THE CHARACTERISATION OF SOUTH AFRICAN AND ETHIOPIAN BREAD AND DURUM WHEAT CULTIVARS FOR DROUGHT STRESS TOLERANCE

BY

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LIST OF ABBREVIATIONS

µg	=micro-gram
µl	= micro litre
ANOVA	=Analysis of variance
APS	= Ammonium per sulphate
Bdl	= Breeding line
CMS	=Cell membrane stability
DF	= Degree of freedom
g	=Gram
GYP	=Grain yield per plant
HM	=High moisture
KN	=Kernel number per plant
LM	=Low moisture
LSD	=Least significant difference
ml	=mililitre
MS	= Mean squares
NS	=Non significant
PEG	=Polyethylene glycol
pH	= Measure of alkalinity or acidity
PKM	= Primary tiller kernel mass
SDS	= Sodium dodecyl sulphate
SDS-PAGE	= Sodium dodecyl sulphate polyacylamide gel electrophresis
SKM	=Secondary tiller kernel mass

SNP	=Spikelets number per spike
SP	=Spike number per plant
SSI	=Stress susceptibility index
STI	=Stress tolerance index
TTC	=Triphenyl tetrazolium chloride

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CHAPTER 1

1 INTRODUCTION

Wheat (*Triticum aestivum L.* and *T. turgidum L.*) is the world's leading cereal grain and one of the most important food crops. 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 (Satorre and Slafer, 1999). Wheat production, however, is affected by drought when it is grown in marginal agro-climatic zones (Briggle and Curtis, 1987).

Drought is among the most important constraints that threaten the food security of people on the globe (Barters and Nelson, 1994). It occurs as Baker (1989) explained when precipitation falls significantly below the long-term average over large areas for an extended period. It has caused a serious fall of cereal stock almost below the level that the FAO considers necessary for global food reserves (William, 1989).

Drought is a multidimensional problem and covers large areas throughout the world (William, 1989). Gupta (1997) estimated that about 26 % (17,255,700 square miles) of the world's total cultivable land falls in arid and semi-arid areas, where water is the limiting factor to crop production. An estimated 32 % of the 99 million-hectare of wheat grown in developing countries experience varying levels of drought stress (Rajaram *et al*, 1996).

Consequently, the development of drought tolerant varieties became an important objective in many plant breeding programs. Morphological and physiological traits that might enhance drought tolerance have been proposed, but only a few of these mechanisms have been demonstrated in the expression of tolerance under field conditions (Ludlow and Muchow, 1990). The selection for drought tolerance while maintaining maximum productivity under optimal conditions has also been difficult (Barters and Nelson, 1994), due to the low heritability of yield in such conditions.

The magnitude of morphological and physiological responses to water stress varies among species and between varieties within a crop species (Kramer, 1980). The success of selecting appropriate genotypes for the stress environment was also limited by inadequate screening techniques and the lack of genotypes that show clear differences in response to well defined environment stresses (Bruckner and Frohberg, 1987).

Moustafa *et al* (1996) also stated that there is a limitation in selecting for drought tolerance and a need to identify drought tolerant techniques that are repeatable and that can be used in a population of high genetic variation because of the multitude of factors that are involved in drought tolerant mechanisms.

It is therefore suggested that along with morphological and physiological knowledge, the biochemical basis of drought tolerance is an essential pre-requisite for enhancing crop tolerance to drought along with a clear understanding of morpho-physiological traits (Bushuk and Zillman, 1989).

Drought tolerance mechanisms have been identified in a number of crop plants, mainly as drought avoidance and drought tolerance (Blum and Ebercon, 1981). Drought avoidance is manifested in a given genotype by a relatively smaller reduction in tissue water potential under conditions of increasing soil or atmospheric moisture deficits. Drought tolerance on the other hand is manifested by the ability of the plant tissue to sustain a smaller reduction in physiological or metabolic activity as its water potential decreases. These drought tolerance mechanisms include many morphological and physiological attributes and are due to multi-genic expressions that involve the whole plant. It is known that a range of drought tolerance mechanisms is present during water stress. The understanding of plant water relations and predicting water stress responses can have a positive effect on crop production and water use efficiency.

Since drought tolerance is process-specific (Blum *et al*, 1990) different physiological processes may show tolerance or susceptibility within a genotype. Thus, screening techniques for drought tolerance need to be rapid, accurate and inexpensive (Winter *et al*, 1988).

The use of physiological responses of plants and their relationship with productivity under water deficit can help the breeder to improve drought tolerance. To explore the complete knowledge of drought tolerance, one has to study the morphological as well as physiological and biochemical responses of crop plants to the water stress process. It is important to study them independently by means of screening methods.

The objectives of this study were:

1. to investigate the effect of moisture stress on yield and yield components and the genetic relationships between yield and yield components,

2. to discriminate between drought tolerant and susceptible bread and durum wheat varieties at seedling stage using the wooden box screening method,

3. to determine the varietal differences of bread and durum wheat in response to water stress under laboratory conditions based on proline content, cell membrane stability (CMS) and 2,3,5 triphenyltetrazolium chloride (TTC) reduction.

4. to determine genetic distances between drought tolerant and susceptible varieties using gliadins in order to determine the best possible combinations for drought tolerance breeding.

CHAPTER 2

2 LITERATURE REVIEW

2. 1 Wheat

2.1.1 Importance and classification of wheat

Wheat (*Triticum aestivum* L. and *T. turgidum* L.) is grown all around the world and is one of the most important food crops. More wheat is produced annually than any other food or feed crop. It is the world's major source of calories and protein (Briggle and Curtis, 1987).

Wheat is grown for various purposes. It is utilized for making bread, flour confectionery products (cakes, cookies, crackers, pretzels), unleavened bread, semolina, bugler and breakfast cereals (Satorre and Slafer, 1999). Wheat is also used as animal feed and as a raw material for various industries. Its diversity of uses, nutritive content and storage qualities have made wheat a staple food for more than one third of the world's population.

Wheat falls into three categories. One group has the usual two sets of chromosomes (diploid), the second group has four sets of chromosomes (tetraploid) and the third group has six sets of chromosomes (hexaploid). The basic number of chromosomes in wheat is seven. Diploid wheats have 14 chromosomes (two sets of seven chromosomes, one set from each parent). Tetraploid wheats have 28 chromosomes (four sets of seven

chromosomes) and hexaploid wheats have 42 chromosomes (six sets of seven chromosomes) (Cook and Veseth, 1991).

2.1.2 Climatic adaptation of wheat

Wheat is grown over a wide range of moisture and temperature conditions. The main wheat regions of the world is between latitudes 30^0 and 55^0 in the Northern temperate zone and 25^0 and 40^0 in the Southern temperature zone, in areas where annual precipitation ranges between 30 and 114 cm (Nuttonson, 1955). The wheat of the more humid areas of the world is generally soft and starchy and those of less humid areas are usually hard. Spring wheat is sown where the winters are dry and cold. Winter wheat grows where plants can survive winter temperatures. The largest amount of the best wheat is produced in countries with cold winters.

The distribution of wheat and the kind of wheat grown in relation to temperature are largely determined by the length of the frost-free period, the minimum winter temperature, the temperature in relation to the average length of day during the growing season and the maximum temperatures immediately preceding harvest. For the most satisfactory growth and development of grain, a cool, moist, growing season, followed by a bright, dry and warm ripening period of 6-8 weeks, with a mean temperature of 18 to19^0 C is necessary (Nuttonson, 1955).

2.2 Importance and reaction of plants to drought stress

Drought stress is defined as a prolonged and abnormal moisture deficiency. It occurs when the water loss through transpiration exceeds the water supply from the soil. The change to the global weather pattern and our exploitations of the environment are the factors that have contributed a lot to the manifestation of drought (William, 1989).

Crop plants are frequently subjected to water stress during the course of their life. Certain stages, such as germination, seedling and flowering are the most critical for drought stress damage. Stress imposed during these stages drastically affects crop yield.

Drought stress reduces plant growth and manifests several morphological, physiological and biochemical alterations in plants, ultimately leading to a massive loss in yield.

Reports indicate that the world's cereal production has declined because of drought for two successive years against a requirement for a sustained increase of almost three percent in developing countries to maintain even current "levels of malnutrition" to the year 2000 and beyond (Baker, 1989).

About 26 % (17,255,700 square miles) of the world's total cultivable land falls in arid and semi arid areas where water is the limiting factor to crop production (Gupta, 1997).

Rajaram *et al* (1996) reported that about 32 % of the 99 million hectare of wheat grown in developing countries experienced varying levels of drought stress. They concluded that it is important to evaluate germplasm under optimal conditions, to utilize high heritabilities and to identify lines with high yield potential under stress conditions to preserve for drought tolerance.

The importance of improving drought tolerance genotypes and identification of their mechanisms of tolerance is therefore suggested as a desirable breeding objective in wheat crops (Keim and Kronstad, 1979; Clarke *et al*, 1992). Drought tolerance mechanisms are mainly escape or tolerance. Drought escape usually involves early maturity to avoid the onset of severe water deficits, whereas tolerance involves either avoidance or postponement of dehydration by maintaining water uptake or reducing water loss, or desiccation tolerance which usually involves osmotic adjustment (Kramer, 1980; Levitt, 1980).

2.3 Effect of drought stress on plant growth and development

Water is an essential resource for plant life. Therefore, any limitation in water availability affects almost all plant functions. The availability of water for all plant biological functions can be impaired by different environmental conditions.

Drought is a multidimensional stress affecting plants at various levels of their organization (Blum, 1996). The effect of and plant responses at the whole plant and crop

level is most complex, because it reflects the integration of stress effects and responses at all underlying levels of organization over space and time.

If the soil water content is below the minimum required for germination, the radical will not emerge from the testa and the seed will eventually be damaged or destroyed by the soil fungi. Drought seedling mortality in a drying seedbed is also a common problem leading to a decreased stand (Johnson and Assay, 1993).

Plant developmental characters that are associated with co-development of seminal and crown roots and leaves would affect seedling establishment under drought stress in grasses (Johnson and Assay, 1993). In wheat, seminal roots are functional for most of the plant's life. Farshadfar *et al* (1993) reported that in wheat, the root system is one of the most important morphological characters, related to drought, which had a highly significant positive correlation with total biomass and showed the highest direct effect. The most common observation concerning roots under drought stress is the increase in root/shoot dry matter weight ratio. The increase in ratio results from the relatively greater decrease in shoot growth than root growth under drought stress (Slatyer, 1969; Blum, 1996). The increase in dry matter root/shoot ratio often implies the development of a larger ratio of root length density to leaf area, which translates into a better capacity for sustaining plant water status under a given evapotranspirational demand (Blum and Askin, 1984).

Sharp (1990) indicated that ABA accumulation in roots under the effect of the substrate water deficit was responsible for reducing shoot growth on the one hand and sustaining root growth on the other. Root growth depends on the active growing region just above its apex. Osmotic adjustment and turgor maintenance in the growing region were also important in sustaining root growth at low water potential. Research have shown a progressive reduction in rate of root elongation as drought is imposed and in some cases root elongation ceases before shoot growth. In addition, as the rates of root elongation are reduced, the rate of suberization exceeds the rate of elongation. The non-suberized zone is reduced until it is virtually eliminated in non-elongating roots. Such a response to severe drought stress greatly reduced the absorbing ability of roots.

Drought stress also have a profound effect on cell enlargement. One of the most important consequences of the sensitivity of cell enlargement to soil water deficits is a marked reduction in leaf area. A reduction in leaf area will reduce crop growth rate particularly during the early stages of growth when there is incomplete light interception. One of the most damaging features of a reduction in leaf area is the fact that the effect is permanent and in the case of a determinate crop there is no scope for compensation *via* an increase in the number of leaves. The effect of inhibited leaf enlargement because of drought is a reduction in the size of the photosynthesizing surface causing a reduced crop growth. However, a reduction in photosynthesis can recover on the relief of stress.

Drought stress can also affect leaf area through its effect in hastening the rate of leaf senescence (Fischer, 1973; Ludlow and Muchow, 1990). In the small grains, the

degeneration of existing tillers and the total cessation of the appearance of the new tillers are also important factors in limiting leaf area under drought stress (Blum *et al,* 1990). However, compared with growth cessation of single leaves on a stem, the control of leaf area by tillers allows an impressive recovery of leaf area as tillers appear at very high rates upon dehydration. Before flowering, the reduction in leaf area index and intercepted radiation under stress are largely a result of impaired leaf expansion and changes in leaf display. In determinate crops such as wheat where the leaf area is fixed at flowering, yield under dry land conditions has been inversely related to the rate of leaf senescence after flowering, which in turn was related to plant water stress. Evidently the control over leaf area viability under drought stress is different before and after flowering (Begg and Turner, 1976).

The negative effect of drought stress is also very important during flowering, seed set and seed filling. It can induce an early switch of plant development from vegetative to reproductive state. If the stress occurred early in seed filling, the yield is reduced without reduction of seed number as a result of a shortened seed filling period (Blum, 1996). Thus, drought stress during seed filling and physiological maturity has the biggest effect on seed yield reductions, resulting in accelerated decline in leaf photosynthetic activity and increase in the mobilization of carbon to nitrogen.

2.4 Screening methods for drought stress

A number of plant components and physiological parameters can be used as screening methods for drought tolerance.

2.4.1 Yield and yield components

There are some reports in literature indicating that water deficits limit yield and/or that irrigation increases yield. Begg and Turner (1976) reviewed an example of this with green peas. The lack of irrigation reduced the total yield by 47 %, but the yield of peas by only 36 %.

Yield reduction by a water deficit or enhancement through irrigation will depend on the growth stage in relation to drought, the degree, duration, and timing of the deficit and on the proportion of the total yield that comprises the economic yield of the crop.

According to Slatyer (1969) there are three stages of growth susceptable to drought stress. These are stages of floral initiation and inflorescence development, anthesis and fertilization and grain filling. A slight drought stress can reduce the rate of the appearance of floral primordial. When a severe drought stress was imposed, the recovery from drought was unsatisfactory and the total spikelet number was greatly reduced. Primordial initiation is more affected by drought stress than spikelet development and thus, stress at the former stage can alter grain number more than at the latter stage. Fischer (1973) found moderate stress before heading contributes a lot to yield reduction in wheat.

Drought stress at anthesis and fertilization will reduce the number of kernels because of the dehydration of pollen grains. Crop plants that shed pollen over an extended period of

time will be more likely to avoid the influence of drought at this stage of growth than crop plants that shed pollen in a relatively short period of time.

Drought stress at the stage of grain filling is pronounced as yield development, expressed by weight per grain, requires the accumulation of photosynthate in the grain.

Drought stress has been shown to reduce translocation from the leaves and as drought hastens maturation, this response in addition to reduced photosynthesis contributes to lower grain yield. Labuschagne (1989) also reported that yield reduction was the result of a reduction of photosynthate due to a reduction of translocation and photosynthesis, which was caused by moisture stress.

The components of yield that are influenced by water stress depend largely on the timing of stress in relation to the development of the portion of the plant utilized for economic yield.

Most determinate annual crops are especially sensitive to water deficits from the time of floral initiation and during flowering. Begg and Turner (1976) reviewed the influences of water stress on inflorescence development, fertilization, and grain filling in cereals.

In the determinate cereals, moisture stress prior to ear emergence also influences the number of grains set per spikelet. Fischer (1973) showed that the period 5-15 days before

ear emergence in wheat was the most sensitive stage and at a potential decrease below – 12 bars, fewer grains were set per spikelet.

Different studies indicated that yield which reflects an integrated effect of many components, is still an important factor to be considered for screening and selecting drought resistant varieties in crop species. Roy and Murty (1970) reported that when using yield to screen for drought tolerance, visual scores and actual yields agreed in 27 of the 33 cases.

In drier environments, variation in rainfall (water supply to the crop) can account for most of the variation in yield. For instance, Austin (1989) reported that 70 % to 80 % of the variance in yield of wheat in South Australia and almost 70 % of the variance in maize yield in semi-arid Kenya are caused by drought. In crops like soybean (De Ronde, 2000) depending on the time of stress, the yield reduction as a result of drought can vary between 13 % (early drought) to 88 % (late drought). In sorghum, Garrity and Gilley (1982) reported a yield reduction of 41 to 45 % resulting from drought deficits at the grain filling stage.

Mederski and Jeffers (1973) reported that under high moisture stress conditions, the yield of the most stress resistant varieties of soybeans was reduced by about 20 % while the yield of the least stress resistant varieties was reduced by 40 %. The absolute reduction in yield for the most stress sensitive varieties was approximately 1000 kg/ha, while the yield of the least stress sensitive varieties was reduced by about 200 to 400 kg/ha.

Begg and Turner (1976) observed a 79 % decrease in grain yield of wheat from a water deficit imposed five weeks prior to ear emergence, but only a 53 % decrease in total dry matter by the same treatment.

On the other hand, water stress influences not only yield, but also yield quality: the effect on yield quality can be either beneficial or determental depending on use. Water stress increases the nitrogen percentage of small grains such as wheat and barley (Begg and Turner, 1976) when water deficit was imposed on wheat five weeks before ear emergence. The nitrogen percentage of the grain was 53 % higher than in well-watered controls.

Yield components are also affected by drought differently, based on growth stage when stress develops. Innes and Quarrie (1987) reported a relatively higher yield reduction during pre-anthesis stress than post anthesis.

Water stress at spike initiation causes the greatest reduction in yield. Potential yield of wheat can only be obtained under well-watered conditions (Oosterhuis and Cartwright, 1983). The water deficit increased the rate of tiller death from $3/m^2$/day in the control to $11/m^2$/day in the stressed wheat and also reduced the number of tillers bearing ears by 55% (Begg and Turner, 1976). Fischer (1973) has reported reductions in ear-bearing tillers in wheat and Blum and Askin (1984) reported a reduction in panicle numbers in sorghum. Richard (1982) also reported the importance of slower pre-anthesis water use

due to its influence on yield by reducing kernel number. Oosterhuis and Cartwright (1983) noticed that the greatest effect of water stress on grain yield was associated with the reduction of kernel number.

Richard (1982) reported that water stress during grain filling caused a reduction in kernel mass. Oosterhuis and Cartwright (1983) and Labuschagne (1989) also reported a larger reduction of kernel mass caused by moisture stress for the secondary tillers than for the primary tillers.

2.4.2 Wheat protein composition

A protein is a primary product of a structural gene and it serves as a marker for that particular gene. Genes are coupled into genetic systems and because of this, proteins also serve as markers for such systems, including chromosomes and the genomes as a whole.

Wheat proteins are composed of five classes of proteins: albumins (soluble in water), globulins (soluble in salt solutions), gliadins (soluble in aqueous ethanol), glutenins (soluble or rather dispersible, in dilute acid or alkali) and an insoluble residue.

Based on molecular size, proteins larger than 100 kDa are considered to be mainly glutenin, between 100 and 25kDa mainly gliadin and less than 25 kDa are considered as albumins and globulins (Eliasson and Larsson, 1993).

Gliadins

Gliadins are defined as the wheat proteins soluble in aqueous ethanol in the classic Osborne extraction procedure, as cited in Eliasson and Larsson (1993). Gliadins are non-aggregating or monomeric proteins and consist of a complex mixture of single polypeptide chains associated by hydrogen bonds and hydrophobic interactions (Shewry and Tatham, 1990). They are a highly heterogeneous group of proteins with molecular weights ranging from 20 to 70 kDa (Southan and MacRitchie, 1999). Fractionation is based on extraction procedures by gel electrophoresis at low pH and are separated into α, β, γ and ω gliadins (Woychik *et al*, 1961). The composition of each class will depend on the exact conditions during the extraction, such as temperature and solvent/ flour ratio.

Gliadin components have been shown to be inherited as linked groups (blocks), co dominantly and in accordance with a gene dosage in triploid endosperm. Blocks include components differing in their electrophoretic mobility and molecular weight (Metakovsky, 1991).

Gliadin polypeptides occur in groups or blocks based on each of the several sets of tightly linked genes coding for the polypetides. These blocks of gliadin genes are located on the short arms of chromosome 1 and 6 (Wrigley, 1992).

Polyacrylamide gel electrophoresis in the presence of SDS has been widely used for detecting polymorphism of wheat storage proteins. This enables a detailed study of the

number, size, distribution and the genetic control of specific proteins at the subunit or block levels (Galili and Feldman, 1983). Variation in the storage protein composition of wheat cultivars has been associated with the presence of allelic genes tightly linked as clusters at each complex loci (Payne *et al,* 1981). Both gliadins and glutenins have demonstrated extensive multiple allelism at their encoding genes and hence storage proteins are highly polymorphic (Metakovsky, 1991).

The heterogeneity of gliadins that constitute 45 % of total wheat proteins was shown by electrophresis as varietal characteristics. These characteristics make these proteins the best and most often used for cultivar identification. Besides, the gliadin electrophoregram is not affected by growing conditions and environmental factors (location, year, climate, fungicides and fertilizers) do not alter this polymorphism (Wrigley, 1982).

For instance, Clements (1987) found no environmental effects on gliadins electrophoretic profile of soft wheat. The electrophoretic similarities of profiles from immature and mature seed provided additional evidences of the intrasigence of seed protein profiles to factors other than genetic changes. Similarly, Huebner and Bietz (1988) were unable to find differences in gliadin electrophoretic profiles of seed lots grown in soils with different sulfur levels.

Wrigley *et al* (1980) found significant changes in relative intensities of gliadin bands when sulfur was severely deficient during growth. Lookhart and Finney (1984) also noticed a slightly different gliadin electrophretic profiles for two wheat cultivars grown in

soils that were severely deficient in sulfur, but no differences could be detected due to various levels of soil nitrogen.

In general, gliadin genetic markers are characterized by high levels of polymorphism and it is a rapid and relatively inexpensive technique to use (Wrigley, 1992). It has also been indicated to be tightly linked with many important agronomic characters such as seed size, heading time, disease and pest tolerance, frost hardiness and plant height and other quantitative characters (Metakovsky, 1991).

Hence, with the above merits, gliadin markers may be relevant and associated with drought tolerant mechanisms to help in the identification or categorization of genotypes based on their similarity.

2.4.3 Survival and recovery of seedlings

During the life span of a plant it can encounter several drought spells that can affect the plant adversely. Components of drought tolerance at the seedling stage such as survival of the plant, root development and recovery from water stress is very important. These components include cellular, developmental and biochemical traits that lead to improved complex traits such as yield under drought conditions. In the wooden box screening method, survival of seedlings after desiccation and the recovery of plants can be used to screen for drought tolerance. The ability of a crop to recover from a mild or severe water

stress and the rate of recovery are linked to drought tolerance and the water use efficiency of the crop.

The woodenbox method is relatively cheap and easy to use and gives reliable results, especially when a lot of plants need to be screened (Singh *et al,* 1999). It is appealing because of the speed and the ease of handling large populations. It would appear to be suitable for screening large populations to improve drought tolerance prior to yield testing. Using these techniques, more uniform drought could be achieved by growing the plants hydroponically with an osmoticum to achieve the desired stress (Sullivan and Ross, 1979). In addition to handling large populations, the method is attractive because the survivors could be vernalized and grown to maturity with minimum effort.

The effectiveness for identifying drought tolerant lines is also among the merits of this technique. Winter *et al* (1988) reported that of the many screening techniques used for evaluating genotypes for drought tolerance in wheat, they found that survival after desiccation is the most suitable for screening large populations of segregating lines.

Singh *et al* (1999) reported the suitability of the wooden box screening method for drought tolerance at the seedling stage. They also found varietal differences for plant response to drought stress in cowpea. They noted that the close correspondence between the results of seedling screening and pot screening further indicates that the phenomenon responsible for drought tolerance in the seedling stage is also manifested at the reproductive stage.

Winter *et al* (1988) reported the variation in survival of seedlings was 13.7 times greater between wheat genotypes. They also noticed that when stress was relieved too soon the majority of all cultivars survived.

Rao and Venkateshwarlu (1989) noted that of the 24 rice cultivars tested for drought tolerance and their ability to recover from moisture stress at the seedling stage, five cultivars showed a high degree of drought tolerance and four cultivars showed fast recovery. They concluded that cultivars that have high drought tolerance with fast recovery could be used for breeding for moisture stress conditions.

2.4.4 Proline content

Water is an essential resource for plant life. The availability of water for all plant biological functions can be impaired by environmental conditions under severe stress. A plant adapts its mechanism and alters its development. Under conditions of water stress, there are changes in many processes as the plant attempts to maintain its metabolism and restore the metabolic conditions needed for growth (Singh *et al,* 1973).

One of the most frequently induced responses in all organisms subjected to water deficits is the accumulation of osmolytes. The amino acid, proline, is the most widely distributed "compatible" osmolyte (Tan and Halloran, 1982). Proline represents a unique class of molecules among the amino acids.

Many studies demonstrated that moisture stress results in changes in various plant metabolic activities. An increase in proline content by water stress was reported and has been suggested as a test of tolerance to water stress (Bates *et al*, 1973; Singh *et al,* 1974). As water stress increases, the plant's pH decreases, enzymes cannot function and high water potential gets lower. Tolerant plants form proline that protects enzymes and high water potential and increase the PH. It is suggested that proline accumulation in water stressed leaves might provide a source of respiratory energy to the recovering plant. This has been subsequently observed in many species including wheat.

Van Heerden and De Villiers (1996) observed a higher proline accumulation during drought stress in drought tolerant spring wheat genotypes than in the more sensitive cultivars.

Narayan and Misra (1989) determined free proline content in 25 wheat genotypes grown either with or without irrigation. All the genotypes accumulated higher proline contents under non-irrigated conditions. Five genotypes with the highest free proline contents gave the highest yields and had high yield stability indices.

Van Heerden and De Villiers (1996) found distinct genotypical differences in wheat, especially during pre-anthesis drought stress than during anthesis. They also found a positive correlation between proline accumulation and drought stress.

Karamanos *et al* (1983) noticed that proline accumulated with increasing water stress before heading and after ear emergence in the leaves, stems and roots of wheat genotypes. The results indicate that proline accumulation during drought stress may be a potential indicator of drought tolerance in spring wheat genotypes.

Wheat yield was also correlated with free proline content in the leaves under drought conditions as reported by Ivanov *et al* (1987). However, Hanson *et al* (1977) reported that in barley grown without irrigation, leaves of drought resistant Excelisor accumulated less free proline than did the leaves of drought susceptible Proctor.

Singh *et al* (1973) reported that barley genotypes that yielded well under drought prone environments showed higher proline accumulation during water stress at the seedling stages than did drought susceptible genotypes. They also indicated the correlation of grain yield stability with the proline accumulating potential of 10 barley cultivars.

De Ronde *et al* (2000) noticed that maximum accumulation of free proline in drought stressed cotton occurred at 11 days without water.

2.4.5 Cell membrane stability (injury)

During drought and heat stress, damage and injury occurs to the plant cells where leakage of electrolytes takes place. This unavoidably has a negative effect on the electron

transport system of the plant. Electrolyte leakage is a measurement of membrane stability (Ruter, 1993).

Cell membranes perform the vital role of regulating the passage of materials into and out of the cell. The technique of using cell membrane stability for screening plant material for potential anti-oxidant activity (stress tolerance) is based on an indirect monitoring of cell membrane intactness after a stress treatment, measuring K^+ leakage with an atomic absorption spectrophotometer or a conductivity meter. Membrane leakage is measured to determine oxidant damage. The anti-oxidant systems in plants act as important stress tolerance mechanisms by protecting membranes against damage caused by the toxic oxygen such as superoxide (O^-_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) produced under environmental and xenobiotic stress conditions. Because of the chloroplast/chlorophyll protection action of a high anti-oxidant activity in tolerant plants compared to sensitive plants, better yields can be obtained in field grown plants subjected to stress, including environmental and chemical stresses.

Cell membrane stability (injury) is estimated by the relative rate of electrolyte leakage from leaf tissue samples after being subjected to stress. Electrolyte leakage is estimated by measuring the electrical conductivity of the medium with which the leaf sample is equilibrated.

Studies indicated that the critical role of cell membrane stability under condition of moisture stress is a major component of drought tolerance. Different authors also reported its significance contribution for other stresses as a screening method in different crops.

The rate of injury to cell membranes in response to moisture stress may be estimated through the measurement of electrolyte leakage from cells (Blum and Ebercon, 1981).

Ruter (1993) reviewed electrolyte leakage as an effective means of measuring membrane thermostability in leaves and followed sigmodial response curves.

Blum and Ebercon (1981) reported that wheat genotypes grown under conditions of moisture stress significantly vary in their membrane injury level. They also noted that an injury level ranged from 16.7 to 70 % when the genotypes were screened artificially using a 30 % PEG solution as a dehydration media.

The effect of growth stages in wheat cultivars on the level of injury is also evident. Blum and Ebercon (1981) found that younger wheat leaf tissue is more tolerant to drought than older leaf tissues.

The same authors also noted the variations between bread wheat and durum wheat cultivars and they reported that bread wheat cultivars consistently suffered greater injury than durum cultivars.

Chu-Yung *et al* (1985) suggested that increased solute leakage is attributable to the loss of membrane integrity through lipid phase transitions and to the effect on membrane-bound transport proteins. These proteins play a role in preventing leakage.

Mark *et al* (1991) also recommended that cellular rupture because of leaked substances are important for assessing freezing injury in alfalfa.

2.4.6 2,3,5-Triphenyltetrazolium chloride (TTC) test as a measure of drought tolerance

Drought tolerance is manifested by the relative ability of the plant to sustain a smaller reduction in physiological or metabolic activity as its water potential decreases. During drought stress, damage and injury occurs to the plant cells where leakage of electrolytes takes place. This unavoidably has a negative effect on the electron transport system of the plants (Laurie, 1999).

Vital staining with TTC is used as a method of viability measurement and can provide information about whether individual cells are functioning or not (De Ronde and Van Der Mescht, 1997).

In TTC staining, 2.3.5-triphenyltetrazolium salt (TTC) is cleaved to formazan by the "succinate-tetrazolium reductase" system that belongs to the respiratory chain of the mitochondrion (Steponkus and Lanphear, 1967) and is active only in viable cells.

Berridge *et al (*1996) reported that reduction apparently occurs in the mitochondria by the tetrazolium salt accepting electrons from the electron transport chain, whereafter formazan is formed. Therefore the amount of formazan dye formed correlates directly to the number of metabolically active cells in the piece of tissue.

Formazan has a red color, which can be monitored spectrophotometrically. The ability of viable cells to reduce tetrazolium salt appears to be a superior measure of heat tolerance for both experimental use and genotype selection (Chen *et al,* 1982).

Studies indicate that evaluating cultivars using TTC assay showed a great promise for screening drought tolerance.

Laurie (1999) found cultivar differences towards drought tolerance in cowpeas using the TTC assay. He also reported a positive correlation between the tolerant and sensitive plants and the greenhouse experiment.

De Ronde and Van der Mescht (1996) found that a heat tolerant cultivar was characterized by having a higher formazan value over time in stress treatment compared to the control.

CHAPTER 3

3. THE INFLUENCE OF DROUGHT STRESS ON YIELD AND YIELD COMPONENTS

3.1 Introduction

The wheat grain is affected by drought stress and a yield reduction of up to 79 % can be caused when drought stress is imposed sometime prior to ear emergence (Begg and Turner, 1976). In drier environments variation in water supply to the wheat crop can also account for most of the variation in yield (Austin, 1989).

Different literature showed that yield which reflects an integrated effect of many components, is still an important factor to be considered for screening and selecting drought tolerant varieties in crop species. Roy and Murty (1970) reported that when using yield for screening of drought tolerance, visual scores and actual yields agreed in 27 of the 33 cases.

Relative yield performances of genotypes in drought stressed and more favorable environments seems to be a common starting point in the identification of traits related to drought tolerance and the selection of genotypes for use in breeding programs or dry environments (Clarke *et al*, 1992).

The importance of improving drought tolerance is suggested as a desirable breeding objective in wheat crop (Keim and Kronstad, 1979; Clarke *et al,* 1992). This study was therefore aimed to determine the influence of drought stress on yield and yield components of bread wheat genotypes.

3.2 Materials and methods

3.2.1 Materials

A total of 44 breeding lines (Bdl) from the CIMMYT drought screening nursery, and 11 South African bread wheat cultivars were used in this study. The pedigrees of these wheat lines are given in Table 3.1.

Table 3.1 The breeding lines and cultivars used in the study and their pedigrees

	Breeding lines/ cultivars	Pedigrees
1	Bdl-1	OPATA/BOW//BAU/B/OPATA/BOW
2	Bdl-2	BCW//BUC/BUL
3	Bdl-3	DHARWAR DRY
4	Bdl-4	MYNA/VUL//JUN
5	Bdl-5	TUI
6	Bdl-6	SITTA*2//PSN/BOW
7	Bdl-7	BABAX
8	Bdl-8	BJY/COC//PRL/BOW
9	Bdl-9	TJB368.251/BUC//CUPE
10	Bdl-10	TJB368.251/BUC//BUC/CHRC
11	Bdl-11	GEN*3/PVN
12	Bdl-12	CHIL//ALD/PVN
13	Bdl-13	BAVIACORA M 92
14	Bdl-14	PFAU/BOW/VEE#9
15	Bdl-15	GEN/3/GOV/AZ//MUS/4/BUC/MOR/5HD2359/3/
16	Bdl-16	FIRETAIL
17	Bdl-17	ATTILA
18	Bdl-18	TAM200/TRAP#1
19	Bdl-19	CHIL/BUC
20	Bdl-20	IL-75-2264/4CAR//KAL/BB/3/NAC/5/GAA
21	Bdl-21	PSN/BOW//SERI
22	Bdl-22	MIMUS
23	Bdl-23	ND/VG9144//KAL/BB/3/YACO/4/CHIL
24	Bdl-24	MRL/BUC//VEE#7
25	Bdl-25	PASTOR
26	Bdl-26	CHIL//ALD/PVN
27	Bdl-27	CHIL/BUC
28	Bdl-28	OPATA/KILL
29	Bdl-29	RL6043/4*NAC

30	Bdl-30	PRINIA
31	Bdl-31	KARIEGA
32	Bdl-32	SITTA
33	Bdl-33	TIA.2
34	Bdl-34	PASTOR*2/OPATA
35	Bdl-35	PASTOR/OPATA
36	Bdl-36	NESSER
37	Bdl-37	PIK/OPATA
38	Bdl-38	IRENA
39	Bdl-39	URES/JUN/KAZU
40	Bdl-40	OPATA/BOW*2//BUC/MOR
41	Bdl-41	URES/PRL
42	Bdl-42	PFAU/VEE#9
43	Bdl-43	F60314.76/MRL//CN079
44	GamtoosDn	GMTO*4/GANDUM I FASAI
45	Harts	SCH6913/AG.ELPW327/S11-11-A1 113* SHASHI
46	Inia	LR 64/SN64
47	Marico	CMT/MO73IITRM
48	Nantes	SST16* 311T4* 5/S67-336
49	Palmiet	SST3 * 211 SCOUT * 5/AG
50	SST 55	Confidential
51	SST 57	Confidential
52	SST 66	LD398/LD357//ST464/3 * FLAM/4/3 * SST16l
53	SST 825	Confidential
54	T4	LR/N/OB//ANE-3E

3.2.2 Methods

3.2.2.1 Growing conditions

The experiment was conducted in a greenhouse at the University of the Free State. The 54 entries were grown in pots containing three kg of soil under two different moisture levels. An optimum growing temperature was maintained throughout the growing period. A completely randomised block design with three replication was used. The soil was fertilised with NPK fertiliser before planting and after emergence a 10 ml nitrogen solution was given when required.

3.2.2.2 Moisture stress

Two moisture levels were used as a treatment when plants reached the two leaf stage. For the high moisture level (control), a moisture level of 80 % field capacity was maintained throughout the experiment. The 80 % field capacity represented a soil and water mass totaling 3529g without plant mass. For the low moisture level or stress treatment, 50 % field capacity was maintained throughout the experiment. The 50 % field capacity represented a soil and mass of 3331g without plant mass. With this procedure it was assumed that the moisture reached different depths for the two treatments and that the plants at the low moisture level would experience stress earlier than those at the high moisture level. The moisture for both treatments was replenished three times weekly.

3.2.2.3 Measurements

The following plant characters were measured for both moisture levels:

Total Yield (GYP): the total mass of all the kernels of primary and secondary tillers

Primary tiller kernel mass (PKM): the kernel mass of the kernels of the primary tiller spike

Secondary tiller kernels mass (SKM): the total kernel mass of all kernels of all the secondary tillers.

Kernel number per plant (KN): the total kernel number of both primary and secondary tillers

Number of spikes per plant (SN): the number of productive spikes per plant

Spikelet number per spike (SNP): The number of spikelets on the spike of the primary tiller of each plant.

3.2.2.4 Statistical analysis

Analysis of variance was done with Agrobase 2000 software. The heritability was calculated by the Agrobase software, and refers to the contribution of the genotype to variability.

Yield based indices the stress susceptibility index (SSI) (Fischer and Maurer, 1978) and stress tolerance index (STI) (Fernandez, 1992) were calculated using the formula:

$SSI=(Y_{HM}-Y_{LM})/ [Y_{HM\ x}(1-(\ _{LM}/\ _{HM})]$ and

$STI = [(Y_{HM})(YLM)]/\ _{HM}^2$, where

$Y_{HM\ and}\ Y_{LM}$ are the yield of a genotype under high and low moisture level respectively.

$_{HM\ and}\ _{LM}$ are mean yields of all genotypes under high and low moisture levels.

3.3 Results

Highly significant variation was found among genotypes and between moisture levels for all the characters measured (Table 3.2). This indicates that genotypes and the different moisture levels had a significant effect on yield and yield components.

Table 3.2 Analysis of variance for yield and yield components

	GYP	PKM	SKM	KN	SN	SPN
Heritability (%)	31.1	64.3	39.7	47.6	69	86.4
Treatment(M)	258.7**	14.38**	285.13**	520.86**	318.96**	415**
Genotype (G)	2.46**	3.48**	2.61**	4.07**	14.76**	4.56**
M x G	1.7ns	0.26ns	1.57ns	1.26ns	4.32ns	40.9ns

** Significant at p=0.01

ns = non significant

Table 3.3 Summarizes the results found for yield and yield components for the 54 lines and cultivars tested.

3.3.1 Grain yield (GYP)

The highest yielding line under control conditions was Bdl-5, followed by Bdl-25 and T4. The lowest yielding lines under control conditions were Bdl-16 and Bdl-40. The highest yielding cultivar under drought stress conditions was T4 followed by Bdl-36. The lowest yielding lines under these conditions were Bdl-8 and Bdl-1.

Figure 3.1 (Appendix A) illustrates the effect of drought stress on yield for the materials tested. It is clear that there was a significant reduction in yield for most of the lines and cultivars tested under drought stress conditions. The highest reduction in yield was found in Bdl-42 with a reduction of 71.5%. It was followed by Bdl-5 (71.4%) and Bdl-1

(69.9%). Three lines showed an increase in their yields when planted under moisture stress conditions. It was however, not significantly different from their control yields. The three lines were Bdl-16 (38.9% increase), Bdl-17 (15.1%) and Bdl-19 (1.7%). Bdl-40 and Harts gave nearly the same yields under control and stress conditions. Thus, although these five lines and cultivars were not the highest yielders, they were relatively tolerant to drought stress.

The heritability according to the ANOVA was 31.1%. It means that the contribution of the genotypes was very low and that drought stress had a significant influence on the yield per plant.

3.3.2 Yield components

3.3.2.1 Primary tiller kernel mass (PKM)

The highest kernel mass for the primary tiller was found in Bdl-26, followed by Bdl-5 under control conditions. The lowest kernel mass under control conditions was found in Bdl-8 and Bdl-29. The highest kernel mass for the primary tiller under stress conditions was also found in Bdl-26. In spite of the drought stress, Bdl-26 still had the highest primary kernel mass. It was followed by Bdl-37 and Bdl-13. These two lines also had high primary kernel mass under control conditions. It would seem that if a line had a high kernel mass under control conditions, it also had a high primary kernel mass under stress conditions. It was also true for the lines with the lowest primary kernel mass, namely Bdl-8 and Bdl-29.

Figure 3.2 (Appendix A) illustrates the effect of drought stress on the kernel mass of the primary tiller. Not many significant differences (positive or negative) were found between the control and stress conditions. There were significantly positive differences between these conditions in Bdl-3, Bdl-5, Bdl-21, Bdl-28, Bdl-34, Bdl-42, Bdl-43 and Nantes. Significantly negative differences were found in Bdl-16, Bdl-19 and Bdl-40 for the control and stress conditions.

The highest reduction in the kernel mass of the primary tiller under stress conditions was found in Bdl-42 (48.8%), followed by Bdl-3 (40.6 %) and Bdl-5 (35.8%). The highest increase in the primary tiller kernel mass under stress conditions was found in Bdl-16 (59.4%), Bdl-19 (43.2%) and Bdl-17 (33.8%). Sixteen lines and two cultivars (Palmiet and SST 825) had a higher kernel mass under drought stress conditions than that was found in the control conditions.

The heritability according to the ANOVA was 64.3%, that means that the drought stress explained only 35.7% of the heritability of the primary tiller kernel mass. More than half of the heritability could therefore be explained by the genotypes.

3.3.2.2 Secondary tiller kernel mass (SKM)

The highest kernel mass for the secondary tiller was found in Bdl-5, followed by Bdl-36 and T4 under control conditions. The lowest secondary tiller kernel mass was found in Bdl-40 and Bdl-16.

On the other hand, the highest kernel mass for the secondary tiller under drought stress conditions was found in T4. It was followed by Bdl-36. The lowest secondary kernel mass was found in Bdl-8 and Bdl-2. Bdl-36 and T4 showed good performance in secondary kernel mass production under both control and drought stress conditions.

Figure 3.3 (Appendix A) shows the effect of drought stress on the kernel mass of the secondary tiller. Significant secondary tiller kernel mass reductions from control to drought stress conditions were found for most of the materials tested. The highest reduction was observed in Bdl-5 (79.6%). This was followed by Bdl-1 (78%) and Bdl-34 (77.3%). Harts (9.2%) showed the lowest reduction. Two lines, Bdl-16 (39%) and Bdl-17 (11.86%) had a higher kernel mass under drought stress than the control conditions.

The heritability estimates of the secondary tiller kernel mass was 39.7% indicating that drought stress had a significant influence on the kernel mass of the secondary tillers.

3.3.2.3 Number of kernels (KN)

The highest kernel number per plant was found in Bdl-36 under control conditions. It was followed by the cultivar Palmiet and the lines Bdl-42 and Bdl-44. Bdl-20 and Bdl-16 produced the least number of kernels per plant under the control conditions. The highest kernel number per plant under the drought stress conditions was obtained from Bdl-36, followed by Harts and T4. Line Bdl-6 had the lowest number of kernels per plant. Bdl-36 thus had the highest kernel number per plant under both conditions.

Figure 3.4 (Appendix A) illustrates the effect of drought stress on the number of kernels per plant. A reduction in kernel number per plant from control to drought stress conditions was observed in all the lines and cultivars tested. On average the kernel number per plant was reduced more than two fold under drought stress conditions compared to the control moisture level. The maximum reduction was found in Bdl-6 (68.9%) and Bdl-1 (68.8%). Lines Bdl-17 (22.3%) and Bdl-46 (24.6%) showed a minimum reduction in kernel number per plant from control to drought stress conditions.

The heritability estimates of kernel number per plant was 47.6% indicating that the drought stress explained 52.4% of the variability of the number of kernels per plant.

3.3.2.4 Spike number per plant (SN)

Under control conditions T4 had the highest number of spikes per plant followed by Bdl-36 and Bdl-8. The lowest number of spikes per plant was found in Inia.

On the other hand, under drought stress conditions T4 again produced the highest number of spikes per plant. It was followed by Bdl-36 and SST-66. Bdl-6 and Bdl-1 had the lowest number of spikes per plant. The performance of T4 and Bdl-36 were found to be superior under both control and drought stress conditions in the number of spikes per plant. This suggests that the ability to produce a high number of spikes per plant is because of the genes of the plant and not because of the environment.

Figure 3.5 (Appendix A) shows the effect of drought stress on the number of spikes per plant. All the lines and cultivars showed a reduction in spike number per plant from control to drought stress condition with an average reduction of more than 56%. The highest reduction was observed in Bdl-1 (72%) and Bdl-8 (67.6%), whereas Bdl-40 (6.6%) showed the lowest reduction in spike number per plant. SST-66 produced the same number of spikes per plant at both control and drought stress conditions.

The heritability estimates for spike number per plant was 69%. It means that the genotypes contribute 69 % of the variation for spike number per plant.

3.3.2.5 Spikelet number per spike (SPN)

The highest spikelet number per spike under high moisture or control conditions was found in Gamtoos DN, followed by Bdl-12 and Bdl-39. The lowest SPN under the same conditions was found in Bdl-20 and Bdl-31.The highest spikelet number per plant under drought stress conditions was found in Bdl-2 followed by Harts, whereas Bdl-36 and Bdl-31 gave the least number of spikelets per spike. Bdl-31 had the least spikelets per spike under both conditions.

Figure 3.6 (Appendix A) illustrates the effect of drought stress on the spikelets per spike. The results revealed a reduction from control to drought stress conditions for most of the lines and cultivars tested. The average reduction from control to stress was only 3.89 %. Three lines Bdl-8, Bdl-19 and Bdl-31 and two cultivars Inia and SST-66 showed an increase in spikelet number per spike when planted under drought stress

conditions. Lines Bdl-27 and Bdl-32 and cultivar SST-825 produced exactly the same number of spikelets per spike.

The heritability estimates of spikelets number per spike was very high (86.4%) suggesting that about 86.4 % of the variation is explained by the genotypes.

3.3.3 Stress sensitivity index

The yield-based stress susceptibility index (SSI) and stress tolerance index (STI) calculated for all the genotypes are presented in Table 3.3. Genotypes showed significantly high variation only for STI. The mean SSI and STI values were 0.227 and 0.541 for all the lines and cultivars respectively.

Values >1 for STI and <1 for SSI values indicate a high level of tolerance to moisture stress, whereas values <1 for STI values and >1 for SSI-values indicates high susceptibility (Fischer and Maurer, 1978).

Bdl-23, Bdl-17 and Bdl-5 had the highest and Bdl-22 had the lowest SSI values. The STI values were above one only for T4 (1.17) and Bdl-36 (1.07). The lowest STI value was for Bdl-8. Based on the SSI value, Bdl-23 Bdl-17 and Bdl-5 could be considered as susceptible lines and Bdl-22 as a tolerant line. Based on the STI values, T4 and Bdl-36 are tolerant to moisture stress, while Bdl-16 and Bdl-20 are susceptible.

Table 3.3 Yield and some agronomic characteristics of bread wheat lines/cultivars grown at both high (H) and low (L) moisture levels

		GYP		PKM		SKM		KN		SN		SNP		SSI	STI
		H	L	H	L	H	L	H	L	H	L	H	L		
1	Bdl-1	10.3	3.1	1.7	1.2	8.64	1.90	287	89	8	2	77	75	0.32	0.35
2	Bdl-2	7.9	3.4	2.0	1.7	5.85	1.71	317	108	7	4	86	88	0.26	0.29
3	Bdl-3	12.1	4.0	1.9	1.1	10.2	2.89	206	102	10	5	86	80	0.29	0.49
4	Bdl-4	9.6	4.2	2.2	1.9	7.43	2.29	338	128	9	4	94	82	0.31	0.40
5	Bdl-5	15.4	4.4	2.8	1.8	12.6	2.57	376	121	11	5	76	68	0.32	0.71
6	Bdl-6	10.6	4.3	1.8	2.1	8.8	2.47	284	88	8	3	84	78	0.24	0.42
7	Bdl-7	12.5	5.2	2.4	2.0	10.2	3.10	312	133	10	5	85	76	0.24	0.65
8	Bdl-8	6.1	2.9	1.0	1.0	5.2	1.84	193	92	12	4	68	69	0.26	0.18
9	Bdl-9	10.6	3.8	1.7	1.1	8.71	2.71	326	118	10	5	87	74	0.29	0.43
10	Bdl-10	7.6	4.7	1.6	1.5	5.93	3.23	300	154	7	4	85	79	0.15	0.36
11	Bdl-11	11.4	7.0	2.3	2.0	9.11	4.97	326	174	10	5	82	80	0.15	0.77
12	Bdl-12	10.9	5.5	2.0	2.1	9.07	3.41	398	149	8	5	98	84	0.23	0.65
13	Bdl-13	8.9	5.5	2.3	2.4	6.63	3.11	292	144	7	5	89	78	0.17	0.51
14	Bdl-14	10.3	4.1	2.3	1.7	8.05	2.40	368	104	8	5	86	81	0.24	0.41
15	Bdl-15	8.7	5.7	1.6	1.5	7.09	4.20	245	183	10	5	79	75	0.15	0.53
16	Bdl-16	3.3	5.4	1.3	2.0	2.01	3.33	136	102	5	4	77	75	0.28	0.20
17	Bdl-17	6.2	7.3	1.4	1.9	4.83	5.48	244	190	9	6	75	70	0.51	0.45
18	Bdl-18	11.9	5.7	1.6	1.8	10.3	3.91	330	156	12	7	89	80	0.20	0.71
19	Bdl-19	5.8	5.9	1.5	2.1	4.33	3.75	201	151	6	4	69	72	0.30	0.33
20	Bdl-20	5.9	3.8	1.6	1.7	4.31	2.09	160	92	6	4	67	61	0.15	0.23
21	Bdl-21	10.6	4.9	2.5	1.8	8.42	3.02	324	144	9	6	90	79	0.18	0.56
22	Bdl-22	8.3	6.9	1.7	1.6	6.67	5.07	310	103	10	6	83	77	0.11	0.59
23	Bdl-23	7.3	5.8	2.2	2.3	5.08	3.47	294	139	8	4	90	81	0.66	0.44
24	Bdl-24	8.4	4.8	2.3	1.9	6.11	2.89	265	128	7	4	90	78	0.16	0.43
25	Bdl-25	14.2	5.7	2.5	2.0	11.7	3.75	369	164	12	5	88	76	0.27	0.86
26	Bdl-26	12.7	6.1	3.3	2.8	9.39	3.28	287	117	7	4	97	82	0.22	0.80
27	Bdl-27	6.7	4.8	1.7	2.0	5.01	2.78	228	150	8	3	74	74	0.14	0.37
28	Bdl-28	10	5.7	2.1	1.4	7.92	4.36	321	156	9	7	83	77	0.17	0.60
29	Bdl-29	6.1	3.7	1.2	1.4	4.91	2.29	256	112	8	4	74	65	0.18	0.25
30	Bdl-30	9.4	5.4	1.8	1.8	7.64	3.57	327	126	9	4	85	79	0.18	0.30
31	Bdl-31	10.8	5.1	1.4	1.1	9.34	4.01	256	117	10	6	56	58	0.23	0.55
32	Bdl-32	5.8	4.1	1.7	1.7	4.11	2.35	228	121	7	4	78	78	0.16	0.25
33	Bdl-33	9.9	4.7	1.6	1.6	8.26	3.63	320	136	11	6	82	79	0.22	0.48
34	Bdl-34	11.8	3.8	2.6	1.7	9.24	2.10	338	132	10	4	81	77	0.29	0.48
35	Bdl-35	12.4	4.7	1.9	1.5	10.5	3.13	310	148	11	5	84	74	0.25	0.55
36	Bdl-36	13.3	7.7	1.5	1.3	11.8	6.40	412	214	12	8	69	59	0.19	1.08
37	Bdl-37	7.5	5.3	2.5	2.4	5.03	2.85	250	152	6	4	89	81	0.14	0.43
38	Bdl-38	8.3	4.8	1.8	2.1	6.42	2.73	262	150	7	4	89	76	0.22	0.40
39	Bdl-39	8.5	5.2	1.8	1.7	6.39	3.49	259	123	6	4	98	85	0.15	0.48
40	Bdl-40	5.3	4.9	1.4	2.0	3.92	2.93	176	125	5	5	80	78	0.12	0.27
41	Bdl-41	9.6	7.5	1.9	2.0	7.72	5.48	318	186	9	6	87	80	0.31	0.72
42	Bdl-42	12.3	3.5	2.5	1.3	9.85	2.26	406	126	9	5	87	71	0.32	0.66
43	Bdl-43	7.8	4.2	2.0	1.3	5.79	2.31	270	126	8	5	83	75	0.26	0.35
44	Gamtoos DN	12.0	6.6	2.3	2.0	9.69	4.62	406	159	9	6	102	83	0.17	0.85
45	Harts	7.8	7.5	1.9	1.5	5.87	5.33	303	202	6	5	89	86	0.16	0.64
46	Inia	6.0	4.1	1.9	1.5	4.14	2.56	185	139	5	4	72	73	0.14	0.26
47	Marico	13.4	6.4	2.1	1.8	11.2	4.60	320	162	8	5	81	74	0.20	0.94
48	Nantes	9.9	4.5	2.2	1.5	7.71	2.97	278	121	9	4	75	66	0.25	0.49
49	Palmiet	12.5	7.1	1.9	2.0	10.6	5.10	406	195	11	6	79	77	0.20	0.95
50	SST 55	11.5	6.3	2.6	2.3	8.9	4.08	324	139	7	4	78	74	0.19	0.78
51	SST 57	11.2	5.4	2.2	1.7	9.02	3.65	354	144	7	4	85	80	0.23	0.59
52	SST 66	10.1	5.5	2.2	1.9	7.91	3.53	291	116	7	7	74	80	0.20	0.63
53	SST 825	10.3	6.1	2.1	2.2	8.15	3.85	343	164	9	4	80	80	0.16	0.70
54	T4	13.6	8.2	1.8	1.7	11.8	6.48	380	197	17	10	73	67	0.14	1.17
55	LSD P=0.05	4.19	2.22	0.64	0.59	3.87	1.93	107	51	3.1	2.1	7.91	9.34	0.22	0.34

3.3.4 Phenotypic correlation

Phenotypic correlations were made within and between characters for the two moisture levels to determine the influence of the characters on each other. The correlation coefficients and its significant level of each character within the two moisture levels are given in Table 3.4.

Table 3.4 Correlation coefficients and significance level of all characters of wheat genotypes grown at low moisture level (upper diagonal) and high moisture level (lower diagonals).

	Yield	PKM	SKM	KN	SN	SPN
Yield		0.5215**	0.9677**	0.7888**	0.4985**	0.2261**
PKM	0.5594**		0.3281**	0.3409**	-0.0050 ^{ns}	0.3645**
SKM	0.9901**	0.4416**		0.7802**	0.5654**	0.1406 ^{ns}
KN	0.7658**	0.3956**	0.7623**		0.3988**	0.2060**
SN	0.5821**	0.0756 ^{ns}	0.6198**	0.4719**		-0.0670 ^{ns}
SPN	0.1615*	0.3931**	0.1081 ^{ns}	0.2923**	-0.15*	

** p =0.01
*p =0.05
ns = non significant

At high moisture levels, yield was correlated positively and significantly ($p<0.05$) with all the characters. Especially, the associations of yield with secondary tiller kernel mass ($r=0.9901$) and kernel mass ($r=0.7658$) were strong compare to the other characters. The correlation of yield with spikelet number per spike ($r=0.1615$) was weak (Table 3.4).

At low moisture levels, yield was still correlated positively and significantly ($p<0.05$) with all the characters. A strong association was observed with secondary tiller kernel

mass (r=0.9677) and kernel number (r=0.7888). A low correlation coefficient was observed with spikelet number per spike (r=0.2261).

Primary kernel mass (PKM) had highly significant, positive correlation coefficients with all the characters except for spike number at both moisture levels. The association of primary kernel mass with spike number was negative (insignificant) at low moisture (r=-0.0050) level and it showed a weak, but positive (r=0.0756) correlation at high moisture level.

Secondary kernel mass (SKM) was positively and significantly correlated with all the characters, except for spikelet number per spike which showed non-significant association at both high and low moisture levels.

Kernel number (KN) was positively (highly significant) correlated with all the characters at both moisture levels. It also had a strong association with yield and secondary tiller kernel mass.

Spike number per plant (SN) was correlated positively and highly significantly with all the characters but showed a negative correlation (non-significant) to primary tiller kernel mass at low moisture level. At high moisture level, spike number correlated positively and highly significantly to yield, secondary tiller kernel mass and spike number. It showed a positive (non-significant) and a negative (significant) association with primary tiller kernel mass and spikelet number per spike respectively.

Spikelet number per spike (SPN) was correlated positively and highly significantly with yield, primary tiller kernel mass, secondary tiller kernel mass and kernel number and it was negatively correlated with spike number at both high and low moisture levels.

3.4 Discussion

At optimum moisture levels, Bdl-5, Bdl-25 and T4 gave the highest grain yield respectively. Under stress conditions, T4, Bdl-6 and Harts were the highest yielders. This implies that selection for genotypes that perform well in non-stress environments may not necessarily identify drought tolerant genotypes. Laing and Fischer (1979) found that direct selection in stress environments would decrease yield in non-stress environments. Blum (1985) and Nasir *et al* (1992) reported similar findings, that stress causes a reduction in the genetic variance and heritability for yield, which consequently limits selection efficiency for yield under stress conditions.

Allen *et al* (1978) showed that the relative magnitude of genotypic variance in different environments is crop specific. For instance in wheat, the genotypic variance in favorable environments was several times greater than in unfavorable environments.

The reduction in yield obtained in this study was very high compared to the 50% yield reduction reported by Fisher and Maurer (1978). However, these results were comparable with the findings of Austin (1989) that moisture stress can cause 70% to 80% reduction of yield in wheat.

The yield reduction reported in this study was probably due to the effect of moisture stress on the formation of yield ascribed to the growth of organs and their final yield (Blum, 1998). Labuschage (1989) also reported that yield reduction could be caused due to a reduction of available photosynthate due to a reduction of translocation and photosynthesis.

Performances of lines Bdl-5 and Bdl-25 were substantially reduced under the stress condition as a result of the severe impacts of the stress on primary and secondary kernel mass (Fig. 4.2 and 4.3, Appendix A) and kernel number per plant (Fig. 4.4, Appendix A). It is not uncommon to find a reduction of grain yield due to a reduction of kernel weight and/or kernel number per plant (Blum, 1996).

Bdl-5, Bdl-25, T4 and Bdl-36 were found among the top yielders and had high secondary tiller kernel mass (SKM) and kernel number (KN). This indicates that these three characters have a strong and positive correlation and improvement of grain yield could be achieved by selecting genotypes based on secondary tiller kernel mass (SKM) and kernel number (KN).

The best performances of T4, Bdl-36 and Bdl-45 (Fig. 4.1), were due to either primary kernel mass or secondary kernel mass (Fig. 4.2 and 4.3, Appendix A) and kernel number (Fig. 4.4, Appendix A). Strong positive and significantly high ($p<0.05$) phenotypic correlations were observed between grain yield and primary kernel mass ($r=0.52$), secondary kernel mass ($r=0.97$) and kernel number per plant ($r=0.78$), respectively, at low moisture level. This indicates that yield under stress conditions is primarily

governed by primary tiller kernel mass, secondary tiller kernel mass and kernel number per plant. Genotypes, which are able to maintain a high number of secondary tillers till maturity, will perform the best under stress conditions.

The results of this study also showed that moisture stress has a significant impact on primary tiller kernel mass, secondary tiller kernel mass and kernel number per plant. Richard (1982) reported a reduction in kernel mass due to moisture stress during grain filling.

Similar studies also found a small reduction of primary kernel mass compared to secondary tiller mass. Oosterhuis and Cartwright (1983) noted that secondary and tertiary tiller kernel mass were reduced within the spikelet, since they filled later than the primary tiller kernel mass. The kernels of the secondary tiller also filled later than the kernels of the primary tiller. Florets and spikelets tended to die at the terminal ends of the spikes. Labuschagne (1989) also supported this idea that the last formed spikes were the most sensitive to moisture stress.

The results of this study also indicated that spike number per plant had a negative association with spikelet number per spike. In similar findings, Moustafa *et al* (1996) explained that the low number of spikelets per spike compensated for the higher number of spikes per plant of a genotype.

Oosterhuis and Cartwright (1983) noted that the development of the wheat spike is extremely sensitive to water stress applied either at the late vegetative and transition phases or during the late internode elongation immediately prior to spike emergence.

It also noted that Bdl-36, Bdl-5 and T4 were found to be high yielders and performed well for most of the yield components. These lines were also found to have the lowest spikelet number per spike under both control and stress conditions. This was further explained by a negative and weak association of spikelet number per spike with most of the yield and yield components (Table 3.4).

Bdl-36 was found to be the highest in kernel number per plant under both control and moisture stress conditions indicating that the line probably has a high level of tolerance to pre-anthesis moisture stress. Innes and Quarrie (1987) reported that pre-anthesis moisture stress has a significant effect on kernel number per plant of wheat genotypes.

3.5 Conclusions

The results of this study showed that moisture stress seriously affected grain yield and yield components of wheat genotypes.

The results also showed that no genotype by moisture level interactions were found for most of the characters studied except grain yield per plant and secondary tiller kernel mass.

Bdl-5, T4, Bdl-5 and Bdl-25 were found to be tolerant and performed well at both optimum and low moisture levels, whereas Inia, Bdl-40, Bdl-16 and Bdl-20 were sensitive to moisture stress.

Under both high and low moisture levels all characters correlated positively and highly significantly with grain yield. However, the correlations of secondary tiller kernel mass and kernel numbers per plant were found to be high compared to the other characteristics and the correlation of grain yield with spikelets per spike was relatively low.

Breeding for moisture stress needs the considerations of most yield components for improving the final yield. Secondary tiller kernel mass and kernel numbers per plant are particularly important characters for improving grain yield of wheat crop.

CHAPTER 4

4. EFFECT OF DROUGHT STRESS ON SEEDLING SURVIVAL AND RECOVERY OF WHEAT

4.1 INTRODUCTION

Wheat is one of the most important cereal crops grown and consumed worldwide. Its cultivation extends to the semi-arid areas where drought is a major production constraint.

Recent reviews indicated available knowledge on different aspects of drought tolerance in crop plants and ways and means to minimise yield losses due to drought (Winter *et al,* 1988).

Major differences among and within crop species have also been reported in wheat (Sapra *et al,* 1991). However, the success in breeding for drought tolerance has not been as pronounced as for the other traits (Singh *et al,* 1999).

This is partly due to the lack of simple, cheap and reliable screening methods to select drought tolerant plants from among a large number of plants and due to the complexity of factors involved in drought tolerance.

Several methods have been used to estimate drought tolerance including physiological and morphological characterization of crop species. Field screening is difficult due to uncertain rainfall patterns and different temperatures in the dry seasons (Singh *et al,* 1999).

Box screening of varieties at seedling stage is a quite reliable method to identify drought tolerance. It is more practical, because of the ease of handling, possibility of controlled environment and ability to screen large number of lines.

Singh *et al* (1999) confirmed the suitability of box screening techniques for drought tolerance and indicated varietal differences for plants response to drought stress in cowpea. They also indicated that the phenomenon responsible for drought tolerance in seedling stage is also manifested at the reproductive stage.

Singh *et al* (1991) in cowpea and Winter *et al* (1988) in wheat have reported the reliability and the importance of screening genotypes at the seedling stages for shoot drought tolerance without the effects of the roots.

This study aimed to screen and discriminate among different bread and durum wheat genotypes for drought tolerance at the seedling stage.

4.2 Materials and methods

4.2.1 Materials

Ten durum and 10 bread wheat genotypes obtained from Ethiopia and CIMMYT were used for this study. The name and sources of these materials are given in Table 4.1(for bread wheat) and Table 4.2 (for durum wheat).

Table 4.1 Bread wheat genotypes used to screen for seedling survival

Genotypes	Sources
Bdl-8	CIMMYT
Bdl-16	CIMMYT
Bdl-36	CIMMYT
Bdl-20	CIMMYT
Bdl-41	CIMMYT
Et-13	Ethiopia
Dereselign	Ethiopia
HAR-604	Ethiopia
HAR-1685	Ethiopia
Israel	Ethiopia

Table 4.2 Durum wheat genotypes used screen for seedling survival

Genotypes	sources
Boohai	Ethiopia
Kilinto	Ethiopia
Tob-66	Ethiopia
Foka	Ethiopia
Cadu-17	Ethiopia
Fetan	Ethiopia
Cocorit-71	Ethiopia
Gerardo	Ethiopia
Quami	Ethiopia
LD-357	Ethiopia

4.2.2 Methods

A method of Singh *et al* (1999) was adapted to conduct this study. The bread and durum wheat lines and cultivars were screened for drought tolerance at the seedling stage in wooden boxes of 130 cm length, 65 cm width and 15 cm depth made of 2.5 cm thick wooden planks. The boxes were kept in a glasshouse of the University of the Free State at optimum temperatures. The boxes were lined with polythene sheets and filled with sieved red soil. The boxes were filled to a 12 cm depth, leaving 3 cm space at the top for

watering. The polythene lining along the sides and bottom of the boxes ensured even distribution of water in the boxes. Equidistant holes were made in straight rows to plant each genotype. Each box contained one row of each of the 10 durum and bread wheat varieties for one replication. Treatments were arranged in two randomized design with two blocks. The boxes were watered daily using a small watering can until they reached the two to three leaf stages, after which watering was terminated. Following this, a daily count of wilted plants in each variety was made until all plants of the susceptible lines started dying.

Watering was then resumed to examine regeneration percentage for each variety. Based on the days taken to wilting and percentage recovery, the varieties were rated as drought tolerant and drought susceptible.

4.2.3 Statistical analysis

The data was subjected to analysis of variance using Agrobase (2000) statistical software.

4.3 Results

Bread wheat

Table 4.3 gives the analysis of variance for percentage of wilting at different days. The percentage recovery is also shown. The variation among genotypes in percentage wilting was highly significant for days seven and nine ($p<0.0.1$) and significant ($p<0.05$) for 11 days. The percentage recovery among bread wheat genotypes was also highly significant ($p<0.01$).

Table 4.3 Analysis of variance for percentage wilting of different days and recovery percentage for 10 bread wheat genotypes

Source of variation	df	D7	D9	D11	% recovery
Blocks	1	11.3	1048.4	147.9	127.5
Genotypes	9	150.3**	737.8**	697.5*	267.3**
Residuals	9	12.1	109.2	159.6	29.3

** significant at p=0.01
* significant at p=0.05

The first symptoms of wilting were found seven days after withholding of the water. Table 4.4 gives a summary of the results found for the materials tested.

Table 4.4 Wilting and recovery percentage of tested bread wheat lines and cultivars

Genotypes	Day 7	Day 8	Day 9	Day 10	Day 11	% recovery
Dereselign	14.6	14.6	43.8	43.8	75.0	85.4
Israel	0	0	16.7	16.7	46.7	90
HAR-604	0	0	20.5	20.5	58.6	82.9
HAR-1685	0	0	30	30	73.4	77.5
Bdl-41	21.6	21.6	58.5	58.5	86.4	67.6
Et-13	0	0	20.6	20.6	50.5	93.3
Bdl-36	8.7	8.7	47.5	47.5	73.4	82.6
Bdl-16	17.8	17.8	72.2	72.2	100	58.3
Bdl-20	8.3	8.3	38.3	38.3	56.1	74.3
Bdl-8	18.3	18.3	61.9	61.9	96.5	63.5
LSD (p=0.05)	6.4	6.4	19.2	19.2	23.2	9.9

Day 7

The highest percentage of wilting was found in Bdl-41, where 21.6% of the plants started to wilt. It was followed by Bdl-8 (18.3%), Bdl-16 (17.8%) and Dereselign (14.6%). There was a significant difference in the percentage of wilting found in Bdl-41 and Dereselign.

Bdl-48, however, did not differed significantly from Bdl-8 and Bdl-16. There was also no significant difference between Dereselign , Bdl-36 and Bdl-24. The percentage of wilting of all these materials were significantly higher than that found in Israel, HAR-604, HAR-1685 and Et-13, where no wilted plants were visible.

Day 8

The percentage of wilting in day 8 was the same as the results found in day 7.

Day 9

Bdl-16 had the highest percentage of wilted plants, namely 72.2%. It was, however, not significantly different from Bdl-8 (61.9% wilted plants) and Bdl-41 (58.5% wilted plants). The percentage of wilting found in these three lines did, however, differ significantly from the other materials tested, except Bdl-36 and Dereselign. The lowest percentage of wilting was in Israel (16.7%).

Day 10

The result of day 10 was exactly the same as the results found for day 9.

Day 11

At day 11 almost all lines and cultivars showed a high level of wilting except for cultivars Israel and Et-13 where 50 % of the plants showed no wilting symptoms. The highest percentage of wilting was found in line Bdl-16 (100% plants wilted) followed by the lines

Bdl-8 (96.5%) and Bdl-41 (86.4%). There was no significant difference between these three lines. Bdl-16 did, however, differ significantly from the other materials tested.

Recovery of the plants

There was variation between the materials tested during the recovery fase. The highest level of recovery was found in cultivar Et-13 (93.3%) followed by Israel (90%). Although these two lines showed a high level of recovery, they did not vary significantly from Dereselign, HAR-604 and Bdl-36. The lowest percentage of recovery was found in Bdl-16 (58.3%) and Bdl-8 (63.5%). All the genotypes showed more than 50% recovery.

Durum wheat

Highly significant variation ($p<0.01$) was found among cultivars tested at all the different days of withholding moisture. No significant ($p>0.05$) variation was obtained for percent recovery of plants after moisture stress (Table 4.5).

Table 4.5 Analysis of variance for percentage of wilting and recovery in 10 durum wheat cultivars.

Source of variation	df	Day seven	Day nine	Day eleven	% recovery
Blocks	1	1.24	188.49	189.73	91.17
Genotypes	9	24.54**	550.83**	869.22**	107.5ns
Residuals	9	1.79	30.55	118.45	55.25

** Significant at p=0.01

The first symptoms of wilting in durum wheat were visible seven days after moisture stress.

Table 4.6 Wilting percentage and recovery of tested durum wheat cultivars.

Genotypes	Day 7	Day 8	Day 9	Day 10	Day 11	% recovery
Kilinto	0	0	24.3	24.3	51.9	79.1
Quami	0	0	27.9	27.9	55.3	69.2
Foka	0	0	35.7	35.7	83.8	80.4
Cadu-17	6.7	6.7	63.4	63.4	100	65.2
LD-357	9.6	9.6	58.4	58.4	93.4	79.3
Tob-66	0	0	20.1	20.1	38.1	89.8
Fetan	0	0	21.5	21.5	48.6	85.7
Gerardo	0	0	24.1	24.1	39.3	89.3
Cocorit-71	0	0	12.2	12.2	37.1	90.4
Boohai	0	0	32.3	32.3	57.3	90.2
LSD (p=0.05)	2.5	2.5	10.1	10.1	19.9	13.6

Day 7

At day seven, the percentage of wilting for cultivars was relatively low. Only two cultivars showed wilting symptoms. The two cultivars were Cadu-17 and LD-357 with a percentage wilting of 6.7% and 9.6% respectively. The remaining eight cultivars had no visible wilting symptoms (Table 4.6).

Day 8

The same results were found as for day 7.

Day 9

Differences between cultivars became more visible and pronounced at day 9. The highest percentage of wilting was again found in Cadu-17 and LD-357 where 63.4% and 58.4 %

of the plants wilted. These two cultivars differed significantly in percentage wilting from all the materials tested. The lowest percentage of wilted plants was observed in Cocorit-71, where only 12.2 % of wilted plants were visible. The majority of the cultivars had 20 to 35.7% wilted plants.

Day 10

Day 10 had the same results as day 9.

Day 11

After 11 days of no moisture in all the cultivars more than 50% plants showed wilting symptoms, except four cultivars, namely Tob-66, Fetan, Gerardo and Cocorit-71. The highest percentage of wilting was found in Cadu-17 where 100% of the plants wilted. It was followed by LD-357 (93.4%) and Foka (83.8%). These three cultivars showed a significantly higher percentage of wilting than the remaining cultivars. The lowest percentage of wilting was observed from Cocorit-71 (37.1% plants wilted) and Tob-66 (38.1% wilted plants). The remaining cultivars had a medium level of reaction to moisture stress.

Recovery of the plants

Durum wheat cultivars showed a non significant variation in the percent recovery after the termination of moisture stress. High level of recovery was found in Cocorit-71 (93.3%). It was followed by Boohai (90.2%) and Tob-66 (89.8%). The lowest amount of recovery was obtained from the cultivars Cadu-17 (65.2%) and Quami (69.2%). In the

rest of the cultivars, more than 79% of the plants surivived, showing that the cultivars have a high potential to revive after the termination of drought stress.

4.4 Discussion

Highly significant variations were found between the materials tested for both bread and durum wheat in their response to moisture stress. This shows that the materials responded differently and based on their percentage of wilting and recovery, susceptible and tolerant materials could be identified at the seedling stage. Stanca (1987) reported in their study in barley genotypes that genetic variability for drought tolerance could be identified at different stages of growth (seed, seedling and adult plants) and possessed some genetic mechanisms for recovering their normal growth rate after moisture stress.

The results of this study showed that the effect of moisture stress was first seen in both cases at about seven days of termination of moisture. Wilting of plants was first observed on the lower leaves and progressively the upper leaves became wilted.

Among the bread wheat lines and cultivars, Bdl-16, Bdl-8 and Bdl-41 showed the highest percentage of wilted plants and it can be concluded that they are the most susceptible to drought stress. They also started to wilt faster than the other genotypes after only seven days. Dereselign and Bdl-36 had the same reaction to drought stress. They also started to wilt after seven days and most of the plants were wilted after 11 days. It can also be concluded that they are more susceptible to drought stress.

Israel, HAR-604 and Et-13 showed no reaction after seven days and Bdl-20 had only a small amount of wilted plants. It can therefore be concluded that these four genotypes are more tolerant than the others tested, as after 11 days the percentage of wilted plants were also lower.

HAR-1685 was able to withstand the drought stress longer than the susceptible cultivars. However, after 11 days of drought stress, the amount of wilted plants was very high. It could be that HAR-1685 has some genetic mechanisms that enables it to withstand drought stress longer, but after prolonged stress it became wilted.

If one looks at the percentage of recovery, it can again be concluded that Bdl-16, Bdl-8 and Bdl-41 were the most susceptible as they had the lowest percentage of recovery. Although Dereselign and Bdl-36 were also thought to be susceptible, they were able to recover when water was again applied.

Israel, HAR-604 and Et-13 that were classified as the most tolerant, were also able to recover better. Bdl-20 had a lower percentage of recovery than these three. If one looks at HAR-1685, it is clear that although the percentage of wilted plants after 11 days was very high, it was able to recover.

The level of variation among genotypes became very small at day 11 when the severity of the stress increased and where most of the genotypes showed more than 50 % wilting (Table 4.4 and 4.5) in both bread and durum wheat genotypes.

Of the 10 bread wheat genotypes, Israel, HAR-604, HAR-1685 and Et-13 showed no sign of wilting at day seven. On the other hand, among durum wheat cultivars, Cadu-17 and LD-357 were found to be the most susceptible. Although the level of wilting seems to be very low, they started to show wilting symptoms after seven days of terminating moisture, where plants of the remaining cultivars continued to survive without any wilting symptoms.

Cocorit-71 had only a low level of wilting after nine and 11 days of moisture stress. The other three cultivars, Tob-66, Fetan and Gerardo also showed significantly small amounts of wilted plants even after 11 days of moisture stress. It can therefore be concluded that these four cultivars are more tolerant to drought stress than the others. The remaining cultivars have a better level of tolerating moisture stress and can be classified as intermediate to withstand moisture stress.

Based on the percentage of recovery, it can be concluded that Cadu-17 and Quami were the most susceptible. Although LD-357 was considered susceptible, it showed a high level of recovery after resumption of watering. Cocorit-71, Boohai, Tob-66, and Fetan were found to be tolerant and had a high potential for recovery when watering was resumed.

This study also indicated that the variations among wheat cultivars in their recovery after termination of stress were relatively low. The reason is probably that either wheat

cultivars in general have a high potential to recover after the stress period is terminated or the stress level that was imposed might not be sufficient to discriminate between cultivars. This was particularly manifested by durum wheat genotypes that exhibited no significant variation among the genotypes.

In conclusion the results indicated, the use of wooden box techniques for screening genotypes for tolerance to drought at the seedling stage is important and reliable as it insures uniform distribution of the stress level to the genotypes tested. It would be more beneficiary for preliminary screening of large number of materials for drought tolerance at the seedling stage.

CHAPTER 5

5. EVALUATION OF BREAD AND DURUM WHEAT GENOTYPES FOR DROUGHT STRESS BASED ON PROLINE, CELL MEMBRANE STABILITY (CMS), AND TRIPHENYL TETRAZOLIUMCHOLORIDE (TTC) TESTS

5.1 INTRODUCTION

Genetic differences for drought tolerance between cultivars have been found in wheat. However, genetic improvement of drought resistance in crop plants requires identification of relevant drought resistance mechanisms and development of a suitable methodology for their measurement in large breeding populations.

Improvement of drought resistance based on selection for yield under conditions of water deficit is difficult, due to the particularly low heritability of yield in such conditions. Screening based on physiological processes such as proline content (Laurie, 1999), cell membrane stability test (CMS) (Blum and Ebercon, 1981) and triphenyl tetrazolium chloride test (TTC) (Laurie, 1999), in response to moisture stress can be considered to be reliable methods in addition to field screening of genotypes for moisture stress. Plant growth is characterized by a wide array of anabolic and catabolic processes that are driven primarily by endogenous factors but can be strongly influenced by adverse environmental conditions. When environmental stress is imposed on a plant, several physiological responses are induced or accelerated.

The use of physiological responses of plants and their relationship with productivity under water deficit can help the breeder to improve drought resistance.

It would therefore be important to determine the drought tolerance of the lines using some laboratory screening methods to supplement the data obtained through field screening so that breeders have the data to make the right decision for their breeding program.

This study was therefore aimed to determine the varietal differences of bread and durum wheat genotypes in response to moisture stress using some laboratory techniques such as free proline accumulation, CMS and TTC.

5.2 Materials and Methods

5.2.1 Materials

Ten bread wheat and 10 durum wheat genotypes were used for this study. The same materials that were used for the wooden box technique (Chapter 4) were used in this study. Please refer to Table 4.1 (bread wheat genotypes) and Table 4.2 (durum wheat genotypes) for the names, and origins of the materials.

5.2.2 Methods

5.2.2.1 Growing conditions

The materials used in this study were planted in pots containing 3kg of soil in a glasshouse with three replications each. Five seeds were planted per pot and were thinned to three after emergence. An optimum temperature was maintained in the glasshouse throughout the growing period.

Proline content

5.2.2.1.1 Inducing drought

Three weeks after emergence of the seedlings, a drought stress treatment was induced by withholding water from all the cultivars at the optimum temperature. The control plants were watered continuously at the optimum temperature regime.

5.2.2.1.2 Leaf sampling

Young leaves were harvested every five days from the stressed and control plants. The leaves were quick-frozen with liquid nitrogen and freeze-dried immediately after sampling. Three replicates were analyzed for each cultivar at each time interval.

5.2.2.1.3 Extraction and determination of proline

The methods of Bates *et al* (1973) were used. Samples of 0.1g freeze-dried wheat leaves were crushed in liquid nitrogen before adding 10ml 3% sulphosalicilic acid. The supernatant was collected and filtrated through filter paper. Acid ninhydrin (2ml) and 2ml acetic acid were combined with 2 ml of the filtrate. The samples were incubated for one hour in a boiling waterbath and thereafter the reaction was terminated on ice. The reaction mixture was extracted with 4ml toluene and vortexed for 15 to 20 sec. The absorbance was measured at 520nm with a spectrophotometer. The proline concentration was determined using a standard curve and expressed as μg proline/g dry weight using the following equation:

$$\frac{(\mu g \text{ proline/ml} * \text{ml toluene})}{[(115.5 \mu g/\mu mole)]/(g \text{ sample}/5)}$$

5.2.1.5 Statistical analysis

The data were subjected to a variance analysis (ANOVA) using Agrobase (2000) statistical software.

Cell membrane stability test

5.2.2.1 Sampling

Leaf samples of about 10mm in diameter were taken from fully expanded young leaves. Five samples were taken from two or three leaves per genotype. Samples were kept in an airtight test tube, wetted with a drop of water and transferred to the laboratory within an hour.

5.2.2.2 Drought tolerance test

The method of Sullivan (1972) was followed. Samples were washed with three changes of distilled water to remove surface-adhered electrolytes. Five leaf samples for the stress treatments were placed in a test tube with 10cc solution of a 20% concentration of polyethylene glycol 6000(PEG). Five samples for the control treatment were placed in 10cc of distilled water. All the samples were incubated at 10^0 C for 24 hours and then equilibrated in a waterbath at 25^0 C and conductivity of the incubation medium was read using a conductivity meter. After reading, the samples were autoclaved for 15 min to kill the leaf tissues and a second conductivity reading was made after the samples reached room temperature at 25^0 C. Calculation of percentage injury due to desiccation was made as follow:

$$\% \text{ Injury} = 1-[1-(T_1 / T_2) / 1- (C1 / C_2)] *100$$

Where T and C refer to mean of the treatment and control reading, respectively and the subscripts 1 and 2 refer to initial and final conductivities.

5.2.2.3 Statistical analysis

The data were subjected to variance analysis (ANOVA) using Agrobase (2000).

Tri phenyl tetra chloride (TTC) test

5.2.3.1 Inducing of drought and extraction of TTC

Leaf samples, 7mm in diameter, were subjected to 3 ml PO_4 buffer to a control treatment and 3 ml 0.5M mannitol buffer for the drought treatment for acclimation, before incubation in 3 ml 1M mannitol solution. Samples were collected three different times each with a 30 min interval. The leaf samples were transferred to a 2.5ml TTC solution. The leaf samples were centrifuged for 10 minutes, before incubation in the dark overnight.

The washed leaf samples were placed in alcohol and then heated on warm plates until all the alcohol evaporated. The samples were replaced in 1ml alcohol when cooled. The reduction of TTC was then determined with the spectrophotometer at 485nm

5.2.3.2 Statistical analysis

Analysis of variance was done on data using Agrobase (2000) statistical software.

5.3. Results and discussion

5.3.1 Proline test

Bread wheat

The results showed that there was no significant ($p>0.05$) variation among the lines and cultivars tested in free proline accumulation at both five and 10 days of moisture stress. Although the variation was non-significant, lines and cultivars differed among each other in free proline accumulation under both conditions (Table 5.1).

Table 5.1 Summary of the results of the proline content μg proline/g dry weight of bread wheat genotypes after five and 10 days of moisture stress.

Genotypes	5 days stress	5 days control	10 days stress	10 days control
Israel	12.84	9.4	22	8.89
Et-13	12.61	8.06	21.33	9.42
HAR-1685	10.55	10.11	20.39	12.14
HAR-604	12.49	11.22	13.16	10.3
Dereselign	11.82	11.31	22.9	10.39
Bdl-41	14.61	11.73	18.19	10.16
Bdl-20	13.28	11.15	15.84	10.53
Bdl-36	12.12	10.48	18.29	11.38
Bdl-8	13.78	12.19	15.54	11.54
Bdl-16	12.31	8.13	15.75	9.6
LSD (p=0.05)	3.29	2.67	7.48	3.08

Six genotypes showed a decrease in the proline content when five and 10 days of optimal conditions were compared. These were Israel, HAR-604, Dereselign, Bdl-41, Bdl-20 and Bdl-8. This shows that under optimal conditions there was a reduction in the production of proline. There was an increase in the proline content of all the lines and cultivars tested after moisture stress was applied. The highest free proline accumulation was found in Bdl-41 after five days of moisture stress. This was followed by Bdl-8 and Bdl-20. The

least increase in proline content was found in HAR-1685 and Dereselign after five days of moisture stress. After 10 days of moisture stress the highest proline content was found in Israel and Et-13. The lowest content was found in Bdl-8.

The results of this study also showed an increase of free proline in all the lines and cultivars tested from moisture stressed to control conditions (Figure 5.1).

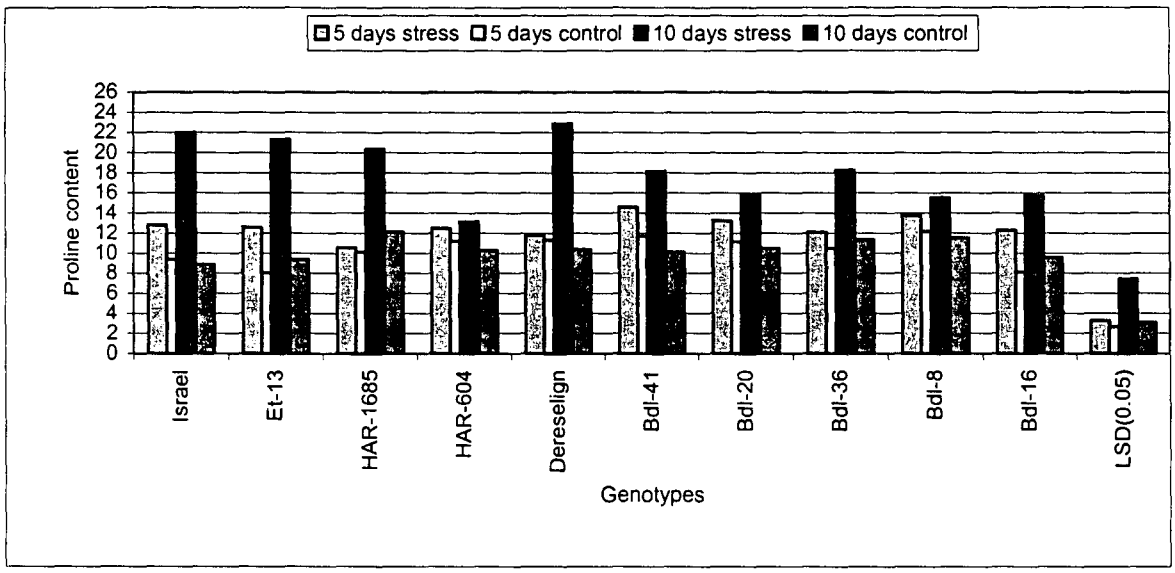


Fig.5.1. Proline content μg proline/g dry weight after five and 10 days of moisture stress for bread wheat cultivars

The highest increase in free proline accumulation was found in Et-13 with an increase of 36.08% after five days of moisture stress. It was followed by Bdl-16 (33.96%) and Israel (26.79%). The smallest increase was found in HAR-1685 (4.17%) and Dereselign (4.31%).

After 10 days of moisture stress, all the genotypes tested showed a significantly higher increase in proline production from the control to the stress treatment. The highest increase was in Israel (59.59%) and Et-13 (55.83%). The lowest reduction was found in HAR-604 (21.73%) and Bdl-8 (25.75%).

Durum wheat

The result showed that there was no significant ($p>0.05$) variation among the cultivars tested in free proline accumulation at both five and 10 days of moisture stress. On the other hand, significant variation was observed between the stressed to the control treatment after 10 days of moisture stress (Table 5.2).

Table 5.2 The proline content μg proline/g dry weight of durum wheat cultivars after five and 10 days of moisture stress.

	5 days stress	5 days control	10 days stress	10 days control
Foka	8.89	8.33	16.51	11.34
Boohai	12.79	8.34	15.08	10.37
Gerardo	11.31	8.63	16.65	12.31
Fetan	11.75	11.27	15.38	8.29
Kilinto	10.94	10.07	15.31	10.6
LD-357	9.86	9.6	15.24	12.58
Cocorit-71-	10.39	12.7	15.95	14.04
Tob-66	13.46	11.36	16.81	14.52
Quami	8.77	9.9	17.82	12.35
Cadu-17	12.58	10.87	15.33	13.67
LSD (0.05)	2.8	2.86	3.53	3.88

All the cultivars produced more proline after 10 days compared to five days of non-moisture stress conditions. Overall, the increase in proline content was much lower in

durum cultivars than the bread wheat lines and cultivars tested after five and 10 days of stress. After five days of moisture stress the highest proline content was found in Tob-66 and Boohai, whereas Quami and Foka had the least increase in proline content. After 10 days of moisture stres, Quami and Tob-66 had the highest proline production whereas Boohai and LD-357 had the lowest in free proline production. Figure 5.2 shows the proline content of durum wheat cultivars after five and 10 days moisture stress.

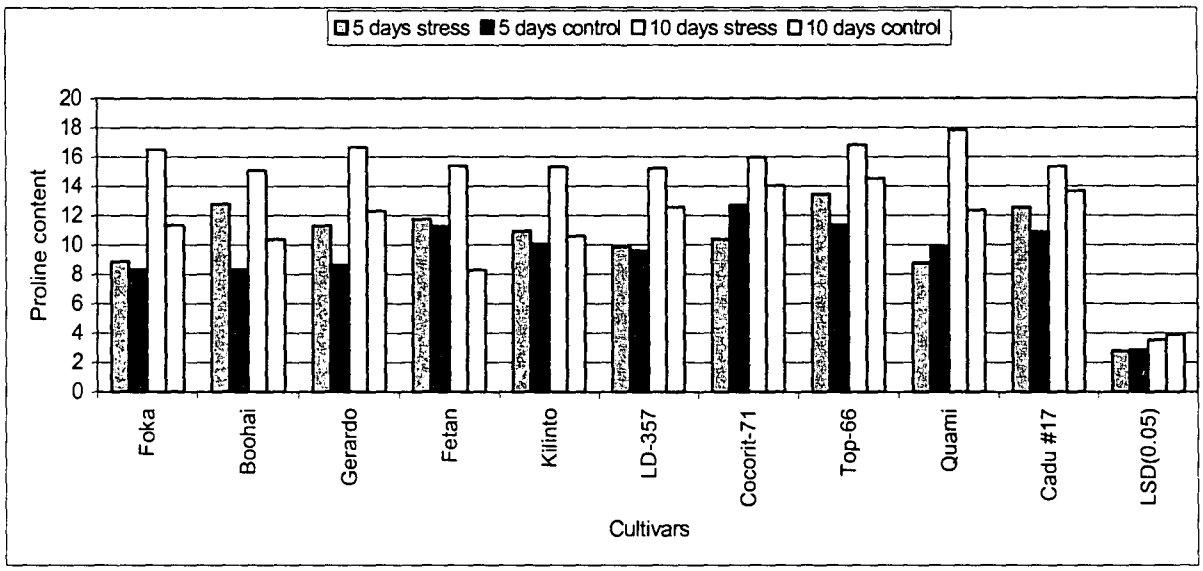


Figure 5.2 Proline contents (μg proline/g dry weight) after five and 10 days of moisture stress for durum wheat cultivars.

All the cultivars, except Cocorit-71 and Quami, showed a reduction in proline content from the stress to control conditions after five days of moisture stress. The highest was found in Boohai with a increase of 34.29 % followed by Gerardo (23.69%). The lowest increase was found in LD-357 (2.64%) and Fetan (4.09%). After 10 days of moisture

stress all the cultivars showed an increase in proline content from moisture stressed to control treatment. The increase was also significantly higher after 10 days compared to five days of moisture stress. The highest reduction was found in Fetan (46.09%) and Kilinto (34.29%) whereas Cocorit-71 (11.97%) showed the minimum reduction after 10 days of moisture stress.

Discussion

Fukutoku and Yamada (1981) and Laurie (1999) reported that total free proline first decreased at mild water stress but then increased as the stress became more severe.

Van Heerden and De Villiers (1996) observed a higher proline accumulation during drought stress in drought tolerant spring wheat cultivars, than in the more sensitive cultivars. Thus, cultivars that produced higher proline under drought than control conditions could be drought tolerant cultivars.

Although no significant variation among bread and durum wheat genotypes were found, differences in proline concentration were found. In bread wheat, it was observed that the stressed plants reacted with an increase in free proline concentration.

The result of this study indicated that proline level increased from five days of moisture stress to 10 days of moisture stress in all the bread wheat lines and cultivars tested. It also indicated that the free proline concentration of the cultivars Israel, Et-13and Dereselign (Figure 5.1) showed a significant increase after 10 days of moisture stress. Bdl-8 showed

a smaller reduction from the stressed to control treatment indicating that it has a minimum reaction to moisture stress treatment.

The control plants of most of the bread wheat genotypes synthesized almost a similar amount of proline and were able to sustain the proline levels needed by these plants.

In durum wheat, the variation among cultivars were found to be insignificant, but two cultivars (Cocorit-71 and Quami), produced higher proline levels under control than stressed conditions after five days of moisture stress. Boohai and Gerardo had the maximum variation between stressed and control plants indicating they were not able to sustain their proline synthesis under these different situations. LD-357 and Fetan showed a similar trend to proline production indicating that they are insensitive to moisture stress variation.

The situation changed when the stress became severe and the concentration of free proline progressively increased in all the cultivars. Cocorit-71 and Quami that had lower free proline under mild stressed conditions showed high proline concentration when the stress level became more severe. Quami and Fetan showed a significantly higher proline concentration. This is probably because when the stress becomes severe, protein breakdown commences causing a drastic rise in free proline, or the total free proline could be a result of the plant's adjusting ability to the stress.

In conclusion, bread wheat reacts differently than durum wheat in the production of proline after moisture stress. More differences between bread genotypes were found after 10 days of moisture stress, while in durum cultivars more differences were visible between cultivars after five days of moisture stress. Bread wheat also produced much more proline in reaction to moisture stress than durum wheat.

To use proline to screen for drought tolerance in wheat cultivars, one must find the appropriate number of days for the stress treatment necessary to induce drought. These findings must, however, be related to yield and yield components in order to use proline as a screening method for drought tolerance.

5.3.2 Cell membrane stability test

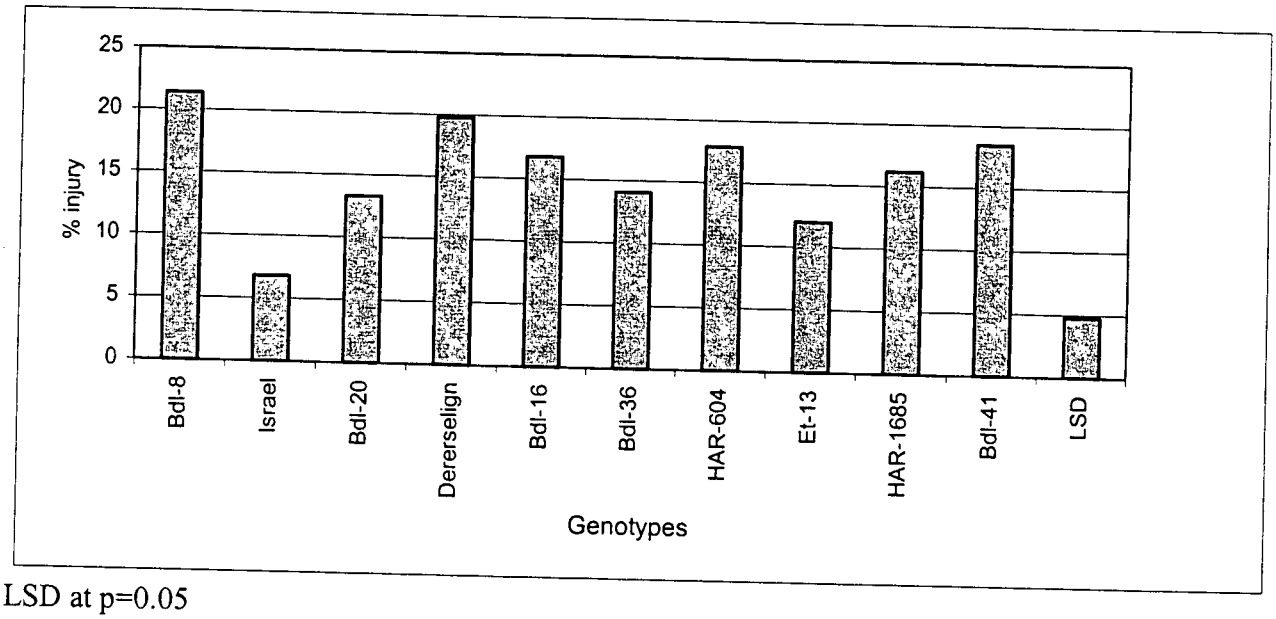
Bread wheat

Table 5.3 Analysis of variance for percentage injury of bread and durum wheat genotypes

Source of variation	df	F-values
Bread wheat		
Genotypes	9	4.03**
Error	30	
Durum wheat		
Genotype	9	4.24**
Error	30	

** Significant at p=0.01

The analysis of variance showed that highly significant ($p<0.01$) variation was obtained in percentage injury among genotypes (Table 5.3). Mean injury for bread wheat genotypes are also provided in Figure 5.3.



LSD at p=0.05

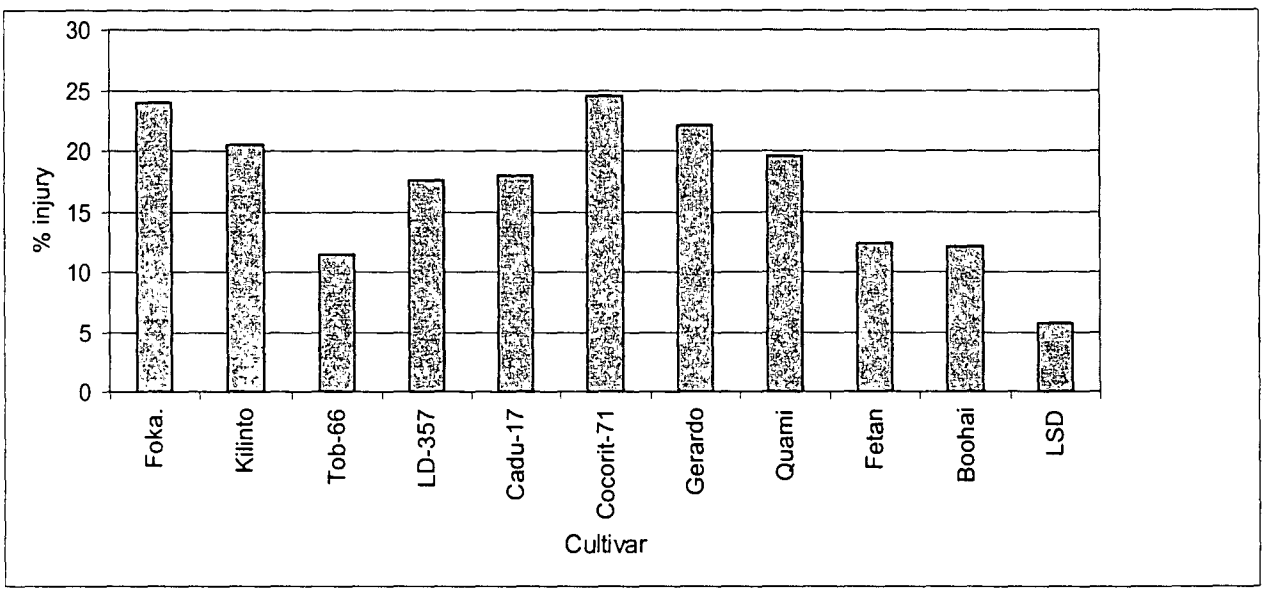
Figure 5.3 Effect of osmotic stress on injury of bread wheat genotypes

The highest injury level was obtained from Bdl-8 (21.5%). This was followed by Dereselign (19.8%) and Bdl-41 (18.4%). Israel (6.8%) showed a low level of injury and was significantly different from the other except three genotypes namely, Et-13, Bdl-24 and Bdl-41 with injury level of 12, 13.3 and 14.1 % respectively.

Although HAR-1685, Bdl-16 and HAR-604 did not differ from Bdl-41 and Dereselign, the injury levels of these genotypes were found to be low.

Durum wheat

Analysis of variance for injury level and mean injury for the durum wheat genotypes are presented in Table 5.3 and Figure 5.4 respectively. The analysis of variance for genotypes revealed that there was significant variation ($p<0.05$) in injury between genotypes. Tob-66, Boohai and Fetan were the lowest with a mean injury level of 11.5, 12.01 and 12.4 %. They differed significantly from Foka (23.97%) and Cocorit-71 (24.55%), which showed the highest amount of injury respectively.



LSD at p=0.05

Figure 5.4 Effect of osmotic stress on injury of durum wheat cultivars

Discussion

The drought tolerance test using CMS under conditions of stress and its significant contribution as a screening method is reported in different crops including wheat (Blum

and Ebercon, 1981; Mark *et al,* 1991). They reported that genotypes, which exhibited a low percent of injury, could be considered as drought tolerant.

Stanca (1987) found high correlation values between grain yield and electrolyte leakage in barley. They concluded that this test was a good indicator for drought tolerant lines.

The results of this study also showed that both bread and durum genotypes differed in injury level. For instance, in bread wheat, three lines Bdl-8, Dereselign and Bdl-41 showed a relatively high level of injury and can be categorized as non-tolerant lines, whereas Israel, Et-13, Bdl-24 and Bdl-36 with a low level of injury can be classified as tolerant lines.

On the other hand, among the durum wheat cultivars, Tob-66, Boohai and Fetan showed a low level of injury, and can be considered as tolerant where as Foka and Cocorit-71 with a high level of injury are susceptible cultivars.

In a similar study, Blum and Ebercon (1981) classified such kind of adaptation as osmotic adjustment. It is the phenomena of cell membrane adjustment to drought stress. The degree of injury to CMS by controlled dehydration was found to decrease in plants subjected to a period of drought stress.

The percentage cell membrane injury level obtained in this study was very low less than 30% in both bread and durum wheat genotypes compared to up to 70% injury level

reported by Blum and Ebercon (1981). The reason is probably that the PEG concentration used in this study was low, 20% to induce osmotic stress, as opposed to the 40% PEG concentration used in their studies.

The results of this study also showed that the bread wheat lines had a low percentage of injury compared to durum wheat cultivars. This finding was not in line with what was reported by Blum and Ebercon (1981). They reported that bread wheat genotypes suffered more with cell membrane injury because of moisture stress than durum wheat.

In conclusion, the effect of drought stress on membrane adjustment in terms of membrane stability, poses a practical problem concerning the desirable environment for genotypic characterization of drought tolerance. Membrane adjustment is related to the rate of stress development (Bewley, 1979). Adjusting genotypes are desirable where drought stress development is slow. A re-evaluation of selected genotypes under conditions of drought stress is desirable for the identification of those that do adjust in terms of membrane stability. Studies also indicated that since growth stages of wheat have a significant effect on the level of injury, it is very important to consider this before classifying genotypes as tolerant to drought or not.

5.3.3 TTC test

Results

Bread wheat

Significant variation ($p<0.05$) between the different times and the lines and cultivars were found in the concentration of formazan (Table 5.4). A non-significant ($p>0.05$) difference was found between the two osmotic stresses. Moisture by time, moisture by lines, time by lines and moisture by time and by line interaction were found to be non-significant ($p>0.05$) indicating that the three treatments did not interact.

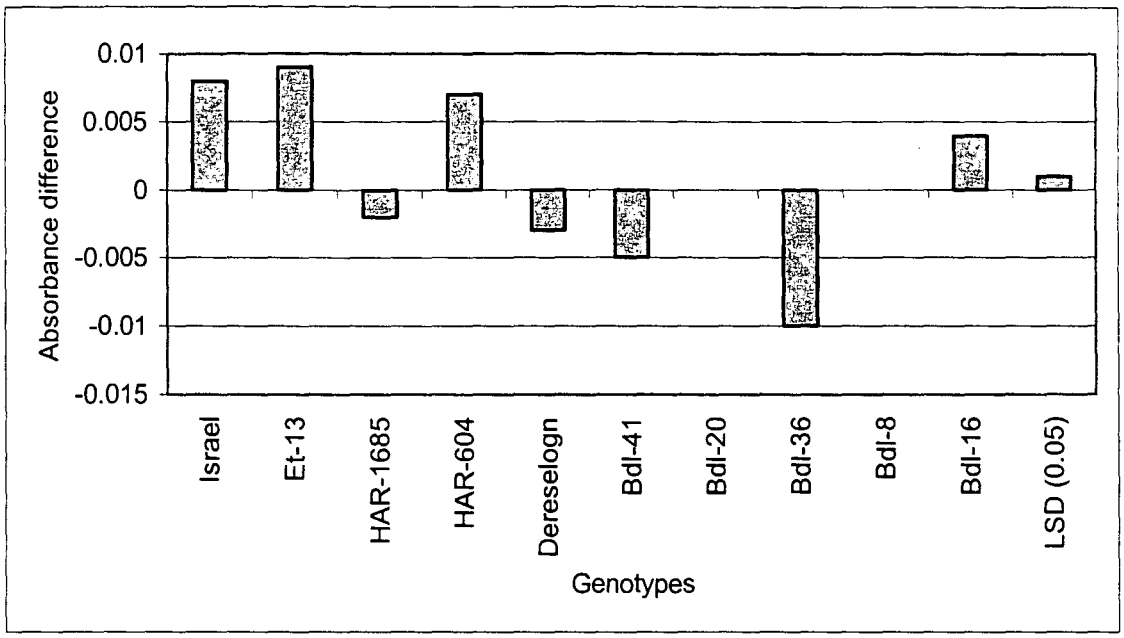
Table 5.4. Analysis of variance for bread wheat lines for TTC reduction

Source	df	F-value	Pr>F
Total	159		
Rep.	1	0.53	0.4705
Moisture	1	0.11	0.7381
Time	3	7.98	0.0001*
Genotype	9	3.09	0.0032*
M * T	3	.0.40	0.7515
M * G	9	0.68	0.7244
T * G	27	1.95	0.0119
M * T * G	27	0.32	0.9992
Residual	79		

M=moisture, T=time, G=genotype

- p=0.05

Although six of the lines and cultivars namely, Israel, Et-13, HAR-1685, HAR-604, Bdl-41 and Bdl-20 did not differ from each other, they were significantly different from the rest of the lines and cultivars tested in absorbance differences.



. Figure 5.5 The absorbance differences of bread wheat genotypes

It is clearly shown that positive and negative absorbance difference values were found for the materials tested (Figure 5.5).

Four of the lines showed a positive difference from the stress to the control treatment. These were Israel, Et-13, HAR-604 and Bdl-16. The highest was found in Et-13. This was followed by Israel, HAR-1685, and HAR-604. No difference between the stressed and the control treatment in absorbance difference values was detected in Bdl-20 and Bdl-8. On the other hand, Bdl-36, Bdl-41, Dereselign and HAR-1685 showed a reduction from stressed to the control treatment.

Durum wheat

Table 5.5 Analysis of variance for durum wheat cultivars for TTC reduction

Source	df	F-value	Pr>F
Total	159		
Rep.	1	6.53	0.0127
Moisture	1	0.16	0.6936
Time	3	1.40	0.2485
Genotype	9	2.56	0.0122
M * T	3	0.18	0.9076
M * G	9	0.96	0.4777
T * G	27	0.88	0.6411
M * T * G	27	0.41	0.9945
Residual	79		

M=moisture, T=time, G=genotype

Except for the cultivars, all the treatment and their interactions showed non-significant variations (Table 5.5).

Figure 5.6 illustrates the absorbance value differences of the durum wheat cultivars. The values increased from control to stress treatment in most of the materials tested. The highest was found in Tob-66. This was followed by Boohai, LD-357 and Fetan. Although the value increased from control to stressed treatment, small differences were found in Kilinto, Cocorit-71 and Quami. Three cultivars, namely Foka, Gerardo, and Cadu-17 showed a higher absorbance difference value in the control than the stress treatment.

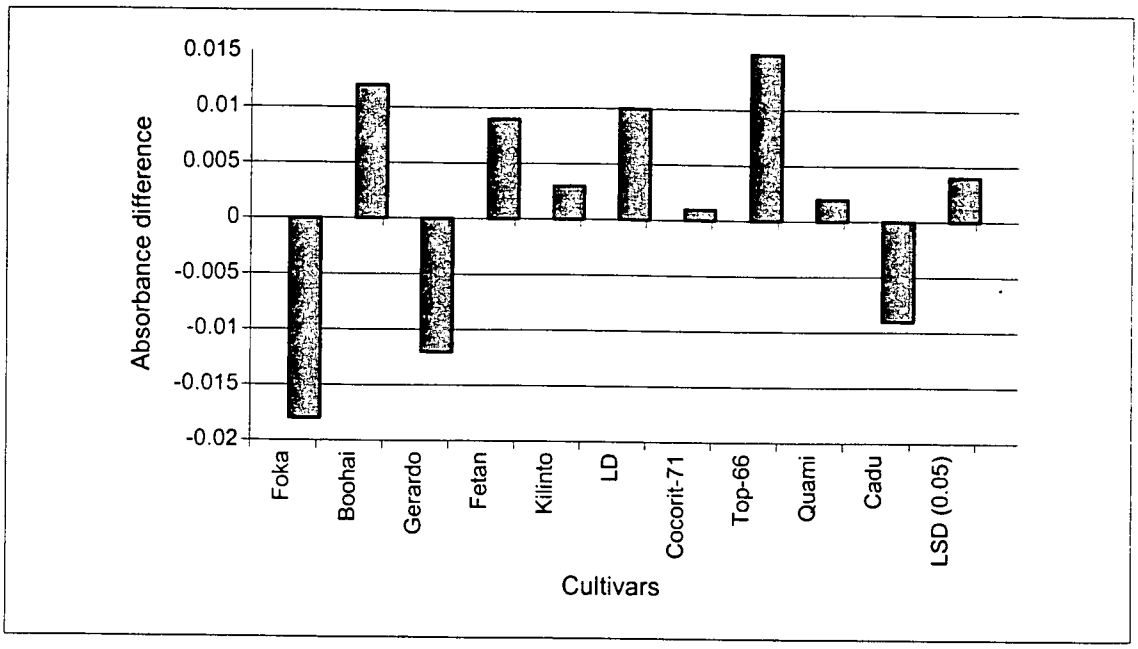


Figure 5.6 the absorbance differences of durum wheat cultivars.

Discussion

The results of this study showed that significant variation was found between the materials tested for both bread and durum wheat in response to simulated moisture stress. This suggests that genotypes varied in their physiological response to react for a given stress and based on the amount of formazan they produced, tolerant and non-tolerant genotypes could be identified. Laurie (1999) suggested that TTC reductions provide information on how cells are performing under normal and stress conditions. De Ronde *et al* (1995) also reported that when the absorbance values of the control treatment exceeded the absorbance of the stress treatment, the cultivar showed sensitivity to that stress. When the absorbance value of the control was less than the stress treatment, the cultivar showed tolerance to that stress.

The results of this study showed a reduction of absorbance values from the control to the stressed treatment in the majority of bread and durum wheat genotypes.

Among the bread wheat genotypes Israel, Et-13, HAR-604 and Bdl-16 showed higher absorbance values in the stressed than control treatment. It could be seen that these materials had a better performance and kept their cells viable under conditions of simulated osmotic stress. HAR-1685, Dereselign, Bdl-41 and Bdl-36 showed a negative effect on the performance of the cells. No differences could be detected between the control and stressed conditions in Bdl-8 and Bdl-20.

All of the durum wheat cultivars except, Foka, Gerardo, and Cadu-17 had a higher absorbance value under the stress than the control conditions.

CHAPTER 6

6. GENETIC DISTANCES BETWEEN SELECTED ENTRIES, AS MEASURED BY GLIADIN BANDS

6.1. Introduction

Proteins are a direct product of gene transcription and translation. Characterization and identification of genotypes by protein electrophoresis is therefore important, as it reflects the genotype and history of the organism (Cooke, 1984). Proteins can thus be regarded as markers for the functional genes that encode them, and methods for comparing protein composition provide a measure of the genetic variation between individuals and populations (Cooke, 1995).

The gliadin electrophoregram is unaffected by growth conditions of the grain, making it ideal for cultivar identification (Lookhart and Finney, 1984). Protein markers can provide a quick and reliable method for estimating genetic relationships between genotypes. Knowledge of genetic relationships will be of value in exploiting the available germplasm to improve drought tolerance. Genetically unrelated cultivars from different clusters, with tolerance where possible, can be used in crosses.

The aim of the study was to determine the genetic distances between genotypes with tolerance, moderate tolerance and susceptibility to drought, in order to determine the best possible combination for drought tolerance breeding.

6.2 Materials and methods

6.2.1 Materials

A total of 20 selected wheat genotypes listed in Table 6.1 were used in this study. Chinese Spring was included as a reference for the analysis of gliadin bands. The mobility of the gliadin bands of the genotypes was scored relative to the mobilities of the Chinese Spring bands.

Table 6.1 Genotypes used for this study and their reaction to drought stress

Breeding lines	Reaction to drought stress
Marico	Tolerant
Bdl-5	Tolerant
T4	Tolerant
Bdl-36	Tolerant
Bdl-25	Tolerant
Bdl-16	Intermediate
Bdl-20	Intermediate
Bdl-19	Intermediate
Harts	Intermediate
GamtoosDn	Intermediate
Bdl-41	Intermediate
Bdl-23	Intermediate
Bdl-32	Intermediate
Bdl-11	Intermediate
Bdl-17	Intermediate
Bdl-1	Susceptible
Bdl-42	Susceptible
Bdl-2	Susceptible
Bd8	Susceptible
Bdl-29	Susceptible

6.2.2 Methods

6.2.2.1 Extraction of gliadins

The method of De Villers and Bosman (1993) was adapted for the extraction of gliadins. Extraction was made from a single kernel of each breeding line. Kernels of each line were crushed and ground to a fine powder with a pestle. Six replications were done for each line. The powder was then transferred to a 1.5-ml eppendorf tube and extracted with an extraction buffer.

The following stock solutions and extraction procedures were employed for the extraction of gliadins.

Stock solutions for extraction

Extraction buffer

1.8 g urea

0.1ml beta-mercapto-ethanol

10 ml distilled water

Sample buffer

1.0 g Tris hydroxymethyl aminomethane (Tris).

90 ml 50 % n-propanol

Titrate to pH 8 with N HCl

Make up to 100 ml with 50% n- propanol.

Add 40-g glycerol, 2 g SDS and 0.02-g bromophenol blue.

Extraction procedure

1 Crush wheat seed into fine powder and transfer to a 1.5-ml -eppendorf tube.

2 Add 500 µl of extraction buffer to the eppendorf tube and put in 60^0 C waterbath for 1 hour. Vortex at 20 and 40 minutes.

3 Centrifuge tubes for 2 minutes at 10,000 rpm.

4 Transfer 80 µl of the supernatant into a new tube containing 80 µl of sample buffer.

5 Mix well and centrifuge as before. Samples are now ready for loading. Fourty µl of the sample was loaded for a single wheat kernel. Of the six-replicated gliadin extracts, three were loaded on one gel and the remaining three on another gel.

6.2.2.2 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

The method was adapted from Singh *et al* (1991).

Gel preparation

A discontinuous pH, two gel systems, which first concentrate the proteins into a narrow starting zone, were used. The discontinuous system of SDS-PAGE is made up of a separating and stacking gel. The following stock solutions were used for the discontinuous gel system.

Stock solutions for the discontinuous two-gel system

Separating buffer (pH 8.88)

Dissolve 45.412 g Tris in 460 ml distilled water.

Titrate to pH 8.88, then add 1.0 g SDS

Make a total of 500ml. Store at 4^0C.

Separating acrylamide (30% Ac/ 1% cross linker)

Dissolve 75-g acrylamide and 0.75 g bisacrylamide in 181-ml water

Make a total of 200ml. Store in dark at 4^0 C.

Stacking buffer pH 6.8:

Dissolve 6.06 g Tris in 190 ml distilled water

Titrate to PH 6.8 then add 0.4 g SDS.

Make a total of 200 ml. Store at 4^0 C.

Stacking acrylamide (35% Ac/1.5% cross linker):

Dissolve 87.5-g acrylamide and 1.32 g bisacrylamide in 181ml distilled water. Store at 4^0 C.

Preparation of separating gel:

For two gels

Separating buffer	38 ml
Separating acrylamide	28 ml
Distilled water	14 ml
Temed	16 µl
Ammonium persulphate (APS 10%)	90 µl

The amount of chemicals mentioned above was mixed and the APS was added just before casting. Then, the gel was poured as vertical slaps between two glassplates clamped to the side of a stand and 3 ml n-buthanol was immediately added on top of the gel for leveling and left to polymerize. After the gel was set, the n- buthanol was removed by washing.

Preparation of stacking gel

For two gels

Stacking buffer	10ml
Stacking acrylamide	2.6ml
Distilled water	7.4ml
Temed	40µl
APS (10%)	100µ

The above chemicals were mixed and the APS was added just before casting the gel. Then, the stacking gel was poured on the top of the separating gel and the slot- forming

comb was inserted to form wells into which the protein samples were loaded for electrophoresis

Electrode buffers:

Cathode buffer

30.28 g Tris,

144g glysine,

10g SDS

Make up to 1 liter with distilled water

Anode buffer

Mix 30.28g Tris

800 ml distilled water.

Then titrate to pH 8.4and

Make up to 1 liter with distilled water and dilute 10x before use.

Running of the gel

Prior to electrophoresis, the sample tubes were centrifuged for 10 minutes and 40µl of the supernatant was loaded into each well. The standard cultivar, Chinese Spring, was loaded at the center as well as at both sides of each gel. The unknown samples were loaded in the remaining wells. Electrophoresis was carried out using a vertical slab gel electrophoresis unit, Model SE 600 system (Hoefer Scientific Instruments, San Franscisco, CA), with the

negative terminal (cathode buffer) in the upper tank and the positive terminal (anode buffer) in the bottom tank at a constant current (66mA) for about 3 hours at 15^0C.

Gel analysis

The gels were analyzed with the help of the " Molecular Analyst Fingerprinting" software of Biorad. The gliadin banding patterns of Chinese Spring were used as a standard. Gels were scanned with the help of Gel Doc 1000 using an UV-gel camera and VGA graphics in 256 colors as recommended. Band pattern migration distances were directly obtained from a gel and put into a computer file, thus obviating the need to manually transcribe data. The analysis procedure consisted of three steps: 1 the conversion of the gel, 2 the normalization of the gel and 3 the analysis of the tracks.

The resolution power was set at 200 points. A densitometric curve of every replication of the cultivars was drawn and from this the migration distances were determined. Only bands with an intensity of 15% were considered. An average value was calculated for the six replications of each cultivar and these values were used to compare the genotypes with each other.

6.2.2.3 Nomenclature and cluster analysis

The nomenclature suggested by Konarev (1979) was used for the purpose of this study. The data was subjected to cluster analysis using the Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998) statistical system for windows. The hierarchical clustering

method was employed, with a group average (unweighted pair-group), Euclidean distance and cut-off point of 1.35.

6.3 Results

The summary of the bread wheat lines and cultivars tested and their gliadin subunit combinations are given in Table 6.2. The banding patterns of all the lines and cultivars are given in Appemdix B.

Table 6.2 Summary of gliadin banding patterns of tested bread wheat lines and cultivars.

Genotypes	α-gliadins	β-gliadins	γ -gliadins	ω -gliadins
Bdl-16	2, 4^{-}, 4, 7, 7^{+}	2, 4	1, 2, 2^{+}, 3, 4, 5	4, 5, 8
Bdl-20	1, 2, 3, 6,7^{-}, 7	1^{-}, 3, 5	1^{-}, 1, 2, 3,4 $^{-}$, 4, 5	5, 8, 10
Bdl-19	1, 2, 3, 4, 7, 7	2, 3, 4	1^{-}, 1, 3, 4 $^{-}$, 4 $^{+}$, 5	1, 4, 5, 8, 10
Bdl-8	1^{-}, 1^{+}, 6^{-}, 6, 7	1, 3, 5	2^{-}, 2, 3, 4 $^{-}$, 4^{+}, 5	3, 5, 7, 8, 10
Marco	1, 1, 2, 3, 6, 6,7	1^{-}, 2, 4	1, 2, 3, 4 $^{-}$, 4 $^{+}$	2, 4, 5, 8
T4	1, 2, 6, 7, 7^{+}	3, 5	1, 2^{-}, 2, 3^{-}, 3, 5	1, 4, 6, 8, 9
Bdl-5	1^{-}, 1, 2, 6, 7	1, 4	1^{-}, 1, 2, 3, 4, 5^{-}, 5	5, 6, 9
Harts	6, 7^{-}, 7, 7^{+}	3	2, 4, 5	1, 2, 4, 6, 9
GamtoosDn	1, 3, 6, 7	1, 2, 4	1, 2, 4, 5	2, 4, 6, 10
Bdl-41	1, 5^{-}, 5, 6, 7	1, 2, 4	2^{-}, 2, 3, 4, 5	3, 6,9
Bdl-1	1, 2^{-}, 2, 3,5^{-}, 5, 6^{-}, 6	2	1^{-}, 1, 2, 3, 5	4, 6, 8, 9
Bdl-42	6, 7	1^{-}, 2, 5	1, 3^{-}, 3, 4, 5^{-}, 5	3, 5, 6, 8, 10
Bdl-23	1, 7^{-}, 7	1^{-}, 4	1, 3, 4	5, 6, 9
Bdl-2	1, 2, 5^{-}, 5, 6, 7, 7^{+}	2, 4	1, 2, 3, 4	3, 6, 8, 9
Bdl-36	1,5, 6^{-}, 6, 7	1^{-}, 2, 4	1, 2^{-}, 2, 3^{-}, 3, 3^{+}	1, 3, 6, 9, 10
Bdl-32	1, 2, 5, 6, 7^{-}, 7	1, 2, 4	1, 2, 5^{-}, 5	2, 4, 6, 9
Bdl-25	1^{-}, 1, 2, 6, 7^{-}, 7	1, 2, 4	1, 3, 4^{-}, 4, 5	4, 8
Bdl-11	1, 1^{+}, 2^{-}, 2, 3, 7, 7^{+}	2, 3	1, 2, 3^{-}, 3, 4	3, 4, 5, 8
Bdl-29	1, 3, 7	1, 2, 5	2, 3, 4^{-}, 4, 5	5, 8, 9
Bdl-17	6, 7	1^{-}, 3, 4	1, 2, 4 $^{-}$, 4, 5	5, 6, 9

α-Gliadins

In some of the materials tested, more than one band was found in the same interval of the nomenclature of Konarev (1979). Some of these bands were a bit higher (indicated with a

$^{+}$) and some of these bands were a bit lower (indicated with a $^{-}$) than the corresponding bands of the literature.

Seven lines namely Bdl-20, Marico, T4, Bdl-5, Bdl-2, Bdl-32 and Bdl-25 had the α-gliadin combinations of 1, 2, 6 and 7. However, Bdl-20, T4, Bdl-32 and Bdl-25 had two bands corresponding to the α-gliadins 7 band. Marico, Bdl-5 and Bdl-25 had two bands each corresponding to α-gliadins 1 band. Marico also had two bands corresponding to α-gliadin band 6.

β-Gliadin

All the bands found in this group corresponds with the nomenclature of Konarev (1979). The only exception was found in Bdl-20 that had higher bands than the β-gliadin 1 band.

Five of the entries tested, had the β-gliadin combination of band 1, 2 and 4. These were Gamtoos Dn, Bdl-41, Bdl-2, Bdl-36 and Bdl-32. Bdl-20, Bdl-8 and Marico had a lower deviation in band 1. β-Gliadin combination of 1, 2, and 5 were found in Bdl-29 and Bdl-42. Bdl-5 and Bdl-24 had the combination of 1 and 4.

γ-Gliadin

The γ-gliadin bands found, correspond to the nomenclature of Konarev (1979). However, most of the materials tested showed bands which were higher or lower than that described by Konarev (1979).

Four lines Bdl-16, Bdl-20, and Bdl-36 had the γ-gliadin combination of 1, 2, 4 and 5. Gamtoos Dn and Bdl-17 had the γ-gliadin combination of 1, 2, 4 and 5. The combination of γ-gliadin 1, 2, 3,4 were found in Marico and Bdl-2.

ω-Gliadin

All bands found in this group, corresponds to the nomenclature of Konarev (1979).

Three lines, Bdl-19, T4 and Harts had the combination of ω-gliadin 1 and 4. The combination of ω-Gliadins 5, 6, and 9 found in Bdl-5, Bdl-23, and Bdl-19. Two cultivars and one line had the combination of ω-Gliadin 2, 4 and 6. These were Harts, Gamtoos Dn and Bdl-32.

Cluster analysis

A dendrogram constructed using the gliadin data is given in Figure 6.1. The results of cluster analysis showed that the 20 lines and cultivars grouped into four clusters in which most of the materials were divided. Three of the lines were not included in any of the four clusters and are considered as a different cluster.

Cluster one consisted of three lines, namely Bdl-41, Bdl-2 and Bdl-36.

Cluster two was also composed of three lines (Bdl-5, Bdl-32 and Gamtoos Dn).

The third cluster had six lines and cultivars, namely Bdl-23, Bdl-17, Bdl-29, Marico, Harts and T4.

The forth cluster comprised of five lines. These were Bdl-16, Bdl-20, Bdl-19, Bdl-11, and Bdl-25.

Three of the lines, Bdl-42, Bdl-8 and Bdl-1, which all originally showed a non-tolerant reaction to drought, were not included in any of the four clusters.

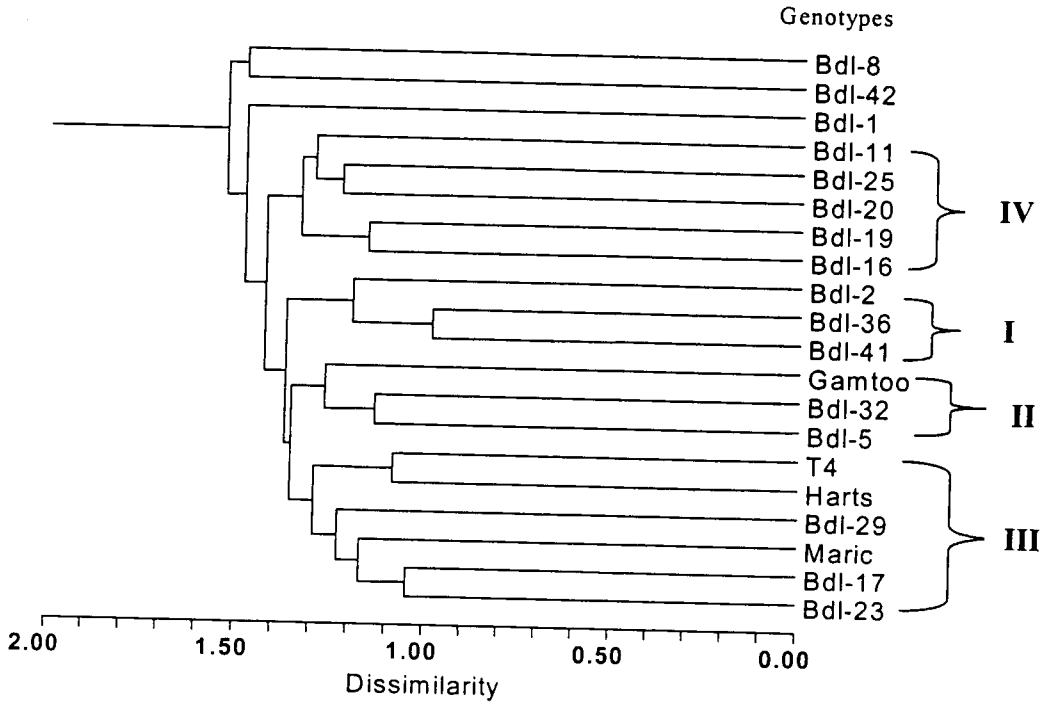


Figure 6.1 A dendrogram of the wheat lines tested based on their electrophoretic gliadin patterns.

6.4 Discussion

The results of cluster analysis showed that genotypes that had different reactions to drought are grouped together. For instance, this is clearly shown in Bdl-36, Bdl-41 and Bdl-2 that were originally categorized as drought tolerant, intermediate and non- tolerant respectively but grouped in the same cluster (cluster one). Similarly, cluster three comprised of lines and cultivars that showed different reactions to drought stress. This indicates that lines and cultivars that respond differently to drought stress had similar banding patterns.

Bdl-36 in cluster one and T4 and Marico in cluster three were characterized as tolerant to drought, whereas those lines in cluster four were known with stable performances for drought stress could be useful for future crossing or breeding programmes for drought tolerance.

In addition, estimates of genetic distance between lines and cultivars were not found to be extensive. This suggests that the materials might not have a wide genetic diversity and it would therefore be difficult to characterize them for drought tolerance, which is polygenic in nature.

The results also revealed that lines and cultivars were not classified according to their original characterization of their reaction to drought stress.

In conclusion, however, the use of a large number of genotypes with a broad genetic background might be important to characterize and identify suitable parentals for breeding drought tolerance.

CHAPTER 7

7. Summary

Drought is a multidimensional problem in all crop species and affects large areas throughout the world. In developing countries nearly 32% of the 99 million hectare of wheat has been affected by varying levels of drought stress. The objective of this study was to characterize wheat lines and cultivars for drought tolerance using different screening methods.

A greenhouse experiment was conducted to screen 54 bread wheat genotypes for tolerance to drought. The genotypes were characterized by measuring their yield and yield components. Significant varietal differences were found in all measured characteristics. T4, Bdl-36and Bdl-47 were found to be tolerant, whereas Bdl-16 and Bdl-8 were sensitive to drought stress.

Grain yield was correlated positively and significantly with all measured characteristics. The correlation of grain yield with SKM and KN per spike was found to be very high. Hence, breeding for moisture stress needs the consideration of most yield components for improving the final yield.

A wooden box study was also undertaken to examine the effects of moisture stress on survival and recovery percentage of 10 bread and 10 durum wheat lines and cultivars at

the seedling stage. The results showed that highly significant variations were found in both the survival and recovery percentage of bread wheat genotypes and durum wheat cultivars. Israel and Et-13 were relatively tolerant whereas Bdl-16, Bdl-8 and Bdl-41 were the most sensitive. Among durum wheat genotypes Cocorit-71, Fetan, Tob-66 and Gerardo were found to be tolerant whereas Cadu-17 and LD-357 showed sensitivity to moisture stress at the seedling stage.

The bread and durum genotypes were also tested with three laboratory-screening methods for drought tolerance. They were proline accumulation, cell membrane stability and 2,3,5-triphenyl tetrazolium chloride reductions.

The effect of drought stress on the accumulation of free proline in leaves of the genotypes was tested. The result showed that proline levels increased in drought stress treatment compare to the control in the majority of bread and durum wheat genotypes.

The results on cell membrane stability also showed that there was significant variation among bread and durum wheat genotypes. Among bread wheat entries, Israel and Et-13 showed a low level of injury whereas Bdl-8 and Dereselign had a relatively high level of injury when they were exposed to simulated osmotic stress. Similarly, among the durum cultivars Tob-66 and Boohai showed a low level of injury and Cocorit-71 and Foka had a high level of injury.

The results on cell viability by using TTC assay showed that among the bread wheat genotypes significant levels of variation were found.

Although the results of the three laboratory techniques seem to be promising in discriminating the cultivars and lines tested, further extensive field screening is required to confirm the results obtained in this study.

An experiment was also carried out to determine the genetic distances between 20 selected bread wheat lines and cultivars for parental selection suited for breeding drought tolerance. The gliadin banding patterns of the lines and cultivars were screened using SDS-PAGE. The results of the study showed that the genetic distances among the entries tested were relatively small. The use of a large number of entries with a broad genetic background might be important to identify suitable parents for breeding drought tolerance.

HOOFSTUK 7

7. Opsomming

Droogte is 'n multidimensionele probleem in alle landbou gewasse en affekteer groot areas oor die hele wêreld. In ontwikkelende lande word amper 32% van die 99 miljoen hektaar koring geaffekteer deur verskillende vlakke van droogte stres. Die doel van hierdie studie was om koring genotipes te karakteriseer vir droogte toleransie met die gebruik van verskillende evaluasie tegnieke.

'n Glashuis eksperiment is gedoen om 54 koring genotipes te evalueer vir toleransie vir droogte. Die genotipes is gekarakteriseer deur hulle opbrengs en opbrengs komponente te meet. Betekenisvolle verskille is tussen inskrywings gevind vir alle gemete eienskappe. T4, Bdl-36 en Bdl-47 was droogte tolerant terwyl Bdl-16 en Bdl-8 sensitief was vir droogte.

Opbrengs was positief en betekenisvol gekorreleer met alle gemete eienskappe. Die korrelasie van graan opbrengs met SKM en KN per aar was baie hoog. Daarom moet, vir teling vir droogte toleransie, meeste opbrengs komponente in ag geneem word om die finale opbrengs te verbeter.

'n Hout kas studie is ook gedoen om die effek van droogte stres op oorlewing en herstel persentasie van 10 brood en 10 durum lyne en cultivars in die saailing fase te meet. Die resultate het aangetoon dat hoogs betekenisvolle variasies gevind is vir beide oorlewing en herstel persentasie van brood en durum korings. Israel en Et-13 was relatief tolerant teen stres, terwyl Bdl-16, Bdl-8 en Bdl-41 die sensitiefste was. By die durum korings was Cocorit-71, Fetan, Tob-66 en Gerardo tolerant terwyl Cadu-17 en LD-357 sensitief was vir droogte stres in die saailing fase.

Die brood en durum genotipes is ook met drie laboratorium prosedures getoets vir droogte toleransie. Prolien akkumulasie, sel membraan stabiliteit en 2,3,4-triphenyl tetrazolium chloried reduksie is gebruik.

Die effek van droogte stres op akkumulasie van vry prolien in blare van die materiaal is getoets. Resultate het aangetoon dat prolien vlakke toegeneem het met droogte behandeling in vergelyking met die kontrole in meeste van die brood en durum korings.

Die resultate van die sel membraan stabiliteit toetse het ook betekenisvolle variasie tussen brood en durum korings aangetoon. Vir die brood korings het Israel en Et-13 'n lae vlak van skade getoon terwyl Bdl-8 en Dereselign 'n relatiewe hoë vlak van skade getoon het as dit blootgestel is aan gesimuleerde osmotiese stres. Net so het die durum cultivars Tob-66 en Boohai 'n lae vlak van skade gehad, en Cocorit en Foka 'n hoë vlak van skade.

Die resultate van sel lewensvatbaarheid deur gebruik van die TTC metode het aangetoon dat daar betekenisvolle variasie is tussen brood korings.

Alhoewel die resultate van die drie laboratorium tegnieke belowende resultate gegee het om cultivars en lyne te diskrimineer vir droogte toleransie, sal verdere uitgebreide veld evaluasies nodig wees om die resultate te bevestig van hierdie studie.

'n Eksperiment is ook gedoen om te kyk na genetiese afstande tussen 20 geselekteerde brood genotipes vir ouer seleksie vir teling van droogte toleransie. Die gliadien band patrone van die lyne en cultivars is getoets met die gebruik van SDS-PAGE. Die resultate het aangetoon dat genetiese afstande tussen inskrywings relatief klein is. Die gebruik van 'n groot aantal inskrywings met 'n wye genetiese agtergrond kan belangrik wees om geskikte ouer materiaal te identifiseer vir die teling van lyne met droogte toleransie.

CHAPTER 8

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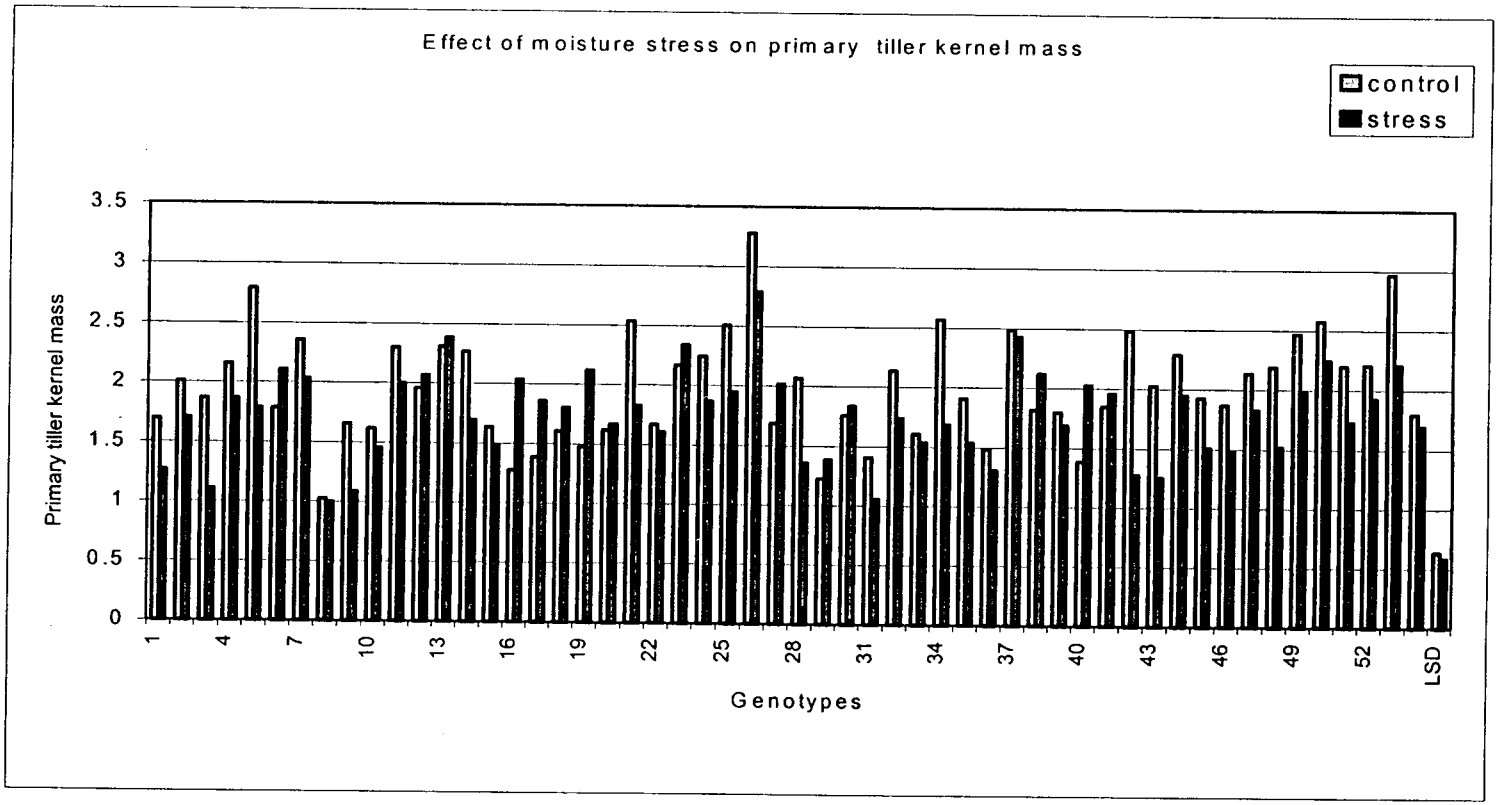

Fig. 3.2 Effect of moisture stress on kernel mass of the primary tillers

Appendix A

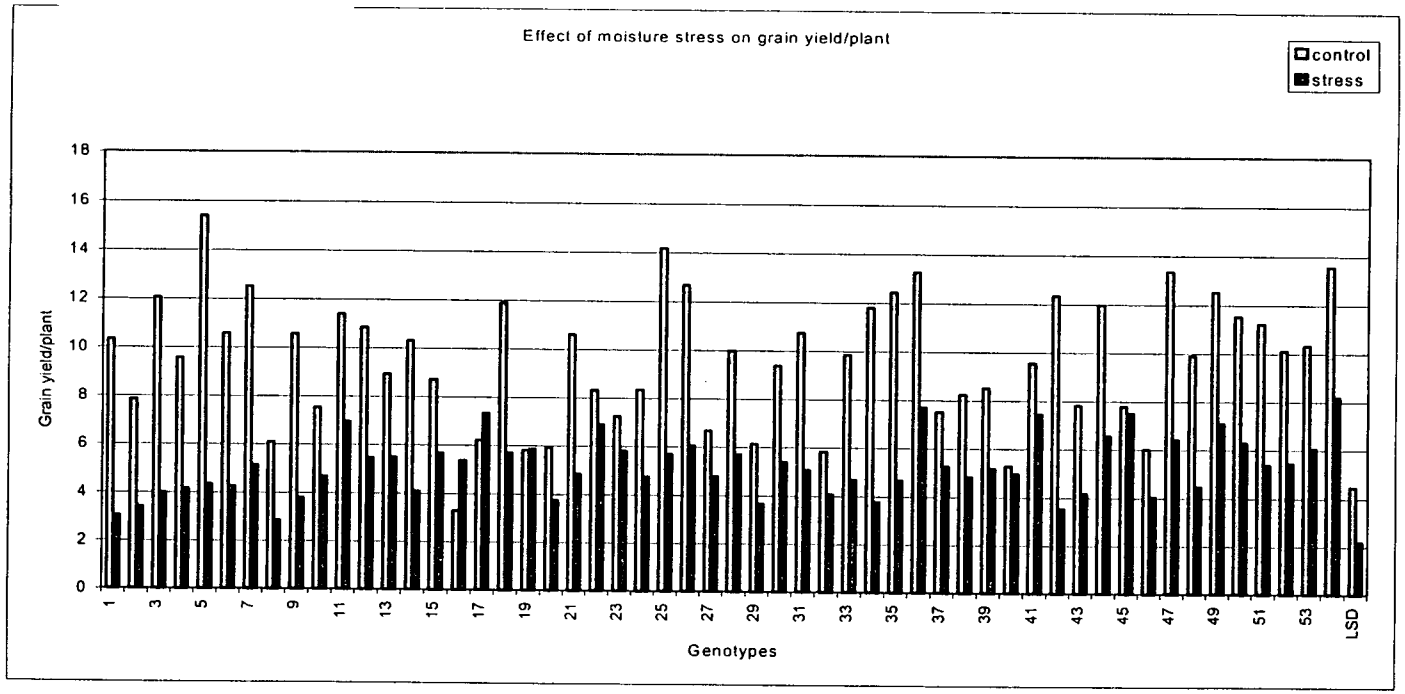

Fig. 3.1 Effect of moisture stress on grain yield/plant

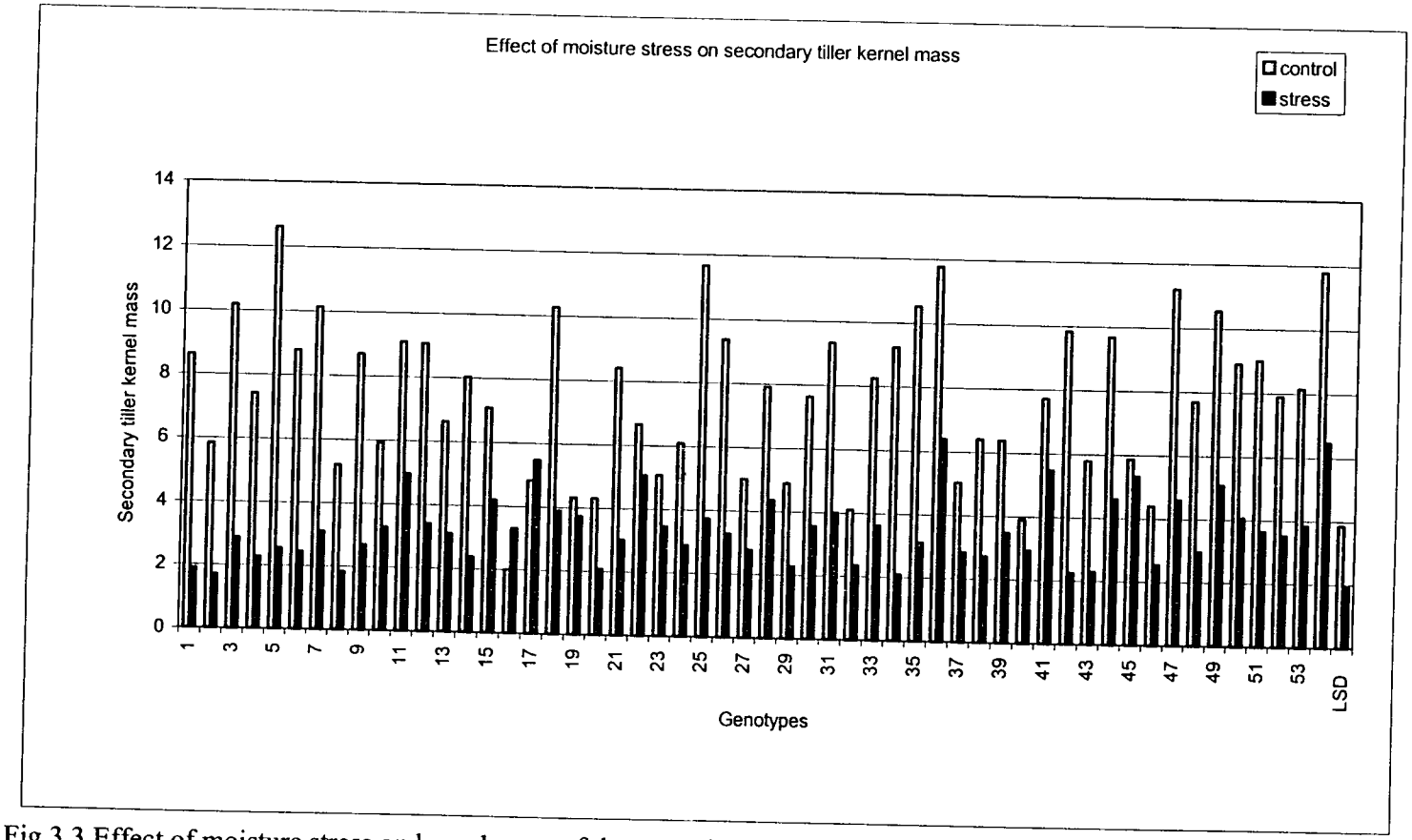

Fig.3.3 Effect of moisture stress on kernel mass of the secondary tiller.

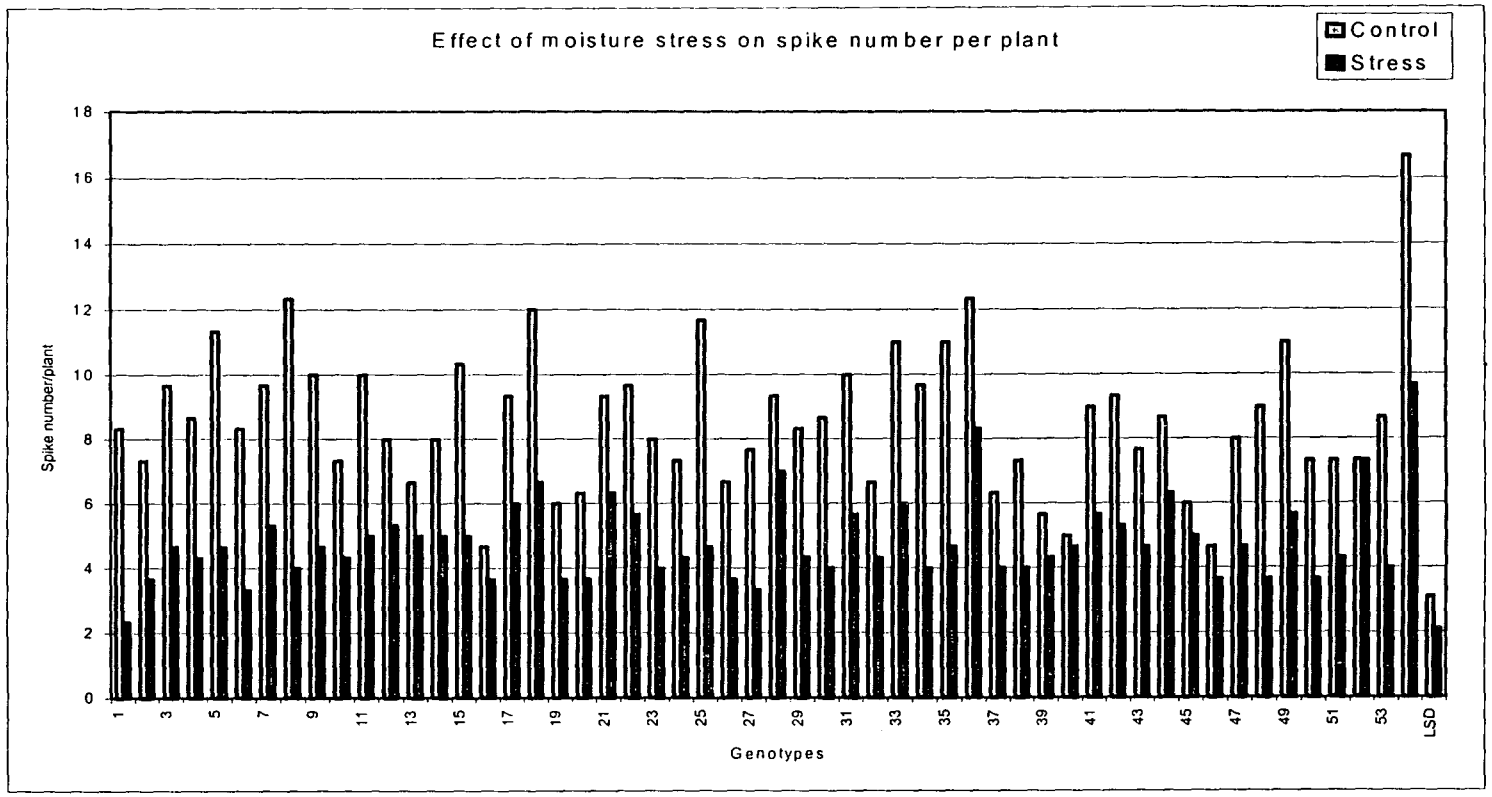

Fig. 3.4 Effect of moisture stress on spike number per plant

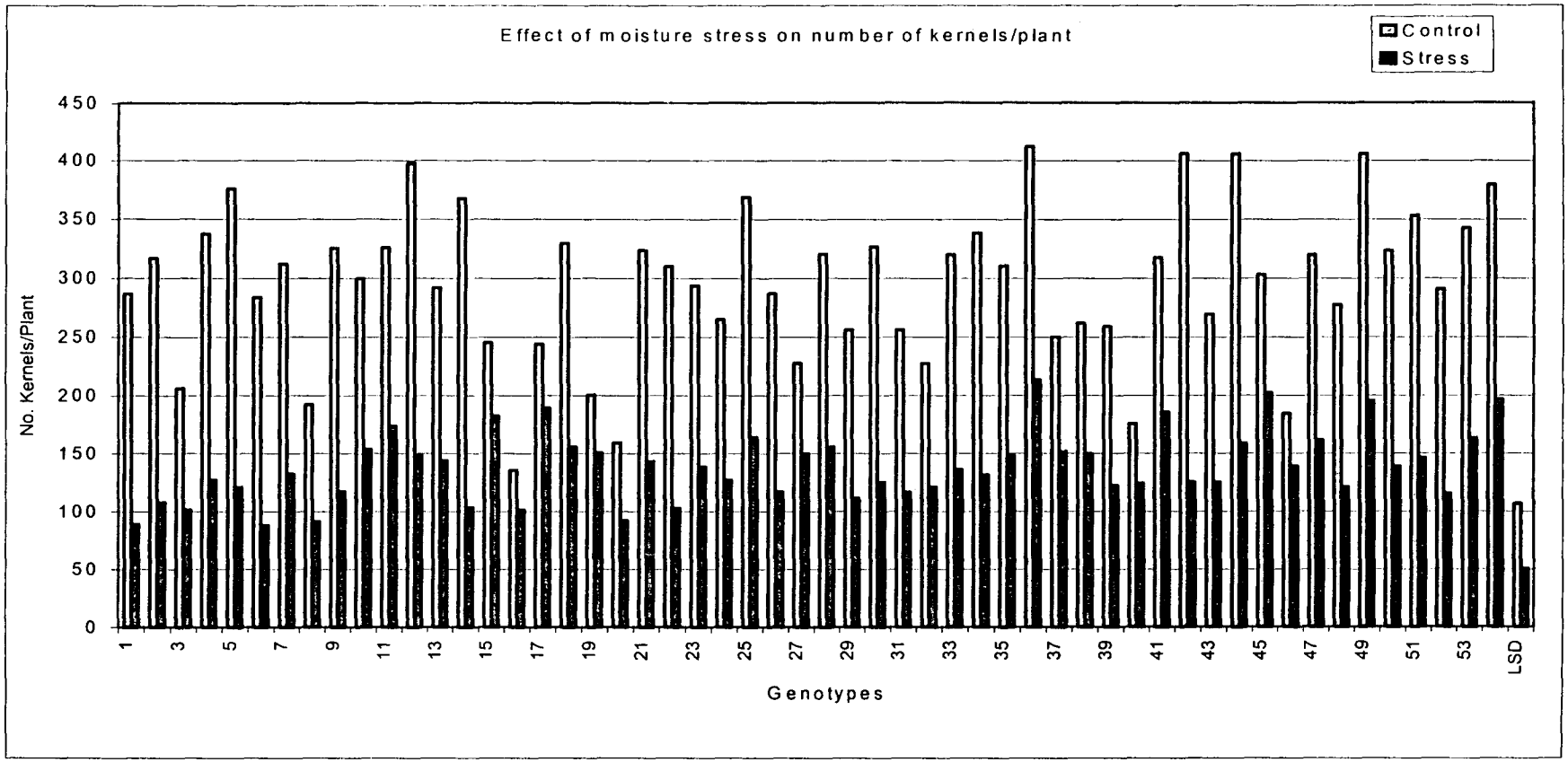

Fig. 3.5 Effect of moisture stress on number of kernels/plant

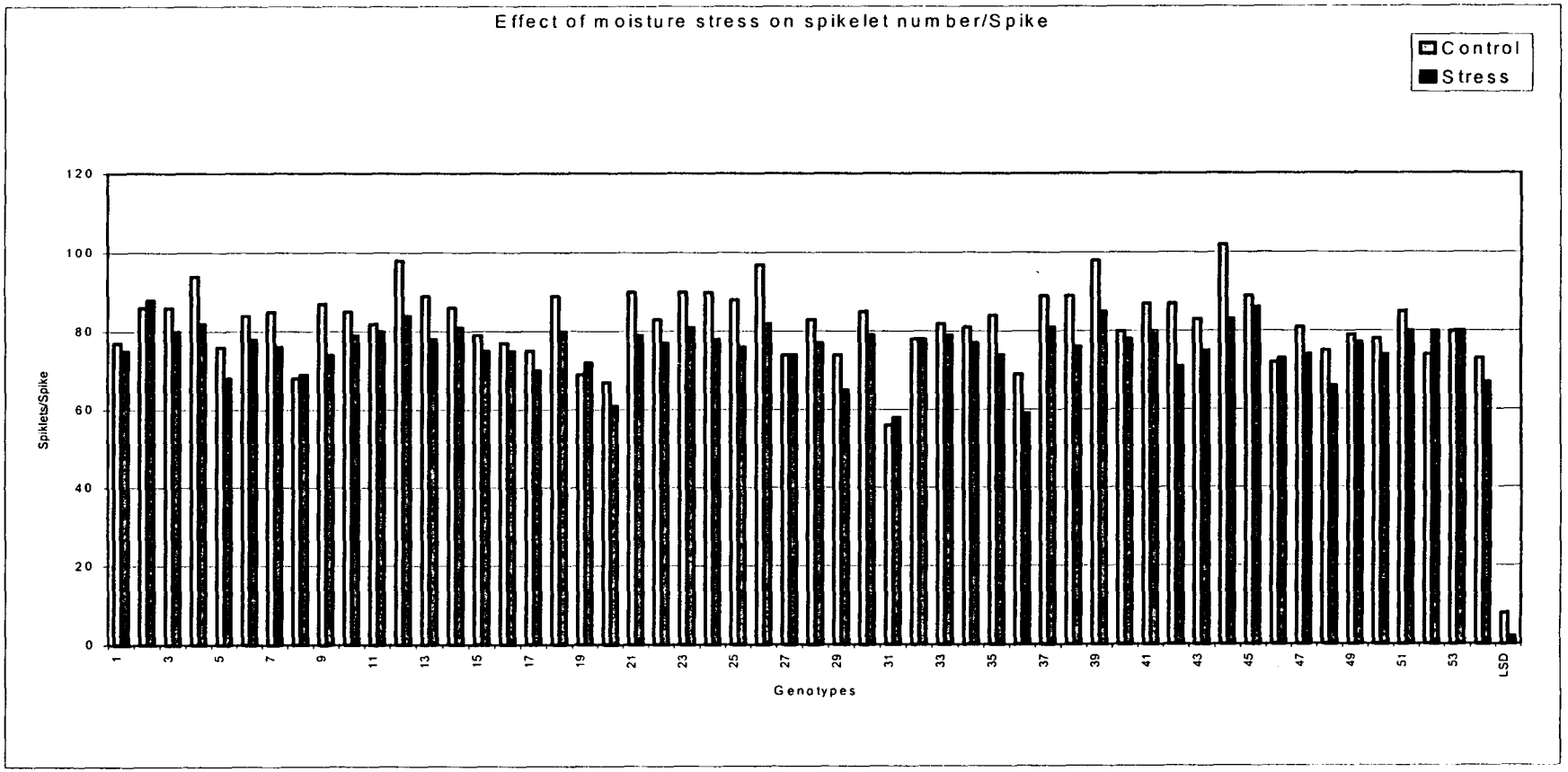

Fig. 3.6 Effect of moisture stress on spikelets number/spike

Appendix-B

Table 4.4 The migration distance of gliadin subunits of Bdl-16

1	2	3	4	5	6	Mean
14	14	14	10	-	10	12.4
27	27	27	24	24	24	25.5
35	35	35	32	32	32	33.5
55	55	55	53	53	55	54.3
-	68		66	68	67	67.3
-	78		76	77	75	76.5
81	84	82	80	81	-	81.6
-	89	90	89	89	87	89
96	96	96	95	95	98	96
105	105	108	106	106	-	106
119	120	120	120	120	118	119.5
128	128	127	128	128	128	127.8
137	137	137	139	138	140	138
165	160	161	162	-	162	162
169	166	166	-	-	168	167.3
189	189	189	188	-	-	189

Table 4.5 The migration distance of gliadin subunits of Bdl-17

1	2	3	4	5	6	Mean
8	9	9	9	10	7	8.7
21	21	21	-	-	23	21.5
30	31	31	28	29	29	29.7
56	56	58	-	58	56	56.8
60	60	61	61	-	61	61
66	69	70	-	68	68	68.2
87	-	89	85	89	89	87.8
96	96	96	98	94	97	96.2
106	106	-	106	107	108	106.6
113	114	114	110	111	112	112
126	127	128	125	126	127	126.5
133	134	134	135	132	134	133.7
140	141	-	-	140	141	140.5

Table 4.6 The migration distance of gliadin subunits Bdl-20

1	2	3	4	5	6	Mean
2	3	3	-	1	1	2
12	12	11	-	13	13	12.2
-	-	30	28	27	26	27.75
57	57	55	-	56	58	56.5
61	61	-	61	62	61	61
69	69	68-	-	-	68	68
72	73	-	76	-	71	72
83	83	83-	85	-	84	83.5
92	91	-	91	90	-	91
98	96	96	-	97	98	97
105	105	104-	105	106	104	105
114	113	-	114	116	115	114.5
128	-	127	125	127	-	127
132	131	132	131	131	130	131
136	137	136-	136	-	137	136.5
-	146	149	148	147	-	147.5
174	171	175	174	-	-	173.5
184	-	185	185	183	185	184.4
193	194	191	192	193	191	192.3

Table 4.7 The migration distance of gliadins subunits of Bdl-29

1	2	3	4	5	6	Mean
8	10	10	7	7	7	6
15	16	17	13	-	13	14.5
26	28	29	25	27	28	27
53	56	57	55	53	54	54.6
65	64	64	65	63	-	64.2
68	67	-	68	66	68	67.4
	77	78	75	77	-	76.8
	90	90	90	88	88	89
101	103	-	103	-	105	103
115	115	116	117	117	118	116.3
-	124	125	124	125	125	125.2
-	129	131	-	129	129	129.5
171	173	172	171	174	174	172.5
-	192	192	193	191	190	191.6

Table 4.8 The migration distance of gliadin subunits of Bdl-19

1	2	3	4	5	6	Mean
4	4	2	4	-	-	3.5
14	14	14	16	-	15	14.6
27	27	27	29	29	28	27.8
35	36	35	32	-	-	34.5
-	49	-	48	48	47	48
57	58	58	59	58	59	58.2
61	62	62	63	-	63	62.2
70	-	69	68	-	70	69.3
78	77	-	77	79	-	77.8
91	92	-	92	93	92	92
98	99	99	-	-	-	99
106	106	106	107	-	-	106
114	-	114	112	114	115	114
121	120	120	120	119	118	120
127	128	128	127	-	129	128
132	132	133	130	131	-	132
162	162	162	164	-	163	162.5
182	182	181	182	183	-	182
186	185	186	185	187	187	186
192	191	191	192	192	-	191.6

Table 4.9 The migration distance of gliadin subunits of Bdl-8

1	2	3	4	5	6	Mean
1	-	2	-	1	1	1
12	11	11	9	-	-	10.8
-	-	18	17	17	17	17
31	30	30	-	-	-	30
-	-	-	37	37	37	37
60	60	60	-	-	-	60
62	62	62	-	-	-	62
69	69	69	69	70	70	69
-	-	-	77	77	77	77
81	80	82	-	-	-	81
93	91	90	90	89	90	90
103	103	102	99	98	99	100.3
119	117	116	110	111	109	113.7
123	122	122	123	124	122	122.7
132	132	131	131	131	130	131
142	141	140	144	144	144	142.5
--	144	145	148	147	147	146.5
192	193	194	195	194	-	193.6
-	196	199	197	199	-	197.8

Table 4.10 The migration distance of gliadin subunits of Bdl-11

1	2	3	4	5	6	Mean
12	13	11	15	15	14	13.3
27	27	25	30	30	29	28
32	33	32	-	-	-	32
36	38	36	37	37	36	36.8
66	65	65	65	65	-	65.4
72	72	73	72	72	74	72.7
81	-	80	79	79	78	79.2
86	86	85	86	85	86	85
99	99	97	100	100	101	100.3
111	113	110	112	-	110	111
117	118	116	118	119	117	117
129	130	127	128	129	127	128.3
139	140	139	138	142	143	140
176	178	176	178	175	176	176.5
185	183	183	182	181	-	182.8
188	188	187	188	186	188	187.5
190	191	190	191	191	192	191
196	199	198	198	197	198	197.7

Table 4.11 The migration distance of gliadin subunits of Bdl-47

1	2	3	4	5	6	Mean
11	12	14	15	15	15	13.7
25	26	29	30	30	29	28.2
33	33	36	37	36	36	35.2
40	44	44	-	42	45	43
62	63	62	64	65	64	62.8
-	67	67	68	67	68	67
71	73	73	74	74	74	73.2
88	86	87	85	87	87	86.7
-	99	101	98	100	101	100
109	109	107	108	109	109	108.5
118	118	116	117	-	115	118.5
126	126	126	128	126	123	125.8
-	131	-	133	-	130	131.3
139	140	142	142	142	143	141.3
145	147	148	150	150	151	148.5
-	171	172	-	173	174	172.5
-	184	184	183	181	182	181.5
187	-	184	183	184	186	184.8
192	192	-	189	190	190	190.6
197	199	199	-	195	199	197.8

Table 4.12 The migration distance of gliadin subunits of T4

1	2	3	4	5	6	Mean
7	6	5	8	7	7	6.7
14	14	14	12	-	11	13
24	23	23	24	23	22	23.2
36	34	33	36	35	34	34.7
47	-	-	42	49	45	45.8
55	54	52	53	54	57	54.2
74	72	75	73	74	72	73.3
78	76	79	79	-	79	77.4
82	81	83	83	81	84	82.3
87	85	-	89	87	-	87
96	92	94	94	93	-	93.8
103	102	100	-	104	100	101.8
115	113	111	-	110	112	112.2
130	128	126	130	129	133	129.3
140	139	136	140	137	138	138.3
148	146	144	144	142	143	138.3
-	184	184	184	181	-	183.3
195	196	198	196	196	198	196.3

Table 4.13 The migration distance of gliadin subunits of Bdl-5

1	2	3	4	5	6	Mean
5	6	7	8	9	9	7.3
20	21	22	23	24	24	22.3
27	28	28	29	29	29	28.3
54	51	52	53	52	53	52.5
57	56	57	57	-	59	57.2
62	59	59	60	61	61	60.3
75	74	70	71	70	-	71.8
-	77	79	78	78	79	78.2
85	84	-	85	-	83	84.2
91	92	91	90	93	92	91.5
96	98	100	100	-	101	99
105	109	108	106	-	-	107
121	120	119	121	120	122	120.5
133	134	135	135	134	135	134.3
141	140	141	140	142	-	140.3
190	190	190	-	-	191	190.3
194	193	-	193	-	195	193.8
199	195	198	199	196	199	197.6

Table 4.14 The migration distance of gliadin subunits of Bdl-25

1	2	3	4	5	6	Mean
10	9	10	16	15	15	12.5
30	29	31	37	36	36	33.2
47	48	49	56	55	55	52
61	61	62	65	65	69	65
-	64	65	69	70	-	69
-	75	77	79	78	77	77
92	92	-	-	90	92	91.5
97	96	96	99	-	-	97
-	109	-	106	106	104	106.3
-	120	119	120	120	120	119.3
121	124	-	126	126	-	124.3
131	130	133	131	130	130	131
138	-	138	138	-	138	138
148	149	150	149	149	149	149
181	181	183	184	181	183	182.2
194	191	193	195	190	192	192.5
-	198	195	199	196	197	197

Table 4.15 The migration distance of gliadin subunits of Bdl-45

1	2	3	4	5	6	Mean
9	10	10	12	-	10	10.2
22	22	23	21	-	-	22
32	32	32	30	31	31	31.3
-	43	-	41	41	42	41.8
50	48	-	49	49	50	49.2
53	54	55	-	-	55	54.3
69	71	70	70	69	-	69.8
81	82	82	80	81	82	81.3
116	115	116	114	113	115	114.8
128	129	130	129	129	130	129.2
135	135	-	134	-	-	135
-	-	136	137	138	138	137.3
143	143	143	142	142	143	142.6

Table 4.20 The migration distance of gliadin subunits of Bdl-1

1	2	3	4	5	6	Mean
7	7	9	-	-	5	7
14	12	-	-	13	14	13
22	23	24	21	22	24	22.7
36	36	38	32	34	36	35.3
54	54	-	52	54	56	54
72	71	74	70	73	71	71.8
81	83	84	82	85	-	83
93	-	93	91	93	-	92.5
98	101	-	97	100	97	98.6
120	121	-	122	125	-	122
145	144	142	-	144	-	143.8
-	148	147	-	149	147	147.8
152	154	151	-	-	153	152.5
157	158	157	-	--	156	156.5
171	170	173	174	175	172	172.5
182	180	184	-	182	183	182.2
187	-	-	-	189	185	187
197	196	199	195	-	-	196.8

Table 4.21 The migration distance of gliadin subunits of Bdl-2

1	2	3	4	5	6	Mean
6	7	6	-	6	7	6.4
10	11	10	12	13	13	11.5
25	26	24	24	24	24	24.5
38	37	36	36	36	36	36.5
61	60	63	61	64	-	61.8
75	77	76	77	77	78	76.6
88	89	90	89	90	91	89.5
93	94	95	-	95	-	93.2
107	108	107	105	105	106	106.3
119	120	119	117	117	118	118
127	128	128	130	127	-	128
134	135	134	-	131	131	133
143	144	143	141	141	142	142.3
152	154	150	148	149	150	150.5
159	160	159	161	-	161	160
183	182	180	-	180	183	181.6
-	-	199	199	199	199	199

Table 4.18 The migration distance of gliadin subunits of Bdl-41

1	2	3	4	5	6	Mean
10	10	10	8	8	8	9
26	26	25	24	24	24	24.8
36	36	36	36	36	36	36
57	58	56	57	57	57	56.8
-	63	64	65	64	64	63.6
77	76	76	76	77	77	76.6
90	90	89	90	91	91	90
98	98	98	98	98	98	98
109	-	107	-	108	108	108
118	118	117	118	118	119	118
124	125	124	122	123	127	124
134	134	133	135	133	133	133.6
145	144	143	142	143	141	143
152	152	151	150	151	150	151
159	158	158	156	156	154	156.8
-	191	-	192	193	192	192

Table 4.19 The migration distance of gliadin subunits of Bdl-36

1	2	3	4	5	6	Mean
1	-	2	4	5	5	3.4
8	7	7	7	8	8	7.5
25	25	25	24	24	24	24.5
36	36	36	36	36	36	36
48	48	-	47	49	-	48
56	56	56	-	-	57	56
-	61	64	63	61	-	62
75	75	74	-	72	-	74
78	79	-	78	77	78	78
-	83	82	84	-	84	83
88	88	87	90	90	90	88.3
98	96	98	-	97	96	97
108	-	-	108	107	108	108
117	117	116	119	120	119	118
127	-	126	127	127	127	126.8
133	132	131	133	133	132	132.3
143	142	142	141	141	140	141.5
151	150	149	150	149	149	149
156	-	-	156	154	155	155.3
-	-	194	191	195	192	193

Table 4.16 The migration distance of gliadin subunits of Bdl-32

1	2	3	4	5	6	Mean
8	9	-	-	7	6	7.5
23	23	24	-	23	-	23.3
33	33	31	32	31	33	32.2
43	-	43	42	43	44	43
-	51	51	50	51	51	51
55	57	57	57	55	58	56.5
83	82	83	81	-	82	82.2
97	97	97	-	99	96	97.2
109	110	110	-	-	111	110
116	116	116	115	116	116	116
125	-	125	-	121	121	123
131	130	130	131	133	134	131.5
137	136	137	136	138	138	137
145	144	144	143	146	145	144.5
151	151	151	-	150	151	151
184	-	182	-	182	184	183
138	-	199	-	199	198	198.5

Table 4.17 The migration distance of gliadin subunits of Bdl-44

1	2	3	4	5	6	Mean
3	3	-	3	2	3	3
24	24	24	-	23	24	24
34	33	33	33	34	34	33.6
-	-	44	45	45	45	45
55	54	55	53	53	53	53.8
69	68	67	-	-	67	67.8
-	81	-	82	83	83	82.3
-	85	-	85	85	86	85
97	97	95	-	94	94	95.4
109	109	107	106	106	106	107
115	115	114	117	117	117	115.8
125	124	122	121	122	121	122.5
137	135	134	135	135	134	135
-	143	-	141	140	140	141
175	176	-	177	179	178	177
-	192	-	194	172	192	192.5

Table 4.22 The migration distance of gliadin subunits of Bdl-42

1	2	3	4	5	6	Mean
4	-	5	-	4	5	4.5
11	11	12	10	10	10	10.7
22	-	-	23	23	23	23
28	28	30	31	32	31	30
-	39	39	38	-	38	38.5
51	52	53	54	54	54	53
58	58	59	58	58	58	58.2
66	67	68	67	67	67	66.8
72	72	73	73	73	73	72.6
74	75	76	-	-	75	75.2
90	91	91	91	91	-	90.8
100	101	102	103	103	100	101.2
116	117	118	117	117	116	117
127	128	128	125	125	126	126.8
134	135	135	-	-	132	134
140	140	141	141	141	141	140.6

Table 4.23 The migration distance of gliadin subunits of Bdl-23

1	2	3	4	5	6	Mean
6	7	8	-	-	9	7.5
19	20	20	-	21	21	20.5
29	30	31	31	27	28	29.3
69	67	67	-	69	69	68.2
78	80	80	81	-	81	80
91	92	93	93	-	-	92.3
104	106	107	104	107	109	106
125	126	127	127	124	125	125.7
133	134	135	-	131	132	133
139	140	139	140	136	138	138
196	198	198	199	-	197	197.6