storage proteins from flour in a manner that does not chemically alter the remaining un-dissolved proteins (Danno *et al.*, 1974). By generating ultrasonic vibrations (frequency of 20 KHz) in a 1.5 ml Eppendorf tube by means of a sonifier, Singh *et al.* (1990b) demonstrated that complete dissolution of unreduced proteins was possible in a 2 % SDS solution (pH 6.9). This technique has several advantages: firstly a very short time (30 sec) is needed for extraction, secondly, small quantities of flour (11 mg) is needed and lastly only very large glutenin polymers (that needs less energy for shear degradation) are degraded. The polymeric and monomeric groups resulting from this process are eluted from the column without affecting the size-based fractionation. The combination of sonication and SE-HPLC has enabled accurate determinations of the individual proportions of the protein classes found in flour samples (Singh *et al.*, 1990a).

Due to variation in grain quality and protein composition, that can be a consequence of variation in the environment or physiological factors, HPLC fractionations have been performed by Huebner *et al.* (1990) to measure changes linked to maturity, kernel size and spike location. By using glutenin samples extracted at various stages of maturity in SE-HPLC analysis, re-dissolving it in phosphate buffer (containing 2 % SDS, 5 % acetonitrile, and 0.01 % dithiothreitol), two major peaks were observed. The peaks corresponded to high (peak B) and low (peak C) molecular weight subunits. Some globulins and albumins were present in peak D. Unreduced material was expressed as a void volume in peak A. As wheat matured only fraction C increased, thus indicating an accumulation of LMW subunits. Parallel synthesis of gliadins may occur. The HMW subunit number, in contrast, remained nearly constant and may be formed out of mainly non-storage proteins. Thus, accumulation rates of both subunits of glutenin during kernel development differ.

Chapter 3

Hydroponics as a tool for drought tolerance breeding

3.1. Introduction

Environmental conditions such as salt loading, drought, and freezing, can cause adverse effects on the growth and productivity of cereal crops, in terms of yield and quality of wheat (*Triticum aestivum* L. and *T. turgidum* L.). The water potential of soil and the availability thereof, is probably the most important factor for any crop to grow and develop. If detection of drought tolerance can be done at an early stage of the wheat plant's development, the time for breeders for accurate selection can be shortened. Turner (2003) stated that for a breeding program in any crop to be successful in selecting for drought tolerance, the breeder needs firstly to identify the type and timing of the stress that the crop might encounter.

Due to the unpredictable nature of drought, breeding for this tolerance is more challenging than for any other abiotic stress. For the progress in cultivar development for dry regions, a number of factors must be considered. Firstly, identifying the prevailing stresses and understanding them better. Secondly, understanding of genetic control of drought tolerance should be improved. Thirdly, refinement of our screening methods for drought tolerance is needed (Pellegrineschi *et al.*, 2002).

Sapra *et al.* (1991) states that it is not possible for breeders to successfully select drought tolerant wheat, by using the limited information about the response of only a few cultivars of wheat to drought. Evaluation for drought tolerance requires a large number of testing sites and seasons. Understanding drought tolerance based on morpho-physiological traits offers the potential to select germplasm based on key-traits linked with grain yield in dry-land.

One of the biggest aspects of drought tolerance research in South Africa is to establish easy and reliable methods for the assessment of water stress in plants. To develop such methods, one should start to obtain a detailed characterization of the plant responses under drought conditions. Previous studies showed that the possible relationship between different stress parameters caused by water stress, namely changes in transpiration rate, leaf water potential, osmotic adjustment, leaf osmotic potential, rate of water loss during drying and leaf diffusive resistance is not always statistically satisfactory. Variations in the above relationships may vary due to the stages in the plant development, differences between species as well as the evaporative demand (Kumar and Tripathy, 1991; Moustafa *et al.*, 1996).

Differences in the response to pre-anthesis water stress of several winter wheat cultivars have been reported by Entz and Fowler (1990). The number of kernels per spike and the ratio of spike dry mass are reported together with the total dry matter at anthesis. This indicates that high water stress conditions pre-anthesis can dramatically reduce the yield due to the reduced dry matter accumulation and kernel production. The accumulation of proline in the leaves of wheat during drought stress is well documented. It was also found that in cases of severe water stress there is a rapid accumulation of proline.

The uniqueness of wheat comes in the second largest storing compound, namely proteins (Mamuya, 2000). These proteins are classified according to their solubility properties into gliadins and glutenins, and account for nearly 85 % of the endosperm proteins in wheat kernels (Osborne, 1907). Glutenin, gliadin, and albumins-globulins can be accurately separated by size-exclusion chromatography (SE-HPLC) (Larroque *et al.*, 1997). By using SE-HPLC, proteins are sorted by size. Accurate molecular weight (MW) estimations can be obtained. Variation in grain quality and protein composition can be a consequence of variation in the environment or physiological factors. HPLC fractionations have been performed by Huebner *et al.* (1990) to measure changes linked to maturity, kernel size and spike location.

The aim of this study was to asses the use of a hydroponic system for accurately screening wheat cultivars for drought tolerance at two growth stages, by measuring yield components as well as fluctuations in polymeric and monomeric proteins.

3.2. Material and methods

3.2.1. Planting, maintenance and sampling procedure in hydroponic systems

Drought tolerance screening was done in two separate trials, the one at two leaf stage, and the other at anthesis. Five South African wheat cultivars, SST 88, Baviaans, Steenbras, SST 876 and Kariega, were germinated in Petri dishes in a controlled environment. After 15 days, the seedlings were transplanted as random sets of eight plants into two identical hydroponic systems. The hydroponic systems consisted of four identical three meter P.V.C. gutter down pipes. These pipes were cut open and filled with swimming pool filter sand. Each system had a 60 l nutrient container. Only 50 l of nutrient solution was circulated at a time through each system. Water and nutrients were provided in each system once a day for 5 min, circulating 6 l of nutrient solution per pipe in each system. The nutrient rich solution drained over a 15 min period for each pipe. A full strength chemicult solution (100 g / 50 l) was made up for both systems and this solution was maintained weekly. The chemicult solution consisted of 6,5 % N, 2,7 % P, 13,0 % K, 7,0 % Ca 2,2 % Mg, 7,5 % S, 0,15 Fe, 0,024 % Mn, 0,024 % B, 0,005 % Zn, 0,002% Cu and 0,001% Mo. The pH of the solution was kept at 5,6.

A second set of the five cultivars was planted two months after the first planting to separate the two stress periods. The first drought stress was induced before grain fill and in the second planting; stress was induced at two leaf stage. This was done simultaneously. Eight plants of each cultivar were planted for each test, 12.5 cm apart, and each plant was considered to be a replication. Drought stress was induced by stopping the supply of water and nutrients for 10 days for each treatment hereafter normal

watering was reestablished. During this time the control treatment received the optimum flow of water and nutrients.

After the 10 day treatment, three plants of each cultivar in each treatment were selected from the systems. The leaves of the selected plants were then used for proline extractions. The remaining plants were kept in the hydroponic systems until full maturity. When maturity was reached, the following measurements were taken: dry mass, number of tillers, number of spikes, number of primary spikelets, number of secondary spikelets, primary kernel number, secondary kernel number, primary kernel mass (g), secondary kernel mass (g), total kernel number and total kernel mass (g). Primary and secondary kernels were combined and ground to flour with a coffee mill. The flour was used for determining the protein concentration and for extract the protein content for SE-HPLC analysis.

In Figure 3.1 both hydroponic systems are visible, showing the wheat plants in the flowering as well as the seedling stages before the start of the drought treatment. On the far left side, the seedling control treatment can be seen. The following tube to the right is the flowering stage control. The seedlings on the far right represent the drought treatment and the wheat plants on their left are the flowering stage drought treatment plants.

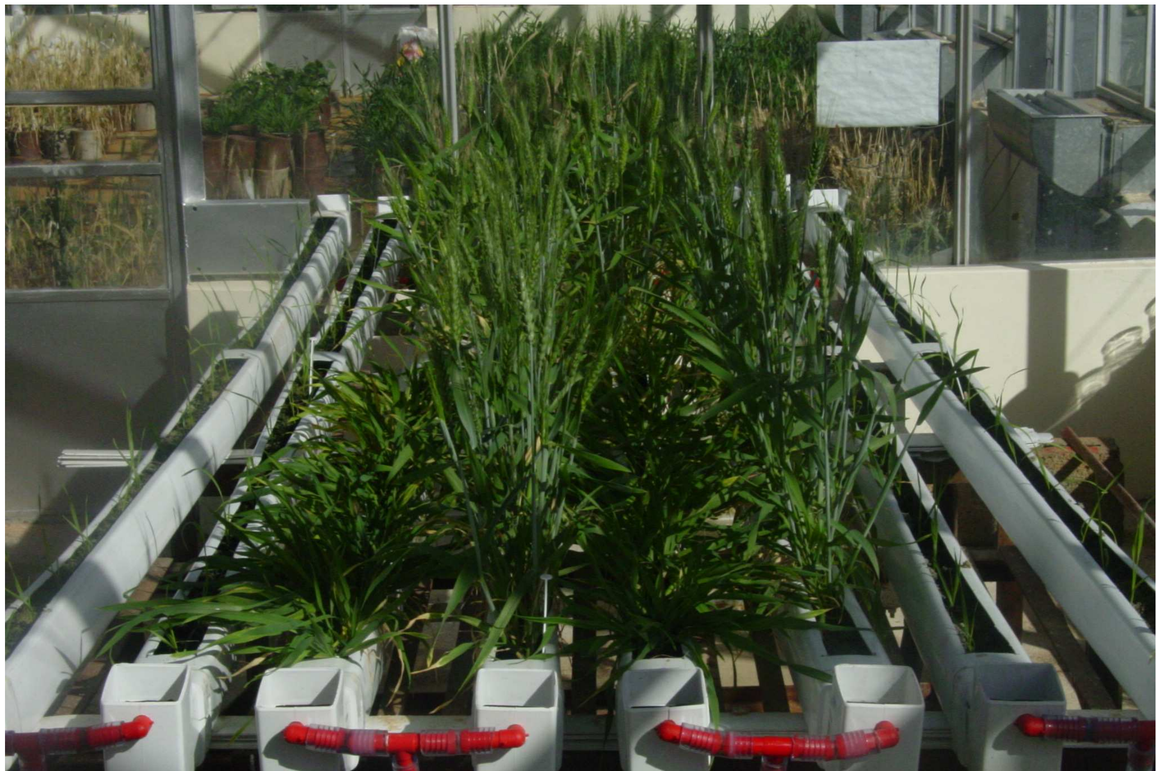


Figure 3.1: Two hydroponic systems before drought treatment started

After drought was induced in the hydroponic system on the right-hand side of Figure 3.2, there was a visible difference between the plants in the two systems. On the right hand side one can see the drought treatment system and on the left the control system. In Figure 3.3, the close up of the drought induced plants shows that the drought treatment that was induced on both the flowering and seedling plants had less of an visible effect on the seedlings.

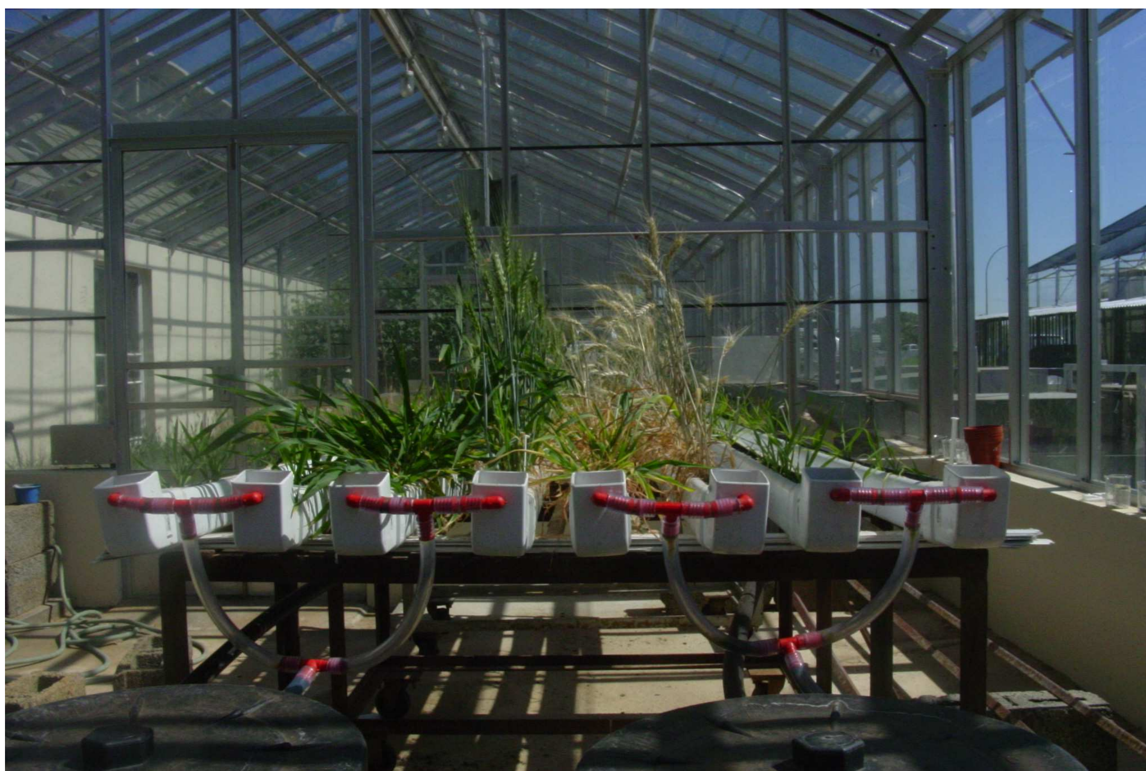


Figure 3.2: Two hydroponic systems after drought treatment stopped.

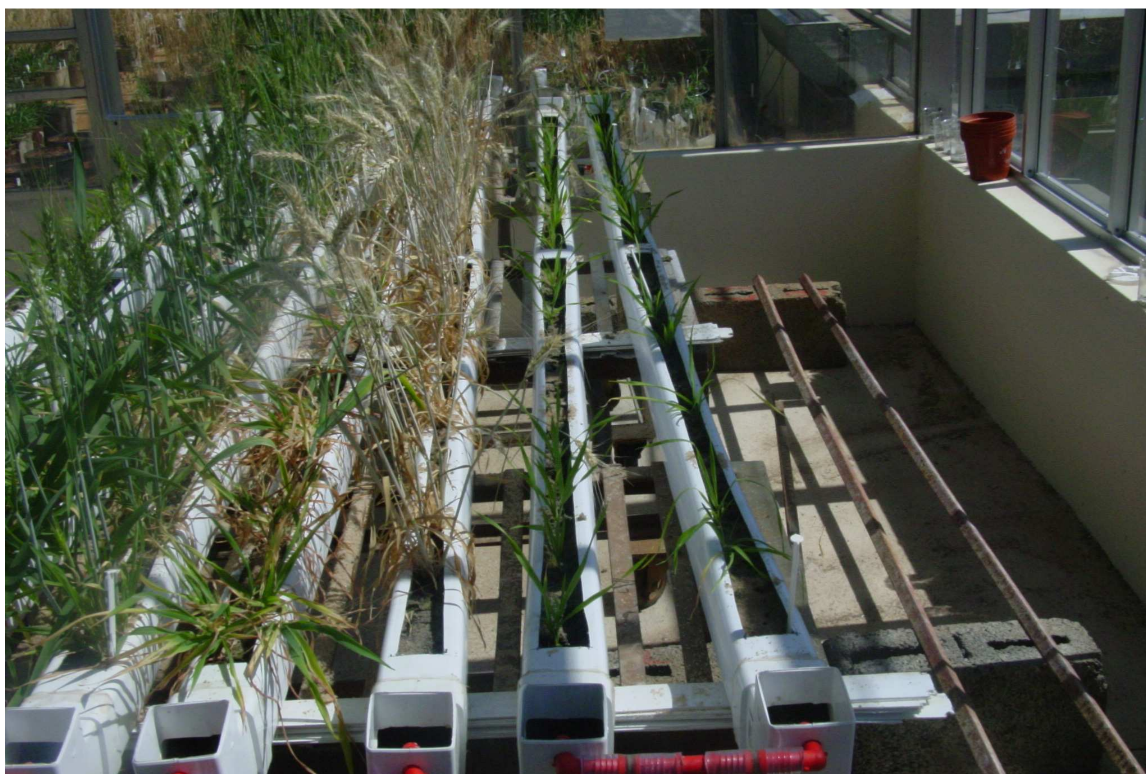


Figure 3.3: View of wheat plants in the drought treatment hydroponic system during the seedling and flowering stage.

The plants in the control system as seen in Figure 3.4, is still visibly healthy. The seedlings have also grown considerably.

Figure 3.4: View of wheat plants in the control hydroponic system during the seedling and flowering stage.

3.2.2. Proline extraction

To extract proline, 0.1 g of freeze dried leaves was crushed in liquid nitrogen to a fine powder. The sample was placed in a test-tube. A 3 % sulphosalicylic acid solution was made up. Ten milliliter of this solution was added to each leaf sample in each test-tube. The samples were then centrifuged at 13000 revolutions per minute for 10 minutes, until the supernatant became clear.

Acid ninhydrin was made up by dissolving 0.25 g of ninhydrin in 30 ml of acetic acid. The solution must be heated to dissolve the ninhydrin completely. After the solution was completely dissolved, it was left to cool to room temperature and 20 ml phosphoric acid

was added. This solution was freshly made daily. Two ml acid ninhydrin and 2 ml acetic acid were combined with 2 ml of the filtrate. The solution was mixed thoroughly and incubated at 100 ^{o}C for one hour.

The reaction was stopped by placing the samples on ice until they returned to room temperature. Then 4 ml of toluene was added to each sample and placed on a vortex for 15 sec. One hundred micro liters of each sample was loaded into the wells of an ELISA plate. Toluene was used as a blank. The samples were read at 520 nm in a spectrophotometer.

There were, however, not enough data obtained for a statistical analysis of the proline concentrations. The proline concentrations were only used in the correlation results.

3.2.3. Total protein analysis

The LECO FP-2000 Nitrogen/Protein Analyser, a non-dispersive infrared microcomputer, was used to determine flour protein content. The samples were placed into a combustion chamber and the furnace and flow of oxygen gas caused the sample to combust. This process converted all elemental nitrogen into N_2 and NO_x. In the catalyst heater all NO_x gases were reduced to N_2. The nitrogen gas in the flour thus assessed the protein quantity. These data was only used in the correlations.

3.2.4. Size exclusion high performance liquid chromatography (SE-HPLC)

Proteins were extracted from wheat flour with a two-step extraction procedure developed by Gupta *et al.* (1993) with a few modifications. The same method is described by Singh *et al.* (1990a) and Batey *et al.* (1991). The first step extracts the proteins soluble in diluted SDS while the second contains proteins soluble after sonication.

Step one:

For the extraction of proteins for the SE-HPLC analysis 0.017 g of flour was weighed in a 1.5 ml Eppendorf tube. A 0.05 M NaH_2PO_4 buffer solution with a pH of 6.9, using distilled water, was made up daily. The buffer was then made up as a 0.5 % SDS buffer.

One milliliter of buffer was added to the flour and was vortexed for 5 min. Samples were then centrifuged for 30 min at 10 000 rpm. Thereafter they were filtered through a 0.5 nm filter. The samples were run through the HPLC machine.

Step two:

The sonication step extracts the rest of the proteins that could not be extracted in the previous step. The pellet was resuspended in 1.5 ml of the extraction buffer by vortexing for 5 min. The samples were sonicated at 5 µm amplitude for 30 sec, then centrifuged for 30 min at 10 000 rpm. The extracts were filtered through 0.45 µm filters before running on HPLC. Aliquots of 20 µl of each extract were injected into a BIOSEP SEC-4000 Phenomenex column on a System Gold HPLC (Beckman Instruments Inc., Fullerton, CA, USA) and run for 30 min with a flow rate of 0.2 ml/min. The elution solvent used was 50% acetonitrile in water (v/v) with 0.1% of trifluoroacetic acid (v/v). The solvent was previously filtered and degassed. Proteins were detected by UV absorbance at 210nm. Areas of the different peaks were calculated.

The measured HPLC fractions were: SDS-soluble and SDS-insoluble, with each chromatogram subdivided into larger polymeric proteins (LPP), smaller polymeric proteins (SPP), larger monomeric proteins (LMP) mainly gliadins, smaller monomeric proteins (SMP) mainly albumins and globulins. The four major peaks were eluted between 9 and 20 min. The percentage of total un-extractable polymeric protein (TUPP) in the total polymeric protein [(SDS-insoluble large and smaller protein polymers)/SDS-soluble and insoluble large and smaller protein polymers)] and the percentage of large unextractable polymeric protein (LUPP) in the total large polymeric protein [(SDS-insoluble large protein polymers) / (SDS-soluble and SDS-insoluble large protein polymers)] was calculated according to the method of Gupta *et al.* (1993).

3.2.5. Statistical analysis

All statistical analyses were done with Agrobase (2000) software. Relations between SE-HPLC protein fractions and quality characteristics were investigated by carrying out analysis of variance (ANOVA) and linear correlation coefficients.

3.3. Results and Discussion

3.3.1. Morphological characteristics of five cultivars under drought stress at seedling stage compared to the control

The effect of treatment was highly significant ($p < 0.01$) for all characteristics except tiller number, number of spikes and primary kernel mass (Table 3.1).

There were significant differences between entries for all characteristics excluding primary spikelet number. There was no significant interaction between entry and treatment for any of the characteristics.

Table 3.1: The mean squares of the measured morphological characteristics of the five cultivars under drought stress treatment during the seedling stage compared to the control

Mean squares of measured characteristics					
TRAIT	TREATMENT	ENTRIES	ENTRIES X TREATMENT	BLOCKS	RESIDUAL
Dry Mass	114.973**	114.383**	6.316	5.08	10.748
Tiller	0.72	6.630**	0.77	0.38	1.275
Spikes	0.18	10.130**	0.23	0.33	1.168
Primary Spikelet	66.91**	4.87	0.03	2.02	2.263
Secondary Spikelet	4588.820**	1266.720**	508.72	105.77	240.183
P/Kernel n	832.320**	302.770**	50.37	47.27	60.008
S/Kernel n	8897.780**	7697.180**	331.48	522.08	1033.805
P/Kernel g	0.306	0.532**	0.082	0.027	0.129
S/Kernel g	7.296**	6.816**	0.199	1.099	0.925
Kernel n	15770.880**	9404.370**	929.83	770.12	1455.875
Kernel g	8.048*	10.134**	0.455	0.949	1.596

*$p < 0.05$, ** $p < 0.01$ Dry mass in gram, Tiller = Total number of tillers, Spikes = Total number of spikes, Primary spikelet = Primary spikelet number, Secondary spikelet = Secondary spikelet number, P/Kernel n = Primary kernel number, S/Kernel n = Secondary kernel number, P/Kernel g = Primary kernel mass (in g), S/Kernel g = Secondary kernel mass (in g), Kernel n = Total kernel number, Kernel g = Total kernel mass (in g).

3.3.2. The mean squares of the measured morphological characteristics, for the five cultivars under drought stress during the seedling stage compared to the control

There were no significant differences between the plants for the measured characteristics. The differences between entries were highly significant ($p < 0.01$) for all the characteristics excluding tiller number and primary kernel mass with a significant ($p < 0.05$) difference.

3.3.3. The measured morphological characteristics, for the five cultivars under drought stress during the seedling stage compared to the control

In comparing the control treatment to the drought treatment, only the SST 88 (control) showed a significant difference in the dry mass and number of tillers compared to drought stressed SST 88 (Table 3.2).

There were no significant differences between the cultivars for number of spikes. Significant differences were found for all the cultivars between the control and drought treatments for number of primary spikelets. Only the control of SST 88 showed a significantly higher secondary spikelet count compared to the drought treated cultivar.

In comparing the primary kernel number only SST 88 did not show a significant difference between treatments. In comparing the secondary kernel number only SST 876 showed a significant difference between treatments. There were no significant differences between treatments for primary and secondary kernel weight. The controls of Baviaans and SST 876 had a significantly higher total kernel number compared to the drought treated cultivars.

Table 3.2: Means of the measured morphological characteristics of the five cultivars during drought treatment in the seedling stage compared to a control

TRAIT	KARIEGA DROUGHT	KARIEGA CONTROL	BAVIAANS DROUGHT	BAVIAANS CONTROL	SST 88 DROUGHT	SST 88 CONTROL	STEENBRAS DROUGHT	STEENBRAS CONTROL	SST876 DROUGHT	SST876 CONTROL	LSD (0.05)
Dry Mass	4.66	6.74	14.14	15.68	6.66	12.25	7.30	10.74	6.88	9.40	3.6318
Tiller	4.40	4.20	6.00	6.00	4.20	5.40	5.20	5.20	3.80	4.00	1.1653
Spikes	4.20	4.00	6.00	6.00	3.80	3.80	4.80	5.00	3.20	3.80	1.1462
Primary Spikelet	13.60	17.20	13.80	17.20	14.40	18.00	14.00	17.40	15.40	18.80	1.5506
Secondary Spikelet	40.20	45.40	60.20	75.80	32.80	76.00	48.00	62.00	30.80	48.60	16.5259
P/Kernel n	32.60	41.00	34.80	48.80	41.60	43.00	35.20	43.20	46.80	55.80	7.8910
S/Kernel n	52.00	59.60	114.40	145.60	57.60	90.60	80.20	105.00	60.80	97.60	36.5947
P/Kernel g	0.56	0.92	1.09	1.43	0.92	0.90	0.71	0.81	1.13	1.14	0.3932
S/Kernel g	0.50	0.77	2.37	3.25	0.73	1.55	0.84	1.73	0.63	1.60	1.0840
Kernel n	84.60	89.60	149.20	194.20	95.80	133.80	115.40	148.20	96.60	153.40	42.9680
Kernel g	1.07	1.64	3.46	4.68	2.28	2.45	1.56	2.54	1.67	2.74	1.4299

Dry mass in gram, Tiller = Total number of tillers, Spikes = Total number of spikes, Primary spikelet = Primary spikelet number, Secondary spikelet = Secondary spikelet number, P/Kernel n = Primary kernel number, S/Kernel n = Secondary kernel number, P/Kernel g = Primary kernel mass (in g), S/Kernel g = Secondary kernel mass (in g), Kernel n = Total kernel number, Kernel g = Total kernel mass (in g).

3.3.4. The mean squares of the protein fractions of the five cultivars under drought treatment during the seedling stage compared to the control

Graphic representations of the SDS-soluble and SDS-insoluble protein elution profiles for wheat are given in Figures 3.12 and 3.13.

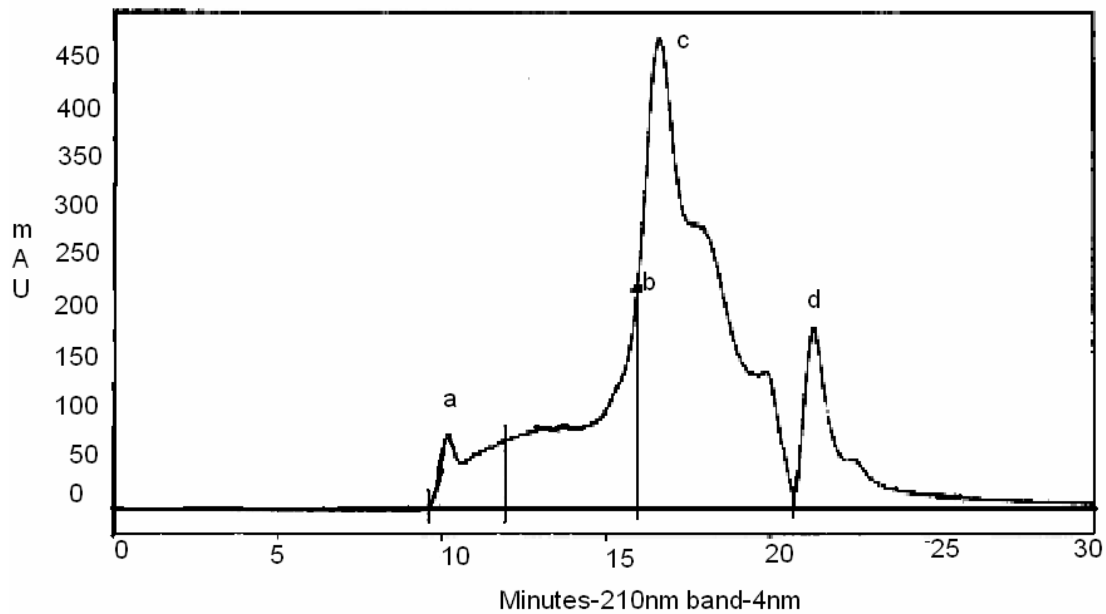


Figure 3.12: SDS-soluble proteins as separated with SE-HPLC where a = larger polymeric proteins (LPP), b = smaller polymeric proteins (SPP), c = larger monomeric proteins (LMP) mainly gliadins, d = smaller monomeric proteins (SMP) mainly albumins and globulins (mAU = milli adsorption units).

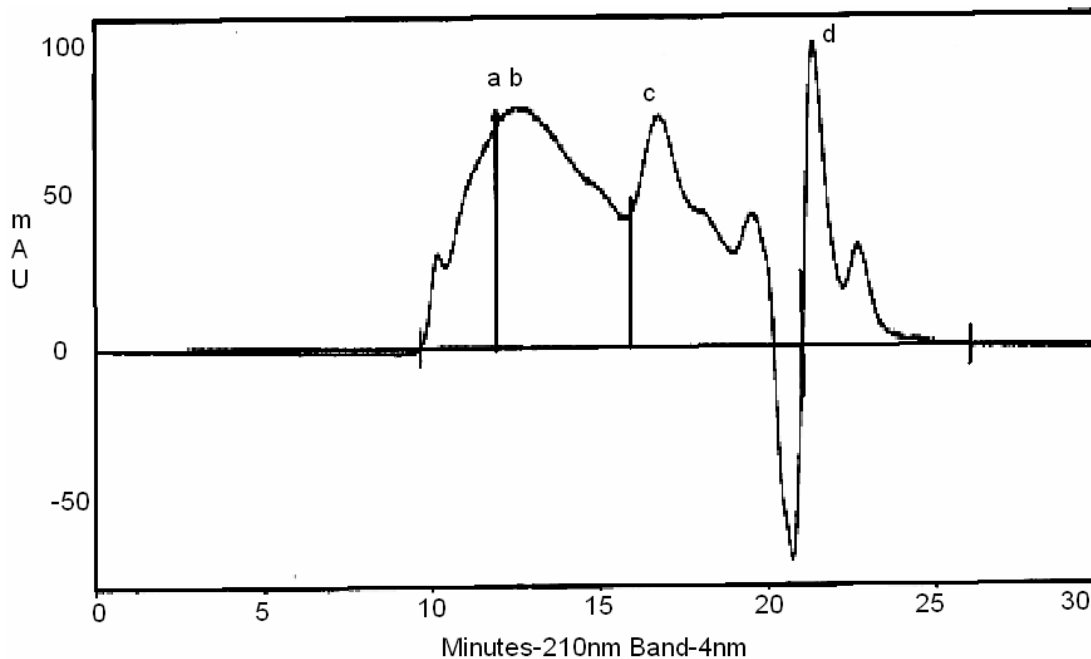


Figure 3.13: SDS-insoluble proteins as separated with SE-HPLC (after sonication) where a = larger polymeric proteins (LPP), b = smaller polymeric proteins (SPP), c = larger monomeric proteins (LMP) mainly gliadins, d = smaller monomeric proteins (SMP) mainly albumins and globulins (mAU = milli adsorption units).

Only LPP1, SMP2 and LMP1 were significantly influenced by the different treatments. There were significant differences between entries for LPP1, LMP1, SMP1 and LMP. There was significant interaction between entry and treatment for LPP1.

3.3.5. The mean squares of the protein fractions of the five cultivars under drought treatment during the seedling stage compared to the control

Significant differences were found between blocks for SMP1, SPP, SPP1, SPP2, SMP2 and PP. Between entries significant differences were recorded for LPP1, LMP, SMP2 and LUPP.

Table 3.3: The mean squares of the protein fractions of the five cultivars during drought treatment together with the control, during the seedling stage.

Mean squares of measured characteristics					
TRAIT	TREATMENT	ENTRIES	ENTRY X TREATMENT	BLOCKS	RESIDUAL
LPP1	4.506**	2.006**	1.106*	0.718	0.163
SPP1	5.238	7.532	0.896	21.261*	3.111
LMP1	10.831*	21.859**	1.79	6.29	1.846
SMP1	5.93	7.669*	3.628	26.221**	1.560
LPP2	1.121	30.215	3.817	11.325	8.787
SPP2	4.47	59.398	23.962	138.133*	24.267
LMP2	0.137	1.363	0.996	0.25	0.473
SMP2	34.325**	2.145	8.437	21.257*	2.625
TUPP	0.001	0.001	0.001	0	0.000
LUPP	30.702	88.35	18.311	5.986	12.529
LPP	1.132	22.756	1.354	17.745	9.672
SPP	19.385	73.052	19.79	267.780**	21.297
LMP	8.535	16.244*	0.933	9.046	3.037
SMP	11.721	8.768	18.353	0.26	6.181
PP	29.888	70.964	20.153	147.658*	29.581
MP	40.26	10.951	15.403	6.238	12.309

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total un-extractable polymeric proteins, LUPP = larger un-extractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins
*$p < 0.05$, ** $p < 0.01$

3.3.6. The protein fractions of the five cultivars under drought treatment during the seedling stage compared to the control

In comparing the different protein fractions of the control treatment cultivars to their drought treated counterparts (Table 3.4), it can be seen that the LPP1 of control treatment cultivars: Kariega, SST 876 and SST 88 were significantly higher than their counterparts that were drought treated. The Kariega (control) had significantly higher SPP1 than the drought stressed cultivar.

Kariega and Steenbras (control) had a lower LMP1 measurement than there counterparts in the drought treatment. These differences were the only significant ones for LMP1. The Steenbras (control) was the only cultivar with a significantly higher SMP1 level compared to the same cultivar's drought treatment value. There were no significant differences between treatments for any cultivar in terms of the LPP2 and SPP2 levels. Kariega (control) was the only cultivar with a significantly higher LMP2 level compared to the drought treatment.

The control treatment cultivars of Kariega and SST 876 had a significantly lower SMP2 measurement than their drought treated counterparts. There were no significant differences between treatments for any cultivar in terms of the TUPP levels. The control treatment of Kariega had a lower LUPP measurement than the drought treatment. This difference was the only significant one for LUPP. There were no significant differences between treatments for any cultivars in terms of the LPP, SPP and LMP levels.

The control treatment of Kariega and SST 876 had a significantly lower SMP measurement than the drought treatment. There were no significant differences between treatments for PP levels. The control treatment of Kariega and SST 876 had a significantly lower SMP measurement than the drought treatment.

Table 3.4: Means of the protein fractions of the five cultivars during drought treatment together with the control, during the seedling stage.

Means for measured characteristics											
TRAIT	KARIEGA DROUGHT	KARIEGA CONTROL	STEENBRAS DROUGHT	STEENBRAS CONTROL	BAVIAANS DROUGHT	BAVIAANS CONTROL	SST876 DROUGHT	SST876 CONTROL	SST 88 DROUGHT	SST 88 CONTROL	LSD (0.05)
LPP1	3.24	5.56	2.85	2.82	3.20	3.07	2.58	4.17	2.05	3.06	0.7694
SPP1	32.87	34.77	29.79	31.48	31.56	32.28	30.34	31.63	33.40	32.94	3.4142
LMP1	31.62	29.17	37.55	34.95	35.76	33.70	35.33	35.90	35.71	34.89	2.4301
SMP1	29.43	30.34	25.91	28.67	26.30	28.27	27.94	25.83	25.32	27.23	2.4764
LPP2	22.40	18.99	21.07	22.68	23.52	24.24	18.72	18.70	26.42	25.15	5.1233
SPP2	48.22	46.51	42.68	47.39	37.63	36.78	47.14	42.46	38.93	46.19	9.2583
LMP2	3.81	5.36	3.60	3.99	3.34	2.92	4.61	3.51	3.12	3.51	1.2713
SMP2	23.68	16.90	20.68	20.72	22.02	19.77	23.81	19.67	19.76	19.79	2.8001
TUPP	0.66	0.62	0.66	0.67	0.64	0.63	0.67	0.63	0.65	0.66	0.0423
LUPP	29.62	22.4	28.48	30.71	30.87	32.37	26.02	23.15	39.41	33.39	6.2345
LPP	25.64	24.55	23.92	25.5	26.72	27.31	21.31	22.87	28.47	28.2	5.3834
SPP	81.09	81.28	72.47	78.86	69.19	69.06	77.48	74.08	72.34	79.12	8.2476
LMP	35.43	34.53	41.15	38.94	39.1	36.63	39.94	39.41	38.83	38.41	3.1989
SMP	53.11	47.24	46.59	49.39	48.32	48.04	51.75	45.5	45.08	47.02	4.4667
PP	106.72	105.82	96.39	104.37	95.91	96.38	98.79	96.95	100.81	107.33	9.5698
MP	88.54	81.78	87.74	88.33	87.41	84.66	91.69	84.91	83.91	85.43	6.4846

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total un-extractable polymeric proteins, LUPP = larger un-extractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

3.3.7. The correlation between all the protein fractions and morphological characteristics of the five cultivars during drought treatment together with the control, at seedling stage

Only significant correlations are discussed. The total dry mass correlated positively with the total number of tillers, total number of spikes, total number of secondary spikelets, secondary kernel number, secondary kernel weight, total kernel number and total kernel weight (Table 3.5). The tiller number correlated positively with the number of spikes, number of secondary spikelets, secondary kernel number and secondary kernel weight. The number of spikes was correlated positively with the secondary kernel number and secondary kernel weight. The number of primary spikelets only correlated positively with the number of primary kernels.

The number of secondary spikelets correlated positively with secondary kernel number, the secondary kernel weight and the total kernel number. The secondary kernel number correlated positively with the secondary kernel weight, the total kernel number and the total kernel weight. The primary kernel mass correlated positively with the total kernel weight. The secondary kernel weight correlated positively with the total kernel number and total kernel weight. The total kernel number correlated positively with the total kernel weight. The total kernel weight correlated negatively with SPP2 and SPP.

In Table 3.6 the correlation between all the protein fractions and morphological characteristics of the five cultivars during drought treatment at seedling stage were significant. There were no significant correlations between any of the protein fractions or morphological characteristics of the five cultivars during the control treatment at seedling stage (Table 3.7).

Table 3.5: The correlation between all the protein fractions and morphological characteristics of the five cultivars during drought treatment together with the control at seedling stage.

	PROLINE	DRYMASS	TILLER	SPIKES	PSPIKELET	SSPIKELET	P/KERn	S/KERn	P/KERg	S/KERg	KERN n	KERN g
DRYMASS	-0.2503											
TILLER	-0.2899	0.8268*										
SPIKES	-0.2088	0.7143*	0.8749**									
PSPIKELET	-0.6051	0.3592	-0.0467	-0.1629								
SSPIKELET	-0.6137	0.8662**	0.8342**	0.6108	0.4769							
P/KERn	-0.2155	0.2736	-0.2582	-0.2442	0.7998*	0.1392						
S/KERn	-0.3744	0.9411**	0.7887*	0.783*	0.3766	0.8199*	0.3456					
P/KERg	0.1085	0.6657	0.2285	0.2864	0.4208	0.3301	0.6888	0.6489				
S/KERg	-0.3179	0.9604**	0.7825*	0.7853*	0.3508	0.8105*	0.3256	0.9782**	0.7051			
KERN n	-0.4246	0.9089**	0.6952	0.6823	0.4753	0.7941*	0.4759	0.9843**	0.6692	0.9541*		
KERN g	-0.2102	0.9205**	0.6751	0.6962	0.3367	0.6929	0.4208	0.9217**	0.807*	0.9654**	0.9135**	
LPP1	-0.3417	-0.087	-0.2027	-0.0747	0.4349	0.0499	0.1436	-0.0679	0.0444	-0.0348	-0.0731	-0.1076
SPP1	-0.2853	-0.1014	-0.1524	-0.1561	0.2268	0.0483	-0.0477	-0.2347	-0.066	-0.0817	-0.2384	-0.0251
LMP1	0.2174	0.2356	0.2169	0.1033	-0.1515	0.0471	0.1367	0.2746	0.1197	0.1562	0.3172	0.2017
SMP1	-0.2252	-0.155	-0.0906	-0.0147	0.175	0.0686	-0.1428	-0.1469	-0.1548	-0.0922	-0.2051	-0.2113
LPP2	-0.1251	0.3896	0.5461	0.353	-0.2151	0.4069	-0.2834	0.258	-0.0289	0.33	0.2292	0.3895
SPP2	-0.0976	-0.5794	-0.4991	-0.6087	0.1551	-0.2589	-0.1184	-0.5857	-0.6066	-0.6373	-0.5619	-0.7564*
LMP2	0.1672	-0.5113	-0.5364	-0.4659	0.1311	-0.4024	-0.0606	-0.5455	-0.2392	-0.543	-0.578	-0.5942
SMP2	0.561	-0.168	-0.0462	-0.0246	-0.5724	-0.2916	-0.302	-0.1536	-0.1888	-0.1708	-0.1745	-0.2203
TUPP	0.2534	-0.2658	-0.0574	-0.254	-0.3251	-0.167	-0.2978	-0.2852	-0.496	-0.3752	-0.285	-0.4288
LUPP	0.0526	0.2373	0.3832	0.2187	-0.321	0.191	-0.2522	0.1258	-0.0472	0.182	0.1101	0.2843
LPP	-0.2896	0.4252	0.5625	0.3874	-0.0745	0.5023	-0.2761	0.2773	-0.0154	0.3765	0.241	0.4169
SPP	-0.1801	-0.5714	-0.5126	-0.6155	0.2149	-0.2263	-0.1257	-0.6194	-0.585	-0.6189	-0.5985	-0.7116*
LMP	0.3119	0.0966	0.0658	-0.0428	-0.13	-0.085	0.1389	0.13	0.0566	-0.0073	0.1684	0.0277
SMP	0.3015	-0.2352	-0.0959	-0.0293	-0.3433	-0.1883	-0.3344	-0.2185	-0.2517	-0.1966	-0.2732	-0.3137
PP	-0.3238	-0.3563	-0.2298	-0.4196	0.1776	0.0255	-0.2618	-0.4777	-0.5894	-0.4279	-0.4748	-0.5002
MP	0.5006	-0.1448	-0.0406	-0.0591	-0.406	-0.2339	-0.2027	-0.1053	-0.1885	-0.1854	-0.1269	-0.2664
TPROT	-0.1714	0.5285	0.6069	0.5645	0.1201	0.5835	-0.1446	0.5537	0.0838	0.4837	0.476	0.2629

Table 3.6: The correlation between all the protein fractions and morphological characteristics of the five cultivars for the drought treatment at seedling stage.

	PROLINE	DRYMASS	TILLER	SPIKES	PSPIKELET	SSPIKELET	P/KERn	S/KERn	P/KERg	S/KERg	KERN n	KERN g
DRYMASS	0.8181*											
TILLER	0.7682*	0.936**										
SPIKES	0.7429*	0.9453**	0.9972**									
PSPIKELET	0.8448**	0.8585**	0.962**	0.9409**								
SSPIKELET	0.73*	0.9495**	0.9927**	0.9978**	0.9251**							
P/KERn	0.8882**	0.8342*	0.9265**	0.8985**	0.9915**	0.8789**						
S/KERn	0.7882*	0.986**	0.9758**	0.9805**	0.905**	0.9849**	0.8721**					
P/KERg	0.9508**	0.9174**	0.9219**	0.9028**	0.9552**	0.89**	0.9689**	0.9198**				
S/KERg	0.7001	0.9667**	0.8388**	0.8655**	0.7062	0.8784**	0.6686	0.9273**	0.7944*			
KERN n	0.8175*	0.9677**	0.9921**	0.9893**	0.9519**	0.9869**	0.9257**	0.9916**	0.9484**	0.8798**		
KERN g	0.8362**	0.9826**	0.9176**	0.9238**	0.8604**	0.9167**	0.8482**	0.9572**	0.9287**	0.9417**	0.9503**	
LPP1	0.7642*	0.8789**	0.9786**	0.9722**	0.9657**	0.9689**	0.9278**	0.9315**	0.8984**	0.765*	0.9584**	0.8457**
SPP1	0.8112*	0.8585**	0.9686**	0.9524**	0.9947**	0.9347**	0.9777**	0.9046**	0.937**	0.7168*	0.951**	0.8664**
LMP1	0.8234*	0.8794**	0.9765**	0.9582**	0.9963**	0.9445**	0.9828**	0.9272**	0.9518**	0.7359**	0.9675**	0.8785**
SMP1	0.8147*	0.8465**	0.9637**	0.9461**	0.9948**	0.9321**	0.9765**	0.8985**	0.9316**	0.6998	0.9448**	0.84**
LPP2	0.7843*	0.8676**	0.9685**	0.9563**	0.9806**	0.9356**	0.9611**	0.9076**	0.9259**	0.7381*	0.9511**	0.889**
SPP2	0.8037*	0.8079*	0.9442**	0.9221**	0.9905**	0.908**	0.975**	0.8689**	0.916**	0.647	0.9215**	0.7973*
LMP2	0.8536**	0.8179*	0.9315**	0.9062**	0.9873**	0.8948**	0.9817**	0.871**	0.9385**	0.6546	0.9206**	0.8014*
SMP2	0.8329*	0.8544**	0.9621**	0.9444**	0.994**	0.9321**	0.9776**	0.9029**	0.9397**	0.7096*	0.947**	0.8439**
TUPP	0.8193*	0.8577**	0.9694**	0.9507**	0.9986**	0.9358**	0.9833**	0.9086**	0.942**	0.7096*	0.9542**	0.856**
LUPP	0.7847*	0.8476**	0.951**	0.9358**	0.9744**	0.9111**	0.9615**	0.8856**	0.9228**	0.7112*	0.9344**	0.8812**
LPP	0.7859*	0.873**	0.9743**	0.9626**	0.9836**	0.9438**	0.962**	0.9146**	0.9273**	0.7446*	0.9564**	0.8884**
SPP	0.8094*	0.8319*	0.9576**	0.9379**	0.9955**	0.9222**	0.9793**	0.8868**	0.9279**	0.6786	0.9369**	0.8291*
LMP	0.8281*	0.8754**	0.9743**	0.9553**	0.9976**	0.9417**	0.9848**	0.9238**	0.9526**	0.7296*	0.9651**	0.873**
SMP	0.8231*	0.8502**	0.9632**	0.9456**	0.9946**	0.9323**	0.9773**	0.9007**	0.9355**	0.7044	0.9461**	0.842**
PP	0.8061*	0.8451**	0.9649**	0.9472**	0.9957**	0.9307**	0.9781**	0.8967**	0.9307**	0.6976	0.9449**	0.8469**
MP	0.8273*	0.8634**	0.9704**	0.9521**	0.9984**	0.9387**	0.9829**	0.9131**	0.9453**	0.7172*	0.9567**	0.8577**
TPROT	0.6632*	0.858**	0.8624**	0.8713**	0.7782*	0.8987**	0.7259*	0.8943**	0.7648*	0.8164*	0.8693**	0.7544*

Table 3.7: The correlation between all the protein fractions and morphological characteristics of the five cultivars for the control at seedling stage.

	PROLINE	DRYMASS	TILLER	SPIKES	PSPIKELET	SSPIKELET	P/KERn	S/KERn	P/KERg	S/KERg	KERN n	KERN g
DRYMASS	-0.8374											
TILLER	-0.7468	0.9049										
SPIKES	-0.4997	0.7218	0.746									
PSPIKELET	-0.1136	-0.1656	-0.4913	-0.6152								
SSPIKELET	-0.805	0.9032	0.9426	0.5113	-0.2283							
P/KERn	-0.2374	0.1969	-0.2313	0.0217	0.712	-0.1457						
S/KERn	-0.7946	0.9141	0.7441	0.8318	-0.112	0.6687	0.4305					
P/KERg	-0.244	0.6211	0.3382	0.5792	0.0029	0.2816	0.6216	0.7305				
S/KERg	-0.7185	0.9311	0.7743	0.8514	-0.194	0.6914	0.378	0.9847	0.7983			
KERN n	-0.8127	0.8727	0.6385	0.7222	0.0785	0.6067	0.5766	0.9813	0.7309	0.9473		
KERN g	-0.6495	0.8991	0.7065	0.8177	-0.1467	0.6298	0.451	0.9666	0.8677	0.992	0.9383	
LPP1	0.9855	-0.7994	-0.781	-0.497	-0.0094	-0.8238	-0.0775	-0.7283	-0.1136	-0.651	-0.726	-0.5654
SPP1	0.7998	-0.4773	-0.2606	-0.3272	-0.4244	-0.2926	-0.5965	-0.6582	-0.2044	-0.5202	-0.7522	-0.4808
LMP1	-0.8198	0.4786	0.2405	0.0501	0.6378	0.4045	0.5786	0.5246	0.1165	0.3993	0.6481	0.3653
SMP1	0.5123	-0.2846	0.0721	0.25	-0.8953	-0.1712	-0.7887	-0.3191	-0.2619	-0.2359	-0.4873	-0.2624
LPP2	-0.7689	0.8179	0.933	0.463	-0.306	0.9811	-0.3112	0.5582	0.1036	0.5739	0.4814	0.4947
SPP2	0.3296	-0.6886	-0.4172	-0.6393	0.0261	-0.3549	-0.6025	-0.7933	-0.9951	-0.8537	-0.7882	-0.9125
LMP2	0.8774	-0.8732	-0.6141	-0.4511	-0.3194	-0.7129	-0.58	-0.8654	-0.6117	-0.8284	-0.9207	-0.8186
SMP2	-0.9446	0.629	0.517	0.3595	0.2735	0.5775	0.3267	0.6716	0.0784	0.5483	0.7279	0.4765
TUPP	-0.6594	0.2496	0.4166	0.0334	0.0134	0.4872	-0.3324	0.1272	-0.5639	0.0153	0.1271	-0.1014
LUPP	-0.8418	0.8357	0.9355	0.5092	-0.2751	0.9708	-0.2521	0.6185	0.0983	0.6118	0.5512	0.5256
LPP	-0.5411	0.7094	0.8796	0.3789	-0.4317	0.923	-0.4745	0.3867	0.084	0.4501	0.2809	0.3858
SPP	0.5122	-0.7447	-0.4433	-0.6595	-0.0928	-0.3966	-0.7005	-0.8873	-0.9439	-0.9037	-0.9085	-0.9454
LMP	-0.6864	0.2363	0.0392	-0.1397	0.6991	0.2117	0.5012	0.3001	-0.1251	0.1511	0.4385	0.1104
SMP	-0.3537	0.302	0.6084	0.6583	-0.7766	0.3833	-0.5973	0.3046	-0.2287	0.2776	0.1637	0.1737
PP	0.2632	-0.4159	-0.057	-0.4722	-0.268	0.0057	-0.8667	-0.6864	-0.8673	-0.6759	-0.7506	-0.7426
MP	-0.804	0.385	0.4036	0.2803	0.1262	0.4143	0.0643	0.4414	-0.2497	0.2968	0.4742	0.1983
TPROT	-0.6998	0.4704	0.6742	0.5516	-0.4142	0.5681	-0.3676	0.4488	-0.2554	0.3664	0.3745	0.2475

3.3.8. Morphological characteristics of five cultivars during drought treatment at anthesis

The effect of treatment was highly significant for all characteristics except primary spikelet number and primary kernel number (Table 3.8). There were significant differences between entries for all characteristics excluding primary spikelet number and kernel number, secondary kernel number, dry mass and total kernel number. There was significant interaction between entry and treatment for dry mass yield, secondary tiller kernel number and mass, primary tiller kernel mass and total kernel number and mass.

Table 3.8: The mean squares of measured characteristics of five cultivars during drought treatment, at anthesis

Mean squares of measured characteristics					
TRAIT	TREATMENT	ENTRIES	ENTRY X TREATMENT	BLOCKS	RESIDUAL
Dry Mass	447.244**	250.341**	51.913*	17.651	17.102
Tiller	115.520**	26.150*	7.87	5.5	8.148
Spikes	141.120**	25.780*	8.92	5.08	7.700
Primary Spikelet	0.18	31.400**	6.98	3.95	3.578
Secondary Spikelet	22514.420**	4671.370*	1365.37	805.47	1221.195
P/Kernel n	246.42	759.77	495.47	50.37	164.133
S/Kernel n	148294.580**	4976.05	20948.530**	6436.55	4355.503
P/Kernel g	2.411**	2.586**	2.102**	0.111	0.145
S/Kernel g	156.220**	3.256	14.299**	3.651	2.079
Kernel n	166464.500**	7258.78	26265.000**	7192.13	5293.728
Kernel g	198.802**	8.976*	26.812**	3.528	2.494

*$p < 0.05$, ** $p < 0.01$ Dry mass in gram, Tiller = Total number of tillers, Spikes = Total number of spikes, Primary spikelet = Primary spikelet number, Secondary spikelet = Secondary spikelet number, P/Kernel n = Primary kernel number, S/Kernel n = Secondary kernel number, P/Kernel g = Primary kernel mass (in g), S/Kernel g = Secondary kernel mass (in g), Kernel n = Total kernel number, Kernel g = Total kernel mass (in g).

There were no significant differences between the blocks. The differences in entries were highly significant for all the characteristics.

3.3.9. The measured morphological characteristics of the five cultivars for drought treatment together with the control at anthesis

SST 88 (drought) was the cultivar with the highest dry mass compared to the other cultivars tested with an average of 20.60 g (Table 3.9). It was significantly higher than all the other lines except SST 88 (control) and Baviaans (control). For all entries except SST 88, the control was significantly higher than the corresponding drought treatment.

The entire control treatment had a higher average amount of tillers and spikes per plant compared to the drought treatment, except for SST 876 and SST 88. The number of primary spikelets was higher in the drought treatment for Kariega, Steenbras and SST 88. These differences, however, were not significant. In Baviaans and SST 876, the results were reversed, with the control having a higher spikelet number, with only Baviaans showing a significant difference.

A higher number of secondary spikelets were recorded for all control treatments compared to the drought treatments, with only Steenbras and Baviaans showing a significant difference. Steenbras and SST 88 were the only drought treated lines that had a higher primary kernel number than their control (not significantly). All the other control treatments had higher values than the drought treatment with Baviaans having the only significant difference.

All the control treatments had a significant higher secondary kernel number, primary kernel mass, secondary kernel mass, total kernel number and total kernel mass than the drought treatments, except for SST 88 where the drought treatment had a higher number of secondary kernels, secondary kernel mass and total kernel mass (not significantly). The drought treated SST 88 also had a significant higher primary kernel mass and total kernel number than the control.

Table 3.9: Means of the measured morphological characteristics of the five cultivars under drought stress and the control at anthesis.

Means for measured characteristics											
TRAIT	KARIEGA DROUGHT	KARIEGA CONTROL	STEENBRAS DROUGHT	STEENBRAS CONTROL	BAVIAANS DROUGHT	BAVIAANS CONTROL	SST876 DROUGHT	SST876 CONTROL	SST 88 DROUGHT	SST 88 CONTROL	LSD (0.05)
Dry Mass	6.90	12.68	6.54	11.65	6.66	19.10	4.70	11.60	20.60	20.28	4.5498
Tiller	6.20	9.80	7.80	10.80	6.40	12.00	4.20	6.40	7.80	8.60	3.0947
Spikes	6.20	9.80	7.40	10.80	5.80	12.00	3.80	6.40	7.60	8.60	3.0016
Primary Spikelet	14.20	13.80	17.40	16.60	15.20	17.60	16.60	17.80	19.80	18.00	2.0055
Secondary Spikelet	74.00	101.80	89.60	148.20	67.60	140.20	45.00	83.80	103.80	118.20	39.0688
P/Kernel n	20.40	31.80	42.60	33.00	23.60	40.20	32.20	48.00	55.20	43.20	13.3817
S/Kernel n	50.00	171.20	80.40	226.60	68.20	262.00	58.40	188.80	170.20	123.20	71.7422
P/Kernel g	0.16	1.13	0.40	0.83	0.16	1.12	0.47	1.45	2.38	1.23	0.3897
S/Kernel g	0.47	4.28	0.45	4.51	0.56	6.47	0.36	4.76	3.74	3.25	1.5896
Kernel n	71.80	203.00	123.00	259.60	91.80	302.20	78.60	236.60	225.60	166.40	78.6729
Kernel g	0.63	5.41	0.85	5.33	0.72	7.59	0.76	6.21	6.12	4.48	1.7240

Dry mass in gram, Tiller = Total number of tillers, Spikes = Total number of spikes, Primary spikelet = Primary spikelet number, Secondary spikelet = Secondary spikelet number, P/Kernel n = Primary kernel number, S/Kernel n = Secondary kernel number, P/Kernel g = Primary kernel mass (in g), S/Kernel g = Secondary kernel mass (in g), Kernel n = Total kernel number, Kernel g = Total kernel mass (in g).

3.3.10. The protein fractions of the five cultivars at anthesis under drought stress, compared to the control

When comparing the effects of the different treatments on the different protein fractions (Table 3.10), most of the fractions were influenced highly significantly. Only LMP1 was influenced significantly at a $p < 0.05$ level. Moisture stress had no significant influences on LUPP and LMP.

There were highly significant differences ($p < 0.01$) between entries for most of the protein characteristics measured except SPP2 and LUPP where there was a significant difference at $p < 0.05$. The treatment had no significant influence on LMP, LMP2, SMP2, SPP, LMP and PP.

There was highly significant interaction between entry and treatment for SPP1, SMP1, LPP2, LPP and MP. A significant ($p < 0.05$) interaction between entry and treatment was also found for LPP, TUPP and SMP.

Table 3.10: The mean squares of the protein fractions of the five cultivars during drought stress at anthesis, compared to a control

Mean squares of measured characteristics					
TRAIT	TREATMENT	ENTRIES	ENTRY x TREATMENT	BLOCKS	RESIDUAL
LPP1	6.017**	1.825**	0.394*	0.407*	0.066
SPP1	23.915**	21.573**	9.259**	35.618**	0.735
LMP1	12.039*	6.098	3.819	2.143	1.961
SMP1	97.055**	20.803**	16.701**	40.135**	1.605
LPP2	79.680**	24.167**	21.018**	57.386**	2.858
SPP2	844.116**	39.997*	18.154	77.902*	7.417
LMP2	14.680**	1.026	0.987	0.486	0.596
SMP2	128.535**	10.308	9.205	0.248	3.405
TUPP	0.015**	0.001**	0.001*	0.001**	0.000
LUPP	9.617	51.300*	24.895	75.743*	8.344
LPP	129.490**	28.535**	26.271**	67.462**	2.816
SPP	583.870**	12.339	7.826	218.871**	8.124
LMP	0.131	7.473	2.729	0.588	2.685
SMP	448.973**	58.079**	47.598*	34.071	7.958
PP	163.432**	32.657	18.979	43.306	8.855
MP	464.436**	63.046**	47.628**	25.706	4.892

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total un-extractable polymeric proteins, LUPP = larger un-extractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

*$p < 0.05$, ** $p < 0.01$

3.3.11. The mean squares of the protein fractions of the five cultivars under drought stress during anthesis compared to a control

The mean squares for blocks were significant for SPP1, SMP1, LPP2, TUPP, LPP, SPP. LPP1, SPP2 and LUPP. Between entries, a highly significant level of difference ($p < 0.01$) was recorded for all the protein fractions, except for LMP2, LUPP and PP.

3.3.12. The protein fractions of the five cultivars under drought stress at anthesis compared to a control

In comparing the different protein fractions of the control treatment to the drought treatment (Table 3.11), it can be seen that in all the control treatments the LPP1 protein levels were higher than under drought stress. Only the difference between the two SST 876 treatments was significant. SST 88 was the only drought stressed cultivar with a higher SPP1 level than the control treatment, although not significantly. All the other control treatments had a significantly higher SPP1 content than under drought stress, except for Steenbras.

All the control treatments measured higher LMP1 levels compared with the stress treatment, significantly for Steenbras and Baviaans. SST 88 (control) was the only entry with a higher SMP1 level than at the stress treatment, although not significantly. For the rest of the stress treatments SMP1 levels were significantly higher than their controls, except for Kariega.

LLP2 levels differed significantly between treatments for all cultivars. The control was higher in all cases, except for SST 88. In the case of SPP2, all the drought treatments were significantly higher than the control.

Both the LMP2 and SMP2 values of the drought treatment were significantly higher than the control, except for Kariega and SST 88. All the TUPP values for the stressed

cultivars were significantly higher than the control. In comparing the LUPP values, only SST 876 (drought stressed) and SST 88 (control) were significantly higher than their counterparts.

All the LPP and SPP values for the different cultivars differed significantly between the control and drought treatments. All the drought treatments had a lower LPP value compared to the control, except SST 88. All the control treatments had lower SPP values compared with the stress treatment. No significant differences were found in the LMP values. Only Steenbras, Baviaans and SST 876 showed significantly lower SMP values for the drought stress treatment compared to the control.

The PP levels of drought stressed Kariega, Steenbras and SST 88 were significantly lower than their controls. Only stressed SST 88 did not show a significantly lower MP value compared to the control.

Table 3.11: Means of the protein fractions of the five cultivars during drought stress at anthesis compared with the control

Means for measured characteristics											
TRAIT	KARIEGA DROUGHT	KARIEGA CONTROL	STEENBRAS DROUGHT	STEENBRAS CONTROL	BAVIAANS DROUGHT	BAVIAANS CONTROL	SST876 DROUGHT	SST876 CONTROL	SST 88 DROUGHT	SST 88 CONTROL	LSD (0.05)
LPP1	3.19	4.42	2.91	2.91	2.74	2.74	1.50	3.39	2.04	2.36	0.4472
SPP1	29.92	32.90	27.76	28.34	28.32	30.00	25.19	32.08	34.26	33.06	1.4832
LMP1	35.10	33.50	35.07	38.18	33.95	37.06	32.35	34.44	34.84	35.89	2.4964
SMP1	28.62	27.07	32.46	27.22	31.47	26.69	36.93	26.39	25.51	25.59	2.2296
LPP2	22.35	26.30	19.11	24.30	20.43	25.60	15.30	24.38	28.00	24.56	2.9306
SPP2	48.22	33.87	50.30	34.68	45.31	33.37	51.89	34.96	38.46	32.34	5.2022
LMP2	3.54	2.45	5.04	2.88	3.80	1.99	5.33	2.28	3.24	2.78	1.3348
SMP2	22.29	19.38	24.46	18.33	26.32	18.73	26.87	19.08	19.36	18.44	3.4525
TUPP	0.68	0.62	0.7	0.65	0.68	0.64	0.72	0.63	0.65	0.62	0.0187
LUPP	29.31	32.28	31.93	32.66	30.21	34.96	26.12	31.54	41.87	34.95	5.0014
LPP	25.54	30.71	20.61	27.22	22.53	28.34	16.79	27.77	30.04	26.91	2.9060
SPP	78.14	66.77	78.06	63.02	73.63	63.37	77.08	67.04	72.72	65.4	5.3735
LMP	38.64	35.96	40.12	41.07	37.75	39.05	37.68	36.71	38.08	38.67	2.9039
SMP	50.9	46.45	56.93	45.54	57.79	45.42	63.8	45.47	44.87	44.03	4.9581
PP	103.68	97.48	98.67	90.24	96.15	91.71	93.87	94.81	102.76	92.31	5.5040
MP	89.54	82.4	97.04	86.61	95.54	84.47	101.48	82.18	82.94	82.7	4.1231

LPP1 = larger polymeric proteins (SDS-soluble), SPP1 = smaller polymeric proteins (SDS-soluble), LMP1 = larger monomeric proteins (SDS-soluble), mainly gliadins (SDS-soluble), SMP1 = smaller monomeric proteins, mainly albumins and globulins (SDS-soluble), LPP2 = larger polymeric proteins (SDS-insoluble), SPP2 = smaller polymeric proteins (SDS-insoluble), LMP2 = larger monomeric proteins, mainly gliadins (SDS-insoluble), SMP2 = smaller monomeric proteins, mainly albumins and globulins (SDS-insoluble), TUPP = total un-extractable polymeric proteins, LUPP = larger un-extractable polymeric proteins, LPP = total larger polymeric proteins, LMP = total larger monomeric proteins, SPP = total smaller polymeric proteins, SMP = total smaller monomeric proteins, PP = polymeric proteins, MP = monomeric proteins

3.3.13. The correlation of all the protein fractions and morphological characteristics of the five cultivars under drought stress during anthesis with the control

Only significant correlations are discussed. The total dry mass correlated positively with LUPP, primary kernel mass, secondary kernel mass, total kernel number, total kernel mass, SPP1, LPP2 and LPP (Table 3.12). Dry mass correlated negatively with SMP1, SPP2, SMP2, TUPP, SMP and MP. Tiller number correlated positively with spikes, secondary spikelet number, secondary kernel number, secondary kernel mass, total kernel number and LMP1. Tiller number correlated negatively with SPP2, SMP2 and SPP.

The number of spikes correlated positively with secondary spikelets, secondary kernel number, secondary kernel mass, total kernel number, total kernel mass and LMP1. The number of spikes correlated negatively with SPP, SMP2 and SMP. The number of primary spikelets correlated positively with the primary kernel number. The secondary spikelet number correlated positively with secondary kernel number, secondary kernel mass, total kernel number, total kernel mass and LMP1. The secondary spikelet number correlated negatively with SMP1, SPP2, SMP2, SPP and SMP.

The primary kernel number correlated positively with primary kernel mass. The secondary kernel number correlated positively with primary kernel mass, total kernel number and total kernel mass. The secondary kernel number correlated negatively with SPP2, LMP2, SMP2, SPP, SMP and MP. Primary kernel mass correlated positively with total kernel mass, SPP1, LPP2 and LUPP. Primary kernel mass correlated negatively with MP. Secondary kernel mass correlated positively with total kernel number, total kernel mass, LPP2 and LPP. Secondary kernel mass correlated negatively with SMP1, SPP2, LMP2, SMP2, TUPP, SPP, SMP and MP. The total kernel number correlated positively with total kernel mass, LPP2 and LPP.

The total kernel number correlated negatively with SPP2, LMP2, SMP2, TUPP, SPP, SMP and MP. The total kernel mass correlated positively with LPP2 and LPP. The total kernel mass correlated negatively with SMP1, SPP2, LMP2, SMP2, TUPP, LPP, SPP, SMP and MP. There was no correlation between LPP1 and any of the characteristics that was measured.

SMP2 correlated positively with proline content. There was no further correlation between LUPP and any of the characteristics that was measured. There was no further correlation between LMP and any of the characteristics that was measured. SMP correlated positively with proline content. There was no further correlation between PP and any of the characteristics that was measured. MP and TUPP correlated positively with proline content.

There were no significant correlations found for any of the protein fractions and morphological characteristics of the five cultivars under drought stress during anthesis (Table 3.13). The same was found to be true in regard to the control (Table 3.14).

Table 3.12: The correlations of all the protein fractions and morphological characteristics of the five cultivars during drought treatment and the control at anthesis.

	PROLINE	DRYMASS	TILLER	SPIKES	PSPIKELETS	SSPIKELETS	P/KERNEL n	S/KERNEL n	P/KERNEL g	S/KERNEL g	KERNEL n	KERNEL g
DRYMASS	-0.4239											
TILLER	-0.6501	0.6126										
SPIKES	-0.6825	0.63	0.9971**									
PSPIKELETS	0.0011	0.5857	0.0926	0.0887								
SSPIKELETS	-0.6874	0.697	0.9409**	0.948**	0.2905							
P/KERNEL n	-0.2277	0.6512	0.1875	0.1947	0.8943**	0.3197						
S/KERNEL n	-0.5241	0.6661	0.8109*	0.8243*	0.3497	0.8265*	0.4636					
P/KERNEL g	-0.2423	0.8165*	0.2996	0.3213	0.6854	0.4108	0.8433**	0.6036				
S/KERNEL g	-0.562	0.7499*	0.766*	0.7899*	0.3288	0.7789*	0.4844	0.9711**	0.6661			
KERNEL n	-0.5466	0.7148*	0.7922*	0.8059*	0.4372	0.8244*	0.5614	0.9924**	0.6736	0.9695**		
KERNEL g	-0.5239	0.8169*	0.7022	0.7271*	0.4383	0.7402*	0.6054	0.9433**	0.7928*	0.9826**	0.9597*	
LPP1	-0.6794	-0.0596	0.3713	0.3913	-0.5877	0.21	-0.2333	0.2533	-0.0699	0.2965	0.2288	0.2287
SPP1	-0.5539	0.7701*	0.325	0.359	0.2703	0.3773	0.5216	0.4046	0.7749*	0.5485	0.468	0.6444
LMP1	-0.6138	0.4618	0.7612*	0.7678*	0.2685	0.8866**	0.1432	0.623	0.1367	0.5348	0.6154	0.4732
SMP1	0.76	-0.8017*	-0.621	-0.6569	-0.2524	-0.7108*	-0.4132	-0.6686	-0.664	-0.7516*	-0.7029	-0.783*
LPP2	-0.6457	0.8192*	0.6553	0.6826	0.2405	0.6926	0.4381	0.7139	0.748*	0.7801*	0.7448*	0.8266*
SPP2	0.6702	-0.7994*	-0.7243*	-0.7512*	-0.2365	-0.774*	-0.4064	-0.8312*	-0.6386	-0.9076**	-0.8372**	-0.9016**
LMP2	0.6776	-0.6905	-0.6649	-0.6995	-0.053	-0.6718	-0.2222	-0.7824*	-0.5159	-0.8727**	-0.7742*	-0.8436**
SMP2	0.7777*	-0.7994*	-0.7139*	-0.7568*	-0.3014	-0.8156*	-0.4749	-0.8078*	-0.6831	-0.8684**	-0.8286*	-0.8811**
TUPP	0.7392*	-0.7628*	-0.6245	-0.6587	-0.1308	-0.6558	-0.3694	-0.6964	-0.6232	-0.814*	-0.7142*	-0.8225*
LUPP	-0.342	0.8616**	0.5035	0.5031	0.6568	0.5929	0.7239	0.5358	0.8411**	0.5495	0.6108	0.6608
LPP	-0.6834	0.7439*	0.6472	0.6799	0.1022	0.66	0.3383	0.7065	0.6795	0.7828*	0.7247*	0.8118*
SPP	0.5923	-0.655	-0.769*	-0.7872*	-0.1738	-0.8077*	-0.2718	-0.8676**	-0.448	-0.897**	-0.8453**	-0.8443**
LMP	-0.1855	0.0038	0.3617	0.3433	0.2608	0.497	-0.0057	0.1171	-0.2318	-0.0498	0.1146	-0.0976
SMP	0.7831*	-0.8158*	-0.6781	-0.7181*	-0.2807	-0.7755*	-0.4507	-0.7491*	-0.6858	-0.8228*	-0.7775*	-0.8457**
PP	0.1301	-0.1556	-0.4022	-0.3949	-0.1336	-0.4416	-0.0355	-0.4767	0.0595	-0.4422	-0.4293	-0.3432
MP	0.7282*	-0.7989*	-0.5879	-0.631	-0.2198	-0.6545	-0.4432	-0.7094*	-0.7217*	-0.8171*	-0.7378*	-0.8497**
TPROT	-0.3936	-0.3592	0.246	0.2474	-0.3405	0.2627	-0.3756	0.0231	-0.5836	-0.0492	-0.0281	-0.1855

Table 3.13: The correlations of all the protein fractions and morphological characteristics of the five cultivars during drought treatment at anthesis.

	PROLINE	DRYMASS	TILLER	SPIKES	PSPIKELET	SSPIKELET	P/KERn	S/KERn	P/KERg	S/KERg	KERN n	KERN g
DRYMASS	-0.1752											
TILLER	-0.7961	0.5883										
SPIKES	-0.8383	0.6192	0.9889									
PSPIKELET	0.0204	0.7753	0.4749	0.4566								
SSPIKELET	-0.7269	0.7688	0.9573	0.9762	0.6046							
P/KERn	-0.1226	0.7675	0.5682	0.5578	0.9882	0.6872						
S/KERn	-0.1244	0.9685	0.6176	0.6202	0.8959	0.7682	0.8848					
P/KERg	-0.0044	0.9643	0.4555	0.4807	0.8847	0.6584	0.8613	0.973				
S/KERg	-0.0806	0.9949	0.5213	0.5479	0.8048	0.7114	0.7843	0.9738	0.9817			
KERN n	-0.1995	0.9517	0.6773	0.6785	0.9098	0.8139	0.9123	0.9946	0.9553	0.9514		
KERN g	-0.0618	0.9891	0.5074	0.5336	0.8372	0.7017	0.8167	0.9788	0.992	0.9979*	0.9587	
LPP1	-0.8345	-0.2252	0.489	0.5091	-0.5254	0.3304	-0.4104	-0.3164	-0.448	-0.321	-0.2644	-0.3608
SPP1	-0.4503	0.9184	0.6943	0.748	0.5036	0.8393	0.5317	0.8174	0.7875	0.8777	0.8081	0.852
LMP1	-0.9662	0.3927	0.8656	0.912	0.0916	0.8379	0.2174	0.3181	0.2013	0.3001	0.3765	0.2737
SMP1	0.5889	-0.7985	-0.7182	-0.779	-0.3073	-0.826	-0.3556	-0.6673	-0.6196	-0.739	-0.6647	-0.7033
LPP2	-0.4671	0.8967	0.7019	0.7527	0.4579	0.8339	0.4865	0.7902	0.7513	0.8524	0.7802	0.8229
SPP2	0.1641	-0.9216	-0.5832	-0.5912	-0.5528	-0.7036	-0.5286	-0.8602	-0.8128	-0.9059	-0.8278	-0.8775
LMP2	0.3559	-0.6472	-0.4546	-0.5063	-0.0315	-0.5462	-0.0372	-0.4711	-0.4404	-0.5977	-0.4341	-0.5458
SMP2	0.5289	-0.86	-0.6431	-0.7312	-0.5103	-0.8256	-0.5657	-0.7517	-0.7658	-0.8174	-0.7576	-0.8053
TUPP	0.4188	-0.8432	-0.676	-0.7088	-0.3611	-0.7739	-0.3758	-0.7331	-0.6721	-0.7983	-0.7143	-0.7594
LUPP	-0.3553	0.9661	0.7709	0.7838	0.7874	0.8938	0.8062	0.9652	0.9069	0.9451	0.9711	0.9384
LPP	-0.4925	0.8441	0.6612	0.7226	0.3507	0.7924	0.3834	0.7114	0.6806	0.7942	0.6987	0.7599
SPP	-0.2502	-0.6997	-0.295	-0.2413	-0.4813	-0.3536	-0.395	-0.7051	-0.6463	-0.7205	-0.6504	-0.6956
LMP	-0.7923	-0.1473	0.5834	0.589	0.0779	0.4655	0.219	-0.0695	-0.177	-0.209	0.0332	-0.1911
SMP	0.5758	-0.842	-0.7012	-0.775	-0.4003	-0.8434	-0.4527	-0.7176	-0.6955	-0.7885	-0.7185	-0.7621
PP	-0.7406	0.5798	0.6086	0.7148	0.1248	0.7295	0.2166	0.4182	0.4174	0.5076	0.4365	0.4819
MP	0.4668	-0.8649	-0.6218	-0.695	-0.3909	-0.7808	-0.4238	-0.7294	-0.7222	-0.8198	-0.716	-0.7909
TPROT	-0.1584	-0.9144	-0.2535	-0.2859	-0.612	-0.4651	-0.5509	-0.8445	-0.8933	-0.9335	-0.7877	-0.9208

Table 3.14: The correlations of all the protein fractions and morphological characteristics of the five cultivars during the control at anthesis.

	PROLINE	DRYMASS	TILLER	SPIKES	PSPIKELET	SSPIKELET	P/KERn	S/KERn	P/KERg	S/KERg	KERN n	KERN g
DRYMASS	0.4219											
TILLER	0.677	0.2996										
SPIKES	0.677	0.2996	1									
PSPIKELET	0.1597	0.4666	-0.2232	-0.2232								
SSPIKELET	0.4454	0.3182	0.8556	0.8556	0.1427							
P/KERn	-0.011	0.2842	-0.6572	-0.6572	0.7939	-0.4735						
S/KERn	0.7724	-0.1478	0.6265	0.6265	0.071	0.5359	-0.2027					
P/KERg	-0.1244	0.104	-0.7803	-0.7803	0.3189	-0.8503	0.8261	-0.4057				
S/KERg	0.9138	0.0513	0.5166	0.5166	0.1262	0.3069	0.0248	0.9143	-0.0781			
KERN n	0.7856	-0.1124	0.5529	0.5529	0.1761	0.4847	-0.0761	0.9918	-0.3058	0.9343		
KERN g	0.8869	0.0727	0.3641	0.3641	0.1868	0.1408	0.1844	0.8317	0.1163	0.9811	0.8709	
LPP1	-0.1825	-0.6507	-0.1453	-0.1453	-0.8681	-0.5362	-0.431	-0.0594	0.1019	-0.0136	-0.1175	0.0066
SPP1	-0.3846	0.2156	-0.6046	-0.6046	-0.1659	-0.7828	0.3414	-0.8073	0.7269	-0.5161	-0.7776	-0.372
LMP1	0.3326	0.2354	0.5944	0.5944	0.4682	0.9086	-0.1556	0.5377	-0.6801	0.2864	0.5279	0.1527
SMP1	0.2131	-0.6708	0.4913	0.4913	-0.6402	0.2915	-0.7395	0.6424	-0.6115	0.4284	0.5573	0.3077
LPP2	0.4319	0.1039	0.4348	0.4348	-0.7201	-0.0833	-0.4924	0.1211	-0.0525	0.3052	0.0593	0.2956
SPP2	-0.1571	-0.9206	-0.2429	-0.2429	-0.1792	-0.2155	-0.0709	0.426	-0.0418	0.2523	0.4235	0.2416
LMP2	-0.7701	-0.1631	-0.1162	-0.1162	-0.0879	0.2215	-0.3314	-0.5149	-0.4561	-0.8111	-0.5675	-0.8971
SMP2	0.0025	-0.3884	-0.3065	-0.3065	-0.6077	-0.7442	-0.0351	-0.0995	0.4999	0.1264	-0.1066	0.2236
TUPP	0.3755	-0.2526	0.5071	0.5071	0.2468	0.7068	-0.2251	0.8189	-0.6276	0.5435	0.8045	0.4184
LUPP	0.4989	0.963	0.5137	0.5137	0.4294	0.5612	0.099	0.0228	-0.1556	0.1334	0.0373	0.1039
LPP	0.1573	-0.2836	0.1791	0.1791	-0.8751	-0.3274	-0.5147	0.0435	0.0201	0.1734	-0.0232	0.1779
SPP	-0.5093	-0.2828	-0.7985	-0.7985	-0.2825	-0.9781	0.3337	-0.6433	0.7718	-0.4225	-0.6122	-0.2709
LMP	0.1729	0.1907	0.5391	0.5391	0.4202	0.8941	-0.2096	0.4119	-0.7234	0.1219	0.3926	-0.0197
SMP	0.1617	-0.6957	0.2102	0.2102	-0.7859	-0.1622	-0.5678	0.428	-0.1997	0.3846	0.3611	0.3444
PP	-0.2499	-0.335	-0.4276	-0.4276	-0.6477	-0.813	-0.054	-0.3978	0.5155	-0.1847	-0.4127	-0.0831
MP	0.2617	-0.115	0.6789	0.6789	0.0926	0.8909	-0.4872	0.6429	-0.873	0.3097	0.5914	0.1383
TPROT	-0.4147	-0.242	0.2051	0.2051	0.0651	0.5909	-0.4052	0.0219	-0.7323	-0.3707	-0.0304	-0.5125

3.4. Discussion

3.4.1. Morphological characteristics of five cultivars under drought stress at the seedling stage compared to a control treatment

The effect of treatment was highly significant for all characteristics except tiller number, number of spikes and primary kernel mass. This is in agreement with the findings of Saleem (2003), who reported that drought stress causes a significant reduction of dry mass. In contrast, there was no significant reduction in the plant height and number of spikes, indicating that there were some recoveries by the cultivars from the drought treatment. There were, however, significant differences between entries for all characteristics excluding primary spikelet number, indicating that the recovery capability of the cultivars were insufficient in closing the gap of the control cultivars. This is supported by the fact that there was no significant interaction between entry and treatment for all characteristics. There were no significant differences between the blocks and the measured characteristics (Table 3.1).

According to Saleem (2003) the average plant reaches its maximum biomass potential under sufficient water availability. Where there is drought, a marked decrease in plant biomass can be noted. Considering that species and genotypes within species differ in their capability of resistance to the degree and time span of the stress they can endure, recovery of stress can therefore also differ. This could explain the highly significant differences in entries for all the characteristics, excluding tiller number and primary kernel mass with a significant difference.

In comparing the control treatments with drought treatments, it is apparent that there are very few significant differences between the treatments in terms of the morphological characteristics. This would suggest that the drought period did not exceed the critical point were the wheat in the drought treatment died. Also the recovery period after the stress period was sufficient for the recovery of the drought stress wheat in terms of their

performance compared to the wheat in the control treatment. In the light of the significant differences between the cultivars in the two treatments is that even stress in an early stage affects the yield of wheat, no matter how long and how perfect the recovery conditions may be.

When comparing the effects of the different treatments on the different protein fractions (Table 3.3), most of the fractions were not influenced significantly. Although the LPP1 levels of the cultivars that was in the control treatment measured a significant higher level of LPP1 compared to the same cultivars in the drought treatment. On the other hand the LMP1 levels of the control test were significantly lower than the cultivars in the drought treatments. This would indicate that drought stress, even in an early stage of wheat development, can influence the protein quality of the mature plant. The influence of drought would therefore lead to a decrease in the quality of the protein in the mature plant.

As already mentioned, according to Gupta *et al.* (1991), HMW glutenin subunits apparently have a larger influence to maximum dough resistance than LMW glutenin subunits. Also, wheat possessing different HMW gluten compositions varies in stability in terms of gluten strength due to variations in the environment (Johansson *et al.*, 2001). They also stated that the differences in HMW glutenins, the storage proteins composition and the differences in the SDS insoluble and soluble PP are very important in the prediction or calculation of the gluten strength of any cultivar. The bread volume is influenced by the gluten strength of a cultivar. Johansson *et al.* (2001) explained that the nitrogen application during cultivation of a cultivar influences the bread volume due to its effect on the gluten strength. In the examination of the above results it is clear that the effect of drought in an early stage does not have a dramatic effect on the differences in HMW glutenins, the storage proteins composition, differences in the SDS insoluble and soluble PP, yet there are differences between cultivars and thus early selection for drought tolerance can be made by using SE-HPLC techniques.

In comparing the different protein fractions of the control treatment cultivars to their drought treated counterparts (Table 3.4), it can be seen that the LPP1 protein levels of Kariega (control), SST 876 (control) and SST 88 (control) were significantly higher than their drought induced forms, indicating that the cultivars in the control most probably have better quality proteins than in the drought induced environment. Kariega (control) was the cultivar with a significantly higher SPP1 level than its counterpart. Kariega (control) and Steenbras (control) had a lower LMP1 measurement than their drought induced forms. These differences were the only significant ones for LMP1; this once again suggests that the drought stressed cultivars would most probably have proteins of lesser quality compared to the same cultivars in the control. Steenbras (control) was the only cultivar with a significantly higher SMP1 level compared to the same cultivar in the drought treatment. There were no significant differences between treatments for any cultivar in terms of the LPP2 and SPP2 levels. Kariega (control) was the only cultivar with a significantly higher LMP2 level compared to its counterpart. Kariega (control) and SST 876 (control) had a significantly lower SMP2 measurement than their apposed cultivars in the drought treatment. There were no significant differences between treatments for any cultivar in terms of the TUPP levels. Kariega (control) had a lower LUPP measurement than its drought induced counterparts. This would suggest that the dough properties of the control treated cultivar would be less elastic compared to its drought induced counterpart, meaning that the drought would affect the mixing time for the dough of a cultivar (Kuktaite, 2004). This difference was the only significant one for LUPP. There were no significant differences between treatments for any cultivar in terms of the LPP, SPP and LMP levels. Kariega (control) and SST 876 (control) had a significantly lower SMP measurement than their respected counterparts in the drought treatment. There were no significant differences between treatments for any cultivar in terms of the PP levels. Kariega (control) and SST 876 (control) had a significantly lower SMP measurement than their drought induced counterparts.

Comparing the results of the morphological characteristics of the cultivar in the control treatment with those in the drought treatment at seedling stage, it is clear that the stability of the cultivars can not ideally be predicted by the differences in the selected cultivars.

Yet in the analysis of the protein fractions, it is apparent that the stability can be measured in the protein quality differences between the control and drought tested cultivars. Clearly drought at an early stage of wheat development plays a significant role in the baking quality of the flour that each cultivar can possibly yield. What is also noticeable is the stability that is found in early drought conditions for the cultivars Baviaans and SST 88.

There is once again a clear indication that dry mass is correlated with the totality of the yield components as seen in Table 3.5. The total kernel weight correlated negatively with SPP2 and SPP.

A basic amount of nitrogen (N) is required by a wheat plant from the soil to accumulate dry mass and nitrogen content in the vegetative tissue to ensure an acceptable yield with sufficient protein content (Dechard *et al.*, 1984). There are numerous limiting factors in protein production, the amount of available soil moisture and mineral nutrients availability (Pomeranz, 1988). Limiting these stress factors to seedlings may cause some effect on the proteins in the plant at an adult stage, but when plants such as the plants in the drought induced experiment have the opportunity to grow under normal conditions, the capability of cultivars to recover can be determined by means of proteins and morphological characteristics. However in examining the results obtained by the correlation of the single experiment of drought induction on the seedling stage, it is clear that the high level of correlation between all the parameters would indicate that all the yield components as well as the protein components are influenced at an early stage of the wheat plants development. This theory is supported by the absence in significant correlations between the components in the correlation of the control.

3.4.2. Morphological characteristics of five cultivars during drought treatment together with the control at anthesis

The effect of treatment was highly significant for all characteristics except primary spikelet number and primary kernel number. This agrees with the findings of Saleem

(2003), that drought stress causes a significant reduction of dry mass. This reduction influences plant height and number of spikes. This also influenced the number of spikelets in each of the tested cultivars. In the same way plant biomass also had a synergistic relationship to grain number and weight (Saleem, 2003). Hence the fact that there were significant differences between entries for all characteristics excluding primary spikelet number and kernel number, secondary kernel number, dry mass and total kernel number. There was significant interaction between entry and treatment for dry mass yield, secondary tiller kernel number and mass, primary tiller kernel mass and total kernel number and mass. The differences between the entries were highly significant for all the characteristics.

The lack of large significant differences between cultivars indicated that drought had the same kind of effect on all the other cultivars and that the differences that was measured can be accredited to the adaptability of each cultivar to the conditions that it was presented with. The same principle was explained by Yang *et al.* (2002), in accordance to high temperature response, stating that the effect of individual plant development is an interaction between the environment and the presence of the genes of the trait affected.

SST 88 (drought) was the cultivar with the highest dry mass compared to the other cultivars tested with an average dry mass of 20.60 g (Table 3.10). It was significantly higher than all the other lines that were tested except SST 88 control and Baviaans control. In all the treatments except SST 88, the control was significantly higher than the corresponding drought treatment. The fact that the drought treatment of SST 88 recovered in terms of its dry mass shows the adaptability of the cultivar to drought conditions before anthesis. These differences in dry mass between control and drought tested cultivars supports the findings of Saleem (2003) that dry mass shows a significant reduction in drought conditions.

The fact that in all the cultivars tested except for SST 88 (for reasons explained above) the morphological measurement of the control treatments superseded those of the drought treated cultivars. These findings also correspond with the findings that drought

influences dry weight and in turn the general yield potential in terms of spikes, spikelets, kernel number and weight (Saleem, 2003). The differences that were noticed between the different cultivars show the differences in adaptability of cultivars to environmental conditions (Yang *et al*., 2002). These findings can be used to measure the amount of drought resistance between cultivars, indicating that the use of the hydroponic system for this purpose was effective. There was no need for chemical interference or the use of a salt strain method for conducting these experiments. The drought conditions can thus be easily and effectively replicated by the breeder for the purpose of drought selection at any time of wheat development.

In comparing the different protein fractions of the control cultivars to their drought treated counterparts (Table 3.10), it is clear that in all the control cultivars the LPP1 protein levels were higher than their counterparts that were drought treated. This would once again suggest as in the seedling test that the cultivars in the control most probably have better quality proteins than their drought induced counterparts. Yet only the difference between the two SST 876 experiments was significant. The results obtained again support the theory that monomeric proteins (gliadins) are the most sensitive to environmental factors (Robert *et al.*, 1996), while the polymeric proteins (glutenins) are less sensitive to the environment and almost totally genotype dependent (Graybosch *et al.*, 1996). Using this information, the breeder can focus on the analysis of the monomeric proteins (gliadins) that are most sensitive to environmental factors. Thus using the information obtained by means of SE-HPLC analysis one can see that the cultivars that were the most stable in terms of their protein fractions, were SST 88 and Kariega.

The total dry mass correlated positively with LUPP, primary kernel mass, secondary kernel mass, total kernel number, total kernel mass, SPP1, LPP2 and LPP (Table 3.12). This would suggest that the increase in the plant mass, and in turn a probable increase in yield potential, corresponds positively with the polymeric proteins. The polymeric proteins (glutenins) are less sensitive to the environment and almost totally genotype dependent (Graybosch *et al.*, 1996), suggesting that by selecting for plant biomass in the

case of bread wheat, one can probably select for yield, quality and drought tolerance at the same time. The dry mass correlated negatively with SMP1, SPP2, SMP2, TUPP, SMP and MP. This could have been expected as monomeric proteins (gliadins) are the most sensitive to environmental factors (Robert *et al.*, 1996). This indicates that, when screening for drought tolerance, it is important to monitor the fluctuations in the monomeric proteins of bread wheat.

All the correlations found in the yield components together with the protein fragments explains why Saleem (2003) found that an increase in the biomass of bread wheat will have a positive effect on the yield potential. From the above results it is clear that an increase in the yield components in general has a negative effect on most of the proteins, especially the non-numeric proteins (gliadins) that are according to Robert *et al.* (1996) the most sensitive to environmental factors. There is therefore an obvious negative correlation in the yield and protein concentration in bread wheat.

TUPP, SMP2, SMP and MP correlated positively with proline content. According to Gupta *et al.* (1991), HMW glutenin subunits have a larger influence on maximum dough resistance than LMW glutenin subunits. Also, wheat possessing different HMW gluten compositions varies in stability in terms of gluten strength due to variations in the environment (Johansson *et al.*, 2001). They also found that the differences in HMW glutenins, the storage proteins composition, differences in the SDS insoluble and soluble PP are very important in the prediction or calculation of the gluten strength of any cultivar. The bread volume is influenced by the gluten strength of a cultivar. Johansson *et al.* (2001) explained that the nitrogen application during cultivation of a cultivar influences the bread volume due to its effect on the gluten strength. In examining the above results it is clear that drought also had a significant effect on the stability of HMW glutenins and soluble PP, therefore also the gluten strength and bread volume.

3.5. Conclusions

For the first trial, the morphological characteristics of five cultivars under drought stress at the seedling stage were compared to a control treatment. The effect of the treatment was in agreement with the findings of Saleem (2003), who reported that drought stress causes a significant reduction in dry mass. It was found that the seedlings in the drought treatment developed slower compared to the seedlings in the control. In contrast to this, there was no significant reduction in the plant height and number of spikes in the mature plants after the growth period was concluded, due to the re-instatement of optimal conditions in the drought treatment system, indicating that there were recoveries to a certain extent by the cultivars from the drought treatment.

There were significant differences between entries for most characteristics, indicating that the recovery capabilities of the individual cultivars were insufficient in closing the gap of the control cultivars. This is supported by the fact that there was no significant interaction between entry and treatment for all characteristics.

When comparing the effects of the different treatments on the different protein fractions, most of the fractions were not influenced significantly. There are numerous limiting factors in protein production namely the amount of available soil moisture and mineral nutrients availability (Pomeranz, 1988). Inducing these stress factors to seedlings, may cause some effect on the proteins in the plant at an adult stage, but when plants in the drought induced experiment had the opportunity to grow under normal conditions, the effect of the treatment as well as the capability of cultivars to recover could be determined by means of proteins, morphological and morphological characteristics.

This was demonstrated by the results that were obtained from the SE-HPLC analysis of the different protein fractions. As already mentioned, according to Gupta *et al.* (1991), HMW glutenin subunits apparently have a larger influence on maximum dough resistance than LMW glutenin subunits. Also, wheats that have a different HMW gluten composition vary in stability in terms of gluten strength due to variations in the

environment (Johansson *et al.*, 2001). They also stated that the differences in HMW glutenins, the storage proteins composition and the differences in the SDS insoluble and soluble PP is very important in the prediction or calculation of the gluten strength of any cultivar. From the above results, it is clear that the effect of drought in an early stage does not have a dramatic effect on the protein composition of wheat cultivars, yet there are differences between cultivars.

In the second trial, the morphological characteristics of five cultivars during drought treatment were compared to the control at anthesis. The effect of treatment was highly significant for most characteristics. This is in agreement with findings of Saleem (2003), expressing that drought stress causes a significant reduction of dry mass. In the same way plant biomass also had a synergistic relationship to grain number and weight (Saleem, 2003).

The results of the second study indicated that a stress condition during a critical stage in the development of wheat is far more detrimental than at an early stage. Drought conditions affected each cultivar tested and entries differed highly significantly for all the measured characteristics. These findings also correspond with the findings that drought influences dry weight and in turn the general yield potential in terms of spikes, spikelets, kernel number and weight (Saleem, 2003). It also shows the differences in adaptability of cultivars to environmental conditions (Yang *et al.*, 2002).

In comparing the effects of the different treatments to the different protein fractions, the most of the fractions were highly significantly influenced. These results once again support the theory that monomeric proteins (gliadins) are the most sensitive to environmental factors (Robert *et al.*, 1996), while the polymeric proteins (glutenins) are less sensitive to the environment and almost totally genotype dependent (Graybosch *et al.*, 1996).

In the context of the results obtained, it is clear that the use of a hydroponic cultivation method for the induction of drought conditions at any stage of development of wheat can

be done very effectively, not only for inducing stress but also for re-establishing and maintaining optimum conditions. This is supported by the yield component results, as well as the protein results that correspond with previous findings.

Chapter 4

Comparison of the influence of nutrition in hydroponics and pots

4.1. Introduction

Hydroponics is the art and science of growing plants without soil by feeding it on chemical solutions with artificial forms of nutrients, which they usually draw from the earth. The basic principle of soilless culture is not new. Over the centuries scientists have been producing plants for morphological experiments. Thus hydroponics has developed from the findings of experiments carried out to determine what substances make plants grow and the composition of plants (Deutschmann, 1998).

Interest in hydroponic culture continued for several reasons. Firstly no soil was needed, and a large plant population could be grown in a very small area. Secondly, when fed properly, optimum production could be attained (Deutschmann, 1998). Hydroponic cultivation was used in the past for controlled environment life support systems (CELSS). This was done because of the optimal environment that could be supplied to the roots of the plant for optimal development, to ensure a high growth rate. Nutrients, water and aeration can be controlled to the highest degree. This platform of control is hard to match in solid media (Steinberg *et al.*, 2000). Today, hydroponics is an established branch of agronomical science (Steinberg *et al.*, 2000).

In all hydroponic systems, the nutrition problem is solved in a similar fashion. Nutrient elements are placed in solution in the amounts and proportions required by various plants. The solution is brought into direct contact with the plant roots. According to Salisbury and Ross (1992) many plants invest 20-50 % of their total weight in roots. In some cases when plants are stressed by insufficient water or mineral nitrogen as much as 90 % of the plant biomass is in the roots. On the other hand, in plants grown hydroponically with adequate water and nitrogen, only 3-5 % of the plant biomass was in the roots.

Emergence is also an important factor for a breeder, because limited time and space also means that a reduction in emergence percentage can cause unnecessary delays within a breeding program. The opportunity to make certain crosses at critical times may also be lost because of emergence problems. Thus, growing wheat hydroponically for plant breeding purposes may hold emergence, yield and seed quality advantages compared to traditional planting methods.

The aims of this study were to evaluate the functionality and/or the practicality of hydroponics for wheat breeding, compared to a conventional glasshouse cultivation method for the parent lines.

4.2. Material and methods

4.2.1. Growth systems

Two South African wheat cultivars, Baviaans and Steenbras, were planted as random sets of eight plants into two identical hydroponic systems and in pots containing exactly 2.3 kg of soil. The two hydroponic systems consisted of four identical 3 m P.V.C. gutter down pipes. These pipes were cut open and filled with swimming pool filter sand. Each system had a 60 l nutrient container. Only 50 l of nutrient solution was circulated at a time through each system. Thirty two seeds of each cultivar were planted in each hydroponic system, 12.5 cm apart. Two seeds of each cultivar were simultaneously planted in 20 pots, where each plant was used as a replication.

A full strength chemicult solution (100 g / 50 l) was made up for one hydroponic system. The chemicult solution consisted out of 6,5 % N, 2,7 % P, 13,0 % K, 7,0 % Ca 2,2 % Mg, 7,5 % S, 0,15 Fe, 0,024 % Mn, 0,024 % B, 0,005 % Zn, 0,002 % Cu and 0,001 % Mo The other system was provided with the solution as specified by Bugbee (1995) (Table 4.1). Both solutions were replenished weekly. The pH of the solutions was kept neutral. An "ebb and flow" method was used to deliver water and nutrients to the systems. Water and nutrients were provided in each system once a day for 5 min, circulating 6 l of

nutrient solution per pipe in each system. The nutrient rich solution drained over a 15 min period for each pipe. Each of the 40 pots containing two seeds, were fertilized with the standard KYNOCH 3:2:0 (25) +0.5 % Zn fertilizer. Pots were watered daily. The duration of watering was estimated according to the level of pot soil dehydration. Nutrients were supplemented weakly with the same chemicult solution used in one of the above mentioned hydroponic systems, as done in normal greenhouse maintenance.

Table 4.1: Hydroponic starter, pre-anthesis and post-anthesis solution for wheat cultivation (Bugbee, 1995)

Wheat Nutrient Solution							
		Starter		Pre-anthesis		Post-anthesis	
Salt	Stock []	ml/100l	Final []	ml/100l	Final []	ml/100l	Final []
$Ca(NO_3)_2$	1M	100	1mM	100	1mM	50	0.5mM
$K(NO_3)$	2M	50	1mM	200	4mM	100	2mM
KH_3PO_4	0.5M	100	0.5mM	100	0.5mM	100	0.5mM
$MgSO_4$	0.25M	200	0.5mM	200	0.5mM	100	0.25mM
K_2SiO_3	0.1M	100	0.1mM	100	0.1mM	0	0mM
$Fe(NO_3)_3$	50mM	20	10µM	5	2.5µM	5	2.5µM
Fe-HEDTA	100mM	25	25µM	5	5µM	5	5µM
$MnCl_2$	60mM	5	3µM	10	6µM	5	3µM
$ZnSO_4$	20mM	20	4µM	10	2µM	10	2µM
H_3BO_3	20mM	10	2µM	5	1µM	2	0.2µM
$CuSO_4$	20mM	5	1µM	5	1µM	3	0.6µM
Na_2MoO_4	0.6mM	15	0.09µM	5	0.03µM	5	0.03µM
Add HNO_3 or KOH as needed to control pH to ± 5.6							

4.2.2. Emergence

Emergence percentages and dates were recorded for a one month period after the emergence of the first seedling in the experiment. When maturity was reached, the following was measured: dry mass, number of spikes, number of spikelets, number of primary packets, number of secondary packets, number of primary kernels, number of

secondary kernels, primary kernel weight, secondary kernel weight, total kernel number and total kernel weight.

4.2.3. Statistical analyses

All statistical analyses were done with Agrobase 2000 software. Relations between quality characteristics were investigated with analysis of variance (ANOVA) and linear correlations.

4.3. Results and Discussion

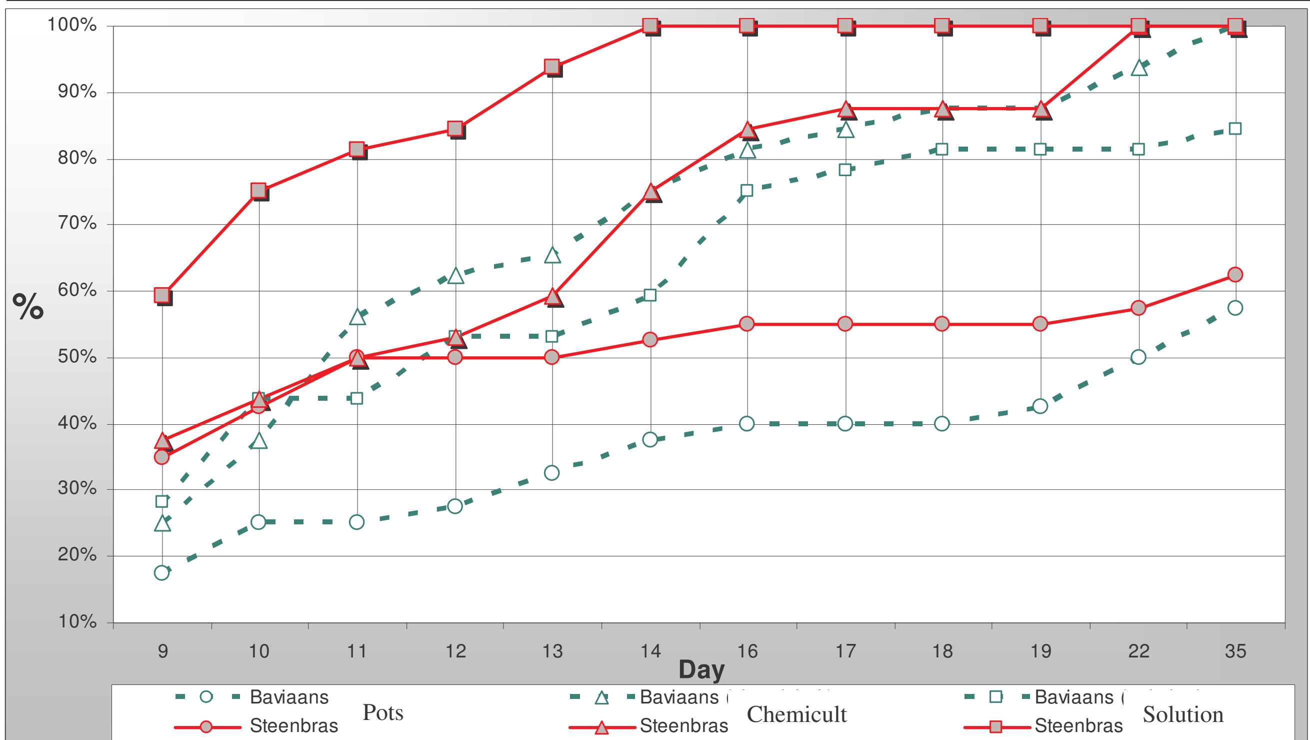
4.3.1. The emergence percentages of wheat planted in the three systems

In Table 4.2 the emergence percentages of Baviaans and Steenbras are illustrated as it was measured in the three different growth systems that were tested (pots, hydroponically in chemicult solution and hydroponically in a published hydroponic solution).

Only Baviaans in the chemicult solution managed 100 % emergence on day 35. Steenbras showed 100 % emergence in the chemicult as well as in the published solution at days 14 and 22 respectively.

Table 4.2: A numerical and graphical presentation of the emergence percentages of wheat seeds planted in three systems per day between days nine and thirty five

The emergence percentages						
Day	Baviaans Pots	Baviaans (chemicult)	Baviaans (solution)	Steenbras Pots	Steenbras (chemicult)	Steenbras (solution)
9	17.50%	25.00%	28.13%	35.00%	37.50%	59.38%
10	25.00%	37.50%	43.75%	42.50%	43.75%	75.00%
11	25.00%	56.25%	43.75%	50.00%	50.00%	81.25%
12	27.50%	62.50%	53.13%	50.00%	53.13%	84.38%
13	32.50%	65.63%	53.13%	50.00%	59.38%	93.75%
14	37.50%	75.00%	59.38%	52.50%	75.00%	100.00%
16	40.00%	81.25%	75.00%	55.00%	84.38%	100.00%
17	40.00%	84.38%	78.13%	55.00%	87.50%	100.00%
18	40.00%	87.50%	81.25%	55.00%	87.50%	100.00%
19	42.50%	87.50%	81.25%	55.00%	87.50%	100.00%
22	50.00%	93.75%	81.25%	57.50%	100.00%	100.00%
35	57.50%	100.00%	84.38%	62.50%	100.00%	100.00%



4.3.2. The mean squares of the measured morphological characteristics, for two cultivars planted in three different systems

The effect of treatment was highly significant for all characteristics except primary kernel weight (Table 4.3). There were significant differences between entries for dry mass. Highly significant differences between entries were observed for primary spikelet number, primary kernel weight, secondary kernel weight and total kernel weight.

Significant interactions were also found for number of spikes and the number of primary spikelets and not only for secondary spikelets.

Table 4.3: The mean squares of the measured morphological characteristics of two cultivars planted in three different systems

Mean squares of the measured morphological characteristics					
	TREATMENT	ENTRIES	ENTRY X TREATMENT	BLOCKS	RESIDUAL
Dry Mass	610.031**	189.844*	15.281	32.644	30.491
Tiller	96.167**	8.167	20.292	6.178	10.361
Spikes	71.885**	10.667	48.948*	6.531	10.499
Primary Spikelet	52.042**	100.052**	8.292*	2.597	1.919
Secondary Spikelet	55789.542**	3432.042	12175.042**	2398.044	2095.108
P/Kernel n	234.385**	58.594	33.219	22.016	42.166
S/Kernel n	222892.448**	3220.167	1159.26	8564.842	5990.474
P/Kernel g	0.219	2.344**	0.594	0.197	0.199
S/Kernel g	153.406**	52.510**	2.26	8.1	6.366
Kernel n	235475.844**	4147.51	954.76	8851.583	6520.122
Kernel g	166.010**	70.042**	2.698	8.122	8.190

*$p < 0.05$, ** $p < 0.01$ Dry mass in gram, Tiller = Total number of tillers, Spikes = Total number of spikes, P/Kernel n = Primary kernel number, S/Kernel n = Secondary kernel number, P/Kernel g = Primary kernel mass (in g), S/Kernel g = Secondary kernel mass (in g), Kernel n = Total kernel number, Kernel g = Total kernel mass (in g).

4.3.3. Means of the measured morphological characteristics of two cultivars planted in three different systems

In comparing the cultivars in the different systems, Steenbras, as well as Baviaans, in the chemicult system, had a significantly higher dry mass compared to the other two cultivating methods (Table 4.6). Both cultivars in the second of the hydroponic systems (the published formula) also had a significantly higher dry mass than their counterparts grown in the pots.

Steenbras as well as Baviaans, in the chemicult system, had a significantly higher tiller and spike number compared to the other two cultivating methods. Only Steenbras, in the solution system, had a significantly higher tiller and spike number than the counterparts grown in the pots. The Baviaans in the pots had a significantly higher spike number compared to the solution system.

The Steenbras in the chemicult system had a significantly higher primary spikelet number compared to the other two cultivating methods. Steenbras in the solution system had a significantly higher primary spikelet number than its counterparts grown in the pots. Only Baviaans in the solution system had a significantly higher primary spikelet number than its counterparts grown in the pots.

Steenbras as well as Baviaans in the chemicult system had a significantly higher secondary spikelet number compared to the other two cultivating methods. Only Steenbras in the solution system had a significantly higher secondary spikelet number than their counterparts grown in the pots.

Only the Steenbras in the chemicult system had a significantly higher primary kernel number compared to the other two cultivating methods. Baviaans showed no significant differences.

Steenbras as well as Baviaans in the chemicult system had a significantly higher secondary kernel number compared to the other two cultivating methods. Both Steenbras and Baviaans in the solution system had a significantly higher secondary kernel number than their counterparts grown in the pots.

The primary kernel mass of the Steenbras in the pots was significantly higher than both its counterparts. No significant differences were obtained for Baviaans.

Only the Steenbras in the chemicult system had a significantly higher secondary kernel weight compared to the other two cultivating methods. Both Steenbras and Baviaans in the solution system had a significantly higher secondary kernel weight than their counterparts grown in the pots.

Steenbras as well as Baviaans in the chemicult system had a significantly higher total kernel number compared to the other two cultivating methods. Both Steenbras and Baviaans in the solution system had a significantly higher total kernel number than their counterparts grown in the pots.

Only the Steenbras in the chemicult system had a significantly higher total kernel weight compared to the other two cultivating methods. Baviaans, in the chemicult system, only had a significantly higher total kernel weight than its counterpart in the pots. Both Steenbras and Baviaans in the solution system had a significantly higher total kernel weight than their counterparts grown in the pots.

Table 4.6: Means of the measured morphological characteristics of two cultivars planted in three different systems

Means of the measured morphological characteristics							
	STEENBRAS POTS	BAVIAANS POTS	STEENBRAS SOLUTION	BAVIAANS SOLUTION	STEENBRAS CHEM	BAVIAANS CHEM	LSD (0.05)
Dry Mass	10.00	14.38	15.25	17.00	19.75	22.06	3.1195
Tiller	7.50	8.75	9.94	8.31	12.19	10.81	1.8086
Spikes	8.50	10.69	10.56	8.38	13.13	11.13	1.9666
Primary Spikelet	16.94	16.06	19.44	16.94	20.38	17.63	0.8741
Secondary Spikelet	71.44	104.44	139.88	107.88	189.63	152.75	26.0201
P/Kernel n	35.38	37.13	35.13	38.63	41.50	40.94	3.8544
S/Kernel n	101.56	123.50	216.00	214.38	270.63	285.06	43.8740
P/Kernel g	1.31	1.38	1.00	1.50	1.00	1.44	0.2388
S/Kernel g	3.00	3.81	5.38	7.25	6.81	8.50	1.3830
Kernel n	136.94	160.63	251.13	253.00	312.13	326.00	45.5806
Kernel g	3.94	5.00	6.38	8.56	8.00	9.88	1.5542

Dry mass in gram, Tiller = Total number of tillers, Spikes = Total number of spikes, Primary spikelet = Primary spikelet number, Secondary spikelet = Secondary spikelet number, P/Kernel n = Primary kernel number, S/Kernel n = Secondary kernel number, P/Kernel g = Primary kernel mass (in g), S/Kernel g = Secondary kernel mass (in g), Kernel n = Total kernel number, Kernel g = Total kernel mass (in g).

4.3.4. The correlation matrix of the measured morphological characteristics of two cultivars planted in three different systems

The total dry mass correlated positively with the total number of tillers, total number of spikes, number of primary spikelets, number of secondary spikelets, primary kernel number, secondary kernel number, secondary kernel mass, total kernel number and total kernel mass.

The total number of tillers correlated positively with the total number of spikes, number of primary spikelets, number of secondary spikelets, primary kernel number, secondary kernel number, secondary kernel mass, total kernel number and total kernel mass. The total number of spikes correlated positively with the number of primary spikelets, number of secondary spikelets, secondary kernel number, secondary kernel mass, total kernel number and total kernel mass.

The total number of primary spikelets correlated positively with the number of secondary spikelets, primary kernel number, secondary kernel number, secondary kernel mass, total kernel number and total kernel mass. The total number of secondary spikelets correlated positively with the primary kernel number, secondary kernel number, secondary kernel mass, total kernel number and total kernel mass.

The primary kernel number correlated positively with the secondary kernel number, primary kernel mass, secondary kernel mass, total kernel number and total kernel mass. The secondary kernel number correlated positively with the secondary kernel mass, total kernel number and total kernel mass. The primary kernel weight correlated positively with the secondary kernel mass and total kernel mass. The secondary kernel weight correlated positively with the total kernel number and total kernel mass. The total kernel number correlated positively with the total kernel mass.

Table 4.7: The correlation matrix of the measured morphological characteristics of two cultivars planted in three different systems.

The correlation matrix of the measured morphological characteristics										
	Dry Mass	Tiller	Spikes	P/Spikelet	S/Spikelet	P/Kernel n	S/Kernel n	P/Kernel g	S/Kernel g	Kernel n
Tiller	0.8148**									
Spikes	0.7688**	0.9038**								
P/Spikelet	0.4621**	0.5244**	0.4336**							
S/Spikelet	0.8719**	0.9275**	0.8539**	0.6264**						
P/Kernel n	0.4592**	0.2734**	0.1722	0.3576**	0.3371**					
S/Kernel n	0.9351**	0.7902**	0.6843**	0.5582**	0.8802**	0.4585**				
P/Kernel g	0.1931	-0.0686	-0.1100	-0.1342	-0.1076	0.4726**	0.1062			
S/Kernel g	0.9451**	0.7015**	0.5988**	0.4138**	0.7729**	0.4530**	0.9281**	0.2804**		
Kernel n	0.9356**	0.7834**	0.6744**	0.5637**	0.8747**	0.5071**	0.9985**	0.1325	0.9284**	
Kernel g	0.9434**	0.6845**	0.5876**	0.4107**	0.7549**	0.5193**	0.9156**	0.3298**	0.9838**	0.9204**

Dry mass in gram, Tiller = Total number of tillers, Spikes = Total number of spikes, Primary spikelet = Primary spikelet number, Secondary spikelet = Secondary spikelet number, P/Kernel n = Primary kernel number, S/Kernel n = Secondary kernel number, P/Kernel g = Primary kernel mass (in g), S/Kernel g = Secondary kernel mass (in g), Kernel n = Total kernel number, Kernel g = Total kernel mass (in g).** $P \leq 0.01$

4.4. Discussion

In Table 4.2 the emergence percentages of Baviaans and Steenbras are illustrated. Baviaans in the chemicult solution was the only one of the three methods that managed a 100 % emergence on the 35th day after planting. In the chemicult as well as in the published solution Steenbras managed 100 % emergence at days 22 and 14 respectively. The fact that there is a huge difference in the emergence percentages of the two cultivars in the three different growth methods, indicates that the problem does not only lie with the seedling vigor but can possibly be related to the differences in the systems. The flexibility within a cultivation system for optimum emergence together with optimum development in a wheat breeding program is essential for the planning and execution of crosses within such a breeding program. Therefore, the use of a suitable system for optimum emergence can eliminate the need for a breeder to replant germplasm for crosses. This does not only save time, but also money.

The differences in the methods used, were illustrated in the results of the yield components that were tested. The differences between the cultivars in the different systems can be attributed to the fact that when plants in the hydroponic systems were fed properly, optimum production could be attained. With most vegetables, growth in hydroponic systems was accelerated and, as a rule, the quality was better than that of soil grown crops. Produce grown hydroponically had a much longer shelf life or keeping quality (Deutschmann, 1998).

There were significant differences between entries for dry mass. The reduction of dry mass has an influence on plant height and number of spikes. This also influences the number of spikelets in tested cultivars. In the same way plant biomass also had a synergistic relationship to grain number and weight (Saleem, 2003). This explains why highly significant differences between entries were observed for primary spikelet number, primary kernel weight, secondary kernel weight and total kernel weight. There was also significant interaction between entry and treatment for the number of spikes and

the number of primary spikelets. There was highly significant interaction between entry and treatment for the number of secondary spikelets.

In comparing each cultivar in the different systems, it is evident that Steenbras, as well as Baviaans, in the chemicult system had a significantly higher dry mass compared to the other two cultivating methods indicating the effectiveness of the method used (Table 4.6). Both cultivars in the second of the hydroponic systems (using the published formula) also had a significantly higher dry mass than their counterparts grown in the pots indicating that although the system was not as effective as the first, the hydroponic cultivation method as a whole is more effective than the traditional pot system.

Hydroponic cultivation was used in the past for controlled environment life support systems (CELSS). This was done because of the optimal environment that could be supplied to the roots of the plant for optimal development, to ensure a high growth rate. Nutrients, water and aeration can be controlled to the highest degree. This platform of control is hard to match in solid media (Steinberg *et al.*, 2000). This explains the results obtained in the discussion above, indicating the effectiveness of any hydroponic system compared to the traditional pot planting method used in wheat breeding practices.

The total dry mass correlated positively with the total number of tillers, total number of spikes, number of primary spikelets, number of secondary spikelets, primary kernel number, secondary kernel number, secondary kernel mass, total kernel number and total kernel mass. These findings support the findings of Saleem (2003), where biomass or the lack there off due to water stress, influences the number of spikes and spikelets in bread wheat.

In terms of biomass and the realization of yield potential it is clear that the effect of the biomass relate to all the yield components, as expressed by Saleem (2003) suggesting that for a plant to produce at optimum levels, it should be provided with the optimum environment. Thus by using a hydroponic method, the optimum conditions can be obtained and maintained. For the breeder this would mean that less of the same lines will

have to be planted to ensure the same amount of crosses, due to the higher emergence percentage and higher yield potential. By making these crosses the breeder will have a better chance of obtaining quality seed from the crosses made. Furthermore the breeder will be able to plant more lines in the same space available, thus being able to produce more varieties in the parental material and have the chance of expanding the genetic diversity of the breeding program at an accelerated pace without further cost or time needed.

4.5. Conclusions

In this study two identical hydroponic systems differing only in the nutrient solution were compared to the traditional potting method used in greenhouse cultivation of wheat for parent lines during wheat breeding. The difference in the emergence percentages of the two cultivars in the three different growth methods indicates that the problem does not only lie with the seedling vigor, but can possibly be related to the differences in the systems. The fact that the two hydroponic systems provided a better emergence environment compared to the potting solution is already significant, indicating that there is already a higher potential number of crosses that can be made in the breeding program due to the higher emergence percentage. The need to replant lines in a breeding program for crosses does not only involve the non-germinating lines but also the planting of the lines that will be used in the eventual crosses. The effectiveness of a cultivation system to provide the ideal germinating conditions is most important. Mainly because the effectiveness of emergence influences the number of seeds that needs to be planted together with the timing of parent plantings for crosses, this also influences the space available for the breeder.

The differences in the effectiveness of the methods used were illustrated in the results of the yield components that were tested. There were significant differences between entries for dry mass. The reduction of dry mass has an influence on plant height and number of spikes. This also influences the number of spikelets in tested cultivars. In the same way

plant biomass also had a synergistic relationship to grain number and weight according to Saleem (2003).

In all these aspects the hydroponic cultivation methods were highly superior to the potting cultivation method. The small differences between the hydroponic units would indicate that the effect of different solutions had a lesser effect on the growth and development of the wheat than the methods itself. What was noticeable was the fact that in both cases Baviaans and Steenbras, in the chemicult solution, superceded the measurements that was observed in their solution-planted counterparts. This would suggest that the use of the lesser complicated or more user friendly method was best suited for the breeder.

It is clear that the effect of the treatments on the cultivars relate to their biomass. In terms of biomass and the realization of yield potential it is clear that the effect of the biomass relate to all the yield components, as expressed by Saleem (2003) suggesting that for a plant to produce at optimum levels, it should be provided that plant with the optimum environment. Thus, by using a form of hydroponic method, the optimum conditions can be obtained and maintained.

These findings would indicate that by using a hydroponic cultivation method instead of the potting method for growing wheat parent lines can increase the amount of crosses that can potentially be made. This is done by increasing the amount of potential crosses, potential success and quality of the crosses made, due to a higher emergence rate and superior development achieved by the use of a hydroponic system for wheat parent line cultivation.

CHAPTER 5

5.1. General conclusions and recommendations

For the first trial, the morphological characteristics of five cultivars under drought stress at the seedling stage were compared to a control treatment. During the second trial, the morphological characteristics of five cultivars during drought treatment were compared with the control at anthesis. The effect of both treatments was in agreement with the findings of Saleem (2003), who reported that drought stress causes a significant reduction of dry mass. In the same way plant biomass also had a direct relationship to grain number and weight.

It was found that the seedlings in the drought treatment developed slower compared to the seedlings in the control. In contrast to this, there was no significant reduction in the plant height and number of spikes in the mature plants after the growth period was concluded, due to the re-instatement of optimal conditions in the drought treatment system, indicating that there were some recoveries by the cultivars from the drought treatment. The results of the second study indicated that a stress condition during a critical stage in the development of wheat is far more detrimental than at an early stage.

There are numerous limiting factors in protein production such as the amount of available soil moisture and mineral nutrients availability (Pomeranz, 1988). Limiting these stress factors to seedlings, may cause some effect on the proteins in the plant at an adult stage but, when plants in the drought induced experiment have the opportunity to grow under normal conditions, the capability of cultivars to recover can be determined by means of proteins, physiological and morphological characteristics. From the results, it is clear that the effect of drought at an early stage does not have a dramatic effect on the protein composition of wheat cultivars, yet there are differences between cultivars.

In the third study two identical hydroponic systems differing only in the nutrient solution were compared to the traditional potting method used in greenhouse cultivation of wheat for parent lines during wheat breeding. The two hydroponic systems provided a better

emergence environment compared to the potting solution. This indicates that there is already a higher potential number of crosses that can be made in the breeding program due to the higher emergence percentage. The differences in the effectiveness of the methods used were illustrated in the results of the yield components that were tested. There were significant differences between entries for dry mass. The reduction of dry mass had an influence on plant height and number of spikes. This also influenced the number of spikelets in tested cultivars. In the same way plant biomass also had a synergistic relationship to grain number and weight according to Saleem (2003).

In the context of the results obtained, the use of a hydroponic cultivation method instead of the potting method for growing wheat parent lines can increase the amount of crosses that can potentially be made. This is done by increasing the amount of potential crosses, potential success and quality of the crosses made, due to a higher emergence rate and superior development achieved by the use of a hydroponic system for wheat parent line cultivation. The hydroponic cultivation method can also be used for the induction of drought conditions at any stage of development of wheat. This can be done very effectively, not only for inducing stress but for re-establishing and maintaining optimum conditions as well. This is supported by the results of both the yield component results as well as the protein results that correspond with previous findings. In addition to the use of hydroponic cultivation, one can also assume that the use of SE-HPLC for protein separation can certainly be used as an early identification tool for drought tolerance in wheat.

The practical implication for the breeder would be that the use of a hydroponic cultivation method can be more time, space and labor efficient. The practical implication of stress conditions can easily be incorporated in the same system without physical modifications. The refinement of selection criteria for wheat breeding can be done on the same system during the time when crosses are not made in the same controlled environment. The results also indicate that by only using the morphological characters the breeder will not be entirely able to detect or predict tolerance for all the stresses that will be tested. Further techniques such as SE-HLPC can also be implemented

successfully in the identification of resistance or tolerance in wheat for practically any external factor.

CHAPTER 6

Summary

Keywords: Hydroponics, wheat, yield components, monomeric proteins, polymeric proteins, SE-HPLC,

ξ The aims of this study were to evaluate the functionality and the practicality of hydroponics in the process of wheat breeding compared to a conventional glasshouse cultivation method. Furthermore for assessing the screening capability of drought tolerance in wheat cultivars using a hydroponic system was used and morphological yield components were measured and protein concentration fluctuations by means of SE-HPLC were analyzed.

ξ In the first study, five South African wheat cultivars, SST 88, Baviaans, Steenbras, SST 876 and Kariega, were planted into two identical hydroponic systems. A second set of the five cultivars where planted two months after the first planting to separate the two stress periods, the first being drought stress before seed fill and in the second planting, stress was induced at seedling stage. This was done simultaneously.

ξ When maturity was reached, several yield component measurements were taken.

ξ The influences of the drought treatment on the different yield components were established.

ξ Protein extracts of wheat flour were analyzed by SE-HPLC.

ξ SDS was used for the first protein extraction step, and the rest of the proteins were extracted by sonication.

- The SE-HPLC method fractionated the storage proteins (both SDS-soluble and SDS-insoluble) into four distinct peaks of decreasing molecular size range, representing mainly larger polymeric proteins (mainly HMW-glutenins), smaller polymeric proteins (mainly LMW-glutenins), larger monomeric proteins (mainly gliadins), and smaller monomeric proteins (mainly albumins and globulins).
- Relationships between the amount and size-distribution of polymeric and monomeric proteins and flour quality properties were established.
- The influence of the drought treatment on protein fractions and quality characteristics was also determined by SE-HPLC.
- The results showed that both the genotype and drought conditions had a significant influence on the yield components and protein characteristics.
- The use of a hydroponic cultivation method for the induction of drought conditions at any stage of development of wheat can be done at a highly significant level, not only for inducing stresses but for re-establishing and maintaining optimum conditions as well.
- SE-HPLC provided a simple and an objective test for measuring the relative size-distributions of wheat storage proteins.
- In the second study two South African wheat cultivars, Baviaans and Steenbras, were planted into two identical hydroponic systems (differing only in the makeup of the nutrient solution) and in pots.
- Emergence percentages and dates were recorded for a one month period after the emergence of the first seedling in the experiment.
- When maturity was reached several yield component measurements were taken.

- The two hydroponic systems provided a better emergence environment compared to the potting solution, indicating that there is already a higher potential number of crosses that can be made in the breeding program using a hydroponic cultivation method.

- The effect of treatment was highly significant for most of the yield characteristics, indicating that the effectiveness of the treatments played a significant role in the yield potential of each cultivar.

- Findings indicated that by using a hydroponic cultivation method instead of the potting method for growing wheat, parent lines can increase the amount of crosses that can potentially be made, thus increasing the amount, potential success and quality of the crosses made, together with the potential of producing seeds that will have the potential of a higher emergence rate.

- The hydroponic cultivation method using the chemicult solution proved to be the most effective method of cultivation during this study.

Opsomming

Sleutelwoorde: Hidroponika, koring, opbrengskomponente, monomeriese proteïene, polimeriese proteïene, SE-HPLC

- Die doel van die studie was die evaluering van die effektiwiteit en die praktiese toepassing van ‘n hidroponiese koring kultiveringsproses teenoor die konvensionele pot-aanplantingsmetode. Die tweede doel was om die doeltreffendheid van ‘n hidroponiese stelsel vir gebruik in ‘n droogtestremmingstoets vir koring te evalueer deur die opbrengspotensiaal asook die fluktuasie van proteïene deur middel van SE-HPLC te meet.

- In die eerste studie is vyf Suid-Afrikaanse lentekoringkultivars nl. SST 88, Baviaans, Steenbras, SST 876 en Kariega, in twee identiese hidroponiese stelsels geplant. ‘n Tweede stel van die vyf kultivars is twee maande later geplant in dieselfde stelsel om die stremmingsperiodes van die studies te skei. Die stremming is toegepas tydens die blomstadium van die eerste proef, en op die saailingstadium van die tweede proef. Die proewe is gelyktydig uitgevoer.

- Na rypwording is opbrengskomponente gemeet en vergelyk.

- Die invloed van die droogtestremming op die opbrengskomponente is bepaal.

- SE-HPLC is gebruik om proteïen-ekstrakte van die meel te ontleed.

- SDS is tydens die eerste ekstraksieprosedure gebruik. Die SDS-onoplosbare proteïene is daarna met behulp van sonifisering geëkstraheer.

- The SE-HPLC metode het die bergingsproteïene (beide SDS-oplosbare en SDS-onoplosbare) in vier duidelike fraksies verdeel volgens hul molekulêre gewig. Die vier fraksies (of pieke) het bestaan uit groter polimeriese proteïene (hoofsaaklik hoë molekulêre gewig gluteniene), kleiner polimeriese proteïene (hoofsaaklik lae molekulêre gewig gluteniene), groter monomeriese proteïene (hoofsaaklik gliadiene) en kleiner monomeriese proteïene (hoofsaaklik albumiene en globuliene).

- Die verwantskappe tussen die hoeveelhede en grootte-verspreiding van polimeriese en monomeriese proteïene en kwaliteitseienskappe is bepaal.

- Die invloed van die droogtestremming is op die proteïenfraksies en kwaliteitseienskappe vasgestel met behulp van SE-HPLC.

- Die resultate het getoon dat beide die genotipe en die droogtestremming gelei het tot betekenisvolle verskille in die kwaliteitseienskappe.

- Die gebruik van 'n hidroponiese kultiveringsmetode in die uitvoering van 'n droogtestudie kan ter enige tyd in die ontwikkeling van koring met groot sukses gedoen word; nie net vir die induksie van stremming nie, maar ook vir die herstel en handhawing van optimale groeitoestande.

- SE-HPLC was 'n relatief eenvoudige en objektiewe metode om die grootte-verspreiding van bergingsproteïene te bepaal.

- In die tweede studie is twee Suid-Afrikaanse lentekoringkultivars nl. Baviaans en Steenbras, in twee identiese hidroponiese stelsels, wat slegs in hul voedingstof samestelling verskil, in potte geplant.

- Die ontkiemingspersentasies is oor 'n periode van een maand na die ontkieming van die eerste saailing gemeet.

- Na rypwording is verskillende opbrengskomponente gemeet.

- Albei die hidroponiese stelsels het beter ontkiemingsomgewings geskep teenoor die potte vir die koring. In ‘n teelprogram sal dit beteken dat daar in ‘n kruisingsblok potensieel meer kruisings gemaak sal kan word.

- Die invloed van die behandelings was hoogs betekenisvol vir die meeste van die opbrengsveranderlikes. Dit sal beteken dat die effektiwiteit van die behandelings ‘n betekenisvolle verskil in die opbrengspotensiaal van elke kultivar sal hê.

- Die resultate toon aan dat die gebruik van ‘n hidroponiese kultiveringsmetode vir koring, in plaas van die tradisionele pot-metode die kruisings potensiaal van ouerlyne kan verhoog. Die verhoging in die aantal kruisings, die potensiële sukses en kwaliteit van die kruisings, tesame met die verhoogde ontkiemingspotensiaal van die kruisingsgenerasie is voordelig in ‘n teelprogram.

- Die hidroponiese kultiveringstegniek wat gebruik gemaak het van die chemicult voedingsmedium, is geïdentifiseer as die mees optimale sisteem tydens die studie.

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