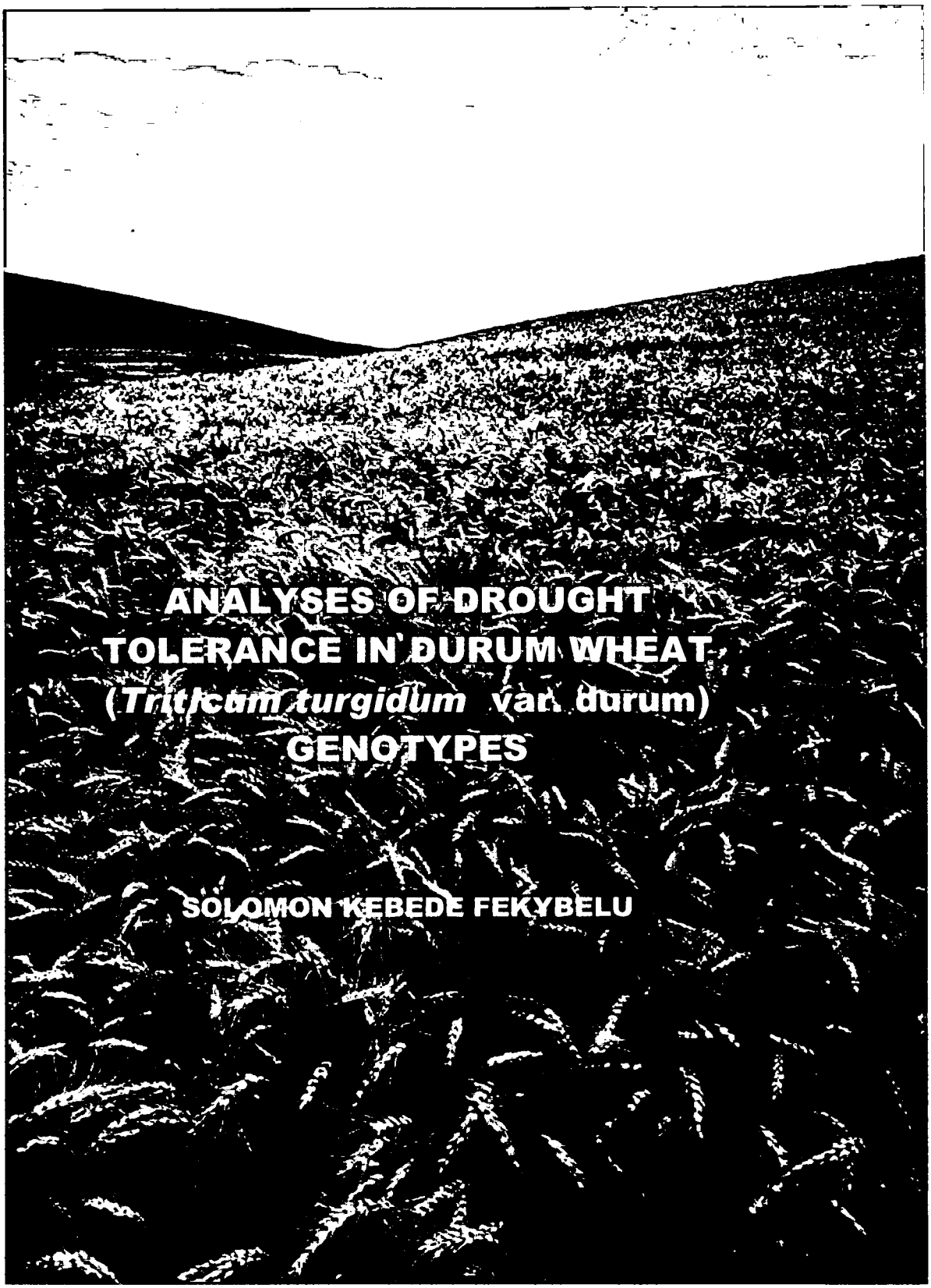

ANALYSES OF DROUGHT TOLERANCE IN DURUM WHEAT (*Triticum turgidum* var. durum) GENOTYPES

SOLOMON KEBEDE FEKYBELU

ANALYSES OF DROUGHT TOLERANCE IN DURUM WHEAT (*Triticum turgidum* var. *durum*) GENOTYPES

By

SOLOMON KEBEDE FEKYBELU

Submitted in the fulfillment of the academic requirements for the degree of

Philosophiae Doctor

In the Department of Plant Sciences (Plant Breeding)

Faculty of Natural and Agricultural Sciences

University of the Free State

Bloemfontein, RSA

Supervisor: Prof. M.T. Labuschagne (Ph.D)
Co-supervisor: Dr. C.D. Viljoen

March 2003

DEDICATION

My parents: Tsgie Yirgashewa and Kebede Fekybelu

My brother, Fantahun Kebede.

My wife, Selamawit Zerihun

My son, Krubel Solomon

DECLARATION

I hereby declare that this dissertation, prepared for the degree of Philosophiae Doctor, which was submitted by me to the University of the Free State, is my own original work and has not previously in its entirety or in part been submitted to any other university. All sources of materials and financial assistance used for the study have been dully acknowledged. I also agree that the University of the Free State has the sole right to the publication of this dissertation.

Signed on March 2003 at the University of the Free State, Bloemfontein, South Africa.

Signature----------

Solomon Kebede Fekybelu

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List of some symbols and abbreviations

ϵ =extinction coefficient of NADPH

ΔA =change in substance concentration

μg = microgram

μl = microlitre

μM = micromolar

A = absorbance

AFLP = amplified fragment length polymorphism

ATP = adenosine triphosphate

bp = base pair

°C = degree Celsius

c = concentration mg per gram of fresh weight

cm = centimeter

CTAB = cetyltrimethyl ammonium bromide

D = Additive genetic variance

d = light path(0.8698 cm)

DAP= Days after planting

DF = days to flowering

DH = days to heading

DM = days to maturity

DMSO = dimethyl sulphoxide

DNA = deoxyribonueclic acid

DNS = dinitrosalicylic acid

DNTP = deoxynucleoside triphosphate

DZARC=Debrezeit Agricultural Research Center

E = Expected environmental variance

EDTA = ethylenediamin tetra acetic acid

ETba = Evapotranspiration after anthesis

ETba = Evapotranspiration before anthesis

F= dilution factor

g = gram

GFP = grain filling period

H_1 = Uncorrected dominance genetic variance

H_2 = Corrected dominance variance

H^2= Narrow sense heritability

h^2=Dominance effect and also broad sense heritability

H_2O = water

HCl = hydrochloric acid

HI = harvest index

KCl = potassium chloride

KD= Number of dominant genes

kg = kilograms

KR = Number of recessive genes in the parents

LAR = leaf area ratio

LSD = least significant difference

LWR = leaf weight ratio

m = meter

M = molar

mg = milligram

$MgCl_2$ = magnesium chloride

min = minute

ml = milliliter

mM = millimolar

mol = mole

MW=molecular weight of the substance

NaCl = sodium chloride

NADP=Nicotinamide adenine dinucleotide phosphate

NADPH= Chemically reduced form of NADP

NaOH = sodium hydroxide

NAR = net assimilation rate

NCSS = number cruncher statistical system

ng = nanogram

nm = nanometre

PCR = polymerase chain reaction

r = correlation coefficient

RAPD = random amplified polymorphic DNA

RFLP = restriction fragment length polymorphism

r_g= genotypic correlation

RGR = relative growth rate

r_p= phenotypic correlation

RSA = Republic of South Africa

SDS = sodium dodecyl sulphate

SLA = specific leaf area

SSR = simple sequence repeat

TAE = Tris, acetic acid and EDTA

Taq = *Thermus aquaticus*

TDM= Total above ground biomass

TE = Tris EDTA

T_G = Transpiration efficiency based on grain yield.

Tris-HCl = (Tris [hydroxymethyl] aminomethane) hydrochloric acid

T_{TDM}= Transpiration efficiency based on TDM

UPGMA = unweighted pair group method using arithmetic averages

V= volume

Wr+Vr = Measure of parental order of dominance

WUE_G = Water use efficiency based on grain yield

WUE_{TDM} = Water use efficiency based on TDM

Yr = Parental size

CHAPTER 1

General introduction

Durum wheat (*Triticum turgidum.* L. var *durum*) has its primary center of origin in the Mediterranean region (Simmonds, 1976; Zeven and De Wett, 1982). An immense genetic diversity, however, has developed after it was introduced to Ethiopia (Zeven and De Wett, 1982; Perrino and Porceddu, 1990). Currently, durum wheat is one of the most important wheat species grown in Ethiopia (Tesfaye *et al.*, 1998). The country is known to have an amazing wealth of genetic diversity and has contributed a lot to the world durum wheat improvement programs (Tesfaye, 1987). Durum wheat is widely grown in the semi arid tropics, mainly as a rainfed crop (Nachit and Quassou, 1988). The area suitable for agriculture in this region is very small and fragile. In the West Asia and North Africa (WANA) region alone, only 8% of the total area is suitable for agriculture. Irrigated land in this region accounts only for 27% of the arable land (35 million ha)(Van Schoonhoven,1989). Production of durum wheat in these environments is often low and variable in both space and time. In WANA region, variation in seasonal rainfall has been reported to account for 75% of wheat yield variation (Blum and Pnuel, 1990). Hence, low and erratic rainfall are the major climatic factors influencing wheat yield variability in space and time (Singh and Bayerelee, 1990).

No other environmental factor limits global crop productivity more severely than water deficit (Boyer, 1982; Fischer and Maurer, 1978). Plant growth and development can be affected by water deficit at any time during the crop life cycle, but the extent and the nature of damage, the capacity for recovery and the impact on yield depends on the developmental stage at which a crop encounters stress (Saini and Westgate, 2000). In general, moisture deficit

occurring during reproductive development stages has been known to cause the most dramatic grain yield reduction. Water stress during vegetative development or during flower induction and inflorescence development in cereals retards the rate of inflorescence development leading to a delay or even complete inhibition of flowering (Saini and Westgate, 2000).

Drought resistance is usually quantified in a crop by its grain yield under stress in the absence of an understanding of specific mechanisms of tolerance (Fisher and Maurer, 1978; Clarke *et al.*, 1992). Genetic variability among genotypes of the same species has been observed in many crops in the degree of sensitivity to drought stress. Various researchers have shown the prevalence of ample genetic diversity with respect to drought tolerance based on grain yield, yield components, and yield-derived indices in both hexaploid and tetraploid wheat species (Narayan and Misra, 1989; Bansal and Sinha, 1991; Clarke *et al.*, 1992; Lan *et al.*, 1993; Simane *et al.*, 1993; Cedola *et al.*, 1994; Dib *et al.*, 1994; Flagella *et al.*,1994; Kheiralla, 1994; Lazar *et al*, 1995; Liu *et al.*, 1996; Rana and Sharma, 1997; Sutka *et al.*, 1997; Simane *et al*; 1998; Ahmad *et al.*, 1999; Ismail *et al.*, 1999a&b).

Relative yield performance of genotypes in drought stressed and more favourable environments seems to be a common starting point in identification of traits related to drought tolerance and selection of genotypes for use in breeding for drought prone environments (Clarke *et al.*, 1992). Yield stability, i·e·the extent of variation in yield between stress and non-stress conditions is widely accepted as a better indicator of genotypic response to drought stress (Fisher and Maurer, 1978; Blum, 1988; Blum *et al.*, 1989).

Because grain yield has low heritability, particularly under stress, indirect selection traits are usually sought by breeders. When drought is the major stress under consideration, earliness is an excellent escape mechanism in drought-prone environments. Remobilization of pre-anthesis assimilates, rooting depth, and stay green are usually proposed indirect selection traits (Parlevliet *et al.*,1991). The most promising indirect selection traits in wheat for drought tolerance other than the growing cycle are, however, osmotic adjustment,

accumulation of carbohydrates (Morgan, 1984; Kameli and Lösel, 1995), air to canopy temperature differences (Blum, 1988; Rees *et al.*, 1993), and ^{13}C discrimination (Farquhar and Richards, 1984; Acevedo, 1993; Austin *et al.*, 1997; Condon *et al.*, 2001). The last two traits are also related to yield potential in wheat.

The development of drought-tolerant cultivars is most important due to increasing economic and environmental concerns associated with irrigated agriculture (Jensen *et al.*,1990; Rhoades, 1997; Howell, 2001). This research was carried out in different phases with the following overall objectives:

1. to evaluate the performance of Ethiopian durum wheat germplasm, when submitted to an extended period of moisture stress.
2. to study the genetics of yield, yield components and drought tolerance under stress and non stress conditions.
3. to examine the physiological reactions of genotypes to moisture stress.
4. to analyse the genetic basis of some physiological attributes associated with drought tolerance, and
5. to assess the AFLP-based DNA polymorphism in relation to drought tolerance.

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CHAPTER 2

Literature review

2.1. Durum wheat production

2.1.1. Adaptation

Tetraploid wheat (*Triticum turgidum* L. var *durum*) is the most important wheat species, cultivated in Ethiopia (Tesfaye *et al.*, 1998). The Mediterranean (Perrino and Porceddu, 1990) region and Ethiopia (Perrino and Porceddu, 1990; Kebebew *et al.*, 2001) are the most important centres for diversity for the species. North Africa and West Asia account for the largest part of its production in developing countries (Srivastava *et al.*, 1987). The Mediterranean type of climate is generally found between the altitudes of 30 and 40^0 on the West coast of continents. It is characterized by a dry, hot summer alternating with humid and temperate winters. In Ethiopia, durum wheat cultivation is largely found in the altitude range of 1800- 2800 meters above sea level (Tesfaye *et al.*, 1998). Landraces having low yield potential but good adaptation to poor growing conditions are the major types grown in the country (Getachew *et al.*, 1993; Tesfaye *et al.*, 1993).

2.1.2. Constraints of durum wheat production

Productivity of durum wheat is often low and variable in both space and time. Many biophysical and socio-economic factors can be ascribed for this variability. The major ones, however, are low and erratic rainfall, low soil fertility, low winter temperature, high temperature during grain filling period, several diseases and pests, particularly in the small scale farming system, such as in Ethiopia (Parr *et al.*, 1990). In a Mediterranean climate, the mean temperature of the coldest

month ranges from 3 to 15 °C, never too cold for plant growth. Therefore, winter cereals grow well in these environments (Acevedo *et al.*, 1999). It rains during winter and plants grow vigorously as soon as the temperature rises in spring. The rain is stored in the soil, which supplies the spring and summer water requirements. The soil water is usually not enough to supply the crop water requirements towards maturity and crops suffer post-anthesis water stress (Santibañez, 1994). Seventy to 90% of wheat grain yield is produced by post anthesis photosynthesis (Austin *et al.*, 1997). Water stress during the grainfilling period reduces carbon assimilation, thus hampering grain filling and yield (Johnson and Moss, 1976). In many regions where durum wheat is grown as a rain-fed crop, year to year variability is too high to establish definite agronomic practices, such as soil management and fallow management and fallow practices that store water in the soil profile. The seasonal water availability and requirement as determined by planting date, crop density and soil fertility, must be balanced (Loomis and Connor, 1992).

2.2. Drought

Crop water stress may be conveniently defined at the soil water level at which evapotranspiration falls below its maximum value (Acevedo *et al.*,1999). It is a common problem in rain-fed agriculture due to irregularity of rainfall and to the largely unpredictable nature of weather within most climatic environments. Major points that need attention according to Acevedo *et al.* (1999) are:

1. drought is a complex problem
2. several disciplines are dealing with it and
3. the problem should be viewed from a system perspective.

Drought is the major factor limiting plant growth and productivity. Much of the injury to plants caused by stress exposure is associated with oxidative damage at the cellular level (Allen, 1995). Under moisture stress conditions, drastic decline in the levels of CO_2 /NADP and increased transfer of electrons to O_2,

leads to the formation of the super radical (O_2^-). The super oxide radical and its dismutation product, hydrogen peroxide, can directly attack membrane lipids and inactivate SH-containing enzymes. This results in lipid peroxidation and membrane injury (Baisak *et al.*, 1994; Alsher *et al.*, 1997; Becana *et al.*, 1998; Sairam and Srivastava, 2001). Water stress inevitably decreases yield. This fact of nature has prompted agronomists, breeders, physiologists, and physical scientists to study the nature of drought, the effect of water stress on plant growth, development and yield, management practices that would alleviate drought and to search for drought resistant genotypes. The common aim is to minimize the effect of drought on yield in cropping systems, and when conditions are extreme, avoid crop failures.

2.2.1. Nature of drought

It is important to be cognizant about the nature of drought in rain-fed agriculture because it bears directly on the strategy adopted to cope with it. Two broad situations can be recognized (Richards, 1982):

1. when a crop grows under current rainfall, that is, the soil profile undergoes recharge and discharge of water during the growing season; and
2. when the crop grows essentially on soil moisture stored prior to sowing.

The first case is typical of the wet monsoonal semi-arid tropics, when stress can occur at any time and with varying intensities between emergence and maturity, especially on lighter soil (Ludlow and Muchow, 1990). The second case is common to cereals grown after a major rainfall period has occurred such as spring-sown crops in Mediterranean environments, the dry season of semi arid tropics in monsoon areas, or in areas with summer rainfall. In both cases, year to year variability is high and so is the risk of drought. In general, the risk increases as seasonal rainfall decreases (Virmani, 1982; Dennet *et al.*, 1984). The critical difference between these two extreme cases is that in case 1, rainfall use efficiency has to be maximized at the moment at which rainfall is occurring,

while in case 2, a strategy should be adopted that would allow completion of the cycle with an already existing and relatively fixed (known) amount of water in the soil profile. The stressful environments are often characterized by the occurrence of more than one physical stress at the same time or through the growing cycle. This complicates improvement either by breeding or crop management. In Mediterranean environments, drought periods during winter may be associated with low temperatures and sub-optimal radiation levels, while terminal drought is generally associated with above-optimum temperatures and excessive radiation (depending on the latitude). Where crops are irrigated, drought and salinity are commonly associated stresses (Acevedo *et al.*, 1999). The soil may impose additional constraints, such as high or low pH, which induces phosphorus and micronutrient deficiencies or toxicity.

2.2.2. Mechanisms of drought resistance

To survive periods of water deficits, higher plants may use one or two main strategies (Levitt, 1980; Blum, 1988). Desert ephemerals and short season annuals have such a short cycle that they germinate after rain, grow rapidly, flower and set seed before the soil water is exhausted in arid environments with low and variable rainfall. These plants are said to escape drought stress. This is a particularly useful strategy under conditions of late season or terminal stress. The cost of such a strategy, however, is the lost opportunity and low yield in better than average seasons (Ludlow and Muchow, 1990). Longer season annuals and perennials survive water stress by one of two drought tolerance strategies. The first group avoids water deficits in tissues in spite of absence of rain and the presence of a hot dry atmosphere by maintaining cell turgor and volume. This can be done by maintaining water uptake, reducing water loss (savers), and changing tissue characteristics, such as osmotic adjustment or an increase in tissue elasticity. The second group resists drought because its tissues are able to tolerate dehydration, usually because of superior protoplasmic tolerance of dehydration (Levitt, 1980; Blum, 1988; Ludlow and Muchow, 1990). Each of these mechanisms includes several morphological,

physiological and biochemical traits that can be used in breeding for drought tolerance. Morphological, physiological and anatomical characteristics have been identified that enable plants to grow and survive in drought prone environments (Taylor *et al.*, 1983; Ludlow and Muchow, 1988;Schultz, 1988). Usually a complex of attributes are present in cereal species that grow and yield under severe drought. In low rainfall environments, crops grow essentially under current rainfall, thus, the best yielding genotypes in favourable environments, usually yield much lower under stress-prone environments. The high yielding genotypes under stressed environments usually are characterized by high crop water use efficiency (Cooper *et al.*, 1987). They also have an early flowering, and fast grainfilling period, which increases yield significantly. High grain yield under drought, high harvest index (HI), high grain weight, early heading, short grainfilling period and high drought resistance index (low drought susceptibility index), prostrate growth habit in winter, dark green leaves before stem extension and light green leaves after stem extension, short stature under drought, high number of tillers, high number of fertile spikes, high C^{13} discrimination, long emergence to double ridge growth stage and short ear initiation and ear growth period are some of the major attributes (Acevedo and Ceccarelli, 1989) that have been identified to help cereals cope with intermittent stress or to temporarily alleviate its effects. These may also include osmoregulation (Morgan *et al.*, 1986; Blum, 1988), accumulation of proline and betaine (Richards, 1983), high carbohydrate accumulation (Kameli and Lösel, 1995); and developmental plasticity (Ludlow and Muchow , 1990).

2.2.3. Quantification of drought tolerance

Drought tolerance in native plant species is often defined as survival, but in crop plant species, it should be defined in terms of productivity (Passioura, 1983). For instance, definition of drought tolerance as the ability of plants to grow satisfactorily when exposed to water deficits (May and Milthorpe, 1962) has little direct application to either quantifying or breeding for the character in crop species (Clarke *et al.*, 1992). Drought tolerance is usually quantified in a crop by

its grain yield under stress in the absence of an understanding of specific mechanisms of tolerance (Fischer and Maurer, 1978; Clarke *et al.*, 1992). Relative yield performance of genotypes in drought stressed and more favourable environments seems to be a common starting point in identification of traits related to drought tolerance and selection of genotypes for use in breeding for drought prone environments (Clarke *et al.*, 1992). Yield stability analysis as proposed by Finlay and Wilkinson (1963) and Eberhart and Russell (1966) is often used when genotypes have been evaluated across environments. However, Lin and Binns (1988) concluded that regression techniques have not contributed to an understanding of genotype by environment interactions. This is because the joint regression analysis part of the genotype by environment interaction accounts for the linear components, but the intercept is highly dependent on potential yield. Furthermore, phenologically unadapted genotypes may present a low regression slope and a high intercept without necessarily implying a high level of stress resistance (Acevedo *et al.*, 1999). Another approach is measurement of drought tolerance in term of minimization of the reduction in yield caused by moisture stress compared to the non-stressed environments (Blum, 1973; Fischer and Maurer, 1978; Langer *et al.*, 1979). Fischer and Maurer (1978) computed drought susceptibility index (S) based on this premise, and 'S' is currently widely used in studies of response of wheat genotypes to drought (e.g. Bruckner and Frohberg, 1987; Ceccarelli, 1987; Clarke *et al.*, 1992). Bruckner and Frohberg (1987) suggested that 'S' is useful for the comparison of performance of genotypes under drought because it accounts for differences in yield potential. However, 'S' does not account for differences in yield potential among genotypes: it measures the ratio of stressed to non-stressed yield in individual genotypes in comparison to the overall ratio for all genotypes in the experiment. Both high and low yielding genotypes can, therefore, have the same 'S' value if both have the same proportional yield change from stressed to non-stressed conditions. Genotypes with low values of 'S' are presumed to be drought resistant or tolerant (Bruckner and Frohberg, 1987), because they exhibit smaller reductions in yield under stress compared

with the non-stress conditions than the mean of all genotypes. Considered in the opposite direction, these genotypes show a smaller than average yield increase in response to improved environment, analogous to the problem in stability analysis in the Finlay and Wilkinson (1963) model. The lack of response to improved environment may be related to lack of adaptation to high moisture conditions due to factors such as lodging or disease susceptibility (Clarke *et al.*, 1992) rather than to the specific drought tolerance traits that are negatively correlated to yield. It is possible that low yielding, nonresponsive genotypes carry traits associated with improved drought tolerance. Sojka *et al.* (1981) suggested that one cultivar might have higher yield than another under stress conditions not because of superior drought tolerance but because of higher yield potential under both stress and non stress conditions. Selection for 'S' would reduce yield potential in favourable environments, just as selection for stress tolerance will usually reduce mean yield and non-stress yield (Rosille and Hamblin, 1981). Lin and Binns (1988) developed a superiority measure (P) to compare productivity of genotypes across environments. This technique utilizes the highest yielding genotypes within each environment as a springboard. Therefore, 'P' is directly related to the agronomic goal (agroecology) of identification of genotypes with high yield potential.

2.3. Selection for drought tolerance

2.3.1. Genetic variation, and selection for yield and yield components

It is necessary to evaluate a wide genetic base germplasm for drought tolerance before beginning to investigate the genetic and physiological basis of drought tolerance. Thus, in the parent selection phase of the breeding program, any technique for measuring drought tolerance is useful, regardless of how cumbersome it may be (Gupta, 1997). Moreover, evaluation and selection has to be conducted in an environment where the would-be cultivar is intended to be grown (Nachit *et al.*, 1989; Gupta, 1997). To this effect, various researchers

have shown the prevalence of ample genetic diversity with respect to drought tolerance based on grain yield, yield components, and yield-derived indices in both hexa and tetraploid wheat species (Narayan and Misra, 1989; Bansal and Sinha, 1991; Clarke *et al.*, 1992; Simane *et al.,* 1993; Lan *et al.*, 1993; Cedola *et al.*, 1994; Dib *et al.*, 1994; Flagella *et al.*,1994; Kaheiralla, 1994; Lazar *et al*, 1995; Liu *et al.*, 1996; Rana and Sharma, 1997; Sutka *et al.*, 1997; Simane *et al;* 1998; Ismail *et al.*, 1999a&b; Ahmed *et al.*, 1999). Sutka *et al.* (1997) have reported that in wheat, relative water content (RWC), relative water loss, drought susceptibility index and phenotypic stability are controlled by genes located on chromosomes 1A, 5A, 7A, 4B, 5B, 1D, 3D, and 5D. They also suggested that alien chromosomes can be used to improve drought tolerance of cultivated wheat species. Al-Hakimi and Jaradat (1998) suggested that improvement of drought resistance in *Triticum turgidum* L. var *durum* is possible through selection not only for morphological traits related to drought but also through direct selection at the F_4 and F_5 generations for yield and yield components from interspecific crosses of durum wheat with *T. dicoccum, T. polonicum and T carthlicum*. In semi arid conditions, where the rainfall distribution is highly variable and usually low, the potential yield under stress is not the best indicator of drought tolerance (Simane *et al.*, 1993). Yield stability, the extent of variation in yield between stress and non-stress conditions, is widely accepted as a better indicator of genotypic response to drought stress (Fischer and Maurer, 1978; Blum, 1988; Blum *et al.,* 1989). Owing to low heritability of grain yield, particularly under stress conditions, selection based on grain yield is less likely to be successful. Simple correlation between grain yield and yield components may also not provide a complete picture of the significance of each of the yield components in determining grain yield (Garcia del Moral *et al.,* 1991). Path analysis of yield components allows the separation of the direct effect of each of the yield components from the indirect influence caused *via* mutual relationships among yield components (Simane *et al.*, 1993). This is because the maximum expression of each of these yield components is sequentially determined according to their order of development. Earlier developing yield components

could affect later developing ones in compensatory fashion during development in the presence of shortage of resources, such as water (Blum, 1983; Fischer, 1985) depending on the growth stage of the crop. Number of kernels per spike in wheat has been shown to have significantly high positive direct effects on grain yield regardless of the timing of stress (Fischer, 1985; Garcia del Moral *et al.*, 1991; Dofing and Knight, 1992; Simane *et al.*, 1993). Number of spikes has a direct positive effect on grain yield. Nevertheless, its indirect effects on yield through kernel per spike and kernel weight are significant but negative (Garcia del Moral *et al.*, 1991; Dofing and Knight, 1992), suggesting a compensatory effect between tillering and grain growth. Cultivars with high tillering capacity would have increased vegetative growth. This would exhaust the limited available soil moisture and reduce the source sink ratio during grainfilling period and could result in reduced HI (Simane *et al.*, 1993). Many believe that either kernels per spike or kernel weight could be used as selection criteria for improvement of grain yield under water limited conditions (Fischer, 1985; Garcia del Moral *et al.*, 1991).

2.3.2. Indirect selection for yield under stress

Because grain yield has low heritability, particularly under stress, indirect selection traits are usually sought by breeders. When drought is the major stress under consideration, earliness is an excellent escape mechanism in drought-prone environments. Remobilization of preanthesis assimilates, rooting depth, and stay green are usually proposed in direct selection traits (Parlevliet *et al.*, 1991). The most promising indirect selection traits in wheat for drought tolerance other than the growing cycle are, however, osmotic adjustment, accumulation of carbohydrates (Morgan, 1984; Kameli and Lösel, 1995), air to canopy temperature differences (Blum, 1988; Rees *et al.*, 1993), and ^{13}C discrimination (Farquhar and Richards, 1984; Austin *et al.*, 1990; Acevedo, 1993). The last two traits are also related to yield potential in wheat.

2.3.2.1. Osmotic adjustment

Osmotic adjustment consists of the active accumulation of solutes in plant tissues as a response to water shortage. This process lowers the osmotic potential and the total water potential of stems, leaves and roots (Turner and Jones, 1980; Girma and Krieg, 1992). As a result plants can absorb water at low soil water potentials and maintain turgor pressure and related physiological processes or activities in plant tissues (Ludlow *et al.*, 1990). Genetic variability and osmotic adjustment have been observed in wheat (Morgan, 1984). Wheat under stress is positively correlated with osmotic adjustment (Acevedo *et al.*, 1999). Glucose has been shown to be the major osmotic factor accounting for up to 85.5% of the total water-soluble carbohydrates of young plants under stress (Kameli and Lösel, 1995). Wheat genotypes with high osmotic adjustment produce high root biomass, higher root length density, extract more soil water, and have higher transpiration (Morgan, 1984). The higher root growth of genotypes adjusting osmotically is related to turgor maintenance as well as to the amount of carbon fixed which in turn is related to the osmotic adjustment of the apex (Turner, 1986). Osmotic adjustment maintains or even increases the harvest index in wheat (Morgan and Condon, 1986). There is little information on the heritability of the trait, even though some information indicates that few genes are involved and the character may be simply inherited (Morgan *et al.*, 1986).

2.3.2.2. Leaf water potential

The whole essence of examining stomatal and cuticular transpiration and resistance to water flow is to maintain a highly negative leaf water potential (Gupta, 1997). Drought-susceptible genotypes have relatively low leaf water potential (Singh *et al.*, 1990; Gupta *et al.*, 2001), high leaf diffusion resistance and low soil moisture extraction, under increasing soil moisture stress, while, drought tolerant genotypes have comparatively high leaf water potential and low leaf diffusion resistance (Blum 1974; Adjei and Kirkham, 1978). Blum (1974) has investigated a sigmoid relationship between leaf water potential and leaf water

saturation deficit as a major aspect of dehydration avoidance in 10 sorghum genotypes. It has been shown that the leaf water potential at which exponential increase in saturation deficit commenced appeared to vary among genotypes (Gupta, 1997). The pm (afternoon) water potential was directly related to yield under various irrigation regimes for a given genotype (Jat *et al.,* 1991; Gupta *et al.*, 2001). Adjei and Kirkham (1978) showed that drought-resistant genotypes of wheat, having high leaf water potential, also had greater stomatal resistance and lower transpiration rates than the sensitive ones. Joubert (1987) suggested that water potential during the flag leaf stage under moisture stress conditions may indicate differences in drought tolerance. Thus, many (O'Toole and Moya, 1978; Blum, 1988; Chen *et al.*,1990; Siddique *et al.*, 1990; Hirusawa *et al.*, 1995; Gupta *et al.*, 2001) believe that leaf water potential could be considered as a criterion for selecting drought tolerant genotypes.

2.3.2.3. Water use efficiency

In environments where water is a limiting factor to productivity, water must be used as efficiently as possible by a crop. Crop water use efficiency is defined as the ratio of grain or biomass yield to water use. Water use is commonly expressed in terms of total water supply (transpiration plus soil evaporation). This allows the evaluation of crop management practices that would increase the use of available water (Cooper *et al.*, 1987). The yield (Y) of a crop grown under dry land conditions can be expressed in terms of transpiration (T), its transpiration efficiency (TE), and harvest index (HI) (Passioura, 1977):

$$Y = T*TE*HI$$

If water use efficiency (WUE) is measured not only in terms of T but total water use, the water balance equation must be considered:

$$E+T+R+D+I-P=0$$

Where, E=soil evaporation, T=transpiration, R=runoff, D=drainage water, I=water intercepted by the canopy, and P=rainfall. So that WUE is expressed as:

$$WUE = (T*TE*HI)/(E+T+R+D+HI)$$

$$WUE= (TE*HI)/1+(E+R+D+HI)/T$$

From this it follows that WUE can be improved through an increase in genotypes' TE and/or by increasing the fraction of ET that is transpired. WUE is inversely related to the vapour pressure deficit (vpd) experienced by the crop during the transpiration period, such that if vpd decreases, WUE increases (Cooper, 1983).

Agronomic practices, such as adjusting planting dates with a period of high available water supply, modification of plant density and spatial arrangements like optimum fertilization and the use of straw mulching can also help improve WUE. Breeding varieties with fast early growth or selecting genotypes with deep roots may also help improve crops' WUE. In semi arid environments, drought tolerant genotypes capable of surviving, and capable of compensating for, or escape damage from wilting and efficient in water use are needed. Water use efficiency indicates the ability to produce the most from every drop of water that becomes available in the plants' environment. By breeding for early harvest, water use efficiency of cowpea (Hall and Grantz, 1981) was increased. It seems probable that careful examination of morphological features by utilizing isogenetic lines will yield valuable information concerning plant characteristics for increased water use efficiency and increased production under semi-arid environments (Gupta, 1997). Once important adaptive features are identified, they can be incorporated into breeding programs. Plants differ in their capacity to regulate how much water is lost per unit of carbon fixed (Condon *et al.*, 2001). This suggests that there may be genetically controlled characteristics that contribute to water use efficiency. Thus, a rapid and simple test for this purpose should be devised to select for the trait and identify genes that contribute to greater water use efficiency (Farquhar and Richards, 1984; Condon *et al.*, 2001). Gupta *et al.* (1997) proposed the use of isogenic analysis to detect genes associated with water use efficiency.

2.3.2.4. Developmental plasticity

Developmental plasticity refers to the mechanisms whereby the duration of the growth period and rate of growth varies depending on the extent of water

availability (Ludlow and Muchow, 1990). Fast early growth when water is available can increase as much as 25% of a crop's seasonal water use efficiency resulting in increased grain and biomass yield (Siddique *et al.*, 1990; Regan *et al.*, 1992; López-Castañeda and Richards, 1994). Comparison between wheat lines in Southern Australia (Turner and Nicolas, 1987; Whan *et al.*, 1991; Regan *et al.*, 1992; Richards, 1992), and durum wheat and barley in Mediterranean type environments (Nachit *et al.*, 1992; van Oosterom and Acevedo, 1992; Cai *et al.*, 1993; Elhafid *et al.*, 1998), have shown the potential for increasing yield of wheat through more vigorous early growth. Whan *et al.* (1991) reported high broad sense heritability for biomass production measured at 49 days after planting. From low to high level of broad sense heritability has also been reported for dry matter production and RGR (Fakorede and Ojo, 1981). Drought-induced early maturity may be advantageous in dry years (Ludlow and Muchow, 1990). Developmental plasticity ensured that the available water was transpired. Developmental plasticity would seem advantageous for genotypes in both modern and subsistence agriculture where unpredictable, intermittent water deficits occur, but it would be of little advantage in terminal stress situations where late rains are unlikely to occur (Ludlow and Muchow, 1990).

2.3.2.5. Leaf area and harvest index

Reduced leaf growth and accelerated leaf senescence are common responses to water deficit, and they both reduce leaf area (Ludlow and Muchow, 1990). Although these responses tend to enhance survival by conserving water, they can be detrimental to productivity upon relief of water stress if leaf area index falls below three (Ludlow and Muchow, 1990) because radiation interception and transpiration as a proportion of evapotranspiration increase up to these values. Consequently, maintaining leaf area is seen as a trait contributing to yield (Turner and Nicolas, 1987; van Oosterom, 1992). However, in the case of terminal stress situations, leaf area maintenance has no effect on the amount of water transpired; a larger leaf area only exhausts soil water more rapidly.

Hence, it may decrease harvest index (HI) if the soil water supply is exhausted before maturity. On the other hand if it allows more time to retranslocate pre-anthesis dry matter, leaf area maintenance could increase harvest index. In intermittent stress conditions, leaf area maintenance would increase the amount of water transpired at leaf area index greater than three, and would increase the HI if this results in greater radiation interception during grainfilling (Siddique *et al.*, 1989; Blum, 1990). In terms of survival determinants, leaf area maintenance would lower dehydration avoidance by maintaining water loss (Ludlow and Muchow, 1990). Harvest index (HI) is defined as the ratio of economic (grain) yield to shoot biomass at maturity. HI depends, among other factors, on the relative proportion of pre-anthesis and post-anthesis biomass production and mobilization of pre-anthesis assimilates to grain yield. The pattern of water supply also has a large effect on HI. Increased HI is usually related to the amount of water available and transpired after anthesis (Passioura, 1977). Crop breeding should aim to maximize transpiration thereby extending canopy cover as long as practical to minimize evaporation (Ludlow and Muchow, 1990). If transpiration efficiency could be improved, there would be direct benefits for grain yield. The best prospects at the moment for improving grain yield of crops appear to be by increasing the amount of water transpired and maintaining HI (Ludlow and Muchow, 1990).

2.4. Genetic variance and heritability

Breeders normally cross two or more varieties or inbred lines to create variability for a character they wish to improve. Evidently, the critical step in this process is the choice of parents. Choice of parents usually causes serious problem when the main character to be improved has a complex inheritance, such as yield and drought tolerance. It was recognized that for quantitative characters parents cannot be selected with confidence on the basis of their own performance. Certain parents combine, 'nick' well when crossed and produce a large number

of superior segregates, while crosses between equally desirable parents can produce an array of disappointing progeny (Dabholkar, 1992). Hence, breeders handle several segregating generations simultaneously. The objective of hybridisation in several crops, such as, maize, cotton, sorghum is to exploit hybrid vigour, whereas, breeders of many self-pollinated crops are primarily interested in combining desirable genes into a single genotype from two or more genotypes/parents. The *per se* performance of neither the parents nor the hybrids is likely to provide a reliable indication regarding the possibility of isolating superior segregates from the hybrid swarm (Dabholkar, 1992). The cross combinations which appear to be promising in the F_1 need not necessarily give large proportions of desirable genotypes in the later segregating generations. The F_1s may exhibit superior performance due to dominance and/ or non -allelic interaction. In advanced generations, however, linkage breaks and new combinations are formed. This leads to dissipation of the superiority because the degree of dominance observed in the F_1 declines and combinations that bestowed superiority due to non-allelic interaction cease to exist. However, if information could be obtained about the genetic system governing the inheritance of attributes to be improved, it should be possible to assess the potential difference of different crosses in F_1 and F_2 generations and predict their performance in subsequent generations. One of the techniques widely used for this purpose is the diallel analysis.

2.4.1. Components of heritable variances

The science of plant breeding has long relied to a large extent on the creation and quantification of genetic variation. It is unquestionable that progress from selection is less likely to be achieved in the absence of genetic variability. Phenotypic values measured on an individual are made up of a genotypic value and environmental deviation. Variation of phenotypic values is therefore estimated from variance due to genotypic values and environmental deviations. Genotypic values are composed of additive (breeding) values, dominance deviations and interaction deviations. Thus, genotypic variance is made up of

variance of these components (Mather and Jinks, 1977; Singh and Chaudhary, 1977; Falconer, 1989). Variation of breeding value is called additive genetic variance, whereas, variances of dominance deviations and interaction deviations are termed dominance and interaction variance, respectively (Mather and Jinks, 1977; Baker, 1978; Falconer, 1989). The prime importance of measuring phenotypic variation is to partition it into components attributable to different causes. The relative magnitude of these components has been well known to determine the genetic structure (property) of a population, particularly, the extent to which various relatives resemble each other. The degree to which progeny will resemble and continue to resemble the parents is determined by the relative amount of additive and non additive (comprising of dominance and interaction variance) components that make up genotypic variance (Dabholkar, 1992). Additive genetic variance determines degree of resemblance between parents and offspring. It is also dictates the observable genetic properties of the population (Mather and Jinks, 1977; Singh and Chaudhary, 1977; Falconer, 1989; Dabholkar, 1992). This component of genotypic variance is therefore of much importance to the breeders. Dominance variance is variation of dominance deviations. Interaction variance is the variance of interaction deviations. It can arise from the interaction between additive by additive variance, additive by dominance or dominance by dominance.

2.4.2. Heritability

The term heritability was originally introduced by Lush (1943) to describe the ratio of variance due to hereditary difference and genotypic variance to the total phenotypic variance. The higher the ratio the more heritable the trait would be. If, conversely, the ratio is smaller, the more the influence of the environment on the phenotypic expression of the trait. The ratio is now known as broad sense heritablity. The concept of broad sense heritability is useful if interest is in the relative importance of genotype and environment in the determination of phenotypic value. However, it does not indicate the progress that might be made through selection with a particular population (Falconer, 1989; Dabholkar, 1992).

This is because the mean genotypic value of progeny is determined by the average effects of genes transmitted by parents in question, that is, it is the breeding value (additive genetic variance) of the parents that determines the genetic properties of the progeny. Thus, the degree of resemblance between parents and offspring is determined by the breeding value. Hence, it is the properties of the phenotypic variation that is made up of the variation attributable to the breeding values. The ratio of additive genetic variance to the phenotypic variance is known as "narrow sense heritability". It measures the extent of correspondence between breeding values and phenotypic values, and expresses the magnitude of genotypic variance in the population, which is mainly responsible for changing the genetic composition of the population through selection (Falconer, 1989; Mather and Jinks, 1977; Singh and Chaudhary, 1977; Nyquist, 1991). It also provides a basis to predict the accuracy with which selection for genotypes could be made based on phenotypic measurements of individuals or groups of individuals (Dabholkar, 1992). Heritability is a property not only of the character being studied, but also of a population being sampled and the environmental conditions to which individuals have been subjected (Dabholkar, 1992). Populations which are genetically more uniform are expected to show lower heritability than the genetically diverse populations. Since environmental variance is also part of phenotypic variance, it also affects the magnitude of heritability. Environmental variance depends on the conditions of management. More variable environmental conditions reduce the magnitude of heritability and more uniform conditions increase it. Therefore, heritablity of a character refers to a particular population under particular environmental conditions (Falconer, 1989; Dabholkar, 1992).

2.5. Diallel analysis

The term diallel is a Greek word. It means all possible crosses among a collection of male and female animals. Hayman (1954) defined " diallel cross" as the set of all possible matings between several genotypes. The genotypes may be individuals, clones, homozygous lines, etc., and if there are 'n' of them there are 'n^2' mating combinations, counting the reciprocals separately. Diallel mating designs permit estimation of the magnitude of additive and non-additive components of heritable variance (Hayman, 1954; Griffing, 1956; Mather and Jinks, 1977). Some other genetic properties of parental lines can also be studied using this design (Hayman, 1954; Mather and Jinks, 1977; Singh and Chaudhary, 1977). When 'n' inbred lines are crossed in a diallel fashion 'n^2' progeny families are produced. Data obtained from such cross combinations can be analysed in several ways, but most commonly, analyses are based on the procedure proposed by Hayman (1954) and Griffing (1956). Hayman (1954) provided graphical and/or numerical approaches based on the following assumptions:

i. diploid segregation
ii. no differences between reciprocal crosses
iii. independent action of non allelic genes, and in the diallel cross
iv. homozygous parents
v. no multiple allelism
vi. genes independently segregated between parents

On the basis of these premises, a test for the validity of the additive-dominance model has been suggested (Hayman, 1954; Mather and Jinks, 1977). It is also possible to obtain the estimates of additive and dominance components of the heritable components of variation from the mean squares of these mating designs. Determination of average degree of dominance and characterisation of parents containing most dominant and recessive genes are possible. This approach has been widely applied in various crops for different genetic studies

(e.g. Chen *et al.*, 1994; Turgurt *et al.*, 1995; Saadalla, 1997; Kara and Esenda, 1997; Kuo *et al.*, 1997).

2.5.1. Combining ability

Griffing (1956) proposed a more general procedure for diallel analysis that makes provision for non-allelic interaction. According to this approach, mean measurement of a cross is partitioned into major components, apart from the general mean (μ) and an environmental variance, namely

i. the contribution of the parents, the general combining ability (GCA)
ii. the excess over and above the sum of the two GCA effects, termed the specific combining ability (SCA) effect.

The only assumption of the diallel analysis based on the Griffing approach is that parents of the diallel crosses are inbred lines. Estimates of the variance components due to GCA and SCA also provide an apt diagnosis of the relative importance of the additive and non-additive (allelic and non-allelic) interaction effects of genes. The GCA and SCA effects help locate parents and crosses that are responsible for bringing about a particular type of gene action (Baker, 1978). Moreover, since it is not restricted to one gene assumptions and operate with limited feasible assumptions, it is more realistic for plant breeding programs (Arunachalam, 1976). The GCA and SCA effects and their variances are very effective genetic parameters of direct utility to decide the next phase of the breeding program (Arunachalam, 1976; Dabholkar, 1992). It also enables a plant breeder to decide about the strategy of the breeding program, for example, whether to breed for hybrids or pure lines. It also helps choose parents for construction of synthetics, selection of suitable F_1s for a multiple crossing or composite breeding program and the possibility of employing an appropriate selection technique like modified mass selection, recurrent selection or reciprocal selection etc. (Baker, 1978; Dabholkar, 1992). The GCA and SCA effects are important indicators of the potential value of inbreds in hybrid combinations. Differences in GCA have been attributed to additive, additive x additive, and higher order interactions of additive genetic effects in the base

population, while differences in SCA have been attributed to non additive genetic variance (Griffing, 1956; Baker, 1978; Falconer, 1989;). Currently, numerous diallel analyses employ Griffing's approach to aforementioned purposes. For instance in wheat various researchers (Dasgupta and Mandal, 1989; Kathiria and Sharma, 1996; Wagoire *et al.*, 1998; Yunis *et al.*, 1998) have studied combining ability and/or the genetic control of various traits.

2.5.2. Heterosis

Heterosis may be defined as the amount by which the mean of an F_1 exceeds its better parent (high parent heterosis) or the mean of its mean parents (mid parent heterosis). Though not clear until now, it has widely been believed that heterosis arises mainly from heterozygosity. There are different views regarding the genetic basis of heterosis.

i. Dominance theory: heterosis results from favorable dominant genetic factors.
ii. True overdominance: heterosis is obtained as a result of two alleles coding properties that make the heterozygote truly superior.
iii. Pseudo–dominance: Inter allelic interaction (epistatic genetic interaction), or partially dominant alleles in the nearby genetic loci, gives rise to superior F_1s over its best parents as a result of repulsive linkage (Stubber, 1999).

Morgan (1998) described heterosis as a result of accumulation of favourable dominant genes, having non-allelic interaction and directional dominance in the F_1 rather than due to heterozygote superiority or complementary gene interaction. However, Flintham *et al.* (1997) stated that heterozygosity is an important prerequisite for heterosis. According to him, heterosis can arise when over dominance at a given locus is a principal cause. Others, however, believe that dominance and epistasis are the underlying genetic basis of heterosis (Coor *et al.*, 1999).

One strategy to improve drought tolerance in many crops has been to exploit heterosis based on the premise that heterosis for yield and yield components increased under stress. Damarany (1994 a,b,c) has reported high heterosis in the F_1 for weight of dry pod per plant, average pod weight, total yield of dry seed and 100-seed weight in F_1 under moisture stress in cowpea. High levels of heterosis for yield, HI, and spike yield index in wheat have also been obtained under stress conditions (Farashadfar *et al.*, 2001). In many other studies a high level of heterosis under drought stress has been documented (Axtell *et al.*, 1999; Virmani, 1999; Yadaw *et al.*, 2000). In some countries, such as South Africa, where terminal stress is a major drought constraint, heterosis has been widely exploited to ameliorate its impact in wheat productivity (Jordaan, 1999).

2.6. Correlation

Frequently, a change in one character is accompanied by a change in another variable. Such association between two characters has been referred to as correlation. The degree of association between two characters is measured by coefficient of correlation (r). Coefficient of correlation can be either positive, when an increase in one variable is accompanied by an increase in another one, or negative when an increase in one character is accompanied by a decrease in another one. While coefficient of correlation expresses the extent of association between two variables, it tells nothing about the casual relationships of a variable, i.e, which variable is dependent, which is independent or the extent of change of one variable resulting from change in another variable (or linkage of both variables to a third variable, or just coincidence) (Dabholkar, 1992). Correlation can be genetic. Genetic correlation between two or more characters may result from pleiotropic effects of genes or linkage of genes governing inheritance of two or more characters (Falconer, 1989). Correlation resulting from linkage is relatively ephemeral, correlation resulting from pleiotropy is long

lasting since it is the net effect of all segregating genes that influence both the attributes (Dabholkar, 1992). For example some genes may increase the expression of both characters, however, among the remaining genes some may increase manifestation of one character but decrease the other. Correlation can also stem from a net effect of environmental agencies, since some environmental effects may increase one but decrease the other. In many cases the value of a variable is determined by many interacting variables, which calls for an extension of the concept of simple correlation and regression to more than two variables. This needs the concept of partial correlation and partial regression. Partial correlation measures the correlation between two variables after eliminating the effect of other variables in the system. Likewise, partial regression determines the rate of change in one character per unit change in the other variable, when the rest of the variables in the systems are held constant. Association between two characters that is observed and measured is phenotypic correlation, hence it is measured from phenotypic values. Phenotypic values are determined by genotypic values and environmental deviations. Thus, one may find correlation between the breeding values of two characters that will provide an estimate of genotypic correlation. Theoretically, it is also possible to find correlation between dominance deviations of characters. Similarly, it may also be possible to find correlation between non-allelic interaction deviation. Environmental correlation comprises correlation due to environmental agencies and that due to non-additive genetic causes (Falconer, 1989; Dabholkar, 1992). If two characters have high heritabilities, correlation due to environmental agencies will be relatively less important. The genotypic and environmental correlation coefficient may bear different signs. Magnitudes of these correlation coefficients may also be different. Differences in signs suggest that the genetic and environmental agencies are probably influencing different physiological mechanisms. In practice, it is the total genotypic correlation that is usually estimated, rather than the correlation between breeding values of two variables. Additive genetic correlation (breeding value) can, however, be obtained from the

correlation between GCA effects and line performance *per se* (Falconer, 1989; Baker, 1978)

2.7. Role of marker assisted selection

Advances in plant breeding depend on sources of genetic variation, technologies to recombine this variation in the generation of new genotypes and technologies for identifying and selecting genotypes associated with the new adapted phenotypes. Several biotechnologies have been or are in the process of being developed, aimed at improving the efficiency or scope of all of these genetic manipulations. The phenotype of the wheat plant is determined by the effects of approximately 30,000 genes, very few (probably only a few hundred) of these have been identified, mapped, and their primary and pleiotropic effects on plant process elucidated and described (Koebner and Snape, 1999). Most of the selection schemes, such as the pedigree selection system normally practiced in wheat breeding is carried out at the phenotypic level without knowing the genotypic constitution of the segregates examined. This restricts direct manipulation of the variation essentially, selecting desirable gene combinations mainly by chance. In principle, selection would be much more efficient at genotype level, rather than phenotype. However, this requires that the individual genes controlling traits of interest be identified and manipulated singly or in groups (Koebner and Snape, 1999). The development of molecular marker technology is a promising technology to this endeavour. It has been possible, using these tools, to identify and manipulate genes controlling both major genes and polygenic traits (so-called quantitative trait loci- QTL). Once identified, they can be mapped, tagged, and thus followed through breeding programs. There are now several examples where complex traits have been dissected to unravel the numbers and location of genes involved. For instance, microsattelites or RFLPs have been used to the genetic dissection of major fusarium head blight QTL in tetraploid wheat (Otto *et al*., 2002), herbicide

tolerance in maize (Sari-Gorla *et al*., 1997), root growth in rice (Price and Tomos, 1997) and the genetic architecture of quantitative traits (Mackay, 2001).

2.7.1. Molecular markers

Many DNA techniques are used to find molecular markers. Two broad classes of molecular markers can be identified.

2.7.1.1. Hybridisation based molecular markers.

The most common one is restriction fragment length polymorphism (RFLP) (Bostein *et al.*, 1980). It was one of the first DNA marker techniques used to characterize and assess genetic diversity (Kim and Ward, 1997; Paull *et al.*, 1998). It is based on the hybridisation of a cloned length of DNA ("probe") to one or more restriction fragments, which are generated by the cleavage action of endonucleases on genomic DNA. The fragment can vary in length either due to point mutations at restriction recognition sites, or via insertion/deletion events in the genomic DNA sequence flanking the probe. The restriction fragments generated by endonucleases can be separated according to their size (length) using gel electrophoresis, and then transferred to a nylon membrane and denatured. Probes are obtained from various sources such as libraries of genomic DNA (gDNA) or reverse transcriptase products of messenger RNA (cDNA). Probes are labelled by attachment of a fluorochrome or an immunological reagent such as biotin, and allowed to bind with the immobilized restriction fragments. When binding occurs due to sequence homology, detection of a signal is concentrated in bands that can be visualized by an appropriate detection system. The number of bands generated usually gives an indication of the copy number of the sequence in the genome, and hence, allows classification of RFLP loci as single or multiple copies. Wheat is hexaploid, consisting of three similar genomes, and hence single copy sequences generate three bands, a pattern typical of cDNA sequence (Koebner and Snape, 1999). Single copy loci have the advantage that their scoring is unambiguous and that allelism (and therefore co-dominance) can be recognized. In contrast, multicopy

loci generate complex banding profiles, which can be useful in DNA finger printing, but usually are avoided in genetic analysis and marker mediated selection (Koebner and Snape, 1999). Nowadays, the relatively low level of polymorphism obtained based on RFLPs in wheat (Bryan *et al.*, 1999) and the complexity and cost of the technique (Manifesto *et al.*, 2001) is increasingly limiting its application even in routine cultivar identification.

2.7.1.2. Polymerase Chain Reaction (PCR)-based markers

Polymerase chain reaction (PCR) involves:

-thermostable DNA polymerase

-the four single nucleotides, dATP, dCTP, dCTP, and dTTP

-DNA-template

-one or more (usually two) short synthetic oligonucleotides, called primers.

Choice of primers usually specifies the reaction to the particular loci or locus. Application is achieved by cycling the reaction temperature a preset number of times, between melting (denaturing) temperature of DNA (about 90 ^{0}C) and the annealing temperature of the primer(s) to the template, which varies from 35 to 70 ^{0}C depending on the primer length and nucleotide sequence. DNA is synthesised by extending from the primers. The reaction products are separated by size on the gel and visualized by staining with either a fluorescent DNA binding dye or biotin (or ethidium bromide). The advantage of this technology is the relatively low requirements both for the quantity and purity of DNA sample. It has generated a variety of molecular marker technologies in plant breeding. The most common markers are simple sequence repeats (SSR), otherwise called, microsatellites (Koebner and Snape, 1999) and amplified fragment length polymorphism (AFLP), which is recently developed and potentially most powerful of all the PCR-based techniques (Vos *et al.*, 1995). The SSR-technique gained rapid acceptance because of its co-dominant nature, reproducibility and high information content (De Loose and Gheysen, 1995). These loci are amplified by PCR using primers (18-25bp) specific for sequences flanking

hypervariable regions of tandem repeats of 2 or 4 bp (Manifesto *et al.*, 2001). The variation in the number of repeats present in these loci determines differences in lengths of amplified fragments. This methodology is useful in identifying genotypes in self-pollinated species with a low level of genetic variability such as soybean (Rongwen *et al.*, 1995) and wheat (Dommini *et al.*, 2000).

The principle of AFLP is to selectively amplify a defined subset of restriction fragments. Following a double restriction by rare-cutting and a common cutting enzyme, a short oligomer of known sequence is ligated to each cut end. The common cutting enzyme will have the same linker at each end; those with different ends will have a different linker ligated to each end; those with rare cutting sites at each end are not expected to be present at appreciable frequencies. PCR amplification products are produced using primers complementary to the ligated sequence and the remainder of the restriction sites, with the addition of one to three selective bases. These additional bases determine the spectrum of restriction fragments to be amplified. The profile of amplified products (up to 100 in number) (Koebner and Snape, 1999) is therefore specific to both the restriction enzyme combination and selective base used, and is visualized on a sequencing (denaturing polyacrylamide) gel or sequencing machine. This technique has successfully been used to evaluate genetic diversity and genetic relationships in wheat (Salamini *et al.*, 1997; Barrett and Kidwell, 1998; Dommini *et al.*, 2000; Manifesto *et al.*, 2001).

2.7.2. Quantitative Traits Loci (QTL)

One of the principal roles of molecular-based maps is to identify genes of interest, such as those controlling quantitative traits, such as yield, adaptation, quality, and to locate these accurately on the genetic map. The principle of marker-mediated location of genes is to derive an association between the segregation of known marker alleles with differences in the phenotypic expression of the traits. This involves the following steps.

i. establishment of an appropriate mapping population (appropriate recombinant population between the genotypes chosen as differing in phenotype for the characters of interest).

ii. characterization of the individuals (lines) in this population for the marker loci dispersed throughout the genome and identified as being polymorphic in the cross from examination of the parents. In practice this will be done by reference to published maps and availability of particular probes/SSRs. Ideally, the target is to generate markers spaced about 20 to 30 cM apart on each chromosome arm, spanning the centromere. This requires at least six polymorphyic markers per chromosome, given an average genetic distance per chromosome of 150 cM (Koebner and Snape, 1999).

iii. evaluation of the recombinant lines in replicated and randomised experiments in an appropriate environment. The environments, whether in a glasshouse (controlled environment), or field, should allow maximum expression of the genotypic variation while minimizing random environmental error.

iv. Provided there are sufficient maps and good agronomic data, the final step is to use statistical procedures to partition the genotypic variation into components attributable to variation at the individual marker loci, either singly or in linked combinations. The difference between two homozygotes is clearly the most informative, and if significant, a QTL linked to the marker locus is inferred. Further statistical analysis can define the exact location of the QTL on the assumption that only one QTL is in the vicinity of the marker. The initial approaches used individual marker loci in turn as landmarks to estimate the position of (assumed) single QTL's (Snape *et al.*, 1985; Luo and Kearsey, 1993). Recently, however, the analytical approach has become more sophisticated, and numerous approaches are now available to locate genes relative to individual

markers or to flanking markers (Haley and Knott, 1992; Zeng, 1993; Hyne *et al.*, 1994).

2.7.3. Marker Assisted Selection

Marker assisted selection (MAS) has been developed based on the premises that once a gene and a particular allele of interest have been identified and located, indirect selection for the trait of interest can be performed by selecting for a marker closely linked to the trait instead of selecting directly for the trait itself. Single traits can also be targeted without prior knowledge of their map location by a procedure called bulk segregant analysis (BSA) (Michelmore *et al*, 1991). In this procedure DNA from individuals from two alternative phenotypes is bulked and the marker profiles of the two bulks are then compared, in search for a marker that differentiates one bulk from the other. Because the "background" of the two bulks is identical, these differences should lie at loci close to that determining the target trait (Koebner and Snape, 1999). For traits with more complex genetic control, an adequate and complete genetic map is a prerequisite for successful MAS (Koebner and Snape, 1999). MAS has considerable potential advantages which are associated with precision, efficiency and speed in a breeding program (Koebner and Snape, 1999). QTL-analysis and MAS are increasingly applied to the identification and improvement of various polygenetically controlled traits that are difficult to select directly phenotypically. QTL analysis has been applied with the purpose of identifying QTL linked with morphological and physiological traits known to be associated with resistance to various abiotic stress factors, such as drought (Quarrie *et al.*, 1997; Ribaut *et al.,* 1997; Schneider *et al.*, 1997; Sari-Gorla *et al.*, 1999; Mackill *et al.,* 1999; Cooper *et al.*, 1999; Courtois *et al.*, 2000; Nachit *et al.*, 2000), submergence/flooding (Nandi *et al.*, 1997; Sripogpangul, *et al.*, 2000) and aluminum (Flowers *et al.*, 2000; Wu *et al.*, 2000).

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CHAPTER 3

Responses of Ethiopian durum wheat (*Triticum turgidum* L. var. *durum*) genotypes to drought stress

3.1 Abstract

The aim of this study was to identify yield components associated with drought tolerance in durum wheat. Twenty six durum wheat genotypes, from different agro-ecologies of Ethiopia, were evaluated under moisture stress and non-stress conditions (35% and 70% available soil moisture) in a glasshouse throughout the growing period. Stress caused an average grain yield reduction of 79.7%; and a harvest index reduction of 45.2%. Drought susceptibility index proved useful to compare genotypes under stressed conditions. Yield was significantly correlated primarily with number of kernels per spike and 100 kernel weight. Further decompositions of simple correlation coefficients into direct and indirect effects showed that number of kernels per spike and 100 kernel weight had the largest direct effects on grain yield, under both stressed and non-stressed conditions. Number of spikes per plant under the high moisture level was correlated significantly, but negatively, with grain yield because of its large indirect effect on number of kernels per spike and 100 kernel weight.

Keywords: *drought susceptibility index , path-analysis, yield components.*

3.2. Introduction

Plant breeders (Blum, 1996) and crop physiologists (Bidinger, Mahalakshmi & Rao, 1987; Garritry & O'Toole, 1994, Richards *et al.*, 1999), believe that better adapted and high yielding genotypes could be bred if attributes that confer yield under water limited conditions could be identified and used as selection criteria. However, consistent relationships between most physiological characteristics and grain yield have rarely been found that can be used as selection criteria to develop stress-tolerant genotypes (Keim & Kronstad, 1981; Ludlow & Muchow, 1990; Saini & Westgate, 2000). Detailed studies to establish relationships among traits, their inheritance pattern, and their relative contributions to the final yield of a crop in the target environment are of paramount importance (Ludlow & Muchow, 1990; Saini & Westgate, 2000). Drought susceptibility index has been suggested as an important tool for the quantification of drought tolerance, comparison of drought levels and performance of genotypes under stress conditions, since it removes the effects of yield potential on drought tolerance (Fischer & Maurer, 1978; Bruckner & Frohberg, 1987). Other authors (Lin & Binns, 1988) argue that relative yield of genotypes should be a primary port of call if the existence of genetic variability in yield potential and morphological features, associated with drought tolerance is to be demonstrated. Thus, evaluation of wide genetic based germplasm both under stressed and optimum moisture levels has to be a point of departure for the identification of elite materials for subsequently detailed investigation into the genetic, and physiological, basis of drought tolerance mechanisms (Clarke *et al.*, 1992). This study was conducted to identify yield components associated with drought tolerance in durum wheat genotypes, when evaluated for more than three-fourth of the life-cycle of the crop under severe moisture deficit stress conditions simulated in a controlled environment.

3.3. Materials and methods

Twenty-six durum wheat (*Triticum turgidum* L. var. *durum*) genotypes, including landraces, old and recently released cultivars, were used. The experiment was conducted in a glasshouse maintained at 24/15°C day/night temperatures throughout the growing period. Plants were grown in 10 l plastic pots that were first filled with 2 kg of gravel to prevent soil leakage. Uniformly air-dried and sieved loam soil (6.222 kg), was filled in each pot. The soil was composed of 18 % clay, 2 % silt and 80 % sand. The soil air-dried moisture content was measured as 2.5% gravimeter. Its field capacity and permanent wilting point was 15.45% and 4.3%, gravimeter, respectively. A solution of potassium nitrate (0.556 mg/100 ml) per pot was added before planting. The soil was watered with a liter of tap water. The next day 12 seeds per pot of each genotype were planted. A hundred ml of the ammonium nitrate was added to each pot every week beginning from the first week of plant emergence until the grain filling growth stage.

Each variety was planted in six randomly placed pots in the green house. Seedlings were thinned to six at two-leaf stage. Pot positions were changed every two weeks to ensure equal exposure to the growing conditions. Treatment combinations were arranged in a 26 by two-factorial in a completely randomized block experimental design. A procedure was devised to maintain moisture levels at 70 % of the available soil water (ASW) for non-stress and 35 % (ASW) for stress treatments throughout the growing period. Tap water (500 ml) was applied every second day to all pots until plants reached four-leaf growth stage. Half of the experimental units (three pots from each variety) were left without water for 10 days, until severe wilting was observed. Pots were then weighed, and watered until the weight of every pot in each moisture level became equal to the weight of the predetermined treatment moisture levels. The amount of moisture depleted from the pots was obtained by weighing pots every second day for high moisture and fourth day the stress moisture treatments until anthesis. From anthesis until maturity, pots were weighed every three days for stress and every day for the high moisture treatments. The loss in weight was restored by watering pots with amount of water equal to the loss in weight.

Data were collected for the number of spike bearing tillers per plant (tillers with spike completely emerged from the flag leaf ligule), mature plant height (cm), days to heading (when spike completely emerged from the flag leaf ligule), days to physiological maturity (when the entire plant turns to yellow), number of spikelets per spike, number of kernels per spike, 100 kernels weight, air dried above ground biomass and grain yield from four plants per pot. Leaf area (cm^2) per plant was measured by a portable area meter at ear differentiation and anthesis from the remaining two plants per pot (one plant at each growth stage). Leaf dry weight was also determined at both growth stages by oven drying at 72°C for 72 hrs. Harvest index was determined as the proportion of grain yield to the overall above ground biomass from four plants per pot. Drought susceptibility index was computed for each genotype according to Fischer and Maurer (1978) as:

$$\frac{(1- Ys/Yp)}{D}$$

Where, S= Drought susceptibility index, Y_p= Potential yield of a genotype under control moisture level, Y_s = Yield of a genotype under stress moisture level, $Y_p$mean = Mean yield of all genotypes under control moisture level, $Y_s$mean = Mean yield of all genotypes under stress moisture level, D = Drought intensity; D=(1-Y_s mean /Y_p mean)

The relative yield under stress was calculated as the yield of a specific genotype under stress divided by that of the highest yielding genotype under moisture stress conditions. ANOVAs were done using Agrobase2000 and MSTATC. Mean separations were also run when necessary. Analysis of simple phenotypic correlations of characters was run with Genstat, version 4.2. Further decompositions of correlation coefficients into direct (unidirectional, 'P') and indirect effects (via alternate 'P'x r) pathways were computed by path analysis technique outlined by Dewey and Lu (1959), using the software Genstat, version 4.2.

3.4. Results and discussion

Mean values of yield and yield related characteristics are summarized in Table 3.1. Stress caused average reduction of 79.7% in grain yield and 45.2% in harvest index. The proportions of yield reductions as a result of stress ranged from 54.5% in the most tolerant cultivar, Boohai'S' to 100% in the most sensitive cultivars, Cadu# 17 and Bahirseded. Cultivars, DZ-393-4 and DZ-966 had the best ranks (highest grain yield) at high moisture treatment conditions (Table 3.1). Moisture stress caused yield reduction by 84.5 % in DZ-393-4 and 83.5 % in DZ-966. As a result, their rank became one of the lowest under moisture stress conditions. Cultivars with poor yield potential, DZ-1928-1, Bahirseded and Cadu #17, showed yield reductions of 90.4%, 100% and 100%, respectively, under stressed conditions. Dramatic yield reduction due to stress occurring during floral initiation and differentiation of floral parts have been reported to occur as result of delay in inflorescence development which could lead to complete inhibition of flowering (Saini, 1997; Saini & Westgate, 2000).

Moisture stress treatments also caused significant changes in all measured morphophysiological characteristics. Averaged over cultivars, stress caused reductions on leaf area by 46.2% at ear differentiation and by 65.5 % at anthesis. Leaf dry weight was reduced on average by 47.6% at ear differentiation and by 65% at anthesis. The height of the tallest cultivar, Boohai's', was reduced by 26.4%, and that of the shortest cultivar, Gerardo by 21.4% due to stress treatment. On average, stress delayed time to heading by 8.11 days. Heading of the fastest variety, Cocorit-71 was retarded by 10 days while that of the slowest cultivar, Cadu # 17, by nine days (data not shown).

Decomposition of simple phenotypic correlations (Table 3.2) among yield and yield components into direct and indirect effects through unidirectional 'P' and alternate ('P' x r) pathways (Table 3.3) showed distinctly the extent of impact and strength of associations between the two moisture levels. The complex cause and effects relationships accounted for the majority of the variance.

Table 3.1.Measured characteristics for 26 durum wheat genotypes grown in the glasshouse under high and stress moisture levels.

Entry	Moisture Level	Spike plant^{-1}	Spikelets Spike^{-1}	Kernels spike^{-1}	100 kernel Weight	Yield (g pot^{-1})	RY[1]	Harvest Index	S[2]
E-26	High	4.250	20.100	14.180	5.100	12.260		0.233	
	Stress	1.333	16.650	9.100	4.683	2.255	0.40	0.120	1.01
DZ-114-08	High	3.500	19.070	20.110	4.807	13.320		0.259	
	Stress	1.833	15.280	5.946	4.074	1.764	0.29	0.087	1.09
AL-138	High	3.750	17.150	13.710	3.582	7.599		0.261	
	Stress	1.000	14.530	14.580	3.341	1.881	0.31	0.161	0.95
A2-69	High	3.917	17.380	18.310	4.225	12.090		0.262	
	Stress	1.833	15.130	5.929	3.507	1.488	0.25	0.087	1.10
CADU#17	High	3.083	19.130	14.240	4.448	7.789		0.255	
	Stress	1.000	20.000	0.000	0.000	0.000	0.00	0.000	1.26
BAHIRSEDED	High	3.167	18.820	13.890	4.374	7.855		0.177	
	Stress	1.000	17.000	0.000	0.000	0.000	0.00	0.000	1.26
BOOHAI	High	2.833	17.050	22.680	5.050	12.970		0.376	
	Stress	3.167	15.020	2.185	4.507	1.287	0.21	0.097	1.13
BOOHAI'S'	High	3.000	17.030	15.810	7.018	13.260		0.305	
	Stress	2.167	15.330	9.254	7.090	6.036	1.00	0.466	0.68
FOKA	High	2.500	16.470	27.540	5.425	14.670		0.389	
	Stress	2.083	15.070	9.463	4.465	3.521	0.58	0.213	0.95
KLINTO	High	2.500	17.350	19.190	6.944	13.260		0.357	
	Stress	1.750	15.120	3.456	7.810	1.796	0.30	0.168	1.09
LD-357	High	3.417	18.530	14.150	4.520	8.715		0.187	
	Stress	1.833	17.130	1.167	6.457	0.452	0.07	0.016	1.19
QUAMI	High	2.250	20.200	23.650	6.168	12.870		0.304	
	Stress	1.250	14.730	8.600	5.915	2.529	0.42	0.164	1.01
TOP-66	High	2.417	18.750	20.83	5.732	11.900		0.301	
	Stress	2.167	15.200	3.315	5.548	1.460	0.24	0.138	1.10
FETAN	High	3.000	17.880	14.290	6.306	10.690		0.301	
	Stress	1.833	15.580	7.143	7.263	3.611	0.60	0.266	0.83
DZ-393-4	High	2.917	17.220	30.290	6.428	16.250		0.441	
	Stress	2.000	15.800	3.957	5.981	2.525	0.42	0.160	1.06
DZ-2023	High	2.000	19.670	33.520	5.644	14.980		0.420	
	Stress	1.333	15.320	21.730	5.150	5.539	0.92	0.445	0.79
DZ-1691	High	2.000	19.900	21.900	5.494	12.360		0.299	
	Stress	2.917	15.750	6.280	5.169	2.411	0.40	0.200	1.01
DZ-2085	High	3.500	14.350	14.800	6.207	12.830		0.354	
	Stress	2.333	13.330	5.028	5.188	2.419	0.40	0.152	1.02
DZ-1050	High	2.667	18.420	13.170	5.963	8.241		0.319	
	Stress	2.917	16.930	3.939	5.816	2.811	0.47	0.213	0.83
DZ-1052	High	2.250	17.400	19.220	6.908	11.790		0.315	
	Stress	2.583	15.170	7.791	5.530	4.279	0.71	0.247	0.80
GERARDO	High	2.750	16.120	17.590	4.942	9.505		0.276	
	Stress	1.917	13.870	5.485	5.703	2.404	0.40	0.178	0.94
COCORIT-71	High	2.833	16.730	18.600	6.102	12.840		0.324	
	Stress	2.500	14.100	6.037	6.094	3.664	0.61	0.316	0.90
DZ-575	High	2.917	16.880	15.580	6.459	11.700		0.317	
	Stress	3.000	15.100	0.777	5.238	0.480	0.08	0.023	1.20
DZ-1640	High	2.250	17.430	28.750	5.310	12.660		0.349	
	Stress	1.333	14.920	11.840	6.800	4.268	0.71	0.372	0.83
DZ-966	High	2.667	18.180	23.480	6.282	15.370		0.374	
	Stress	2.000	15.800	4.917	6.538	2.557	0.42	0.179	1.05
DZ-1928-1	High	3.083	18.350	10.670	5.840	7.689		0.265	
	Stress	2.417	15.220	1.499	5.139	0.736	0.12	0.069	1.14
SE(±)$^{\mp}$		0.033	0.097	0.024	0.099	0.045	0.04	0.018	0.03
LSD $_{0.05}$ $^{\mp}$		0.390	1.134	2.811	1.154	0.525	0.07	0.030	0.04
SE(±)^{I}		0.038	0.112	0.278	0.114	0.052	-	0.003	-
LSD $_{0.05}$ ^{I}		0.064	0.186	0.461	0.190	0.086	-	0.006	-

$^{\mp}$ = Standard error and LSD for entry

^{I} = Standard error and LSD for moisture level

[1]RY= Yield of a genotype under stress moisture level divided by the highest yielding genotype under stress conditions

[2]S=(1-Ys/Y)/(1-meanYs/meanY), where Ys=yield of a genotype under stress and Y=yield of a genotype without stress.

Only 16.2% and 27.8 % for the control and stress moisture regimes, respectively, stemmed from residual effects.

Under optimal conditions, number of spikes per plant was negatively correlated with yield (Table 3.2). However, it had a large positive direct effect on yield (Table 3.3). On the other hand, the indirect negative effects of spike per plant via kernels per spike and 100-kernel weight on yield under stressed conditions were reduced.

Table 3.2. Phenotypic correlation coefficient matrix for yield and yield components for 26 durum wheat genotypes grown under stress and high moisture levels in the glasshouse.

Characters correlated	Moisture level	Spikes plant^{-1}	Spikelets spike^{-1}	Kernels Spike^{-1}	100 kernel weight (g)	yield (g pot^{-1})
Spikes plant^{-1}	High		-0.117	-0.591**	-0.436**	-0.284*
	Stress		-0.271*	-0.329**	0.308**	0.053
Spikelets spike $^{-1}$	High			0.059	-0.160	-0.096
	Stress			-0.307**	-0.426**	-0.335**
Kernels spike^{-1}	High				0.151	0.734**
	Stress				0.157	0.722**
100 kernel weight (g)	High					0.490**
	Stress					0.513**

* and ** significant at p= 0.05 and p = 0.01, respectively.

Consequently, spikes per plant had a positive (not significant) association with yield. This suggested that the ability to maintain a high number of grain bearing tillers until maturity was an important trait contributing to yield under drought stress (Keim & Kronstad, 1981). All yield components, in general, had positive direct effects on yield. Kernels per spike and 100-kernel weight, however, had the largest direct positive effects on yield at both moisture levels (Table 3.3).

Performances of DZ-393-4, DZ-966 and DZ-2023 were found to be best (Table 3.1) under favorable moisture conditions due to either high kernel number per spike or high kernel number per plant (as a result of high spike number per plant), and/or high 100 kernel weight. Under stressed conditions, good performance of varieties such as DZ-2023 and Boohai's' was also due to a higher number of kernels per spike or 100 kernel weight (Table 3.1). The poor performance of DZ-393-4 and DZ-966, which were best under optimum moisture conditions, was due to the severe impact of

stress either on the number of kernels per spike or 100-kernel weight (Table 3.1).

Table 3.3. Path-coeffcient analysis of the direct and indirect effects of yield components on yield for 26 durm wheat genotypes grown under high and stress moisture levels in the glasshouse.

		Indirect effects via				
Relationship between yield and:	Direct effect	Spikes plant^{-1}	Spikelets spike^{-1}	Kernels spike^{-1}	100 kernel weight(g)	Total(r)
	Control					
Spikes plant^{-1}	0.5395		-0.0004	-0.5701	-0.2532	-0.284
Spikelets	0.0032	-0.0629		0.0569	-0.093	0.0959
Kernels	0.9645	-0.3189	0.00020		0.0897	0.7337
100 kernel	0.5803	-0.2545	-0.00054	0.14610		0.5803
	Stress					
Spike plant^{-1}	0.2270		-0.0338	-0.2558	0.1157	0.053
Spikelets	0.1248	-0.0615		-0.2387	-0.1598	-0.335
Kernels spike^{-}	0.7764	-0.0748	-0.0384		0.0587	0.722
100 kernel	0.3750	0.0700	-0.0532	0.1216		0.513

Moisture stress occurring between floral initiation and differentiation, anthesis and/or grain filling stages, primarily affects the number and/or the size of kernels through floral abnormalities (Westgate *et al.,* 1996). Yield component compensation under stress was observed for Boohai 's' due to the reduction in the number of kernels per spike and other components and increased 100 kernel weight. The increase in 100-kernel weight caused an increase in harvest index of 52.8%. It is not uncommon to encounter similar yield component compensations either due to reduced number or size of panicle for instance in sorghum (Blum, 1996). A decrease in spike number and/or kernel number per spike, causes an increase in kernel weight (Keim & Kronstad, 1981).

Yield under stress was significantly ($P<0.05$) and positively correlated ($r=0.50$) with the mean yield of cultivars under high moisture treatments. 'S' values were, however, negatively and significantly ($r= -0.25$; $P<0.05$) correlated with mean control yield. Clarke *et al.* (1984) also found 'S' to be negatively correlated with mean yield for 10 hexaploid genotypes, but a significant positive correlation for eight tetraploid genotypes was found in the same study. In the most susceptible cultivars, Cadu#17 and Bahirsede, with susceptibility indices of 1.26, 100% reduction of yield and harvest indices were observed. However, harvest index was increased by 52.8 % in

Boohai'S' and by 4.7 % in DZ-2023. Reduced yield performance of varieties under stress conditions was found to be associated with higher drought susceptibility indices. Yield under stress conditions was significantly and negatively correlated with drought susceptibility index. On the other hand, there was a significantly positive correlation between "S" and days to heading. This suggested that when the varieties were exposed for extended periods to moisture stress, the most sensitive stages, the reproductive, and grain initiation stages, were likely to be severely affected.

Variability in growth stage differences, such as days to heading was observed. A significantly negative correlation between yield and days to heading, and significantly positive correlation between days to heading and drought susceptibility index, may indicate yield advantages of early maturing cultivars. Thus, drought tolerance due to escape might have been involved as a mechanism for drought tolerance. Nevertheless, yield adjustment for drought escape using mean high moisture level days to heading as covariate did not confirm yield advantages of early maturing genotypes. The apparent sensitivity of long maturing genotypes might be due to the relatively severe cumulative effects of drought injury.

Genotypes with low values of 'S' are presumed to be drought tolerant. 'S' measures the ratio of stressed to non-stressed yield in individual genotypes in comparison to the overall ratio for genotypes in the experiment. Thus, 'S' accounts for differences in yield potential among genotypes (Bruckner & Frohberg, 1987). It seems in this study that some genotypes with poor yield potential under optimum moisture conditions showed low 'S' values as result of relatively lower yield reduction. However, some with poor and good yield potentials under favorable conditions happened to be susceptible (high values of 'S') because of comparatively higher yield reduction under stressed conditions. Negative correlations between 'S' and yield potential imply that selection for low values of 'S', would allow identification of elite materials which possibly possess drought tolerance traits without altering performance under favorable conditions. The materials could be useful in breeding endeavors for the improvement of yield in drought stressed conditions. This, however, does not guarantee improved response of the selected materials in favorable environments.

3.5. Conclusions

In conclusion it can be said that some genotypes with the best yield potential under stressed conditions could tolerate the stressed conditions better than the other relatively susceptible, but good yield potential, genotypes.

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CHAPTER 4

Expression of drought tolerance in F_1 hybrids of a diallel cross of durum wheat (*Triticum turgidum* L. var. *durum*).

4.1. Abstract

Durum wheat is indigenous to Ethiopia and is planted extensively by small-scale farmers. Drought stress is one of the major constraints faced by farmers. The main aim of this study was to evaluate the expression of drought tolerance in the parents and F_1 progeny of a six by six half diallel cross, made with two drought tolerant, two intermediate and two susceptible parents. Parents and the 15 F_1 hybrids were evaluated at a maximum of 35 and 70% available soil moisture in a glasshouse. Analysis of variance revealed significant variability for yield, yield components, and drought tolerance measurements. Drought tolerance was expressed in the crosses involving tolerant parents, and was controlled predominantly by additive genes. The diallel analysis revealed that mean squares for both GCA and SCA were significant at both moisture levels. GCA: SCA ratios indicated predominance of additive gene actions for all characteristics measured, although non-additive genes also played a role, particularly in the absence of stress. GCA effects were mainly positive. GCA effects as well as their rankings were significantly affected by moisture stress. SCA effects were also mainly negative. Considerable variability in SCA values between the two moisture levels were found

***Key words**: durum wheat , moisture stress, combining ability*

4.2. Introduction

Drought tolerance in crop species should be defined in terms of productivity (Passioura, 1983; Blum, 1996). With the lack of sound information on specific drought tolerance and/or adaptation mechanisms, selection for drought tolerance is still largely guided by grain yield and its stability under dryland conditions (Fischer and Maurer, 1978; Lin and Binns, 1988). Methods proposed to measure and quantify drought tolerance in terms of grain yield, drought susceptibility index (Fischer and Maurer, 1978) and superiority measure (Lin and Binns, 1988), are widely used in studies of the responses of wheat genotypes to drought stress (Bruckner and Frohberg, 1987; CeccarelII, 1987). Endeavors to combine drought tolerance with high and stable yield performance under drought prone environments require demonstration of expression and inheritance of drought tolerance in progenies obtained from susceptible and resistant parents. Traditionally, analysis of combining ability has been employed to assess and exploit the type of gene action, thereby designing the appropriate breeding strategy. Sufficient additive genetic variances should be prevalent, amenable to genetic fixation by subsequent inbreeding, for improvement of most important traits.

The aims of this study were to evaluate the expression of drought tolerance in the F_1 generation of a diallel cross and to examine the effect of moisture stress on the GCA and SCA of yield and yield related characteristics in durum wheat.

4.3. Materials and methods

A six by six diallel cross without reciprocals was made in January to June 2001 at Debrezeit Agricultural Research Center, Ethiopia. Two drought tolerant varieties, Boohai'S' and DZ-2023, two moderately resistant varieties, DZ-1691 and DZ-320, and two susceptible varieties, Klinto and LD-357, were used as parents.

In the subsequent season, two sets of material consisting of parents and their 15 F_1 progeny were grown in the glasshouse in a randomized block design with three replications, at an average of 25/15^{0}C day and night temperatures. Entries were planted in 10 l capacity plastic pots. Pots were filled with 2 kg of gravel (to prevent soil leakage), and 7 kg of steam sterilized and air dried sandy loam soil with a composition of 80, 2 and 18 % of sand, silt and clay, respectively. The air-dry moisture content of the soil was determined as 15.45 % (field capacity) and 4.43 % (permanent wilting point) gravimeter. Genotypes were sown 12 seeds per pot. Seedlings were thinned to nine per pot at the two-leaf growth stage. Pots were maintained at about 70 % available soil moisture for a month (up to about four-leaf growth stage). Half of the experimental unit was then left without water until severe wilting was observed. Application of stress continued by maintaining the moisture level at 35% available soil moisture until maturity. Evaporated water was monitored by weighing the unplanted pots (six per block). The amount of water transpired was determined from planted pots per block. The amount equivalent to the loss in weight was replaced once every two and five days for the control and stress treatments, respectively, until anthesis. From anthesis onwards, the frequency was lowered to every other and third day for the control and stress treatments, respectively. Measurement of physiological parameters started from early boot stage and continued until ripening.

Number of spikes per plant (spikes completely emerged from flag leaf ligule) and spikelets per spike were counted for all plants. At maturity, three plants per pot were harvested and left to air dry for 10 days. Grain and biomass yield were determined as average of the three plants. Number of kernels per spike was calculated as total number of kernels to total number of spikes per plant basis. Kernel weight was determined from total yield to total number of kernels, and expressed in milligram.

Drought susceptibility indices (S) were computed according to the formula of Fischer and Maurer (1978),

$$S = \frac{1-(Ys/Yp)}{D}$$

Where, S = Drought susceptibility index, Y_p = Potential yield of a genotype under control moisture level, Y_s = Yield of a genotype under moisture stress

level, $Y_p$mean = mean yield of all genotypes under control moisture level, Ysmean = mean yield of all genotypes under moisture stress level, D = drought intensity; D=(1-Ys mean/Yp mean).

Superiority measures or relative yield was calculated from the formulas of Lin and Binns (1988). The relative yield under moisture stress was calculated as the yield of a specific genotype under stress, divided by that of the highest yielding genotype under moisture stress conditions.

Data were subjected to analysis of variance using Agrobase 2000. General (GCA) and specific combining ability (SCA) were computed according to Griffing (1956) Method II, fixed effects model.

4.4. Results and discussion

Yield and yield components were significantly influenced by the stress treatments (Table 4.1). Better performance of progenies in terms of yield and yield components were obtained from crosses involving the two tolerant parents, DZ-2023 and Boohai 'S'.

Parameters employed to quantify and measure drought tolerance in terms of yield under stress conditions were drought susceptibility index and relative yield or superiority measure. Higher values indicated a higher degree of susceptibility, and *vice versa* (Fischer and Maurer, 1978; Bruckner and Frohberg, 1987; Ceccarelli, 1987). On the other hand, higher relative yield values correspond with a higher degree of tolerance (Lin and Binns, 1988; Nasir *et al.*, 1992). F_1s obtained from crosses involving susceptible with susceptible parents had higher susceptibility index values and lower relative yield values. Contrary to this, F_1s from all other combinations had lower susceptibility index values and higher relative yield values (Table 4.1).

Analysis of variance for GCA and SCA for all measured characteristics were highly significant (Table 4.2) at both moisture levels. Ratios for GCA:SCA were greater than one (Table 4.2). This confirmed that additive genes controlled the inheritance of most of these characteristics.

Table 4.1.Means for yield and related characteristics for durum wheat grown under stressed and control conditions in the greenhouse

Entry	Moisture level	Yield (g plant^{-1})	Kernel spike^{-1}	Kernel wt(mg)	Spikele spike^{-1}	Spike plant^{-1}	RY	S
DZ-2023	Control	2.72	28.03	29.76	12.53	3.25		
	Stress	1.14	13.43	52.21	11.83	1.63	0.83	0.79
Boohai'S'	Control	2.60	15.75	50.89	11.72	3.25		
	Stress	1.37	11.29	50.32	10.35	2.25	1.00	0.65
DZ-1691	Control	2.44	15.20	35.96	15.25	4.50		
	Stress	0.52	5.65	40.35	10.50	2.33	0.38	1.07
DZ-320	Control	1.62	15.20	24.18	10.40	4.50		
	Stress	0.46	9.59	31.50	12.23	1.58	0.34	0.97
Klinto	Control	3.31	17.08	30.31	11.33	6.50		
	Stress	0.42	9.96	27.93	11.15	1.50	0.30	1.19
LD-357	Control	1.51	27.67	21.95	9.58	2.50		
	Stress	0.23	6.29	31.66	8.75	1.15	0.16	1.18
1*2	Control	2.83	24.50	47.96	13.67	2.42		
	Stress	1.33	11.12	43.81	10.61	2.75	0.97	0.72
1*3	Control	3.02	31.65	35.87	13.94	2.67		
	Stress	0.84	18.50	45.17	11.44	1.00	0.61	0.99
1*4	Control	4.44	40.83	47.21	17.78	2.33		
	Stress	0.96	9.11	30.81	12.48	2.83	0.70	1.07
1*5	Control	2.76	12.35	44.66	11.26	4.17		
	Stress	0.92	5.88	40.44	10.00	3.84	0.67	0.91
1*6	Control	3.36	18.24	45.90	11.92	4.10		
	Stress	1.23	21.75	46.43	13.67	1.25	0.90	0.86
2*3	Control	2.66	21.52	43.62	14.31	2.92		
	Stress	0.63	10.40	43.81	9.72	1.89	0.46	1.04
2*4	Control	3.89	32.00	42.30	13.85	2.87		
	Stress	0.99	12.58	40.22	10.50	2.44	0.72	1.02
2*5	Control	1.67	10.34	39.49	9.52	4.14		
	Stress	0.70	6.33	33.69	11.00	3.33	0.51	0.79
2*6	Control	3.42	17.66	47.30	12.54	4.13		
	Stress	0.87	10.94	56.95	11.27	1.72	0.63	1.02
3*4	Control	3.81	18.70	40.71	11.10	5.00		
	Stress	0.42	10.56	22.32	10.87	1.83	0.31	1.21
3*5	Control	2.69	14.06	51.22	11.18	3.52		
	Stress	0.36	5.25	52.21	10.44	1.33	0.26	1.18
3*6	Control	2.59	15.27	43.06	10.80	4.00		
	Stress	0.67	12.63	22.69	10.08	1.89	0.49	1.01
4*5	Control	1.14	6.65	55.09	10.48	3.17		
	Stress	0.39	4.67	45.22	10.14	2.00	0.28	0.90
4*6	Control	2.46	19.56	41.08	10.39	3.07		
	Stress	0.48	10.54	35.39	8.75	1.75	0.35	1.10
5*6	Control	2.46	9.51	38.32	9.83	3.73		
	Stress	0.43	6.05	24.69	12.66	2.25	0.31	1.13
Mean	Control	2.73	19.60	40.80	12.05	3.65		
	Stress	0.1276	10.12	38.94	10.88	2.03	0.53	0.99
LSD(0.05)*	Control	0.2085	2.80	5.73	0.86	0.54		
	Stress	0.1276	2.42	4.72	0.09	0.52	0.091	0.09
LSD(0.05)**		0.0373	0.56	1.14	0.21	0.11		

LSD for entries * and ** LSD between moisture levels
1=DZ-2023, 2=Boohai'S', 3=Klinto, 4=DZ-1691,5=LD-357 and 6=DZ-320
RY = relative yield, S = susceptibility index

Similar studies have confirmed the predominance of additive genetic effects for yield and yield related characters in bread wheat both under moisture stress and non-stress conditions (Labuschagne and Van Deventer, 1989). The GCA:SCA ratios for yield, number of kernels per spike and kernel weight were higher under stress than control conditions. Expressions of non-additive genetic effects

Table 4.2. Estimates of GCA effects and GCA:SCA ratio's for durum wheat varieties grown in the greenhouse under stressed and control conditions

Parents	Moisture Level	Yield (g plant^{-1})	Kernels Spike^{-1}	Kernel weight (mg)	Spikelet Spike^{-1}	Spike Plant^{-1}	S	RY
DZ-2023	Control	0.3409	5.798	-0.5601	1.1133	-0.4238		
	Stress	0.3047	2.7973	4.8138	0.7135	0.0924	-0.1002	0.2224
Boohai'S'	Control	0.0649	0.0315	4.6037	0.3754	-0.3261		
	Stress	0.2662	0.3886	5.8181	-0.2949	0.3066	-0.1305	0.1943
DZ-1691	Control	0.1848	1.3601	1.5853	1.1708	0.0168		
	Stress	-0.1040	-1.5091	-2.2395	-0.3023	-0.1678	0.0649	-0.0759
DZ-320	Control	-0.1983	-3.3265	-2.6984	-1.0063	0.3060		
	Stress	-0.0632	1.2808	-2.9276	0.5901	-0.2697	0.0166	-0.0461
Klinto	Control	0.2819	-0.2368	-1.3129	-0.0413	0.6916		
	Stress	-0.1697	0.8013	-3.8272	-0.1632	-0.4051	0.1113	-0.1239
LD-357	Control	-0.6743	-3.6264	-1.6176	-1.6121	0.2310		
	Stress	-0.2341	-3.7588	-1.6375	-0.5432	0.1078	0.0379	-0.1709
LSD(0.05)	Control	0.085065	1.140405	2.338245	0.349701	0.221405		
	Stress	0.052034	0.987491	1.926485	0.44656	0.210932	0.034683	0.037966
GCA:SCA	Control	2.311	1.495	0.690	4.684	1.715		
	Stress	24.647	4.071	1.544	1.718	1.249	7.3	24.778

RY = relative yield, S = susceptibility index

were higher under optimum than under stressed conditions. This suggested that non-additive genes also played a significant role in the inheritance of yield, kernels per spike and kernel weight. The inheritance of 1000 kernel weight with stem rust infection has been found to be both under additive non-additive genetic control (Csösz *et al.*, 1995).

On the basis of GCA effects, Boohai'S' and DZ-2023 should be the best parents to improve drought tolerance and yield performance in moisture stressed environments. Drought tolerant parents, DZ-2023 and Boohai'S had positive GCA effects for yield and yield components, except for Boohai'S', which had negative values for spikelet per spike under stress moisture levels. On the other hand, the susceptible parent, LD-357, had negative values for yield and yield components except for spike per plant at both moisture levels. The susceptible parent, Klinto, had positive GCA values for grain yield and spike per plant under control conditions. However, all values under stressed

conditions were reduced and became negative, except for kernels per spike (Table 4.2). GCA effects for drought susceptibility index were generally positive for all parents except for the tolerant ones, DZ-2023 and Boohai'S'. GCA effects for relative yield, however, were negative for all parents, except for DZ-2023 and Boohai'S'. This suggested that DZ-2023 and Boohai'S' are likely to transfer drought tolerance to their progenies. The ratio of GCA: SCA was also positive and very high (Table 4.2). This indicated that genes with additive effects primarily control drought tolerance.

SCA estimates for all measured characteristics were largely negative or close to zero, with some exceptions. SCA effects for yield were positive for Boohai'S' x DZ-1691, Boohai'S' x DZ-320, Klinto x DZ-1691 and LD-357 x DZ-320 under control conditions. The best combination for yield was DZ-2023 x DZ-1691. Relatively higher SCA effects for yield under stress conditions were found only for DZ-2023 x DZ-320 and DZ-2023 x LD-35. SCA effects for number of kernels per spike were positive and relatively higher only for DZ-2023 x Klinto at both moisture levels and DZ-2023 x DZ-1691 and DZ-2023 x DZ-320 under control and stress moisture levels, respectively. At the control moisture level, SCA effects for the number of kernels per spike were positive for all combinations of Boohai'S' with the rest of the parents, except for the cross with LD-357. However, under stress conditions, SCA values for number of kernels per spike were substantially reduced. Considerable variability in SCA effects for kernel weight, number of spikelets per spike and number of spikes per plant, were also observed between the two moisture levels. An increase in SCA effects for kernel weight was observed in DZ-2023 x Klinto, Boohai'S' x Klinto, Boohai'S' x DZ-1691, Boohai'S' x DZ-320 and Klinto x LD-357. More than 50% of the values under both conditions were positive (Table 4.3). The best values, however, were obtained for LD-357 x Klinto, Boohai'S' x DZ-320, and LD-357 x DZ-1691 under both conditions. More than 50% of SCA effects values for the number of spikelets per spike were positive (Table 4.3). The highest values were for DZ-2023 x DZ-1691 at control and LD-357 x DZ-320 at the stress moisture level. Values showed a considerable variation between the two moisture levels, many with a decreasing tendency, though not consistently so. SCA values of spikes per plant, relative yield and susceptibility index

were also close to zero. Reports on similar studies in bread wheat indicated that SCA effects for yield and yield related characteristics have largely been negative (Labuschagne and Van Deventer, 1989).

High SCA effects for yield were observed when SCA effects for yield components, particularly for kernels per spike, spikelets per spike and kernel weight were higher (Table 4.3).

Table 4.3.SCA estimates for yield, yield components and drought tolerance measurements for durum wheat grown in the greenhouse under stressed and control conditions

Combination	Moisture Level	Yield g plant^{-1}	Kernel spike^{-1}	kernel wt (mg)	Spklt spike^{-1}	Spike plant^{-1}	RY	S
1*2	Control	-0.313	-0.94	3.12	0.131	-0.487		
	Stress	0.032	-2.19	-5.76	-0.689	0.325	0.023	-0.038
1*3	Control	-0.334	6.48	-3.06	0.817	-1.252		
	Stress	-0.028	4.78	5.24	0.013	-0.714	-0.020	-0.041
1*4	Control	1.181	14.07	5.38	3.052	-0.880		
	Stress	0.027	-2.30	-10.70	1.190	0.547	0.020	0.116
1*5	Control	0.363	-9.43	6.04	-0.285	1.168		
	Stress	0.116	-3.28	-1.68	-1.050	1.611	0.084	-0.015
1*6	Control	0.487	-3.84	8.36	-0.234	0.564		
	Stress	0.261	7.55	5.6	1.483	-0.599	0.191	-0.043
2*3	Control	-0.426	2.12	-0.48	1.929	-1.103		
	Stress	-0.200	-0.91	2.87	-0.698	-0.038	-0.146	0.072
2*4	Control	0.903	11.0	-4.70	0.257	-0.438		
	Stress	0.093	3.58	-2.30	0.217	-0.057	0.676	0.095
2*5	Control	-0.454	-5.67	-4.30	-1.294	1.040		
	Stress	-0.066	-0.42	-9.43	0.958	0.893	-0.048	-0.110
2*6	Control	0.823	1.35	4.59	1.127	0.491		
	Stress	-0.068	-0.85	15.12	0.092	-0.343	-0.050	0.144
3*4	Control	0.606	2.03	-0.36	-2.077	0.671		
	Stress	-0.032	1.14	-10.55	0.452	0.044	-0.024	0.047
3*5	Control	0.345	-1.68	13.35	0.790	-0.591		
	Stress	0.036	-1.91	18.65	0.270	-0.399	0.026	0.041
3*6	Control	-0.224	-0.77	6.27	-0.196	0.651		
	Stress	0.174	0.43	-9.49	-1.223	0.539	0.127	-0.107
4*5	Control	-1.104	-10.70	14.32	-1.129	-0.237		
	Stress	-0.004	-0.19	10.16	0.109	-0.302	-0.003	-0.192
4*6	Control	-0.259	1.92	1.39	-1.818	-0.876		
	Stress	-0.080	0.651	1.61	-2.418	-0.175	-0.059	0.026
5*6	Control	0.601	-3.14	1.84	0.398	0.004		
	Stress	-0.007	-1.59	-9.68	1.733	0.386	-0.005	0.083
LSD(0.05)	Control	0.170	2.281	4.68	0.699	0.443		
	Stress	0.1047	1.975	3.85	0.893	0.422	0.076	0.068

1=DZ-2023, 2=Boohai'S', 3=Klinto, 4=DZ-1691, 5= LD-357 and 6= DZ-320
RY = relative yield, S = susceptibility index

4.5. Conclusions

Drought tolerance was improved when drought tolerant parents were involved in the crosses. Measurement of drought tolerance in terms of yield performance under stress conditions appeared to be valuable to distinguish drought tolerance from yield potential. However, stability of yield performance should be evaluated, possibly across target environments and over years.

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CHAPTER 5

Diallel analysis of growth response of durum wheat (*Triticum turgidum* L. var. *durum*) genotypes to drought stress

5.1. Abstract

The impact of moisture stress on phenology and growth varies for genotypes and the growth stages at which stress is encountered. The aim of this study was to determine the effect of moisture stress on growth, heritability of characteristics and relationships between them. A 6x6 half diallel cross of durum wheat genotypes differing in their responses to moisture stress, was grown under moisture stress and control moisture levels. Data on major phenological components, growth rate and components of growth rate were collected and analysed. Differences in days to heading, anthesis and physiological maturity were highly significant among genotypes at the two moisture levels. Drought stress was found to delay major growth stages and shorten the grain filling period. Variability in relative growth rate (RGR), components of RGR, net assimilation rate (NAR) and leaf area ratio (LAR) was significantly high. Drought tolerant genotypes had fast early growth, whereas susceptible ones had slow RGR initially. Variation in RGR was associated with NAR and LAR. Differences in hybrid performance were due to significant GCA and SCA effects. Interactions of GCA and SCA with moisture level were also highly significant. Moderate to high levels of broad sense heritability estimates were found for most of the traits. Significantly high genetic and phenotypic correlations between NAR and RGR, and LAR and RGR were found. The genetic and phenotypic correlations of grain yield with total dry matter, harvest index, RGR and LAR were significant. Findings in this study suggest that grain yield, HI and dry matter production

under stress conditions can be improved by indirectly selecting for components of RGR and LAR.

Keywords: *Drought tolerance, heritability, durum wheat*

5.2. Introduction

The impact of moisture stress on phenology and growth varies depending on the crop species, the crop variety and the growth stages at which stress is encountered. In durum wheat, early season stress has been found to retard time to anthesis and physiological maturity. Mid and terminal stress did not affect the time to anthesis but shortened grain filling period (Simane *et al.*, 1993).

In order to improve performance under drought conditions, phenological development should match seasonal water use, as water deficits during grain filling period will limit grain yield (Turner and Nicolas, 1987; Nicolas and Turner, 1992; Simane *et al.,* 1993).

Adaptive response whereby plants respond to moisture stress, thereby changing their rate of growth has been termed as ontogenetic flexibility or developmental plasticity (Van Andel and Jager, 1981). Fast early growth under moisture deficit stress may increase grain yield (Turner and Nicolas, 1987; Whan *et al.*, 1991) by reducing soil evaporation and increasing water use efficiency of the crop. Plants with high relative growth rate have the opportunity to acquire a large share of the limiting resources, such as moisture, than the slow growing ones (Poorter, 1989). Thus, in the semi arid environment where water availability is highly variable, cultivars with high physiological and/or morphological plasticity with respect to relative growth rate (RGR) are better adapted. Under such environments, RGR components rather than RGR *per se* are the target for selection. It is therefore important to identify the association of morphophysiological components of RGR that can easily be used in a breeding programme (Simane *et al.*, 1993). In order to maximise selection efficiency for any characteristic, it is first necessary to determine the size and the nature of genetic variation for the character; and the extent to which genotype x environment interaction may reduce gain from selection. Moreover, it would be

necessary to assess whether gain is expected to be greatest via direct or correlated response to selection (Dudley, 1997). To this effect, diallel crosses among random mating inbred lines, have long been used to understand the nature of genetic variation and the potential values of inbred lines (Mather and Jinks, 1971). Information of phenotypic and genotypic correlation between characters would help not only to devise a selection strategy but also help determine whether indirectly selected traits will increase or decrease or remain unchanged in further selection cycles (Tekouano *et al.*, 2002).

The aims of this study were therefore 1) to evaluate the growth response of genotypes under moisture stress and control moisture regimes 2) to examine the genetic control of relative growth rate and its components 3) to assess the genotypic and phenotypic association among components of growth, yield, HI and total dry matter production using a 6x6 half diallel cross of durum wheat genotypes differing in their responses to drought stress.

5.3. Materials and methods

Plant culture

A six by six diallel cross without the reciprocals was made in January to June 2001 at Debrezeit Agricultural Research Centre, Ethiopia. Two drought tolerant varieties, Boohai'S' and DZ-2023, two moderately tolerant varieties, DZ-1691 and DZ-320, and two susceptible varieties, Klinto and LD-357, were used as parents.

In the subsequent season, two sets of material consisting of parents and their 15 F_1 progeny were grown in the glasshouse in a randomised complete block design with three replications in July to December 2001 at the Free State University. They were grown at an average of 25/15^{0}C day and night temperatures. Entries were planted in 10 l capacity plastic pots. Pots were filled with 2 kg of gravel (to prevent soil leakage), and 7 kg of steam sterilised and air dried sandy loam soil with a particle composition of 80%, 2% and 18% of sand, silt and clay, respectively. The air-dry moisture content of the soil was determined as 13.3% (field capacity) and 4.0% (permanent wilting point) gravimeter. Pots were equally spaced and placed. Each genotype was sown into

six pots. Twelve seeds at equal spacing were planted at about equal depth. Seedlings were thinned to nine per pot at the two-leaf growth stage. Pots were maintained at 70-100% available soil moisture for a month (up to about four-leaf growth stage). Half of the experimental unit was then left without water until severe wilting was observed. Application of stress was continued by maintaining the moisture level at 25-35% available soil moisture until maturity. The amount of water evaporated was monitored by weighing daily the unplanted pots placed between planted pots both for the stress and the control treatments in each block (six in each block). The amount of water transpired was determined by subtracting the weight of bare pots from the weight of planted pots. Pots were replenished with the amount of water equivalent to the loss in weight to bring them to the predetermined level of moisture whenever the weight of pots fell to the lower limit established for the treatments. The moisture level set up was 25-35% and 70-100% available soil moisture for the stress and control treatments. Equal exposure to the growing conditions of the glasshouse was ensured by changing the positions of pots every week.

Data collection

Plants were sampled from 45 days after planting (DAP), every 15 days four times during the growing period. Plants were separated into stem, leaves and spikes. Leaves were excised from leaf sheath above the leaf ligule. Only green leaf blade area was measured at each sampling time using leaf area meter, LI-3100 (LI, COR, Lincoln, Nebraska, USA). Dry weight measurements were made after drying samples for 72 hours at 72^0C in an oven. Leaf area, leaf dry weight, stem dry weight, spike dry weight and total dry matter production was measured per plant basis at each sampling time. Relative growth rate (RGR) is the increase of above ground plant dry matter per unit of dry matter present per unit time and expressed as mg g^{-1} day^{-1}. Net assimilation rate (NAR) is the increase of above ground dry matter per unit of assimilatory material (leaf area) present per unit time and expressed as gm^{-2} day^{-1}. Leaf area ratio (LAR) is the assimilatory material present per unit of plant material and expressed as m^2 kg^{-1}. Leaf weight ratio (LWR) is the weight of green leaf area per unit of total dry matter produce and expressed as g g^{-1}. Specific leaf area (SLA) is total leaf area present per unit of green leaf dry weight and denoted as m^2 kg^{-1}. RGR and the

various components were calculated according to Radford (1967): RGR=(1/TDM)*(dTDM/dT), where TDM is above ground total dry mater and T is time. NAR=(1/A)*(dTDM/dT), where A is leaf area. LAR=A/TDM, LWR=LDM/TDM, where LDM is leaf dry matter. SLA=LA/LDM, where LA is leaf area.

Statistical analysis

The data were subjected to multivariate analysis of variance using the MANOVA option of the GLM procedure in SAS (SAS, 1999). Data were initially combined over environments, considering moisture levels as environments. Subsequently, specific effects of moisture levels were analysed separately. The MANOVA option produces sum of squares and cross product (SSCP) matrices for each component of the statistical model used (SAS, 1999). Mean squares and cross products matrices (MSCP) were then computed from SSCP by dividing the appropriate degrees of freedom. The resulting values were equated to their expectation, treating moisture level as a fixed effect and genotypes as random, in order to obtain the variance-covariance matrices. Hence, the variance-covariance matrices for genotypes define the genetic relationships among parameters. The matrices were then fitted to the equation for calculating genetic correlation according to Chaudhary and Singh (1977):

$$r_{g(x,y)} = \frac{\sigma_{g(x,y)}}{\sigma_{g(x)}\,\sigma_{g(y)}}$$

where: $r_{g(x,y)}$ is the genetic correlation coefficient between any two traits, X and Y, $\sigma_{g(x,y)}$ is the genetic covariance of X and Y, $\sigma_{g(x)}\,\sigma_{g(y)}$ are the root of the genetic variance of X and Y respectively. Significance levels were determined based on the tabulated values (Fisher and Yates, 1963). Phenotypic correlation can also be calculated from the same matrices and formulae by substituting genetic variance and covariance for phenotypic variance and covariance. However, phenotypic correlation was estimated using Pearson's simple correlation analysis (NCSS, 2000) based on the pooled genotypes values over environment and replications.

Broad sense heritability estimates were obtained from:

$$h^2 = \sigma_{g(x)} / \sigma_{p(x)}$$

where; $\sigma_{g(x)}$=genotypic variance and $\sigma_{p(x)}$= phenotypic variance calculated from a mixed effect model, where, moisture level is considered as fixed and genotype is considered as a random effect according to Chaudhary and Singh (1977). Standard error deviation of the heritability estimates were computed as SE $(h^2)=[2/n_1+2+2/n_2+2](1-h^2)$, where n_1 and n_2 = are degree of freedom of the genotypes and error variances following Tekouano *et al.* (2002).

Combining ability was analysed based on the fixed effect model, method II of Griffing (1956). GCA by environment and SCA environment interactions were analysed using DIALLEL-SAS1 program described by Zhang and Kang (1997) for five parents and extended to six parents. The two moisture levels were considered as two environments thus confounding the effect of environment and genotype.

The model was: $Y_{ijkl} = \mu+c_k+bl_l+g_i+g_j+s_{ij}+gc_{ik}+gc_{jk}+s_{ijk}+\varepsilon_{ijkl}$, where Y_{ijkl} = observed trait value from each parent i and j, environment k; block l; μ= population mean; c_k = effect of environment; bl_l = effect of block ; g_i GCA effect of parent i;g_j=GCA effect of parent j; s_{ij} = SCA effect of the ij^{th} HS family; gc_{ik} = interaction between GCA effect for parent I and environment k; gc_{jk} = interaction between GCA effect for parent j and environment k; s_{ijk} = interaction between the SCA effect for the ij^{th} F_1 hybrid and environment k; ε_{ijkl} = residual effect.

Mid parent heterosis was computed as:

Heterosis(MP)(%)=(F_1-MP)/MP*100, where F_1= performance of F_1, MP= mid parent value.

5.4. Results

Genotype performance

Analysis of variance showed that there was significant variability among genotypes for all traits analysed both within the same moisture level and between the moisture levels. The interaction effects of moisture by genotypes were also highly significant for all characteristics (Table 5.1).

The mean days to heading, anthesis and physiological maturity were found to be 59, 66 and 106, respectively, under control conditions. Under stress conditions, however, time to heading (DH) and anthesis (DF) was delayed on average seven days. Days to physiological maturity (DM) were retarded on average by four days. Regardless of the moisture level, drought tolerant parents and their F_1 progenies took the longest time to reach specific growth stages (Table 5.2). Mean grain filling period (GFP), averaged over genotypes, in the absence of stress was found to be 42 days. Delay in the developmental stages due to stress led to the shortening of the grain filling period. The susceptible parents, Klinto and LD-357 were found to have the shortest grain filling period (Table 5.2).

Mean squares for the analysis of RGR and the different components of RGR computed over different growth stages were significantly different for the different genotypes under both moisture conditions. The interaction effects of moisture by genotype were also significantly high (Table 5.1). Differences in the values of RGR and its components between growth stages were also significant ($P<0.05$; data not shown). Averaged over cultivars, over age, RGR declined from 97.08 mg $g^{-1}d^{-1}$ between growth period 45 to 60 DAP to 13.28 mg $g^{-1}d^{-1}$ during the period between 75-90 DAP at control moisture levels. Under stress conditions, RGR declined from 74.12 mg $g^{-1}d^{-1}$ in the growth stage between 45-

Table 5.1. Mean squares for phenology, relative growth rate, and components of relative growth rate of durum wheat genotypes grown under moisture stress and control conditions.

		Means squares								
SOURCE	Df	DH	DF	DM	GFP	RGR	NAR	LAR	SLA	LWR
TOTAL	125									
REP(ML)	4	1.35604	4.973588	5.42652	1.6910865	5.15792	0.524685	2.079741	8.95673	0.000113
ML	1	1665.521**	1510.706**	466.5238**	298.205918**	3239.872**	103.4924**	3484.698**	20976.1**	0.965913**
Genotype	20	57.20486**	63.84323**	108.1218**	21.1774415**	117.8281**	31.16824**	29.87329**	274.9411**	0.008878**
Genotype*ML	20	10.05763**	12.18636**	10.52333**	15.6532425**	71.45695**	21.36606**	38.23901**	83.12998**	0.005689**
GCA	5	85.57778**	93.65949**	166.5424**	18.2296797**	236.1816**	29.386**	59.61252**	286.9904**	0.003186**
SCA	15	47.74722**	53.90456**	88.64825**	22.1600288**	78.37696**	31.76233**	19.96022**	270.9247**	0.010775**
GCA*ML	5	11.35876**	16.41025**	14.27718**	13.6183364**	121.2196**	19.16434**	18.74312**	151.9132**	0.001745**
SCA*ML	15	8.863005**	10.43853**	9.505762**	14.5804759**	57.0523**	23.33912**	46.85357**	58.68167**	0.007319**
GCA:SCA	Control	1.80:1	1.89:1	1.80:1	0.76:1	0.89:1	1.10:1	0.81:1	1.06:1	0.20:1
	Stress	1.55:1	1.49:1	1.88:1	0.89:1	4.62:1	0.88:1	2.00:1	1.54:1	0.10:1
Error		2.034185	3.463234	3.522447	3.497479	5.680395	0.659698	4.45497	19.72919	0.000392
CV%		2.26	2.68	1.75	4.53	6.08	10.97	12.47	10.46	5.13

**Significant at P=0.01

DH = days to heading, DF = days to flowering, DM = days to maturity, GFP = grain filling period, RGR = relative growth rate, NAR = net assimilation rate, LAR = leaf area ratio, SLA = specific leaf area, LWR = leaf weight ratio

Table 5.2. Mean days to heading, anthesis, physiological maturity and grain filling period for durum wheat genotypes grown under control (C) and moisture stress (S) conditions

Genotype	Name	ML	DH	DF	DM	GFP
1	DZ-2023	C	61	69	111	42
		S	70	77	116	39
2	Boohai'S'	C	63	69	113	44
		S	70	77	117	40
3	Klinto	C	57	63	102	39
		S	66	71	108	36
4	DZ-1691	C	62	69	109	40
		S	69	76	114	37
5	LD-357	C	56	63	98	35
		S	61	67	103	35
6	DZ-320	C	57	66	108	42
		S	69	77	116	39
7	1*2	C	58	69	110	41
		S	64	73	112	39
8	1*3	C	64	71	112	42
		S	69	76	111	36
9	1*4	C	61	68	105	38
		S	65	71	110	39
10	1*5	C	50	59	101	42
		S	62	68	101	33
11	1*6	C	60	66	105	39
		S	67	73	104	30
12	2*3	C	63	73	108	35
		S	68	74	113	39
13	2*4	C	63	71	109	37
		S	69	76	114	39
14	2*5	C	53	61	100	39
		S	62	70	103	33
15	2*6	C	54	61	102	41
		S	62	66	108	41
16	3*4	C	60	65	106	41
		S	64	72	109	38
17	3*5	C	60	67	109	42
		S	62	70	109	39
18	3*6	C	59	66	111	45
		S	68	76	114	38
19	4*5	C	57	64	104	40
		S	67	74	111	37
20	4*6	C	57	64	104	40
		S	65	75	111	36
21	5*6	C	59	64	106	42
		S	67	74	111	38
Mean	Ml	C	59	66	106	40
		S	66	73	110	42
S.E±	Ml		0.251	0.332	0.337	0.333
	Entry		0.814	1.075	1.093	1.077
$LSD_{0.05}$	Ml		0.418	0.552	0.561	0.553
	Entry		1.355	1.789	1.818	1.792

60 DAP to 7.46 mg $g^{-1}d^{-1}$ in the growth period between 75-90 DAP. The pattern of dry matter accumulation and rate of growth was different for the different genotypes. In the absence of stress, RGR in DZ-2023, LD-357 and DZ-320 showed a declining trend with ontogenetic drift over age. On the other hand, the rest of the parents showed an increasing trend till the growing period 60-75 DAP, but declined sharply thereafter. Their hybrids showed similar trends in most cases. However, deviations in some hybrids for RGR pattern could not be ruled out due to genetic interactions. Striking differences in RGR due to moisture stress were observed. Differences were largely due to differences in drought tolerance. Drought tolerant parent, DZ-2023, had high RGR in the beginning, but its rate of growth declined sharply in the later growth stages. However, its RGR at the end of the growth period remained unaffected by the stress. All F_1's derived from DZ-2023, had high RGR initially, regardless of the reaction of the other parent to the stress. Decreasing RGR was observed in some of the hybrids in the later growth stages. Hybrids from crosses of DZ-2023 with Klinto, DZ-1691 and DZ-320, however, showed an increasing tendency till the second phase of the growth period (Table 5.3). The other drought tolerant parent, Boohai'S', had RGR even higher than the control treatment in the first phase of the growth cycle. Nevertheless, RGR declined dramatically in the subsequent growth period. Like the other resistant parent, Boohai'S' RGR was not significantly affected by the stress in the end. All but the F_1 Boohai'S' x DZ-320 showed slow initial RGR but sustained minimum reductions in RGR at the end of the growth period, except for the F_1 Boohai'S' x LD-357 in which RGR was significantly reduced by the stress in the end. As compared with the control treatments, the susceptible parent, LD-357 had slow initial RGR, but increased with increasing rate during the middle growth period, and had one of the highest RGR among stress treatments at the end of the growth cycle (Table 5.3). Trends in net assimilation rate (NAR) over growth stage also showed significant variability among genotypes both within the same moisture level and genotypes between different moisture levels. In the period between 45-60 DAP, NAR was 9.79 and 6.28 $gm^{-2}d^{-1}$ at control and stress levels, respectively. NAR increased more for the control treatments, reaching its highest level of 20.35 and 8.3 $gm^{-2}d^{-1}$, at the

Table 5.3. Mean relative growth rate (RGR) and net assimilation rate (NAR) measured over different growth stages for durum wheat genotypes grown under moisture stress (S) control (C) conditions

Genotype	Name	ML	RGR($mg\ g^{-1}d^{-1}$) Growth Stages (DAP*)			NAR($g\ m^{-2}d^{-1}$) Growth Stages (DAP*)		
			45-60	60-75	75-90	45-60	60-75	75-90
1	DZ-2023	C	122.54	54.76	10.61	12.91	14.54	6.01
		S	112.89	28.87	10.74	8.57	5.07	2.94
2	Boohai'S'	C	72.94	124.84	10.34	7.33	32.48	6.46
		S	105.50	60.39	6.75	6.84	11.58	2.53
3	Klinto	C	92.92	115.54	19.58	9.62	24.12	5.94
		S	65.98	24.69	6.15	5.51	4.50	1.92
4	DZ-1691	C	86.00	164.77	8.97	7.57	37.32	4.08
		S	59.70	22.52	5.45	5.55	3.60	1.58
5	LD-357	C	111.60	77.71	20.45	12.42	23.80	14.76
		S	27.44	98.07	15.71	2.48	13.82	4.39
6	DZ-320	C	132.80	57.75	13.55	13.54	13.06	6.81
		S	88.55	16.91	1.77	9.10	2.67	0.48
7	1*2	C	100.31	84.03	6.95	9.05	22.66	5.87
		S	70.89	66.84	4.78	6.56	10.17	1.24
8	1*3	C	85.33	169.56	9.65	8.84	39.48	4.34
		S	78.00	105.60	6.79	7.53	18.95	4.21
9	1*4	C	109.25	98.83	8.47	12.48	24.01	3.83
		S	72.21	98.24	1.33	6.34	18.49	0.65
10	1*5	C	97.29	72.29	11.19	9.64	18.58	3.98
		S	82.43	31.33	5.21	7.77	5.04	1.27
11	1*6	C	86.34	87.41	20.21	8.41	20.25	9.30
		S	70.02	79.56	4.37	6.18	15.80	1.36
12	2*3	C	57.84	82.74	14.51	5.29	17.33	5.68
		S	50.36	81.91	15.80	3.75	11.23	5.95
13	2*4	C	56.02	90.94	10.55	5.46	19.08	4.21
		S	47.59	85.84	7.31	3.07	12.42	2.13
14	2*5	C	63.95	105.35	6.41	5.55	22.44	3.08
		S	38.64	91.04	1.68	4.46	16.83	0.47
15	2*6	C	134.92	58.97	10.02	13.23	13.55	4.02
		S	93.59	35.18	10.47	7.85	5.32	2.46
16	3*4	C	82.21	40.27	16.48	7.55	8.56	6.82
		S	91.83	9.23	7.43	6.58	1.75	3.60
17	3*5	C	97.83	63.55	19.77	9.91	16.28	8.18
		S	94.76	14.36	7.98	9.21	2.32	3.23
18	3*6	C	85.97	35.52	17.70	8.71	7.95	7.94
		S	85.38	22.34	3.78	6.49	2.91	0.83
19	4*5	C	87.24	110.45	15.81	10.31	28.21	5.59
		S	86.17	28.30	8.02	6.54	3.65	3.13
20	4*6	C	181.05	43.00	7.17	17.62	8.25	2.26
		S	69.50	40.15	9.15	6.21	7.60	2.38
21	5*6	C	94.33	63.45	20.55	10.12	15.31	7.70
		S	65.16	4.78	15.93	5.24	0.65	3.25
Mean	Ml	C	97.08	85.80	13.28	9.79	20.35	6.04
		S	74.12	49.82	7.46	6.28	8.30	2.38
S.E±	Ml		1.491	2.042	0.419	0.172	0.380	0.161
	Entry		4.832	6.612	1.356	0.556	1.232	0.523
$LSD_{0.05}$	Ml		2.481	3.397	0.696	0.285	0.633	0.269
	Entry		8.038	11.00	2.567	0.925	2.05	0.870

* DAP= days after planting

control and stress levels, respectively, in the time interval between 60-75 DAP. Thereafter, it declined sharply, reaching its lowest level at the time between 75-90 DAP, 6.04 and 2.4 $gm^{-2}d^{-1}$ at control and stress level, respectively. The difference between moisture levels and among genotypes within the same moisture level were also significantly high (Table 5.3). In the absence of moisture stress, NAR was highest for DZ-1691 x DZ-320 at the time interval between 45-60 DAP. Under stress conditions, NAR was highest at 45-60 DAP for F_1s from the cross of the two susceptible parents, LD-357, Klinto and the moderately tolerant parent, DZ-320. Nevertheless, the parents' NAR values were among the lowest at the same growth stage. The susceptible parent, LD-357's NAR value, however, was one of the best in the growth period between 75-90 DAP. The variation in leaf area ratio (LAR) was significantly high between moisture levels and genotypes within the same moisture levels. The interaction between moisture and genotypes with growth stages was also significant ($P<0.01$; data not shown). High values for LAR were recorded in the first growth period 45-60 DAP in all cases, though there existed significant differences among genotypes in response to moisture treatments. Like RGR and NAR, LAR also declined with age, but the decline of LAR was much faster than it was for NAR. The average reduction in LAR due to moisture stress at the early stage was about 50%. Drought tolerant genotypes were able to maintain relatively higher LAR during early age. In the beginning, the reductions in LAR were more pronounced in drought sensitive genotypes than in less sensitive and tolerant genotypes (Table 5.4). In later growth stages, however, effect of moisture stress diminished due to the increasing effect of ageing (Table 5.4). Genetic trade off was observed between NAR and LAR. Some genotypes with high investment in leaf area had a low rate of net biomass accumulation per unit area. For instance, high RGR of Boohai'S' under stress conditions initially was not due to high NAR, but rather to high LAR. Whereas, high RGR in the middle of the growing period was because of a high LAR, rather than a high NAR (Table 5.3 and 5.4). In some genotypes, however, changes in RGR were due both to changes in NAR and LAR. For example, reduction of RGR in DZ-2023 was due to reductions in both NAR and LAR (Table 5.3 and 5.4).

Table 5.4. Mean leaf area ratio measured over different growth stages for durum wheat genotypes grown under moisture stress (S) and control (C) conditions

Genotype	Name	ML	LAR ($m^2 kg^{-1}$) Days after planting			
			45	60	75	90
1	DZ-2023	C	38.13	10.97	5.26	2.36
		S	29.97	7.40	4.37	3.04
2	Boohai'S'	C	31.92	13.65	4.62	2.18
		S	35.80	6.28	4.45	1.62
3	Klinto	C	33.35	12.10	7.89	5.68
		S	18.69	8.23	3.55	2.85
4	DZ-1691	C	49.60	11.85	6.83	2.77
		S	14.94	8.19	4.79	2.46
5	LD-357	C	35.73	10.26	4.26	1.89
		S	13.44	9.24	5.54	2.31
6	DZ-320	C	35.48	12.47	6.45	2.45
		S	14.57	7.21	5.54	2.35
7	1*2	C	47.88	13.34	4.24	1.21
		S	20.37	7.07	6.43	2.35
8	1*3	C	49.29	10.39	7.32	2.58
		S	15.32	8.39	3.81	0.54
9	1*4	C	44.99	9.36	7.43	2.50
		S	22.46	7.10	4.09	0.77
10	1*5	C	47.20	10.56	5.87	5.44
		S	17.02	7.69	5.08	3.34
11	1*6	C	51.40	10.70	7.11	2.73
		S	21.90	6.96	3.84	2.66
12	2*3	C	57.82	10.23	9.00	2.82
		S	22.53	9.37	5.92	1.09
13	2*4	C	37.92	12.52	7.43	3.41
		S	30.89	8.59	5.79	2.06
14	2*5	C	34.31	16.83	5.75	2.98
		S	14.13	5.65	5.35	2.13
15	2*6	C	44.09	11.92	6.55	3.83
		S	21.88	7.83	5.81	3.21
16	3*4	C	43.82	12.30	7.40	3.42
		S	30.57	7.86	4.00	0.86
17	3*5	C	40.90	10.27	6.27	3.84
		S	19.30	6.57	6.06	0.79
18	3*6	C	34.67	12.41	6.49	3.15
		S	20.34	9.55	6.24	3.92
19	4*5	C	28.66	10.72	5.91	5.47
		S	19.51	9.97	6.38	0.79
20	4*6	C	39.69	13.02	8.49	4.75
		S	19.64	7.03	3.90	4.13
21	5*6	C	33.07	11.56	6.06	4.82
		S	17.44	9.67	5.51	4.45
Mean	Ml	C	40.95	11.78	6.51	3.35
		S	20.99	7.90	5.07	2.27
S.E±	Ml		0.743	0.661	0.084	0.069
	Entry		2.408	0.466	0.271	0.222
$LSD_{0.05}$	Ml		1.236	0.240	0.139	0.114
	Entry		4.006	0.779	0.451	0.370

Increase of RGR in Klinto at the control level was due to increased rate of net biomass accumulation but reduced rate of investment in leaf area. While, the reduction of RGR at stress moisture level was mainly due to reduced LAR (Table 5.3 and 5.4).

Combining ability

The mean squares due to GCA were highly significant for all traits analysed (Table 5.1). Variation among F_1 hybrids was also due to significantly high SCA effects for all traits. The interaction between GCA and moisture levels was significant for all parameters analysed. Significant SCA by moisture level interaction also occurred for all traits. F- values of GCA were mostly larger than F- values of SCA. This suggested the preponderance of additive genetic effects in the inheritance of most of those traits. The significance of SCA, however, emphasized the significant role of the non-additive effects. In some cases such as grain filling period and LWR under both moisture regimes and NAR under stress conditions, dominance was found to play the major role in the genetic control of the traits. On the basis of GCA effects for days to heading, anthesis and physiological maturity, the susceptible parent, LD-357 contributed most to earliness under both stress and control situations. The contribution of the resistant parents as far as earliness is concerned was the lowest under both moisture treatment conditions. LD-357 (susceptible parent), had significant but negative GCA effects for grain filling period under stress conditions. The tolerant parent, Boohai'S', on the other hand, had significant and positive GCA effects under stress conditions. GCA effect of DZ-320, at the control moisture level, however, was positive and significant. In general, moisture stress caused considerable and in some cases significant changes in the sign and magnitude of GCA effects (Table 5.5). GCA effects for RGR were mostly reduced from the control to stress level, pointing to the diminishing importance of additive genetic effects under control conditions in the genetic control of the inheritance of relative growth rate. Under control moisture conditions, all but DZ-2023, showed negative, mostly insignificant GCA effects for RGR. At the stress moisture level, drought tolerant parents, DZ-2023 and Boohai'S' showed the highest GCA effects while all the rest showed negative GCA effects (Table 5.5). At the control moisture level, the GCA effects for NAR of DZ-2023 were relatively high

regardless of the moisture level, whereas DZ-1691 had the smallest GCA effects for NAR at the control moisture level, and DZ-320 and LD-357 had the lowest GCA effects for NAR under stressed conditions. All except DZ-2023 and DZ-1691 had either negative or positive but insignificant GCA effects for specific leaf area (SLA) at control moisture level (Table 5.5). GCA effects for SLA of DZ-2023, Boohai'S' and DZ-320 were positive and high under stress conditions.

Table 5.5. GCA effects of phenology, RGR and components of RGR for durum wheat genotypes grown under moisture stress and control conditions.

	DH	DF	DM	GFP	RGR	NAR	SLA	LWR	LAR
					Control moisture level				
DZ-2023	0.3619	1.0479	1.414	0.3661	2.8893	0.7603	2.8639	0.0134	1.6326
Boohai'S'	0.8153	1.3921	1.3161	-0.076	-1.6492	0.3316	0.1136	-0.0162	-0.3487
Klinto	1.2799	0.6129	0.6536	0.0407	-0.059	-0.4602	-0.5368	0.0067	0.6435
DZ-1691	1.2344	0.915	0.1657	-0.7493	-1.2519	-0.4985	3.00095	0.0045	0.5817
LD-357	-2.6393	-2.7417	-3.5668	-0.8251	0.257	-0.0106	-2.7223	-0.0137	-1.7582
DZ-320	-1.0522	-1.2263	0.0174	1.2436	-0.1863	-0.1225	-2.7279	0.0053	-0.7509
S.E±	0.38781	0.43268	0.4901	0.5995	0.5581	0.2122	1.3482	0.007	0.6958
LSD	0.753515	0.840697	0.952264	1.164829	1.084388	0.412305	2.619553	0.013601	1.351939
					Stress moisture level				
DZ-2023	0.6611	0.4513	-0.2417	-0.6929	5.1379	1.691	2.8553	-0.009	0.6206
Boohai'S'	0.5403	0.2421	1.5083	1.2263	3.5528	-0.1602	3.0567	-0.0094	2.0413
Klinto	-0.0301	-0.08	0.1129	0.1929	-0.4975	1.3765	-3.8695	0.0083	-0.2799
DZ-1691	0.7932	1.15	1.5088	0.3588	-2.5709	-0.4515	-0.3622	0.007	0.2307
LD-357	-2.5435	-2.6867	-3.8904	-1.2038	-2.9459	-0.5381	-4.3767	-0.0008	-2.0248
DZ-320	0.574	0.9233	1.0021	0.0788	-2.6764	-1.9178	2.6964	0.0039	-0.588
S.E±	0.4343	0.6245	0.58896	0.47275	0.797	0.31414	1.2126	0.00397	0.50822
$LSD_{0.05}$	0.843845	1.213404	1.144349	0.918553	1.548571	0.610374	2.356082	0.007714	0.987471

DH = days to heading, DF = days to flowering, DM = days to maturity, GFP = grain filling period, RGR = relative growth rate, NAR = net assimilation rate, SLA = specific leaf area, LWR = leaf weight ratio, LAR = leaf area ratio

All except Boohai's and LD-357 had positive GCA effects for leaf weight ratio (LWR) in the absence of stress. Only Boohai'S' and DZ-2023 had significantly high negative GCA at the stress level. Klinto had the highest positive GCA effect of LWR under stress conditions (Table 5.5).

DZ-2023 and Boohai'S' were the best combiners for LAR at control and stress conditions, respectively. However, the GCA effects of the susceptible parent, LD-357 were negative under stress conditions. The GCA effects of the other susceptible parent, Klinto, were also negative under stress conditions. Klinto, however, had higher GCA effects for LAR at control moisture level than under stress conditions (Table 5.5).

DZ-2023 and Boohai'S' were regarded as the best combiners with respect to RGR for they also showed the best GCA effects for components of RGR such as LAR and NAR.

Heterosis

Significantly high negative heterosis was obtained in most cases under both stress and control moisture conditions for days to heading, flowering and maturity. At the stress moisture level, F_1s reached heading and flowering earlier than their parents compared to the control moisture level (Table 5.6). This reflected differential responses of hybrids to moisture treatments. The level of heterosis for grain filling period ranged between –15 to 15% and –15 to 7% under the control and the stress moisture level, respectively. At the control moisture level, Klinto x LD-357 and Klinto x DZ-320 had the highest heterosis for GFP. Klinto x LD-357 had the highest mid parent heterosis for GFP under stress conditions.

The level of heterosis for RGR under control conditions was mostly lower than at the stress level. The maximum was from DZ-2023 x DZ-1691 (23.7%) The highest level of heterosis for NAR under control conditions was 59%. Heterosis estimates of LAR ranged from –24 to 66%. The highest being for Boohai'S' x Klinto, and the lowest was for DZ-1691 x LD-357. At the control moisture level, hybrid combinations with DZ-2023 as parent had mostly significant and positive heterosis for RGR, and for its morphological component, LAR. All of these combinations also had significant and positive mid-parent heterosis estimates for NAR, except for the cross combination between DZ-2023 x LD-357 and DZ-2023 x DZ-320. Hybrid combinations involving Boohai'S' and/or Klinto *versus* all the rest had either significant but negative or positive but insignificant levels of heterosis for RGR and its components, except for Boohai'S' x DZ-320, which had significant and positive mid parent heterosis (32%) for NAR. LD-357 x DZ-1691 and DZ-320 x LD-357 gave either significant but negative or

Table 5.6 Mean of percentage of mid-parent heterosis calculated for different characteristics of durum wheat grown under moisture stress and control conditions

	DH		DF		DM		GFP		RGR		NAR		LAR	
HYBRID	CONTROL	STRESS	CONTROL	STRESS	CONTROL	STRESS	CONTROL	STRESS	CONTROL	STRESS	CONTROL	STRESS	CONTROL	STRESS
1*2	-5.73	-9.53	-0.03	-5.71	-1.69	-4.10	-4.25	-0.58	18.60	-9.93	44.02	20.13	31.79	-33.66
1*3	7.73	2.02	7.34	1.95	4.41	-0.75	0.11	-5.37	16.18	39.32	20.74	336.44	41.99	-46.17
1*4	-0.67	-7.08	2.29	-3.61	-0.20	-0.18	-4.31	5.93	23.68	25.19	58.99	208.35	3.91	19.44
1*5	-13.94	-5.93	-10.49	-5.93	-2.66	-6.90	11.25	-7.83	11.65	2.13	-34.24	16.88	17.39	24.22
1*6	0.73	-2.84	2.04	1.33	1.75	-5.17	1.28	-15.46	18.79	17.44	-9.80	27.60	43.06	52.04
2*3	5.06	-0.07	2.89	-0.08	0.11	0.83	-15.34	2.34	-7.45	22.72	-21.83	129.93	65.97	-19.13
2*4	1.01	-0.58	-8.16	-1.43	-2.16	-0.69	-10.26	0.76	-21.10	5.41	-34.10	1.46	-4.00	22.47
2*5	-11.35	-5.62	-9.30	-3.71	-5.94	-6.09	-2.32	-9.23	-16.26	-14.48	-32.57	7.60	4.13	-39.00
2*6	-10.89	-10.33	-1.53	-14.19	-7.93	-7.58	-5.73	4.83	1.26	11.78	-27.32	1.02	32.14	-6.43
3*4	1.52	-5.39	2.06	-3.08	0.16	-1.27	3.27	2.07	-13.41	50.32	-8.58	93.36	3.37	61.13
3*5	5.51	-2.85	3.09	-2.41	5.24	1.14	11.03	7.43	-1.66	25.51	-19.86	98.11	-0.68	22.66
3*6	3.15	0.43	-3.10	5.50	7.46	4.29	14.76	1.96	-17.60	8.78	-36.47	-28.68	0.09	50.07
4*5	-3.36	3.24	-0.37	3.28	0.78	2.77	7.84	1.68	-6.15	28.17	-30.36	94.27	-24.17	23.27
4*6	-4.49	-5.92	-0.37	4.12	1.22	1.99	3.86	-1.57	4.49	11.10	-36.70	-31.18	17.31	46.21
5*6	3.32	3.30	-0.28	2.03	2.51	1.92	7.20	1.81	-9.37	7.81	-40.27	-26.61	0.31	36.06
SE±	0.83	1.23	1.41	1.74	1.03	1.11	2.32	2.41	2.97	5.38	4.92	16.51	7.43	11.79

DH = days to heading, DF = days to flowering, DM = days to maturity, GFP = grain filling period, RGR = relative growth rate, NAR = net assimilation rate, SLA = specific leaf area, LWR = leaf weight ratio, LAR = leaf area ratio.
1= DZ-2023, 2= Boohai'S', 3= Klinto, 4= DZ-1691, 5= LD-357 and 6= DZ-320.

positive but not significant mid-parent heterosis for RGR and its components (Table 5.6). Mid-parent heterosis for RGR in general ranged between –14.5 to 50.32% at the stress moisture level. The highest mid-parent heterosis estimates for RGR were obtained from the crosses Klinto x DZ-1691 (50%) and DZ-2023 x Klinto (39%). Under stress conditions, except for DZ-2023 x Boohai'S' and Boohai'S' x LD-357, mid-parent heterosis estimates for RGR were all positive and mostly significant. Mid-parent heterosis estimates for NAR were also either significant and positive or insignificant but negative. Mid-parent heterosis as high as 336% for NAR was found in DZ-2023 x Boohai'S'. The level of heterosis observed under stress conditions was much higher than the heterosis level at the control moisture level. Mid-parent heterosis for LAR were also significant and in most cases positive, except for DZ-2023 x Boohai'S' and DZ-2023 x Klinto, in which heterosis was significant but negative. Estimates of heterosis for LAR were found to range between –46 to 61%. The smallest was for DZ-2023 x Klinto and the highest was for Klinto x DZ-1691. The level of heterosis for LAR was also higher under stress conditions than control conditions (Table 5.6).

Heritability, genetic and phenotypic relationships among traits

A summary of broad sense heritability, genotypic and phenotypic correlation coefficients is presented in Table 5.7. Broad sense heritability computed from the variance components partitioned based on combined analysis of variance revealed that the extent of genetic control varied considerably among the traits studied. Grain yield showed the highest value followed by NAR and TDM. The lowest was obtained for GFP period. Broad sense heritability estimates for all other phenological characteristics, however, were fairly high (Table 5.7). The genetic and phenotypic correlation coefficients among phenology traits were quite high. The association between any of those traits with yield, total dry matter production or harvest index were too low to be significant. Most of the correlations between phenology characteristics and relative growth rate and components of relative growth rate were small (Table 5.7). The genotypic correlations between grain yield and RGR; and grain yield and LAR were positive and significant. The phenotypic correlations between grain yield and RGR and

Table 5.7. Broad sense heritability ($h^2\pm$) diagonal and bold, genetic correlation (r_g) above diagonal and phenotypic correlation (r_p) below diagonal of phenology, RGR and Components of RGR for durum wheat genotypes grown under moisture stress and non stress conditions

	YIELD	RGR	NAR	SLA	LWR	LAR	TDM	HI	DH	DF	DM	GFP
YIELD	**0.94±0.01**	0.54**	0.21	0.31	0.18	0.80***	0.88***	0.82***	0.28	0.19	0.19	0.12
RGR	0.55**	**0.77±0.03**	0.79***	-0.06	0.41†	0.51*	0.46*	0.56**	0.14	0.09	0.06	-0.03
NAR	0.19	0.73***	**0.89±0.01**	-0.33	0.29	0.14	0.15	0.28	0.26	0.28	0.19	-0.06
SLA	0.31	-0.17	-0.54*	**0.69±0.01**	-0.54**	0.28	0.10	0.49*	0.25	0.30	0.38†	0.37
LWR	0.21	0.51*	0.28	-0.50*	**0.79±0.02**	0.39†	0.20	0.05	-0.02	-0.07	-0.22	-0.41
LAR	0.76***	0.47*	0.08	0.37†	0.30	**0.49±0.06**	0.66**	0.78***	0.38†	0.34	0.27	0.02
TDM	0.87***	0.48*	0.16	0.16	0.30	0.64**	**0.82±0.02**	0.48*	0.14	0.05	0.11	0.17
HI	0.79***	0.57**	0.25	0.49*	0.05	0.69**	0.45*	**0.81±0.02**	0.38†	0.33	0.27	0.04
DH	0.32	0.13	0.18	0.22	-0.09	0.39	0.11	0.49*	**0.82±0.02**	0.96***	0.85***	0.30
DF	0.20	0.05	0.18	0.25	-0.17	0.32	-0.02	0.44*	0.90***	**0.74±0.03**	0.93***	0.40
DM	0.10	-0.06	0.05	0.31	-0.40†	0.13	-0.06	0.24	0.76***	0.85***	**0.83±0.02**	0.72
GFP	0.07	0.00	-0.04	0.18	-0.33	-0.06	0.04	-0.04	0.19	0.21	0.64**	**0.46±0.06**

†, *, **, ***, Significantly different from zero at P=0.1, 0.5, 0.01 and 0.001, respectively.

RGR = relative growth rate, NAR = net assimilation rate, SLA = specific leaf area, LWR = leaf weight ratio, LAR = leaf area ratio , TDM = total dry mass, HI = harvest index, DH = days to heading, DF = days to flowering, DM = days to maturity, GFP = grain filling period

grain yield and LAR, were significant. Significant and positive genetic correlations between RGR with NAR, LAR and LWR, respectively, were reflected in significantly high and positive phenotypic correlations. Neither the genotypic nor the phenotypic correlation between LAR and NAR was significant. The genetic correlation between LAR and LWR; and the phenotypic correlation between LAR and SLA was significant and positive. The phenotypic correlation between NAR and SLA was significant but negative.

5.5. Discussion

Genetic variation in growth duration is one of the most obvious means of matching seasonal transpiration with water supply and thus maximising water transpired. Early flowering can lead to better yield and yield stability advantage, if rain does not occur during the latter half of the growing season. Thus, development of short season varieties provides benefits where rainfall is predictable, but in unpredictable environments, potentially transpirable water may be left in soil at maturity in better seasons and yield may be sacrificed. The variability observed in growth duration among genotypes within the same moisture level in this experiment was not large enough to discern any yield-associated variability either due to earliness or lateness because of stress. The reason is that genotypes were selected based on their grain yield response under stress conditions rather than their diversity for growth duration and development. It was, however, observed that stress generally caused significant retardation in major phenological development and shortened grain filling period. Stress occurring during pre-anthesis growth stages in durum wheat has been reported to delay anthesis and maturity (Simane *et al.*, 1993). Under both moisture regimes, F_1s took fewer days to reach heading, flowering and physiological maturity, suggesting earliness is a dominant character. In most cases mean squares of GCA effects were larger than SCA under both moisture regimes, indicating that additive genetic effects played a paramount role in the inheritance of these characters. Nevertheless, because SCA is significant, the

magnitude of the ratio is too small to rule out the importance of non-additive genetic effects. It can be concluded that the characters were governed by both additive and non-additive genetic effects. This was in agreement with other findings (Kathiria and Sharma, 1996). Significant interactions between GCA by moisture and SCA by moisture for growth stages studied in this experiment were in agreement with earlier reports (Kathiria and Sharma, 1996). It is suggested that selection for these characters should be carried out under both moisture conditions. Tremendous genetic variation was observed for RGR for genotypes both within the same moisture level and between the moisture levels. Differential responses of genotypes particularly between susceptible and resistant groups between the two moisture levels were evident from significantly high genotypes by moisture levels interaction for RGR and RGR related traits. This confirms the findings of Turner and Nicolas (1988) and Simane *et al.* (1993). Reductions in RGR at early growth stages due to moisture stress was very high in susceptible genotypes, while the opposite held true for the resistant genotypes (Turner and Nicolas, 1988; Simane *et al.*, 1993). This probably allows drought tolerant genotypes to conserve soil moisture at the expense of direct soil evaporation and make available considerable moisture for later growth periods. The differences in RGR among genotypes were both due to the physiological components of growth (NAR) and the morphological components (LAR). Decrease in RGR has been reported in wheat due to a decrease in LAR (Simane *et al.*, 1993) and due to both LAR and NAR (Van den Boogaard *et al.*, 1996). A decrease of NAR reflects a decrease in rate of photosynthesis or an increase in respiration (Cheeseman, 1988; Rawson *et al.*, 1988). Under field conditions, a higher leaf area and a higher soil cover will reduce evaporation from the soil surface and hence increase the amount of water that can be used for plant growth. Early ground cover and biomass were related to high grain yield in wheat (Turner and Nicolas, 1987) and barley (Van Oosterom and Acevedo, 1992). Whan *et al.* (1991) found this association to be important only under dry conditions. Significant genotypic and phenotypic correlation between grain yield with LAR and RGR in this study suggests the same. GCA and SCA

mean squares were highly significant under both moisture levels for all growth related traits. In most instances, means squares due to GCA were higher than mean squares due to SCA. Thus, both additive and non- additive genetic effects contributed to the observed genetic variation in the different parameters considered. High levels of mid parent heterosis estimates were found for most traits under both conditions, though the estimates tended to increase at the stress level. This suggests the increasing importance of non-additive genetic control under stress conditions. This agreed with the findings of a study in corn (Dehghanpoor *et al.*, 1997) and tomato (Kilchevsky and Babak, 2000). The genetic correlations between RGR and NAR, and RGR and LAR were higher than the phenotypic correlation, indicating antagonistic effects of genotypes and environment on the phenotypic relationships between those traits. The phenotypic correlations between SLA and NAR and SLA and LAR, however, was greater than the genotypic correlation coefficients, implying synergistic effects of genotypes and environments on the phenotypic associations between SLA and other traits· The genetic and phenotypic correlation between grain yield and RGR; and grain yield and LAR were significant and very close, suggesting selection for high RGR and/or LAR can improve grain yield. Greater ground cover early in the season should also reduce light availability beneath the canopy and improve crop competitiveness with weeds (Lemerle *et al.*, 1996). Besides, it has been demonstrated that when leaf area develops rapidly and high leaf area is sustained during grainfilling, duration of grain leaf area affects wheat yield positively (Siddique *et al.*, 1989; Blum, 1990). Leaf weight ratio was found to be positively and significantly correlated with both LAR and RGR. Broad sense heritability estimates for these traits were found to be moderate to high, indicating the possible genetic advance if selection is done. However, significantly high genotype by environment interaction would mean selection should be undertaken both under stress and non-stress conditions. High broad sense heritability estimates for dry matter production was in agreement with that reported by Whan *et al.* (1991). Lower broad sense heritability estimates of LAR was in accordance with smaller broad sense heritability estimates reported for

leaf area in barley (Cai *et al.*, 1993), rice (Redona and Mackill, 1996) and wheat (Rebetzke and Richards, 1999). The genetic correlation between NAR and RGR was high. The broad sense heritability estimate of NAR was also high. Nevertheless, lack of any significant genotypic association between any of the morphological components of RGR or dry matter or grain yield would complicate its usefulness as selection tool for the improvement of RGR under any of the environment.

5.6. Conclusions

Differences in growth and growth related characters observed among drought tolerant and susceptible genotypes in this experiment demonstrated the difference in developmental plasticity between drought tolerant and susceptible genotypes. Fairly high genetic correlation coupled with moderate to high broad sense heritability estimates for most of these traits indicates the prevalence of genetic variation which could be exploited for improvement of drought tolerance. However, further studies should focus on the assessment of the relevance and importance of early vigour traits, preferably over locations.

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CHAPTER 6

Variation in water use and transpiration efficiency among durum wheat genotypes grown under moisture stress and non-stress conditions

6.1. Abstract

Durum wheat genotypes with different responses to moisture stress were studied in a glasshouse under moisture stress and non-stress conditions to investigate differences in water use and transpiration efficiency and interrelationships among water use and transpiration efficiency and associated traits. Significantly high genotypic variability in the cumulative amount of water used before (ETba) and after (ETpa) anthesis was observed. Susceptible genotypes used higher amounts of water before anthesis and lower amounts after anthesis. In contrast, tolerant genotypes used a higher proportion of water during the post-anthesis period. Significantly high variability among the genotypes was observed for various measures of water use and transpiration efficiency, total dry matter and harvest index. Ranking of cultivars for water use efficiency based on grain yield (WUE_G) and transpiration efficiency based on grain yield was consistent with ranking of cultivars for drought susceptibility indices. Drought susceptibility index was significantly but negatively correlated with harvest index, WUE_G and grain yield. However, it was positively and significantly correlated with the ETba:ETpa ratio. A high positive correlation of WUE_G with harvest index and grain yield with harvest index was found. It was concluded that selection for lower ETba:ETpa ratios up to about 0.8 could indirectly lead to improved WUE_G and HI, hence improved grain yield. Selection for

increased WUE_G and/or grain yield would increase yield in water-limited environments.

Keywords: *Drought tolerance, ratio of pre-anthesis to post-anthesis water use, water use efficiency*

6.2. Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is traditionally grown as a rainfed crop in the semi arid tropics (Nachit & Quassou 1988). Rainfed crop production in this climate depends strongly on both the amount and distribution of rain. In this environment, however, rainfall is low and generally poorly distributed. In the West Asian and North African (WANA) region, variation in the amount of rainfall has been reported to account for 75% of year-to-year variability in wheat yield (Blum & Pnuel 1990). Periods of water deficit stress can occur any time in the reproductive growth stages, and particularly during the grain filling period (Oweis *et al.* 2000). Consequently, crop yield and water use efficiency (WUE) are generally low and variable. One approach to improve and stabilize production is the application of supplemental irrigation (Oweis *et al.* 1998; Zhang *et al.* 1998). However, excessive application of water in the form of supplemental irrigation has resulted in a decline of aquifers and deterioration of water quality in many areas (Oweis *et al.* 2000).

Genetic manipulation to improve water use efficiency indeed seems to be more sustainable. For example, increasing the portion of water used for plant transpiration via a large and early canopy can increase water use efficiency (Richards *et al.* 2002). Genetic improvement of water use efficiency has been discussed in detail elsewhere (Condon *et al.* 2002).

Depending on the importance of the crop product, WUE has been defined as a given level of biomass or dry matter or grain yield per unit of water used by the crop (Hatfield *et al.* 2001; Howell 2001). When grain yield is the important economic trait, yield is expressed as a product of water use (W), WUE, and harvest index (HI) (Passioura 1977; Blum 1988). Similarly,

the amount of water used can be expressed as evapotranspiration (transpiration and evaporation), or as a transpiration component only.

Prevalence of genetic variation for water use efficiency among wheat cultivars, particularly of *Triticum aestivum* species has been well documented (Fischer & Turner 1978; Siddique *et al.* 1990). Attributing to such genetic variability are flowering time (Richards 1991), deep root system, variation in relative growth rate, leaf thickness, canopy structure and harvest index (Cooper *et al.* 1987). Presence of sufficient variation has long been noted as a prerequisite for the improvement of WUE. This is because it may result in the identification of new parental lines to generate greater variability in key targeted traits for selection (Richards *et al.* 2002).

The aims of the present study were: 1) to study the genetic variability for water use and transpiration efficiency, and 2) to examine the relationships among measures of water use and transpiration efficiency and associated traits in durum wheat lines selected for their different response to moisture stress and progenies obtained from their combinations.

6.3. Materials and methods

A six by six diallel cross without the reciprocals was made in January to June 2001 at Debrezeit Agricultural Research Centre, Ethiopia. Two drought tolerant varieties, Boohai 'S' and DZ-2023, two moderately tolerant varieties, DZ-1691 and DZ-320, one moderately susceptible variety, Klinto, and one susceptible variety, LD-357, were used as parents.

In the subsequent season, two sets of material consisting of parents and their 15 F_1 progeny were grown in the glasshouse in a randomized complete block design with three replications in July to December 2001 at the Free State University, South Africa. They were grown at an average of 25 and 15^{0}C day and night temperatures, respectively. Entries were planted in 10 litre capacity plastic pots filled with 2kg of gravel (to prevent soil leakage), and 7kg of steam sterilized and air dried sandy loam soil with particle composition of 80%, 2% and 18% of sand, silt and clay, respectively. The air-dry moisture content of the soil was determined as 13.3% (field capacity) and

4.0% (permanent wilting point) gravimeter. Pots were equally spaced. Each genotype was sown into six pots. Twelve seeds at equal spacing were planted at equal depth. Seedlings were thinned to nine per pot at the two-leaf growth stage. Pots were maintained at 70-100% available soil moisture for a month (up to about four-leaf growth stage). Half of the experimental unit was then left without water until severe wilting was observed. The amount of water evaporated was monitored daily by weighing unplanted pots placed between planted pots in both the stress and the non-stress treatments in each block (six in each block). The amount of water transpired was determined by subtracting the weights of unplanted pots from the weights of the planted pots. Pots were replenished with the amount of water equivalent to the loss in weight to bring them to the predetermined level of moisture whenever the weight of pots fell to the lower limit established for the treatments. The moisture levels were 25-35% and 70-100% available soil moisture for the stress and control treatments, respectively until maturity. Equal exposure to the growing conditions of the glasshouse was ensured by changing the positions of pots every week. Total leaf water potential was measured using a pressure chamber model (PMS, Instrument Company, Corvallis, Oregon). Two-thirds of flag leaf lamina was excised and placed immediately into the rubber stopper, and used for reading of water potential according to Fischer *et al.* (1977). One plant per pot was used to determine water potential. Water potential was measured at all growth stages during mid-day between 12h00-13h00.

At maturity, three plants per pot were harvested and left to air dry for 10 days. Grain and biomass yield were determined as average of the three plants. Harvest index was calculated as the ratio of grain yield to biomass yield. Drought susceptibility indices (S) were computed according to Fischer & Maurer (1978). Cumulative water use (ET) was obtained from summation of water applied over the entire growth period. Transpiration (T) was calculated from the difference between ET and Evaporation (E). Both ET and T were expressed as g/plant. Water use efficiency (WUE), was determined as a function of grain yield (WUE_G), and biomass, i.e, above ground dry matter, (WUE_{TDM}). Transpiration efficiency (T) was also expressed on the

basis of grain yield (T_G) and biomass (T_{TDM}). WUE and T were computed as follows:

WUE_G = Grain yield (g plant^{-1})/ET(kg plant^{-1})

WUE_{TDM} = TDM(biomass) (g plant^{-1})/ET(kg plant^{-1})

T_G = Grain yield (g plant^{-1})/T(kg plant^{-1})

T_{TDM} = TDM(biomass) (g plant^{-1})/T(kg plant^{-1})

Data were subjected to analysis of variance. Regression and correlation analyses were computed using Agrobase (Agronomix Software Inc, Winnipeg, Canada).

6.4. Results

Total Dry Matter (TDM), Harvest (HI) and Drought Susceptibility Indices (S)

Averages of TDM, HI and S for the various treatments are summarized in Table 6.1. Moisture stress caused average reductions of 64.4% and 31.9% in TDM and HI, respectively. TDM production was reduced by 53.3% and 49.9 % in the tolerant parents DZ-2023 and Boohai 'S', respectively. In moisture stress-susceptible parents, LD-357 and Klinto, the reductions in TDM were 49.9% and 51.9%, respectively. The reduction in HI in DZ-2023 was only 11.4%. In the other tolerant parent, Boohai 'S', however, an increase of 3.6% in HI was found. Susceptible parents, Klinto and LD-357, showed reductions in HI of 74.5% and 81%, respectively.

Grain yield was found to be significantly and positively correlated with TDM and HI. S was significantly but negatively correlated with total dry matter, and HI (Table 6.3.).

Evapotranspiration (ET)

Grain yield, TDM and HI were significantly affected by the variation in cumulative water use or ET. A direct relationship between ET and TDM was observed (Fig. 6.1). Grain yield was also positively and significantly correlated with ETpa. The amount of water utilized before anthesis (ET_{ba}) and after anthesis (ET_{pa}) was statistically significantly different between

different genotypes and moisture levels (Table 6.1.). The amount of ET_{ba} used by the susceptible parents, LD-357 and Klinto, was about 2.5 and 2 times as high as the amount used by the tolerant parents DZ-2023 and Boohai 'S', respectively.

Table 6.1. Mean values of harvest index (HI), total dry matter (TDM), drought susceptibility index (S), cumulative water use before anthesis (ET_{ba}), post-anthesis (ET_{pa}), and ratio between these two for six durum wheat varieties and their diallel crosses grown under stress and non-stress conditions.

Name	Moisture level	HI	TDM (g^{-1}pt)	S	ETba (g^{-1}pt)	Etpa (g^{-1}pt)	ETba:ETpa
DZ-2023	C	0·47	5·83		508	1353	0·38
	S	0·41	2·75	0·79	496	688	0·72
Boohai 'S'	C	0·48	5·54		645	1106	0·59
	S	0·49	2·77	0·65	670	875	0·77
Klinto	C	0·52	6·48		719	1503	0·48
	S	0·13	3·12	1·19	1318	568	2·32
DZ-1691	C	0·39	6.30		722	983	0·74
	S	0·25	2·61	1·07	797	849	0·94
LD-357	C	0·56	2·68		708	1855	0·38
	S	0·11	2·10	1·18	1249	642	1·95
DZ-320	C	0·34	4·77		791	1382	0·57
	S	0·23	2·03	0·97	811	973	0·83
1*2	C	0·42	6.66		795	1149	0·70
	S	0·41	3·27	0·72	645	843	0·77
1*3	C	0·49	6·23		999	1047	0·95
	S	0·45	1·87	0·99	455	726	0·63
1*4	C	0·55	8·15		799	1348	0·59
	S	0·37	2·61	1·07	642	765	0·84
1*5	C	0·45	6·11		993	946	1·05
	S	0·36	2·53	0·91	557	681	0·82
1*6	C	0·47	7·16		961	1137	0·85
	S	0·47	2·64	0·86	684	955	0·72
2*3	C	0·51	5·24		950	1077	0·88
	S	0·37	1·70	1·04	455	597	0·76
2*4	C	0·58	6·69		865	1205	0·73
	S	0·44	2·25	1·02	591	772	0·77
2*5	C	0·36	4·59		1035	990	1·05
	S	0·31	2·27	0·79	479	691	0·70
2*6	C	0·46	7·43		593	1693	0·35
	S	0·41	2·14	1·02	672	919	0·73
3*4	C	0·44	8·65		779	1402	0·56
	S	0·32	1·34	1·21	1026	673	1·53
3*5	C	0·46	5·84		1114	1426	0·79
	S	0·19	1·95	1·18	1082	711	1·52
3*6	C	0·53	4·91		1097	1537	0·71
	S	0·27	2·55	1·01	804	791	1·02
4*5	C	0·22	5·15		1134	1213	0·94
	S	0·22	1·77	0·90	467	738	0·64
4*6	C	0·56	4·37		821	1352	0·61
	S	0·25	1·96	1·10	595	718	0·83
5*6	C	0·53	4·67		834	1382	0·54
	S	0·21	1·99	1·13	904	973	2·01
Mean		0·39	4·08	0.99	792	1021	0·86
S.E.D(D.F.)		0·029(82)	0·286(82)	0·051(4	25(82)	57·305(	0·045(82)

C = control, S = stress.

The amount of ET_{pa} used by DZ-2023 and Boohai 'S', however, was 1.2 and 1.5 times higher than LD-357 and Klinto, respectively (Table 6.1.). Similar trends and marked variability in the amount of pre-anthesis and post-anthesis water use were observed (Table 6.1.). This resulted in relatively higher ET_{ba}:ET_{pa} ratios for the susceptible parents LD-357 and Klinto. The ratios for the resistant parents DZ-2023 and Boohai 'S' were 0.72 and 0.77, respectively. The average ET_{ba}:ET_{pa} ratio pooled over cultivars were 0.69 and 1.04 for the control and stress treatments, respectively. HI increased exponentially with decreasing ET_{ba}:ET_{pa} ratio (Fig.6.2a). The variation for ET_{ba}:ET_{pa} among genotypes under non-stress conditions was minimized. The major share of the variation in ET_{ba}:ET_{pa} *vs* HI relationships (Fig.6.2a) was due to the stress treatments. Drought tolerant and moderately tolerant genotypes had ET_{ba}:ET_{pa} ratios less than one. Susceptible parents along with susceptible hybrids fell in the right extreme of the ET_{ba}:ET_{pa} *vs* HI negative exponential relation ships (Fig.6.2a).

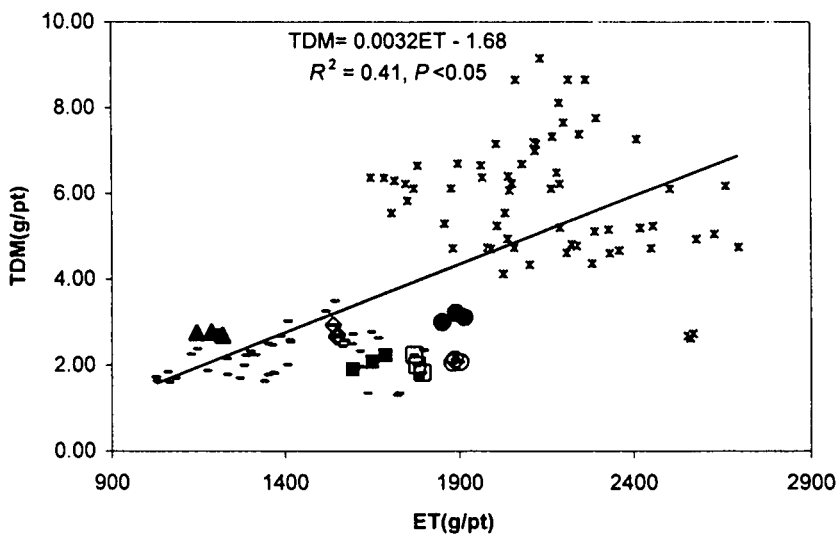

Fig.6.1. Relationships between total dry matter (TDM) production and cumulative water (ET) use per plant for durum wheat grown under optimum and stress moisture conditions in glasshouse.Keys: * and – refers to control and stress moisture treatments, respectively. Parental lines under stress moisture level are: ▲ = DZ-2023, ◊ = Boohai 'S', ■ = DZ-1691, □ = DZ-320, ● = Klinto, and ○ = LD-357.

Water Use Efficiency (WUE)

WUE both for total dry matter production (WUE_{TDM}) and grain yield (WUE_G) were significantly different between cultivars and moisture levels. The interaction effects were also statistically significant. High genotypic variability among the test genotype in WUE_{TDM} and WUE_G was observed (Table 6.2.). Average WUE_{TDM} for the control treatments was 2.8g/kg whereas WUE_{TDM} for the stress treatments was 1.57g/kg The F_1 derived from the crosses DZ-2023 X DZ-1691 and DZ-1691 X Klinto had the highest WUE_{TDM} at the control moisture level. The least amount of WUE_{TDM} was recorded for the susceptible parent LD-357. Tolerant parents DZ-2023 and Boohai 'S' produced 3.14 and 3.17g/kg, respectively. Under stress conditions, the highest amount of dry matter per kilogram of water used was produced by the tolerant parent, DZ-2023 and F_1 DZ-2023 X Boohai 'S'. The least amount was obtained from the F_1, Klinto X DZ-1691. In terms of grain yield production, WUE_G showed more than 40% reduction from the control to the stress moisture level. The reduction was more pronounced in stress-sensitive parents and their hybrids (Table 6.2.). WUE_G for tolerant parents, DZ-2023 and Boohai 'S', was four to eight times higher than that of the susceptible parents, Klinto and LD-357. WUE_{TDM} was positively and significantly correlated with TDM and HI. However, it was insignificantly but negatively correlated with ET_{ba}:ET_{pa}. WUE_G was positively and significantly correlated with TDM (Table 6.3.). HI increased with increasing WUE_G. Under stress conditions genotypes had mostly lower WUE_G, and hence, tended to have in most cases lower HI. Nevertheless, drought tolerant genotypes had higher WUE_G under stress conditions compared to susceptible and moderately tolerant types. As a result, they had higher HI (Fig. 6.3). WUE_G was strongly correlated with grain yield and inversely proportional to ET_{ba}:ET_{pa} ratio below 1.5 (Fig 6.2b). Genotypes under non stress conditions mostly had higher WUE_G at ET_{ba}:ETpa ratio less than 1·0. The tolerant parents fell close to the control treatments while the susceptible parents fell to the extreme right (Fig 6.2b).

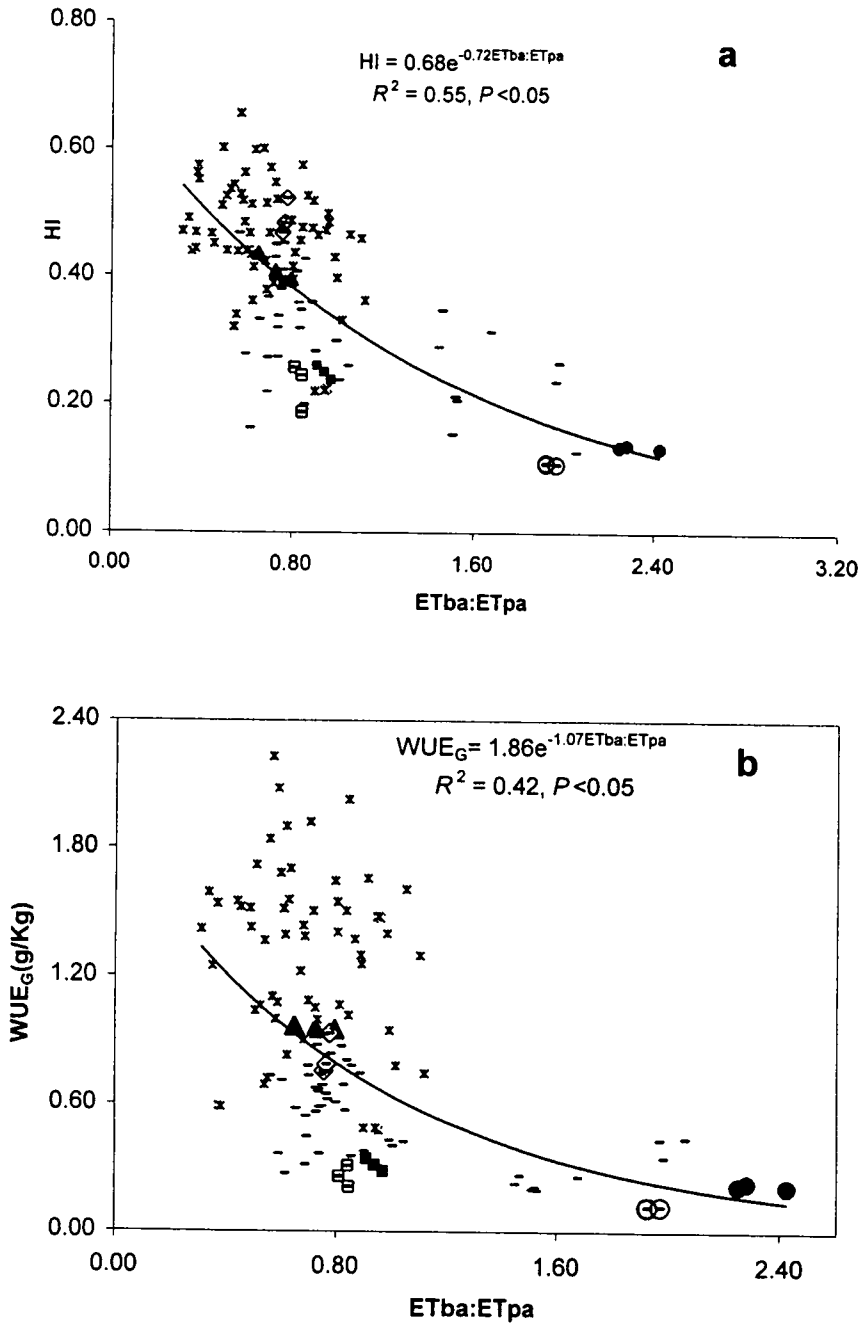

Fig. 6.2. Relationships between the ratio of pre-anthesis to post-anthesis water use (ETba:ETpa) and (a)HI, and (b) WUE_G for durum wheat gown under stress and non-stress moisture levels in glasshouse.
* and – refers to control and stress moisture treatments, respectively. Parental lines under stress moisture level are: ▲ = DZ-2023, ◊ = Boohai 'S', ■ = DZ-1691, □ = DZ-320, ● = Klinto, and ○ = LD-357.

Table 6.2. Mean values of water use efficiency based on total dry matter and grain, transpiration efficiency based on total dry matter and grain yield for six durum wheat varieties and their diallel crosses grown under stress and non stress conditions.

Name	Moisture level	WUE_{TDM} ($g^{-1}pt$)	WUE_G($g^{-1}pt$)	T_G($g^{-1}pt$)	T_{TDM}($g^{-1}pt$)
DZ-2023	C	3·14	1·46	5·10	10·90
	S	2·33	0·96	2·02	4·73
Boohai 'S'	C	3·17	1·48	5·65	12·20
	S	1·79	0·83	2·14	4·62
Klinto	C	2·91	1·49	4·35	8·46
	S	1·65	0·22	0·40	3·02
DZ-1691	C	3·70	1·43	5·16	13·30
	S	1·26	0·32	0·75	3·02
LD-357	C	1·05	0·59	2·27	4·04
	S	1·11	0·12	0·29	2·60
DZ-320	C	2·20	0·75	2·35	6·89
	S	1·14	0·26	0·44	1·81
1*2	C	3·44	1·46	5·44	12·82
	S	2·19	0·89	2·02	4·96
1*3	C	3·05	1·48	4·87	10·03
	S	1·58	0·71	1·45	3·24
1*4	C	3·80	2·07	6·00	11·03
	S	1·85	0·68	1·37	3·74
1*5	C	3·18	1·44	3·73	8·24
	S	2·05	0·74	1·60	4·42
1*6	C	3·41	1·60	4·38	9·32
	S	1·61	0·75	1·52	3·25
2*3	C	2·59	1·31	4·17	8·23
	S	1·61	0·60	1·55	4·18
2*4	C	3·25	1·89	5·22	8·98
	S	1·65	0·73	1·96	4·46
2*5	C	2·27	0·83	2·13	5·84
	S	1·94	0·60	1·90	6·21
2*6	C	3·26	1·50	4·86	10·58
	S	1·35	0·55	1·71	4·18
3*4	C	3·97	1·75	5·40	12·29
	S	0·79	0·25	0·80	2·53
3*5	C	2·30	1·06	3·02	6·55
	S	1·09	0·20	0·63	3·39
3*6	C	1·87	0·99	2·57	4·87
	S	1·60	0·42	0·79	3·00
4*5	C	2·20	0·49	1·44	6·49
	S	1·49	0·32	0·91	4·52
4*6	C	2·01	1·14	3·00	5·35
	S	1·49	0·37	0·90	3·66
5*6	C	1·96	1·03	3·04	5·76
	S	1·47	0·41	1·11	4·67
Mean		2·19	0·91	5·47	6·25
S·E·D(D·F·=82)		0·176	0·063	2·679	0·778

C = control, S = stress, WUE_{TDM} = water use efficiency for total dry mass, WUE_G = water use efficiency for grain yield, T_{TDM} = water used for total dry matter, T_G = water used for grain production.

Table 6.3. Pooled correlation coefficient matrix for water use and transpiration efficiency measures for durum wheat grown in the glasshouse.

	TDM	HI	S	ETba	ETpa	ETba: Etpa	WUE_{TDM}	WUE_G	T_{TDM}	T_G
Grain Yield	0·94**	0·74**	-0·78**	0·08	0·72**	-0·49*	0·86**	0·96**	0·81*	0·93**
TDM		0·52*	-0·49*	0·20	0·67**	-0·37	0·92**	0·89**	0·87*	0·89**
HI			-0·64**	-0·24	0·64**	-0·68**	0·47*	0·75**	0·43*	0·68**
S				0·59**	-0·45*	0·64**	-0·65**	-0·74**	-0·45*	-0·72**
Etba					0·08	0·64**	-0·05	-0·09	-0·06	-0·08
Etpa						-0·62**	0·42*	0·56**	0·45*	0·58**
ETba:Etpa							-0·39	-0·51*	-0·39	-0·50*
WUE_{TDM}								0·92**	0·94**	0·91**
WUE_G									0·85**	0·96**
T_{TDM}										0·94**

TDM = Total dry matter, HI = Harvest index, S = Drought susceptibility index, ETba = Pre-anthesis water use , ETpa = Post-anthesis water use, WUE_{TDM} = water use efficiency for total dry mass, WUE_G = water use efficiency for grain yield, T_{TDM} = water used for total dry matter, T_G = water used for grain production
P=0.05 and 0.01

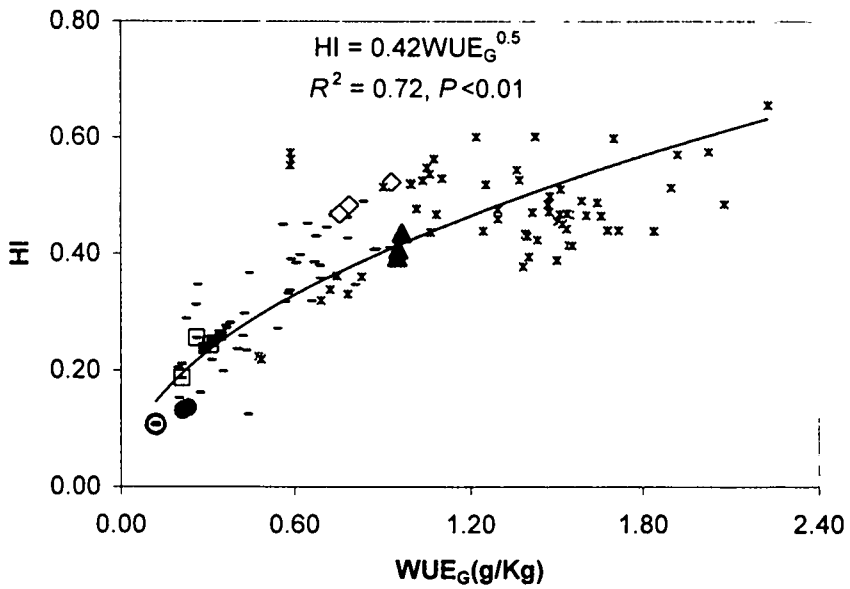

Fig. 6·3. Relationships between WUE_G and HI for durum wheat grown under stress and non-stress moisture levels in glasshouse. * and – refers to control and stress moisture treatments, respectively. Parental lines under stress moisture level are: ▲ = DZ-2023, ◊ = Boohai 'S', ■ = DZ-1691, □ = DZ-320, ● = Klinto, and ○ = LD-357.

Transpiration efficiency (T).

The amount of water exclusively used by plants for TDM production (T_{TDM}) and grain production (T_G) was significantly different between the two moisture levels and among cultivars. The interaction of moisture level with cultivar was also significant. T_{TDM} pooled over cultivars was 3.82 and 8.67 for stress and control moisture levels, respectively, which was significantly (P=0·05) different. At the stress moisture level, the highest amount of TDM per kilogram of water transpired was produced by the F_1 from the cross between the susceptible cultivar LD-357 and the tolerant cultivar Boohai 'S'. The least amount was produced by DZ-320. The amount of TDM produced at the optimum moisture level was very high for DZ-1691. T_{TDM} was positively and significantly correlated with TDM and HI. Its relation with ETba:ETpa, however, was negative but non significant. T_G ranged between 1.44 for F_1 DZ-1691 X LD-357 to 6.0 for F_1 DZ-2023 X DZ-1691 at the control moisture level. The amount under stress conditions ranged between 0.286 for LD357 to 2.135 for Boohai 'S'. The average T_G pooled over cultivars was 4.01 and 1.25 for the control and stress moisture levels, respectively. The difference was significant (P=0·05). In terms of grain yield, transpiration efficiency of resistant parents DZ-2023 and Boohai 'S' reduced by 60.4% and 62.2 %, respectively, from the optimum to stress moisture levels. In the susceptible parents, Klinto and LD-357, the reductions were 90.7% and 87.4%, respectively. Similarly, reductions were more severe on stress-sensitive genotypes than they were for the tolerant types (Table 6.2.). T_G was strongly and positively correlated with grain yield, TDM, HI, WUE_{TDM} and WUE_G. However, it was negatively correlated with ETba:ETpa and S (Table 6.3.).

Water Potential (ψ_p)

Total leaf water potential (ψ_p) measured at various growth stages was significantly affected by cultivar and by moisture level. The interaction of cultivar by moisture level was also significant. Further analysis of the stress and control treatments separately revealed that ψ_p was not statistically significantly different for genotypes at the control moisture level (Table 6.4.). The average ψ_p for control treatments was -19.4, -20.74 and -25.15 bars at

heading, anthesis and ripening growth stages, respectively. There was, however, significantly high variability among genotypes under stress conditions (Table 6.4.). The average ψ_p was -20.8, -24.42 and -30.8 bars at heading, anthesis and ripening, respectively. Stress-tolerant parents' and progenies ψ_p declined relatively slowly as compared to the stress-susceptible ones (Table 6.4.). The tolerant parents, DZ-2023 and Boohai 'S', had relatively higher ψ_p, particularly at anthesis and ripening growth stages. The decline in ψ_p was also relatively slower from heading to anthesis compared to the other parents. The decline from anthesis to ripening growth stage, however, was slow only for DZ-2023. The decline in ψ_p from anthesis to ripening for the tolerant parent Boohai 'S' and the susceptible parent Klinto was rapid and comparable (Table 6.4.). The susceptible parent LD-357 had higher ψ_p at heading. However its ψ_p declined dramatically, reaching -53 bars at ripening stage. Average ψ_p pooled over cultivars showed that ψ_p declines with age, however, the decline was much more rapid under stress conditions than it was under normal conditions (Table 6.4.).

Table 6.4. Mean of total leaf water potential (Ψ, –bars) measured at various growth stages for six durum wheat varieties and their diallel crosses grown under control and stress moisture levels in glasshouse.

	Control			Stress		
Name	Heading	Anthesis	Ripe	Heading	Anthesis	Ripe
DZ-2023	20·00	21·33	25·50	21·75	22·25	25·00
Boohai 'S'	18·17	20·25	24·33	17·25	21·00	32·17
Klinto	19·33	20·67	24·50	22·83	24·83	37·00
DZ-1691	21·67	21·83	26·50	22·83	24·67	29·33
LD-357	19·50	20·83	25·00	18·50	32·83	53·00
DZ-320	18·83	22·00	24·67	20·50	24·00	27·83
1*2	18·00	20·00	26·50	19·00	20·00	29·00
1*3	18·50	20·17	25·00	23·00	26·00	30·50
1*4	19·00	20·33	24·33	22·50	25·00	26·50
1*5	18·75	21·00	25·00	22·92	28·50	31·00
1*6	20·75	21·17	26·58	20·83	22·67	25·50
2*3	20·00	19·83	26·00	16·00	20·25	30·33
2*4	18·25	20·75	25·08	21·67	23·17	27·50
2*5	19·33	21·00	24·00	19·83	20·75	26·75
2*6	20·50	21·50	24·25	20·50	22·00	27·00
3*4	21·75	21·00	26·17	16·67	21·00	33·42
3*5	18·50	20·33	25·33	28·83	33·00	35·67
3*6	19·50	20·00	24·42	18·83	25·17	28·50
4*5	19·33	20·33	24·25	23·50	26·33	28·67
4*6	18·00	21·17	24·67	19·25	25·83	30·83
5*6	19·67	20·00	26·00	19·67	23·51	31·25
Mean	19·40	20·74	25·15	20·79	24·42	30·80
S·E·D(D·F=40)	1·261	0·655	0·931	1·148	0·939	1·422

6.5. Discussion

In semi-arid tropics, water supply is a limiting factor for crop production due to the low and often erratic distribution of rainfall. Crop management practices should play a major role to alleviate the problem. Environmental and economical sustainability, however, is likely to be better achieved if breeding endeavours to develop efficient water-use varieties are successful.

The good correlation between grain yield, total dry matter and harvest index agreed with findings from field studies (Oweis *et al.* 2000) and glasshouse studies (Passioura 1977; Simane 1993). The extent to which yield production is limited, particularly in stress-sensitive genotypes, is indicated by the ratio of ETba:ETpa. These findings are comparable with the values found in previous studies (Passioura 1977; French & Scultz 1984; Simane 1993; Oweis *et al.* 2000). Significantly high negative correlation between ETba:ETpa and grain yield, as well as inverse relationships between ETba:ETpa with WUE_G and HI in the present study, suggest that grain production is largely dependent upon the availability of moisture for the accumulation and subsequent partitioning of the dry matter to harvestable grain yield and/or the genetic capacity of the genotypes to efficiently utilize the available moisture for grain production. This confirms results of other authors (Fischer & Turner 1978; Tanner & Sinclair 1983; Ludlow & Muchow 1988; Simane 1993; Oweis *et al.* 2000; Angus & van Herwaarden 2001). The probable reason for this is that availability of moisture at anthesis may prevent abortion of florets, thereby improving the size and the number of seed setting. Most importantly, it allows the plant to increase its photosynthesis, and gives the plant extra time to translocate carbohydrate reserves to the grain (Zhang *et al.* 1998; Richards *et al.* 2002). Findings in the present study show that drought susceptibility index is significantly but negatively associated with measures of WUE and T. Consistency in the rankings of genotypes based on susceptibility index and WUE_G and T_G was observed in most instances. This affirms that water use efficiency is better

expressed in terms of grain yield and/or above ground dry matter production (Tanner & Sinclair 1983; Simane 1993).

Prevalence of considerable high genetic variability both for harvest index and WUE_G, and also a strong association between WUE_G, and harvest index in the present study suggest the existence of an opportunity for improvement of grain through selection for harvest index and WUE_G (Fischer & Turner 1978; Siddique *et al.* 1990). This is because morphological and physiological traits thus far have been known to contribute to greater yield through increased biomass. At maturity, a proportion of this biomass of grain crops is the grain, and a high proportion is desirable to achieve high yields via harvest index (Richards *et al.* 2002). Though not simple, genetic manipulation of water use and water use efficiency would be possible and effective to increase and stabilize yield under moisture-limited environments (Richards *et al.* 2002). Harvest indices of susceptible cultivars Klinto and LD-357 were the best (>50) under optimum conditions. However, their harvest indices were dramatically reduced by about 74% and 80% in Klinto and LD-357, respectively, under stress conditions. Two separate determinants of HI that can be genetically manipulated to maximize harvest index to achieve high grain yield have been noted (Richards 1991). Moisture-stress-dependent determinants of harvest index depend largely on the availability of moisture, particularly during the grain filling period and also on pre-anthesis partitioning between structural and soluble carbohydrates (Richards 1991). The absence of these features, probably best explained by their relatively low utilization of water during the grain filling period, could be the reason for the very low harvest indices in the susceptible varieties Klinto and LD-357. On the other hand, tolerant varieties, such as DZ-2023 and Boohai 'S', had the best harvest indices and they might possess these features, given that they had the lowest ETba:ETpa ratios in the present study. The fact that these parents also had a good harvest index under optimum conditions would suggest that selection for their high HI even under stress conditions could be promising. Selection for yield in cereal programmes under favourable conditions has indirectly resulted in higher HI at all environments, including unfavourable ones (Sayre *et al.* 1995; Richards *et al.* 2002). The major environmental variable affecting transpiration is air saturation deficit. Data

from pot experiments may be used as a valid determination of transpiration efficiency (Fischer & Turner 1978; Tanner & Sinclair 1983). Since water loss via evaporation is important (Fischer & Turner 1978; Cooper *et al.* 1987), definition of WUE based on evapotranspiration and transpiration alone are essentially different.

Opportunities for increasing T and decreasing evaporation through both management and breeding are good. Altering sowing dates, thereby breeding new late flowering varieties, for instance, can improve WUE (Gomez-Macpherson & Richards 1995). Inherently, stress-tolerant varieties can have high WUE, and thus high T (Simane. 1993). Findings from the present study in which tolerant parents and their progenies had better WUE and hence better T in terms of grain yield production clearly demonstrated the same.

Total leaf water potential has been used as a selection criterion under drought-prone environments (Blum 1988; Siddique *et al.* 1990). In the present study significant variability among genotypes under stress conditions and the decline in Ψp with age is in agreement with the findings of Fischer & Maurer (1978). Nevertheless, lack of consistency in the variability observed among cultivars could not justify use of total leaf Ψp as a screening tool under stress conditions (Turner 1981; Simane 1993).

6.6.Conclusions

Results in the present study revealed that yield of durum wheat may be increased by improving the relative proportion of post-anthesis water use, WUE and HI. The observed genetic variation in ETba:ETpa, WUE and HI could be employed as possible selection criteria to screen genotypes for better WUE, and hence, increased grain yield under moisture stress environments.

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CHAPTER 7

Inheritance of water use and transpiration efficiency in a diallel hybrid population of durum wheat (*Triticum turgidum* L. var. *durum*)

7.1. Abstract

Inheritance of water use and transpiration efficiency was studied in a hybrid population obtained from six parents, selected for their different responses to moisture stress. Plants were grown in 10 l pots at control and stressed moisture levels in the glasshouse. GCA and SCA effects were significant at both the moisture levels. The interactions of GCA and SCA with moisture levels were also highly significant. GCA effects were the major components of the genetic variance of the biological and economic yield of water use efficiency (WUE) and transpiration efficiency (T) measures. Further analysis of the genetic components of variation demonstrated that WUE and T were under the control of additive and dominance type of genes. Narrow sense heritability estimates for water use and transpiration efficiency based on grain yield (WUE_G) were higher at the moisture stress level. Heritability estimates of water use and transpiration efficiency based on biological yield, however, were reduced from the higher to the lower moisture levels. Measures of water use and transpiration efficiency showed significantly high and positive genotypic and phenotypic correlations among them as well as with grain yield and harvest index. In most cases, genotypic correlations were higher than phenotypic correlations. Findings in this study suggest that selection for high water use and transpiration efficiency in terms of grain yield should be undertaken under both stress and optimal conditions. Selection in early segregating generations may lead to effective identification of desirable genotypes among the recombinant types.

***Key words**: moisture stress, durum wheat, water use efficiency*

7.2. Introduction

Improvement of drought resistance is a major objective for all breeding programmes in the semi-arid tropics. This is particularly true for durum wheat because of its importance as a food crop (Hakim *et al.*, 1996). An important aspect of improvement of drought resistance is the improvement of water use and transpiration efficiency. Water use efficiency can be defined in several ways (Tanner & Sinclair, 1983). The basic definition, however, is the ratio of the total biomass produced per unit of water consumed.

There has been increased research demonstrating the existence of genetic variability for water use and transpiration efficiency in different crop species, including both tetra- and hexaploid wheats (Fischer & Turner, 1978; Siddiquie *et al.* 1990; Richards, 1991). Variation in water use efficiency has been shown to be associated with differences in the ability of plants to discriminate against ^{13}C compared to ^{12}C during CO_2 diffusion and fixation (Farquahar & Richards, 1984; Hubick & Farquahar, 1989; Acevedo, 1993). Carbon isotope discrimination is correlated with internal leaf CO_2 pressure, which is regulated by both stomatal aperture and ribulose bisphosphate carboxylase (RuBisco) activity. Because water use efficiency is also correlated with stomatal aperture and RuBisco activity, water use efficiency and carbon isotope discrimination are empirically negatively correlated with each other (Farquahar *et al.*, 1982; Condon *et al.*, 1987). Various other morphophysiological bases for between and within species variation in water use and transpiration efficiency have been discussed in detail (Cooper *et al.*, 1987; Acevedo & Ceccareli, 1989; Turner, 1997).

Utilisation of morphophysiological traits in breeding for water use efficiency in drought prone environments according to Hakim *et al.* (1996) is related to (i) ease of measurement (ii) the existence of genetic variability, and (iii) high values of heritability. Possibility for manipulation of water use and

transpiration efficiency via agronomic practices and/or breeding procedures is quite high (Richards *et al.*, 2002). Crop improvement programmes that emphasise the improvement of water use and transpiration efficiency have been limited. Lack of simple, rapid, and reliable screening criteria and measurement techniques for crop plants has greatly impeded progress in this critical area of crop improvement (Hall *et al.*, 1990). Lack of understanding of the genetics of the traits involved is also a crucial aspect of the problem (Acevedo, 1993).

The purpose of this study was to investigate the genetics of various components of water use and transpiration efficiency in a durum wheat hybrid population obtained from a half diallel cross of parents, differing in their responses to moisture stress.

7.3. Materials and Methods

A six by six diallel cross without reciprocals was made in January to June 2001 at Debrezeit Agricultural Research Center, Ethiopia. Two drought tolerant varieties, Boohai'S' and DZ-2023, two moderately tolerant varieties, DZ-1691 and DZ-320, and two susceptible varieties, Klinto and variety, LD-357, were selected based on their yield and yield component response to moisture stress from 26 randomly selected durum wheat genotypes from Ethiopia. These were used as parents in the crossing programme.

In the subsequent season, two sets of experiments consisting of parents and their 15 F_1's were grown in the glasshouse as a randomised complete block design (RCBD) with three replications in July to December 2001 at the University Free State. They were grown at an average of 25/15^{0}C day and night temperatures. Entries were planted in 10 l capacity plastic pots. Pots were filled with 2 kg of gravel (to prevent soil leakage), and 7 kg of steam sterilised and air dried sandy loam soil with particle composition of 80%, 2% and 18% of sand, silt and clay, respectively. The air-dry moisture content of the soil was determined as 13.3% (field capacity) and 4.0% (permanent wilting point) gravimeter. Each genotype was sown

into six pots. Twelve seeds at equal spacing were planted at about equal depth. Seedlings were thinned to nine per pot at two leaf growth stages. Pots were maintained to 70-100% available soil moisture for a month (up to about four-leaf growth stage). Half of the experimental units were then left without water until severe wilting was observed. Application of stress continued, thereby maintaining the moisture level at 25-35% available soil moisture until maturity. The amount of water evaporated was monitored by daily weighing unplanted pots placed between planted pots in each block both for stress and control treatments (six in each block). The amount of water transpired was determined by subtracting the weight of bare pots from the weight measurements of planted pots. The amount of water equivalent to the loss in weight was replenished in pots to bring to the predetermined level of moisture whenever the weight of pots fell to the lower limit established for the treatments. Equal exposure of the growing conditions of the glasshouse was ensured by changing the positions of pots every week. At maturity, three plants per pot were harvested and left to air dry for 10 days. Grain and biomass yield were determined as average of the three plants. Harvest index was calculated as the ratio of grain yield to biomass yield. Cumulative water use (ET) is obtained from summation of water applied over the entire growth period. Transpiration (T) was calculated from the difference between ET and Evaporation (E). Both ET and T were expressed as g plant^{-1}. Water use efficiency (WUE), was determined as a function of grain yield (WUE_G), and biomass or above ground dry matter (WUE_{TDM}). Transpiration efficiency (T) was also expressed on the basis of grain yield (T_G) and biomass (T_{TDM}). WUE and T were computed as follows:

WUE_G = Grain yield (g plant^{-1})/ET (kg plant^{-1})

WUE_{TDM} = TDM (biomass) (g plant^{-1})/ET (kg plant^{-1})

T_G = Grain yield (g plant^{-1})/T (kg plant^{-1})

T_{TDM} = TDM (biomass) (g plant^{-1})/T(kg plant^{-1})

Data analysis

GCA effects were analysed using the fixed effect model, method II of Griffing (1956). GCA and SCA by moisture interactions were analysed using SAS

software according to Zhang & Khang (1997). The validity of basic assumptions for diallel (Jinks-Hayman) type of analysis was tested according to Hayman (1954) and Singh & Chaudhary (1977). Genetic parameters were estimated and analysed based on Hayman (1954) and Mather & Jinks (1971). The components' genetic variance and genotypic and phenotypic correlations were computed according to Singh & Chaudhary (1977) and Falconer (1989). Significance of correlation coefficients was tested according to Fischer & Yates (1963).

7.4. Results and discussion

Mean squares for GCA and SCA for ETba:ETpa, total dry matter, harvest index and the various measures of water use and transpiration efficiency were significant (Table 7.1). Mean squares for the interaction of GCA by moisture levels and SCA by moisture levels were also highly significant (Table 7.1). The GCA to SCA ratios of WUE_G, T_G and HI were higher under stressed conditions than at the control moisture level. The ratios for TDM and water use and transpiration efficiency measures based on TDM were higher under the control moisture level than under stress. This indicated that non-additive genetic effects were more important under stress conditions for dry matter production and efficiency components based on dry matter production. SCA effects were higher for HI and ETba:ETpa ratio under the control level. In most other cases, however, the additive components of the genetic variation were of paramount importance. In all cases, the ratio of GCA to SCA was positive. This suggests that the GCA or additive effects played the major role in the observed variation. Preponderance of GCA effects as an important component of genetic variation in the inheritance of both vegetative and economic yield measures of WUE in barley has been reported (Gorny, 1999). Moisture treatments caused considerable changes in the sign and magnitude of GCA effects, particularly for water use and transpiration efficiency measures (Table 7.2). There was, however, a general agreement between the sign of GCA effects of parental lines and their

individual means, suggesting the performance of their hybrids may be predicted using the mean parental performance. Drought tolerant parents, DZ-2023 and Boohai'S', were generally found to be the best combiners, and their positive contributions were particularly apparent under stress. On the other hand, the susceptible parents, LD-357 and Klinto, were the poorest combiners as evidenced by their negative or negligible GCA effects for water use and transpiration efficiency measures under stress situations. GCA effects of ETba:ETpa ratio for the resistant parents were the least, whereas those of the susceptible parents were the highest under stress. ETba:ETpa was reported to be negatively associated with total dry matter, HI and WUE_G (Simane 1993; Oweis *et al.*, 2000).

Table 7.1. Mean squares for GCA, SCA and ratios of GCA:SCA for ETba:ETpa, WUE_{TDM}, WUE_G, TDM, HI, T_{TDM} and T_G of durum wheat grown under stress (S) and optimal (C) moisture levels in the glasshouse.

Source	ML	ETba:ETpa	WUE_{TDM}	WUE_G	TDM	HI	T_{TDM}	T_G
GCA	C	0.012*	1.568*	0.432*	3.722*	0.002*	21.909*	5.336*
	S	0.557*	0.372*	0.231*	0.326*	0.040*	2.213*	1.224*
SCA	C	0.055*	0.238*	0.086*	1.273*	0.009*	3.169*	0.711*
	S	0.155*	0.068*	0.007*	0.199*	0.003*	0.749*	0.062*
GCA*ML		0.809*	1.684*	0.371*	4.784*	0.0572*	28.809*	4.633*
SCA*ML		0.479*	0.642*	0.128*	2.373*	0.01189*	7.800*	1.236*
GCA:SCA	C	0.218:1	6.588:1	5.023:1	2.924:1	0.222:1	6.914:1	7.505:1
	S	3.594:1	5.471:1	33.00:1	1.638:1	13.333:1	2.955:1	19.742:1
Error	C	0.001	0.024	0.003	0.065	0.000	0.471	0.067
	S	0.001	0.008	0.001	0.014	0.000	0.156	0.014
CV%	C	7.829	9.518	7.337	7.528	7.369	13.710	11.229
	S	5.186	9.819	10.159	9.096	11.108	17.794	16.621

*Significant at P= 0.05

ETba = Evapotranspiration before anthesis, ETba = Evapotranspiration after anthesis, WUE_{TDM} = Water use efficiency based on TDM, WUE_G = Water use efficiency based on grain yield, TDM= Total above ground biomass, HI = harvest index, T_{TDM}= Transpiration efficiency based on TDM, T_G= Transpiration efficiency based on grain yield

Further analysis of the genetic components of variation for water use and transpiration efficiency measures into additive and non-additive components revealed that the additive (D) and uncorrected dominance variance (H_1) and corrected dominance variance (H_2) were statistically significantly different from zero (Table 7.3). Since D, H_1 and H_2 were different

from zero, it can be concluded that water use and transpiration efficiency traits, defined in terms of total dry matter and grain yield, are all under the control of additive and dominance gene effects. The existence of additive and dominance type of gene effects in this study is in agreement with other findings (Ehdaie *et al.*, 1993; Gorny, 1999; Malik *et al.*, 1999). Dominance-recessive (KD/KR) ratio in all cases (Table 7.3) was close to unity.

Table 7.2. GCA effects of ETba:ETpa, WUE_{TDM}, WUE_G, TDM, HI, T_{TDM}, and T_G of durum wheat grown under stress (S)and control (C) moisture levels in the glasshouse

Parent	ML	ETba:ETpa	WUE_{TDM}	WUE_G	TDM	HI	T_{TDM}	T_G
DZ-2023	C	0.0112	0.0327	0.2374	0.6025	0.0063	1.5655	0.8203
	S	-0.2551	0.3652	0.2574	0.3135	0.0829	0.2618	0.4056
Boohai'S'	C	0.0105	0.1979	0.1084	0.0678	0.0031	1.2657	0.633
	S	-0.2516	0.1643	0.1713	0.1589	0.0874	0.7852	0.5827
Klinto	C	0.0049	0.0021	0.0608	0.335	0.025	-0.2306	0.0853
	S	0.3547	-1296	-0.1273	-0.0306	-0.0452	-0.5717	-0.3419
DZ-1691	C	0.0101	0.3815	0.1397	0.5569	-0.0163	1.2533	0.4165
	S	-0.0983	-1514	-0.0827	-0.2253	-0.0154	-0.2529	-0.1619
LD_357	C	0.0381	-0.6964	-0.3818	-1.1785	-0.0141	-2.4703	-1.2687
	S	0.2874	-0.0964	-0.1413	-0.15	-0.0893	0.2648	-0.2528
DZ-320	C	-0.0749	-0.3322	-0.1645	-0.3838	-0.0041	-1.3836	-0.6864
	S	-0.0371	-0.1521	-0.0774	-0.0683	-0.0204	-0.4872	-0.2317
SE±	C	0.0155	0.0446	0.0275	0.1278	0.0099	0.3433	0.1299
	S	0.0156	0.0768	0.0152	0.0596	0.0102	0.1978	0.0599
$LSD_{0.05}$	C	0.0302	0.0867	0.0533	0.2482	0.0193	0.6670	0.2523
	S	0.0302	0.1492	0.0296	0.1158	0.0197	0.3843	0.1165

ETba = Evapotranspiration before anthesis, ETba = Evapotranspiration after anthesis, WUE_{TDM} = Water use efficiency based on TDM, WUE_G = Water use efficiency based on grain yield, TDM= Total above ground biomass, HI = harvest index, T_{TDM}= Transpiration efficiency based on TDM, T_G= Transpiration efficiency based on grain yield.

Table 7.3. Genetic parameters and some ratios for water use and transpiration efficiency measures for durum wheat grown under different moisture levels

	WUE_G		WUE_{TDM}		T_G		T_{TDM}	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
D	0.169**±0.012	0.122**±0.001	0.862**±0.084	0.206**±0.028	1.748**±0.386	0.699**±0.019	12.455**±1.979	1.091**±0.224
F	0.066*±0.028	0.011*±0.003	0.211±0.205	0.223*±0.067	-0.686±0.943	0.146*±0.047	4.838±4.836	0.380±0.547
H1	0.248**±0.029	0.023**±0.003	0.887**±0.213	0.526**±0.07	3.316*±0.98	0.177**±0.049	16.713*±5.025	1.934*±0.569
H2	0.210**±0.026	0.021**±0.003	0.683*±0.19	0.361**±0.062	2.910*±0.876	0.146*±0.044	15.451*±4.489	1.764*±0.508
h^2	0.187**±0.018	0.026**±0.002	0.003±0.128	0.238**±0.042	1.922*±0.589	0.316**±0.029	9.497*±3.021	1.378**±0.342
E	0.003±0.004	0.001±0.001	0.023±0.032	0.008±0.01	0.067±0.146	0.014±0.007	0.461±0.748	0.027±0.085
H^2	0.56±0.008	0.90±0.001	0.69±0.064	0.43±0.017	0.64±0.286	0.85±0.016	0.51±0.3	0.43±0.135
H2/4*H1	0.212	0.232	0.193	0.172	0.219	0.208	0.231	0.228
KD/KR	1.382	1.225	1.274	1.170	0.751	1.520	1.403	1.301
rYr(Wr+Vr)	-0.776	-0.894	0.086	-0.159	0.510	-0.929	0.880	-0.323
h^2/H2	0.89	1.22	0.004	0.66	0.66	2.16	0.62	0.78

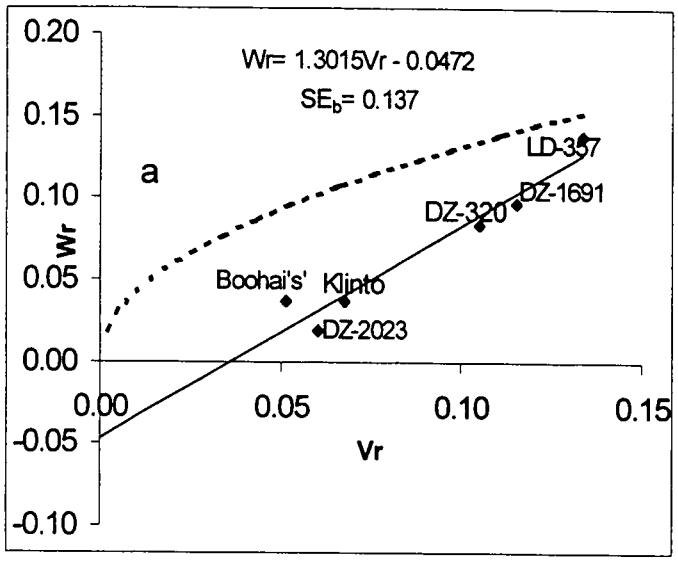
* and **, significant at p = 0.05 and 0.01, respectively.

D = Additive genetic variance, H_1 = Uncorrected dominance genetic variance, H_2 = Corrected dominance variance, h^2=Dominance effect (as algebraic sum over all loci in heterozygous phase in all crosses), E = Expected environmental variance, H^2= Narrow sense heritability, KD= Number of dominant genes and KR = Number of recessive genes in the parents, Yr = Parental size, Wr+Vr = Measure of parental order of dominance, r = correlation coefficient for Yr versus Wr+Vr

The estimates of the proportion of positive and negative genes ($H_2/4H_1$) in the parents (Table 7.3) were according to the normal gene distribution (Hayman, 1954; Mather & Jinks, 1971; Singh & Chaudhary, 1977; Yildirim *et al.*, 1995). This reaffirms the fact that H_2 was not significantly different from H_1. Estimates of the measure of dominance effects (h^2) as algebraic sum over all loci in heterozygous phases in all cross combinations was also significantly different from zero in all cases and circumstances, implying dominance is largely unidirectional, the direction of dominance being towards increasing level of measurements. Dominance effects (h^2) for WUE_{TDM} under control conditions was not significant, suggesting dominance was not unidirectional (Table 7.3). Moreover the mean of covariance of additive and dominance effects (F) were also statistically high in all cases, except for WUE_{TDM} and T_G under the control moisture level and T_{TDM} under both stress and control conditions (Table 7.3). This suggests that most of the characters were under the influence of dominance genes.

Narrow sense heritability estimates (H^2) are also shown in Table 7.3. Heritability estimates of WUE_G under stress conditions were relatively high, while they were moderate at the control level. Ranking of drought susceptibility indices have been reported to coincide with the WUE_G under stress conditions in other parts of the study and elsewhere (Simane 1993). Strong association of WUE_G and HI with grain yield under stress (Simane 1993; Oweis *et al.*, 2000), suggests WUE_G under stress is likely to have a strong additive genetic control and hence higher heritability. Thus, the presence of traits associated with drought resistance, and hence, high WUE_G under stress conditions in this material can, at least in part, be a probable reason for the high estimate of heritability under stress conditions. However, the observed reductions in heritability estimates for WUE defined in terms of total dry matter production from control to the stress level is in agreement with other findings (Gorny, 1999). Heritability estimates for T_G under stress conditions was higher than it was in control conditions. However, the value reported here is very similar to previous findings (Malik *et al.*, 1999).

The correlation coefficients (r=-0.78 and –0.89) between the parental order of dominance (Wr+Vr) were high and negative for WUE_G and T_G, respectively, under stress conditions.



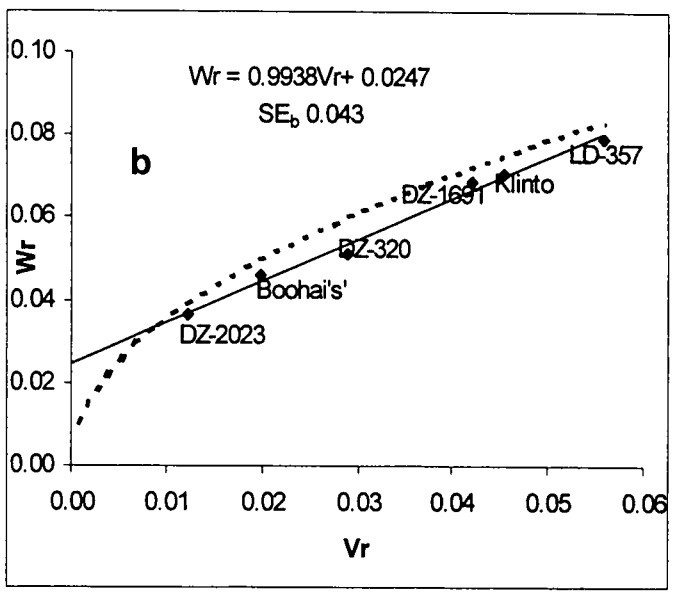
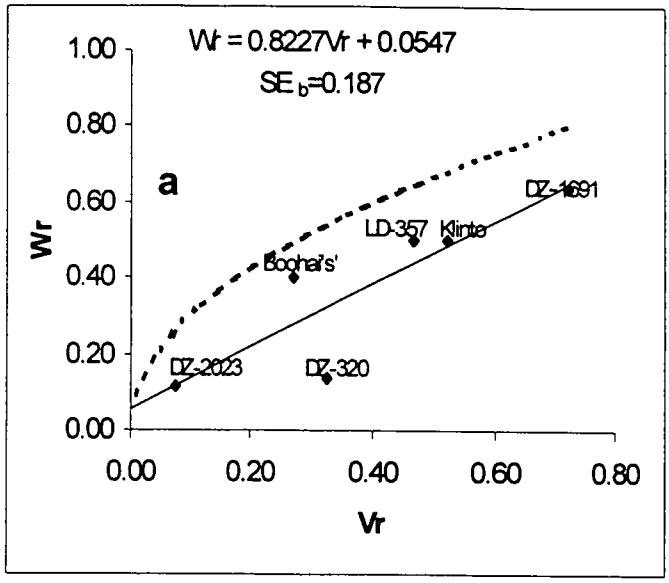


Fig. 7.1. Variance-covariance (VrWr) regression of WUE_G for durum wheat grown under (a) control and (b) moisture stress conditions

This suggests that those parents with high values of WUE_G and T_G may carry the most dominant and favourable genes for these traits (Yildirim *et al.*, 1995). On the other hand correlations were 0.5 and 0.8 for WUE_{TDM} and T_{TDM}, respectively, under stress situations, indicating the hybrids had lower WUE and T for total dry matter production under stress conditions. In all other cases, however, (r) was negligible, probably because equal proportions of the dominant genes are positive and negative with respect to the traits. In most cases, at least a pair of genes with dominance effects was found to control the characters. At least two pairs of genes with dominance effects were found to regulate T_G. The observed over dominance in WUE_G at control moisture level could be accountable for the underestimation of gene number with dominance effects (Hayman, 1954; Mather & Jinks, 1971).

Additional information about the mode of inheritance of the different water use and transpiration efficiency characteristics was inferred from WrVr graphs. The inheritance of WUE_G was under the control of over-dominance and partial dominance, at control and stress moisture levels, respectively (Fig 7.1). The inheritance of WUE_{TDM} was, however, due to partial dominance and over-dominance, under control and stress moisture levels, respectively (Fig 7.2). The different transpiration efficiency measures were all under partial dominance under both moisture regimes (Fig 7.3 and 7.4). Partial dominance of genes in the inheritance of WUE_G and WUE_{TDM} has been reported (Gorny, 1999). Along the regression line in the WrVr graph (Fig 7.1), the tolerant parents, DZ-2023 and Boohai'S', were found to reside close enough to the origin under both stress and control conditions. It can be concluded that these parents could carry the most dominant genes for WUE_G. Under stress conditions, moderately tolerant parents DZ-1691 and DZ-320, were found to lie in the middle along the regression line (Fig 7.1b). This is probably because they may have an equal proportion of dominant and recessive genes controlling the inheritance of WUE_G. Furthest from the origin in the same axis were the two susceptible parents, Klinto and LD-357, which could carry the most recessive genes for WUE_G. The location of Klinto was changed from its position under control conditions (Fig 7.1a), possibly because of its sensitivity to the stress conditions. Parents, DZ-2023 and DZ-

320 appeared to contain the most dominant genes for WUE $_{TDM}$ at control moisture level. Klinto and DZ-1691 were located distantly from the origin, and hence, they are likely to possess an excess of recessive genes for WUE $_{TDM}$. At the stress level, Boohai'S' was the closest to the origin, while LD-357 was the most distant from the origin. The relative position of DZ-2023 and DZ-1691 remains unchanged even under the stress level (Fig 7.2). Inside the parabola limit of the T_G WrVr graph (Fig 7.3), the most susceptible parents, LD-357 and Klinto were very close to the origin at control moisture level while Boohai'S' and DZ-1691 were the most distant. The situation, however, was changed when stress was applied. DZ-2023 and Boohai'S' were observed to lie nearest to the origin, and hence, bore the most dominant genes. On contrary, the two susceptible parents appeared to posses the least dominant genes. Variance-covariance regression graphs for WUE_G and T_G were very similar, indicating the genetic capacity to yield a unit amount of grain per unit of water either consumed (evapotranspiration) or transpired (evapotranspiration-evaporation) are very similar. In terms of dry matter production per unit of water transpired, LD-357 and DZ-2023 were likely to have the most dominant genes, whereas Boohai'S' and DZ-1691 were found to contain the most recessive genes in the absence of stress. In the presence of stress, however, Boohai'S' followed by DZ-320 were found to possess the most dominant genes. LD-357 was located furthest from the origin probably because of its highest number of recessive genes. Klinto on the other hand maintained intermediate positions under both stress and control conditions (Fig 7.4). The observed differences between WUE $_{TDM}$ and T_{TDM} graphs for some of the parental lines can be attributed to the probable genetic difference in terms of growth rate and vigour which lead to inherent variability to minimise soil evaporation and maximise transpiration efficiency (Richards *et al.*, 2002).



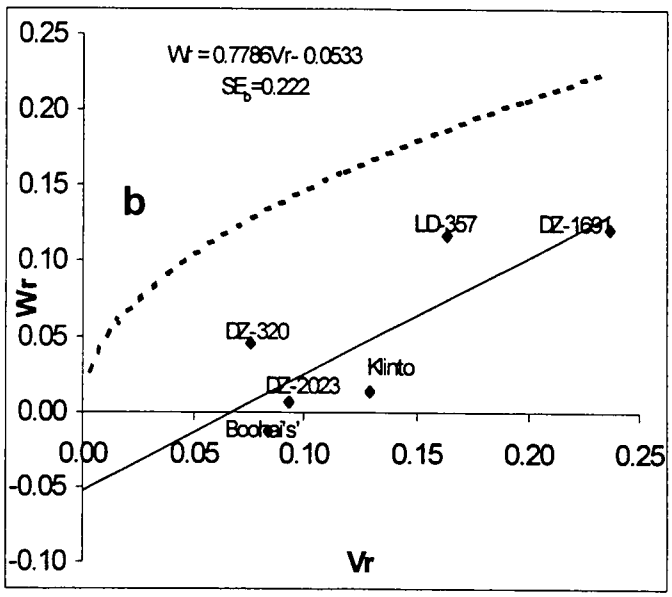
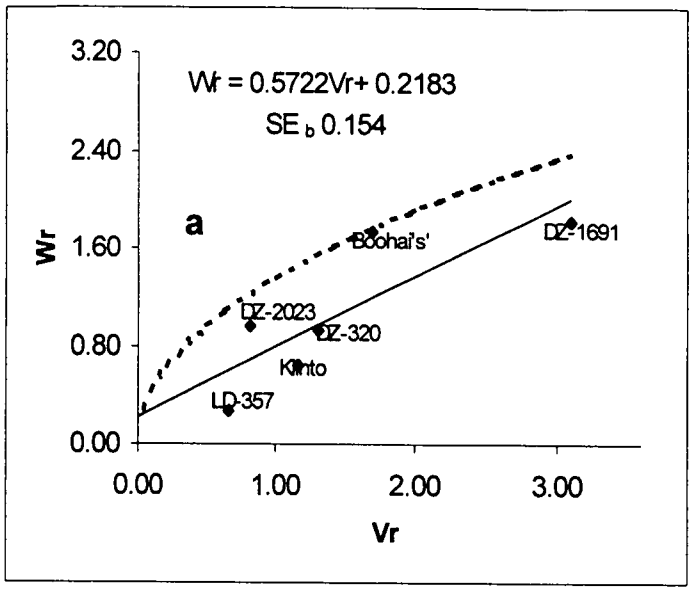


Fig. 7·2. Variance-covariance (VrWr) regression of WUE_{TDM} for durum wheat grown under (a) control and (b) moisture stress conditions

Genotypic and phenotypic correlations, pooled over cultivars and moisture levels, are presented in Table 7.4. Negative genotypic and phenotypic associations between ETba:ETpa ratio with TDM, HI, grain yield and all water use and transpiration efficiency measures were as expected. Reports are available indicating performance of genotypes under water limited environments are to a large extent limited by the genetic capacity of genotypes to utilize and apportion available soil moisture between vegetative growth and grain filling periods (Simane 1993; Oweis *et al.*, 2000). The genotypic correlations between grain yield, HI, TDM and water use and transpiration components were all positive and significant. The phenotypic correlation coefficients were also positive and significant, except for the association between T_{TDM} and HI, which was still positive but insignificant. The genotypic correlation coefficients were higher than the phenotypic correlation coefficients. Grain yield genotypic and phenotypic correlations with WUE_G (r_g=0.94 and r_p=0.92) were high and very close, implying the observed performance of the genotypes truly reflects the genetic potential. Similarly, the phenotypic and genotypic associations between grain yield and the amount of water transpired to produce a gram of grain was quite high. Thus, defining water use efficiency in terms of economic yield and grain has indeed practical significance. Generally, all measures of water use and transpiration efficiency showed significantly high and positive genotypic and phenotypic correlation with grain yield. It can therefore be concluded that some of the genes appear to pleiotropically influence all of these traits. Selection for higher water use efficiency and transpiration efficiency should also improve dry matter production with high HI, and hence high grain yield.



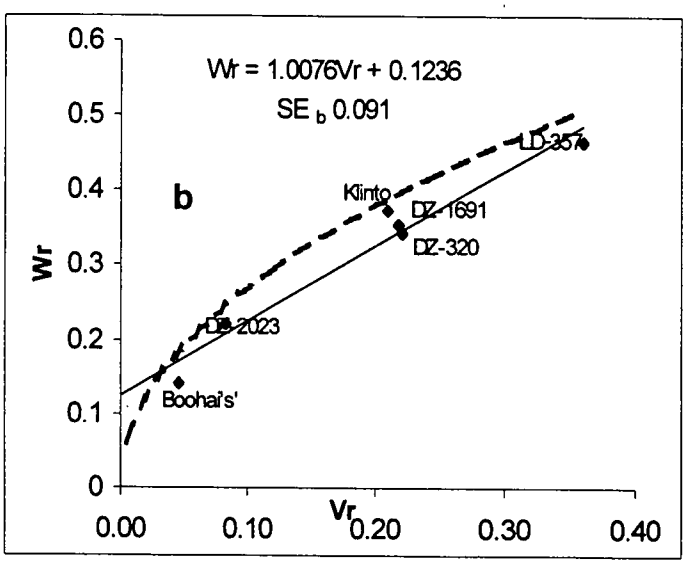
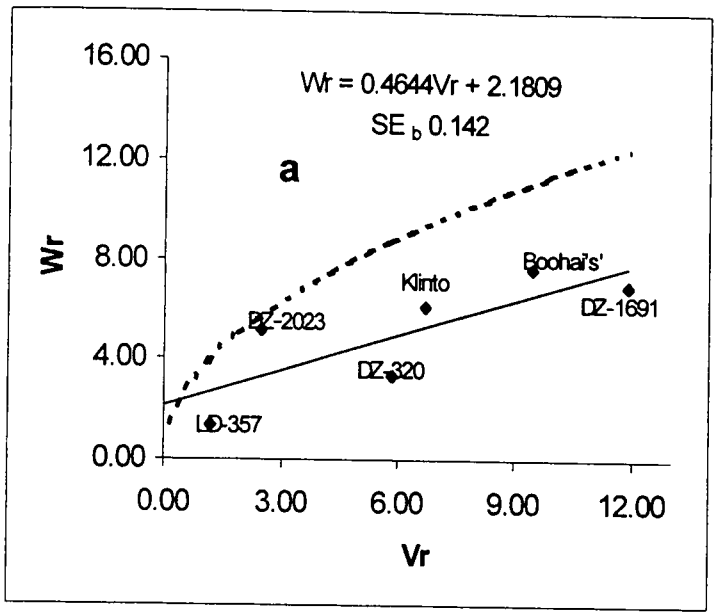


Fig. 7.3. Variance-covariance (VrWr) regression of T_G for durum wheat grown under a) control and b) moisture stress conditions



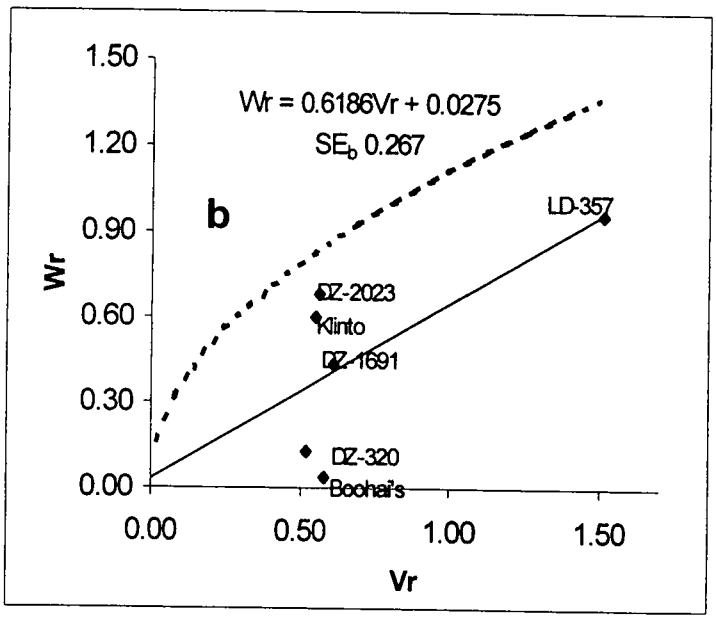


Fig. 7.4. Variance covariance (VrWr) regression of T_{TDM} for durum wheat grown under (a) control and (b) moisture stress conditions

Table 7.4. Pooled genotypic (r_g, above the diagonal) and phenotypic (r_p, below the diagonal) correlation for water use and transpiration efficiency and related characters for durum wheat grown under stress moisture conditions.

Trait	Grain yield	ETba:ETpa	TDM	HI	WUE_{TDM}	T_{TDM}	T_G	WUE_G
Grain yield		-0.28	0.88**	0.82**	0.80**	0.65**	0.87**	0.94**
ETba:ETpa	-0.27		-0.21	-0.42*	-0.44*	-0.45*	-0.45*	-0.43*
TDM	0.81**	-0.20		0.48*	0.86**	0.72**	0.77**	0.81**
HI	0.77**	-0.38*	0.30		0.59**	0.50*	0.81**	0.86**
WUE_{TDM}	0.72**	-0.37	0.85**	0.39*		0.89**	0.89**	0.91**
T_{TDM}	0.54**	-0.33	0.68**	0.27	0.87**		0.90**	0.78**
T_G	0.82**	-0.39*	0.69**	0.70**	0.83**	0.85**		0.95**
WUE_G	0.92**	-0.39*	0.74**	0.78**	0.85**	0.70**	0.92**	

* and ** significant at p=0.05 and 0.01, respectively.

ETba = Evapotranspiration before anthesis, ETba = Evapotranspiration after anthesis, TDM= Total above ground biomass, WUE_{TDM} = Water use efficiency based on TDM, WUE_G = Water use efficiency based on grain yield, T_{TDM}= Transpiration efficiency based on TDM, T_G= Transpiration efficiency based on grain yield

7.5.Conclusions

It may be inferred that water use and transpiration efficiency traits studied in this experiment are largely under the control of additive-dominance type of genes. Genotypic correlations among the various parameters were generally significant and higher than the phenotypic correlation coefficients. Moreover estimates of narrow sense heritability were found to range from moderate to high levels for most of the characters. This suggests that success in selection and fixing desirable recombinant breeding lines for improved water use and transpiration efficiency, and hence high HI and grain yield may be carried out early in the segregating generations (Weber, 1983). The high proportion of additive genetic variance observed in this study also suggests that selection may be effective in predicting the properties of recombinant lines that can be derived from these crosses. Nevertheless, it is worthy to bear in mind that the materials studied

may not represent the whole species. Therefore generalisation about the whole species is likely to be questionable. Further studies should therefore be geared towards similar genetic studies, possibly in multi-environments on hybrid populations originating from breeding programmes. It would also be important to establish whether the differences in water use and transpiration efficiency attributes observed in this study translate to differences in water and transpiration efficiency in water limited environments in the field.

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CHAPTER 8

Differences in the level of D-glucose and sucrose among durum wheat (*Triticum turgidum* L. var. *durum*) genotypes differing in their responses to moisture deficit stress

8.1. Abstract

The effect of moisture stress on the content of water soluble carbohydrate (sucrose and D-glucose) was investigated in durum wheat (*Triticum turgidum* L. var. *durum*) above ground organs to assess and characterise the responses of genotypes with differing responses to drought stress. Six durum wheat genotypes varying in their responses to moisture deficit stress were grown at optimum and stress moisture levels in a glasshouse. Total D-glucose and sucrose level of leaves, stems and spikes were determined and analysed over different growth stages. In all organs examined, drought tolerant genotypes accumulated more glucose and sucrose, particularly at an early age. Stress caused an overall increase in the level of these carbohydrates, but the levels were highest in the stems compared to other organs. Compared to the optimum moisture level, sucrose levels of leaves at the stress level was reduced at early growth stages, but increased in later growth stages. Drought tolerant genotypes had higher levels of sucrose than the susceptible types throughout all growth stages. D-glucose accumulation in all organs of the tolerant genotypes was highest at early growth stages while it was highest in later growth stages of the susceptible types. Moderately tolerant genotypes had accumulation patterns more or less in between the tolerant and susceptible groups. The accumulation of sucrose in the spikes of stress tolerant genotypes increased after anthesis as the level of sucrose in the stems declined. Level of drought (drought susceptibility index, 'S') was strongly related to the level of carbohydrates in the

various plant organs. The correlation between 'S' and carbohydrate levels of leaves and stems, however, was very strong and of special value as a physiological marker to the screening of drought tolerant genotypes.

***Key word**: Carbohydrate, drought susceptibility index, leaf, spike, stem*

8.2. Introduction

Moisture deficit stress occurring during critical growth stages has been noted to severely affect crop productivity. Drought has been documented to negatively affect photosynthesis, dark respiration, carbon allocation and partitioning as well as carbohydrate accumulation (Huang and Jiang, 2002). Carbohydrates serve as important structural components and energy reserves of cells and are often associated with osmotic adjustment during stress in many species (Zhang and Archbold, 1993; Rekika *et al.*, 1998). Thus, carbohydrate metabolism plays an important role in plant tolerance to various environmental stresses, such as drought and heat stress (Lafta and Lorenzen, 1995; Savin and Nicolas, 1996). Drought stress reduces the rate of assimilation export from leaves (Deng *et al.*, 1989) and inhibits the conversion of glucose into starch (Wang *et al.*, 1996). Under drought stress, the allocation of carbon to leaves and rapidly expanding tissues is often reduced, while the proportion of carbon allocated to stems and roots is increased (McCoy *et al.*, 1990). The effect of drought on the accumulation of different fractions of non-structural carbohydrates varies with plant species and organs. Volaire *et al.* (1998) reported that fructans accumulated in drought sensitive cocksfoot during drought stress; as drought stress progressed, total soluble carbohydrate increased and then stabilised. Sucrose concentration has been reported to decrease in mature leaves and stems of apple, yet increased in young leaves and roots as leaf water potential declined (Wang *et al.*, 1995). Sucrose concentration has been found to increase

in root nodules of stressed soybean (Muller *et al.*, 1996). Tan *et al.* (1992) also reported that soluble carbohydrate content, mainly glucose and fructose, increased in *Picea mariana* with vigorous growth under drought in the field. Among the water-soluble carbohydrates, sucrose and fructans (glucose and fructose) have a potential role in adaptation to various environmental stresses (Mckersie and Leshema, 1994). Sucrose can act in water replacement to maintain phospholipids in the liquid crystalline phase to prevent structural changes in soluble proteins. Glucose participates in cross linking with protein by a complex glycosylation reaction between amino and carbonyl groups known as the Millard reaction (Koster and Leopold, 1988). As respiratory substrates, monosaccharides promote respiration and mitochondrial electron transport, which would seem to oppose the onset of quiescence and favour metabolism, energy production and formation of oxygen radicals (Leprince *et al.*, 1993).

Understanding of physiological responses under moisture stress conditions may help devise selection strategies and tools for the development of drought tolerant genotypes. Thus, the objectives of this study were to assess the carbohydrate accumulation pattern of genotypes differing in their responses to drought stress and examine the association of carbohydrate levels of the various organs with their drought tolerance levels.

8.3. Materials and Methods

Plant materials

The plant materials consisted of two drought tolerant varieties, Boohai'S' and DZ-2023, two moderately tolerant varieties, DZ-1691 and DZ-320, and two susceptible varieties, Klinto and LD-357 (Solomon *et al.*, 2003). Materials were grown in glasshouse in a randomised complete block design with three replications in July to December 2001 at the Free State University. They were grown at an average of 25/15^{0}C day and night temperatures, respectively. Entries were planted in 10 l capacity plastic pots filled with 2 kg of gravel (to

prevent soil leakage), and 7 kg of steam sterilised and air-dried sandy loam soil with particle composition of 80%, 2% and 18% of sand, silt and clay, respectively. The air-dry moisture content of the soil was determined as 13.3% (field capacity) and 4.0% (permanent wilting point) gravimeter. Pots were equally spaced. Each genotype was sown into six pots. Twelve seeds at equal spacing were planted at equal depth. Seedlings were thinned to nine per pot at the two-leaf growth stage. Pots were maintained at 70-100% available soil moisture for a month (up to about four-leaf growth stage). Half of the experimental unit was then left without water until severe wilting was observed. The amount of water evaporated was monitored by weighing daily the unplanted pots placed between planted pots both for the stress and the non-stress treatments in each block (six in each block). The amount of water transpired was determined by subtracting the weight of bare pots from the weight measurements of planted pots. Pots were replenished with the amount water equivalent to the loss in weight to bring them to the predetermined level of moisture whenever the weight of pots fell to the lower limit established for the treatments. The moisture levels were 25-35% and 70-100% available soil moisture for the stress and control treatments, respectively until maturity. Equal exposure to the growing conditions of the glasshouse was ensured by changing the positions of pots every week.

Sampling

Plants were sampled at 45, 60, 75 and 90 days after planting (DAP). The first sampling coincided with stem elongation and the last with ripening. Plants were separated into leaves (leaf blade), stems and spikes (when appropriate). Collection was made in liquid nitrogen and samples were put in a deep freezer at –20 ^{0}C until extraction.

Determination of sucrose and D-glucose

Extraction of carbohydrates (sucrose and D-glucose) was carried out as the procedure outlined by Boehringer Mannheim (1997). A gram of freezed plant sample (leaves, stems and spikes) was grounded in liquid nitrogen and homogenised by adding 80% ethanol using a mortal and pestle. The enzymatic reaction was stopped by heating in a water bath at 80^0C for 15 minutes. Original volumes were restored by adding 80% alcohol. Extracts were then centrifuged at 12000 rpm for 10 minutes at ambient temperature. Supernatants were removed and 1 ml of aliquot of each replicate was transferred to new eppendorf tubes and allowed to dry in an oven at 37 ^{0}C. The pellet was dissolved in 1 ml of distilled water. Glucose and sucrose levels were then determined according to Boehringer Mannheim (1997) using Boehringer Mannheim Biochemical Kit No. 139 041. The process was replicated three times within each replication and nine times per treatment combination. D-glucose concentration was determined before and after the enzymatic hydrolysis of sucrose. The concentration determined before hydrolysis was used to estimate the quantity of D-glucose present, while the after hydrolysis was used to estimate the concentration of sucrose after subtracting the glucose which was present.

$$\text{D-glucose+ATP} \xrightarrow{\textit{Hexokinase}} \text{glucose-6-phosphate+ADP}$$

$$\text{Glucose-6-phosphate+NADP}^+ \xrightarrow{\textit{G-6-PDH}} \text{gluconate-6-phosphate+NADPH+H}^+$$

The NADPH formed in this reaction is stoichiometric with the amount of D-glucose and is measured by means of its absorbency at 340 nm. At pH= 4.6 sucrose is hydrolysed by the enzyme *β-fructosidase (invertase)* to D-glucose and D-fructose.

$$\text{Sucrose} + H_20 \xrightarrow[\text{pH=4.6}]{\textit{β-fructosidase (invertase)}} \text{D-glucose} + \text{D-fructose}$$

The determination of D-glucose after inversion (total glucose) was carried out according to the principle outlined above. Concentrations were calculated as:

$$C\ (mgg^{-1}) = \frac{VxMW\ x\ \Delta AxF}{\epsilon xdxvx1000}$$

where c=concentration mg per gram of fresh weight, V=final volume (ml), molecular weight of the substance, ΔA =change in substance concentration, F= dilution factor(4), ϵ=extinction coefficient of NADPH (5.4099 $mmol^{-1}\ cm^{-1}$), d = light path(0.8698 cm), v = sample volume (ml).

Data analysis

Data were pooled within replications for each treatment. Three way analysis of variance (ANOVA) was performed using the GLM procedure of SAS (1999).

8.4. Results

Mean squares for the effect of various treatment combinations are summarised in Table 8.1 and Table 8.2. Model fitness of the analysis (R^2) ranged from 94 to 99%. Genetic variations for glucose and sucrose contents in all organs were significant, except for the glucose level in the spikes. Moisture treatments had significantly high effects in all cases, except for the level of sucrose in the spikes. The accumulation pattern of carbohydrates was significantly different for the various growth stages. Content of carbohydrates in various plant organs was also significantly different among genotypes between the two moisture levels. The interaction effects of growth stage x genotype, growth stage x moisture level, and genotype x moisture level x growth stages significantly affected the pattern of accumulation.

Table 8.1 Mean squares for total D-glucose and sucrose content of leaf measured over different growth stages (time) for durum wheat grown under different moisture regimes

		Mean squares	
Source	df	glucose	Sucrose
Total	143		
Block	2	4.641	4.056
Genotype	5	1034.456**	575.87**
Moisture	1	331.303**	120.521**
Time	3	2513.977**	711.747**
Time*Genotype	15	291.687**	70.215**
Time*moisture	3	750.494**	2003.747**
Moisture*Genotype	5	116.445**	212.842**
Time*Genotype*Moisture	15	379.946**	150.012**
Residual	94	7.281	2.909
R^2		0.97	0.98
CV%		7.21	7.87

** Significant at P=0.01

Averaged over genotypes, glucose content in the leaves was 29.93 mg g^{-1}FW at control and 29.70 mg g^{-1}FW at stress moisture levels at 45 DAP. In later growth stages, the glucose content was reduced by 7.5 and 1.8% at 60 and 75 DAP, respectively, due to stress. By 90 DAP, however, glucose at the stress level was increased by 74%. However, the pattern and the level of glucose accumulation in the leaves were different for the various genotypes and growth stages depending the availability of moisture (Fig 8.1a and b). Drought tolerant genotypes, DZ-2023 and Boohai'S' had an increased level of glucose at early growth stages when compared to the control moisture level. Accumulation of glucose in these genotypes declined to the level in the control moisture treatment by 90 DAP. Susceptible genotypes Klinto and LD-357, on the other hand had slow accumulation of glucose, and the level surpassed the control level after 90 DAP in Klinto and 75 DAP in LD-357. Compared to Klinto, LD-357 had a lower glucose level at the stress moisture level. Moderately tolerant genotypes, exhibited more or less intermediate reactions between the tolerant and the susceptible groups (Fig 8.1b).

Table 8.2. Mean squares for total D-glucose and sucrose content of stem and spike measured over different growth stages (time) for durum wheat grown under different moisture regimes

		Mean squares			
		D-glucose		Sucrose	
Source	df	Stem	Spike	Stem	Spike
Total	107				
Block	2	0.206	36.068	0.065	0.05
Genotype	5	1366.221**	3.477ns	368.221**	186.898**
Moisture	1	5062.894**	2388.867**	2502.768**	0.681ns
Time	2	3506.084**	11160.71**	755.811**	1464.926**
Time*Genotype	10	669.256**	157.485**	315.108**	48.742**
Time*moisture	2	2077.977**	125.532*	471.916**	5.887*
Moisture*Genotype	5	1163.81**	284.689**	150.618**	20.677**
Time*Genotype*Moisture	10	412.246**	276.916**	192**	89.429**
Residual	70	7.929	29.518	2.002	1.51
R^2		0.99	0.94	0.99	0.98
CV%		6.99	12.55	7.09	8.48

ns = not significant P > 0.05

*, ** are significant at P=0.05 and 0.01, respectively.

Moderately tolerant genotypes, DZ-320 and DZ-1691, had a lower level of glucose at the stress level than at the control throughout growth, except at 90 DAP. Nevertheless, compared to the susceptible genotypes, the glucose content was higher at all growth stages (Fig 8.1 a & b). A significantly high (r = -0.69; P<0.05) negative correlation between glucose content of leaves pooled over growth stages and drought susceptibility index ('S') showed that the capacity to accumulate glucose under stress conditions was dependent on the ability of the genotype to tolerate moisture deficit stress. The level of glucose in the stem under stress generally was higher than the level in the leaves. The level under control moisture level, however, was comparable. Averaged over cultivars, the level of glucose in the stems under stress was twice that of the control treatments at 60 DAP. Increase due to stress by 75 DAP was only 45%. After 90 DAP, however, stem glucose decreased by 10% as a result of the stress treatment.

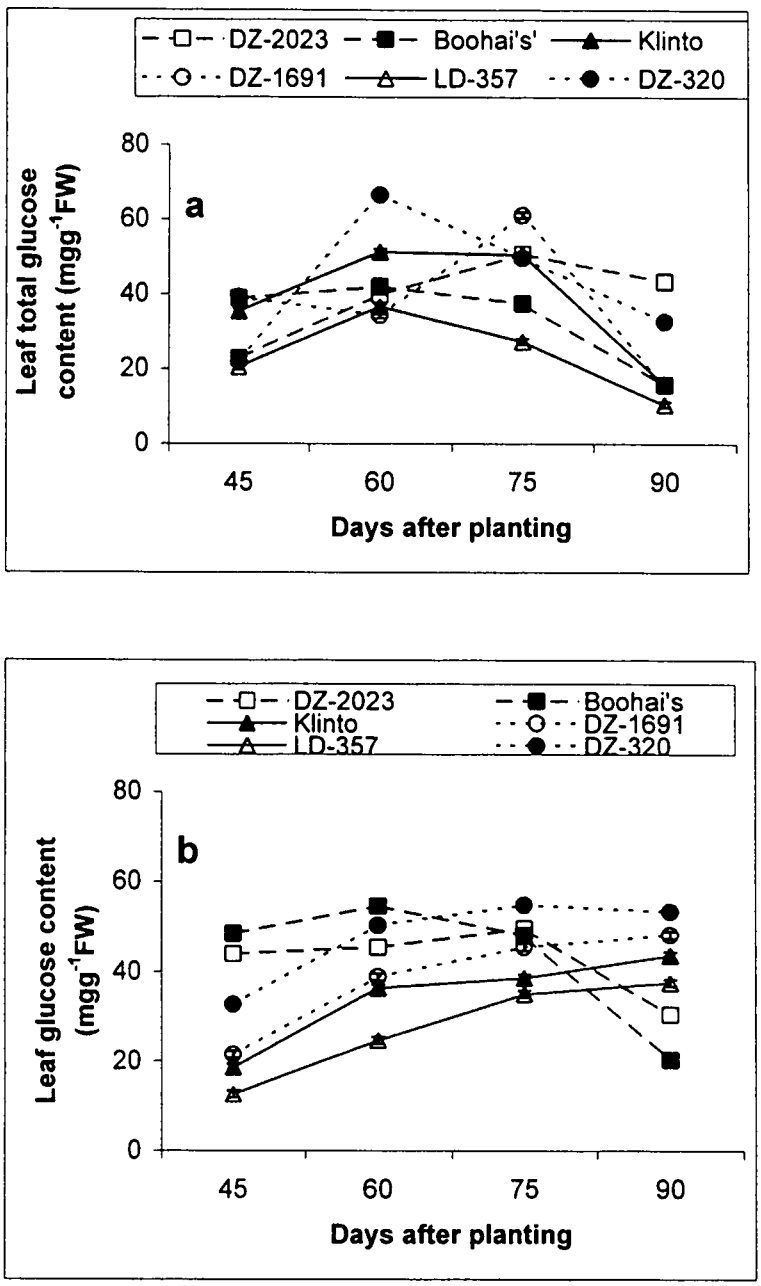


Fig.8.1. Glucose content of leaf determined over different growth stages (time) for durum wheat genotypes grown under control (a) and stress (b) moisture levels. Values are means of three replications with (±SE).

Drought tolerant genotypes had nearly three times the level of glucose of the control treatments at 60 DAP and 75 DAP, while the level at 90 DAP was also

higher than the control treatments. The accumulation of glucose in the stems of drought sensitive genotype, Klinto was significantly higher than the control only at 60 DAP. The level of glucose in the stem of LD-357 was higher than the control level at all times, and it reached its highest level by 90 DAP. The level of glucose in both susceptible genotypes were lower than both the tolerant and intermediate groups at all growth stages, except for LD-357 in which its glucose level was the highest at 90 DAP. The moderately tolerant genotype, DZ-1691 had a higher level of stem glucose than both the control treatment and the susceptible genotypes at the stress moisture level at 60 and 75 DAP. The level at 90 DAP was also higher than the control treatment and all genotypes in the stress treatments, except for LD-357. DZ-320, on the other hand, had nearly equal levels at both stress and control conditions, but its glucose level was higher than the susceptible groups under stress conditions (Table 8.3). The correlation between 'S' and stem glucose content was highly significant but negative ($r= -0.73$; $P<0.01$). The genotypic difference in the glucose content of spikes was not statistically significant. However, the accumulation pattern of glucose in the spike was significantly different among growth stages. Glucose content of the spike was increased by 25, 22 and 26 % at 60, 75 and 90 DAP, respectively, under stress. The level and pattern of glucose accumulation was also significantly affected by the interaction of genotype x moisture level, moisture level x growth stage, genotype x growth stage and genotype x moisture level x growth stage (Table 8.2). Glucose content in the spike under stress was generally higher than the level in the leaves at 60 and 75 DAP and stems only at 60 DAP. The level in later growth stages was however, lower than the level in both leaves and stems. Moderately tolerant genotypes had the highest glucose level in their spikes at 60 and 75 DAP under stress. The glucose content of these genotypes was also higher than that of the control treatments. Susceptible genotypes, on contrary, had a low level of glucose in their spikes at 60 DAP, but had the highest level at 90 DAP (Table 8.4). Marked genetic variation in the accumulation of sucrose in the leaf both at control and stress moisture levels was found. The variation was significantly different between the different growth

stages. Pooled over growth stages and genotypes, 23 mg g^{-1}FW sucrose was found in the leaf under stress. This was slightly and yet significantly higher than the average level of sucrose (21 mg g^{-1}FW) under control conditions.

Table 8.3. Total D-glucose and sucrose contents of stem measured over various growth stages (time) for durum wheat genotypes grown under control and stress moisture levels

	Moisture level[§]	Glucose (mg g^{-1}FW)			Sucrose (mg g^{-1}FW)		
		Growth stages (Days after			Growth stages (Days after		
Entry		60	75	90	60	75	90
DZ-2023	C	24.12	27.45	15.09	6.77	12.56	10.66
	S	68.77	65.51	20.25	40.37	36.36	5.90
Boohai'S'	C	24.84	29.99	28.48	9.45	18.27	14.93
	S	73.88	74.96	14.61	32.63	46.01	8.86
Klinto	C	37.80	43.43	22.94	5.73	12.50	13.38
	S	35.25	47.88	10.51	10.40	15.19	16.51
DZ-1691	C	18.67	36.12	33.85	5.48	27.46	27.07
	S	54.37	56.00	43.69	18.10	25.64	27.64
LD-357	C	23.78	33.86	18.27	7.96	16.16	17.91
	S	34.53	45.22	46.38	21.02	19.52	32.65
DZ-320	C	45.58	68.68	39.19	18.12	24.68	25.71
	S	45.58	68.68	39.19	17.68	45.00	26.27
Means	C	26.54	41.24	32.59	8.52	18.61	18.28
	S	52.3	59.92	29.22	23.37	31.29	19.64
$LSD_{(0.05)}$	ENTRY	2.56	2.95	2.70	1.53	1.40	1.19
	ML	1.48	1.70	1.56	0.88	0.81	0.69

[§] = moisture levels are C=control treatments and S=stress treatments.

On average over genotypes, stress caused sucrose reduction in the leaves by 54, 11.6 and 3.6% at 60, 75, 90 DAP, respectively. After 90 DAP, sucrose level in the leaves, however, showed a three-fold increase over the control treatments. Nevertheless, accumulation of sucrose in the leaves was significantly affected by the interaction effect of genotype x moisture level x growth stages (Table 8.1). Drought tolerant genotypes increased the level of sucrose in their leaves relatively rapidly and exceeded the control level by 75 DAP and continued to accumulate at a higher level than the control throughout the growing period.

In all other genotypes, sucrose accumulation was slow and it could not exceed the control level in most cases until 90 DAP. Among the moderately tolerant genotypes, DZ-320 had the highest accumulation of sucrose in the leaves through all the growth stages, until its sucrose content dropped by the

last growth stage (Fig 8.2 a & b). Strong correlation between sucrose level of leaves and 'S' showed that drought resistant genotypes had higher levels of sucrose at lower values of 'S' (Fig 8.3). The level of sucrose in the stem was also significantly different for the various treatment combinations (Table 8.2). Across all genotypes, the level of sucrose in the stem was increased by 174% at 60, 68% at 75 and 7% at 90 DAP because of stress. Sucrose was highest in the stem as compared to any of the organs analysed under stress, particularly at an early growth stage. At an older growth stage, the level of sucrose in the stem was lower than the level in the leaves, and yet it was higher than the level in the spikes. Significantly high genotype x moisture level x growth stage interaction effect (Table 8.2) showed that there was differential response of cultivars in sucrose accumulation over the different growth stages due to moisture level treatments. Initially, close to six fold in DZ-2023 and four fold increase in Boohai's sucrose levels of stems due to stress treatment was found.

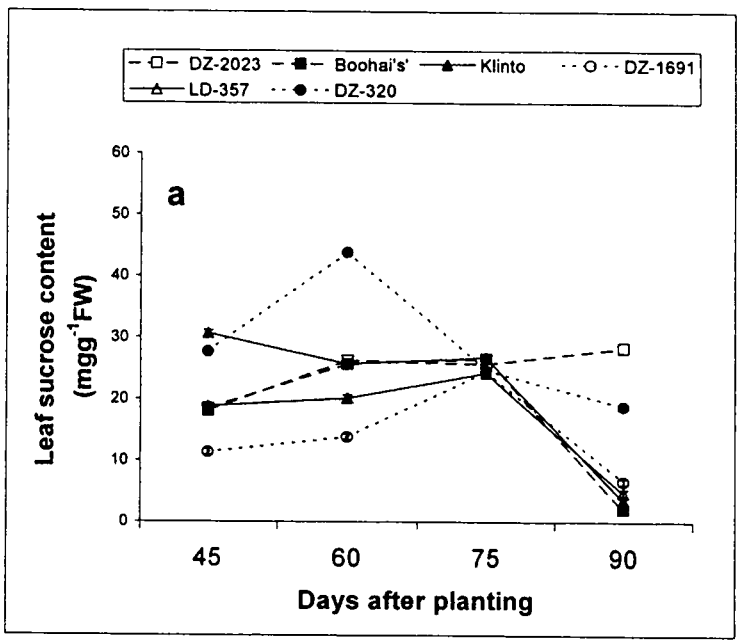
The accumulation gradually declined by nearly two fold in both genotypes after 90 DAP growth stage. Sucrose accumulation in the stem was slow in the rest of the genotypes at early growth stages. As plants matured, moderately tolerant genotypes showed rapid increase in the sucrose levels of their stems. Susceptible types accumulated sucrose very slowly and continued to increase the level above the control level even at 90 DAP when the level in the tolerant and moderately tolerant groups dropped back to the control or below the control level (Table 8.3). Significantly high correlation between average level of sucrose in the stem and genotype 'S' values ($r = -0.68$; $P<0.05$) reflected genotypic dependent characteristic of the pattern of sucrose accumulation. There was no significant difference in the level of sucrose in the spike between the two moisture levels. Nevertheless, significantly high variation among genotypes, between growth stages and the various interaction effects was shown in Table 8.2. The level of sucrose in the spike of drought tolerant genotypes, DZ-2023 was reduced by 31 and Boohai'S' by 75% due to stress at early growth stages.

Table 8.4. Total D-glucose and sucrose contents of spikes measured over various growth stages (time) for durum wheat genotypes grown under control and stress moisture levels

	Moisture level[§]	Glucose (mg g^{-1}FW)			Sucrose (mg g^{-1}FW)		
		Growth stages(Days after planting)			Growth stages(Days after planting)		
Entry		60	75	90	60	75	90
DZ-2023	C	62.88	41.11	22.55	15.72	21.88	17.71
	S	68.78	40.55	22.41	10.91	21.23	20.46
Boohai'S'	C	52.57	52.64	25.83	8.34	19.56	20.02
	S	59.83	50.73	18.31	2.06	21.88	23.65
Klinto	C	64.54	41.99	12.52	1.39	14.12	10.09
	S	54.60	54.03	36.60	3.36	20.34	7.61
DZ-1691	C	37.90	43.94	31.08	4.43	21.95	20.26
	S	71.51	54.88	20.36	20.59	20.61	13.82
LD-357	C	47.68	38.67	21.04	3.13	19.25	17.29
	S	56.66	58.57	37.67	5.47	19.49	6.98
DZ-320	C	42.24	42.92	12.61	12.21	25.28	6.06
	S	74.39	60.65	23.50	6.94	21.34	10.11
Means	C	51.3	43.55	20.94	7.54	20.34	15.85
	S	64.29	53.24	26.48	7.96	20.52	14.76
$LSD_{0.05}$	Entry	5.93	6.53	3.02	0.52	1.77	0.94
	Moisture level	3.42	3.77	1.74	0.30	1.02	0.54

[§] = moisture levels are C=contol treatments and S=stress treatments.

Due to stress induced accumulation, the sucrose level in the stress treatment of these genotypes became higher than the control levels by 90 DAP. Though lower than the most tolerant genotypes, the level of sucrose in the spike of moderately tolerant genotypes showed a similar pattern, but unlike the tolerant ones the level sharply declined after 75 DAP. Among the susceptible types, LD-357 had the highest sucrose level in the 60 DAP, but the level declined quickly after 90 DAP. Klinto had a low initial level of sucrose in its spikes and had reached its peak at 75 DAP but the sucrose content by 90 DAP dropped below the control treatment (Table 8.4).



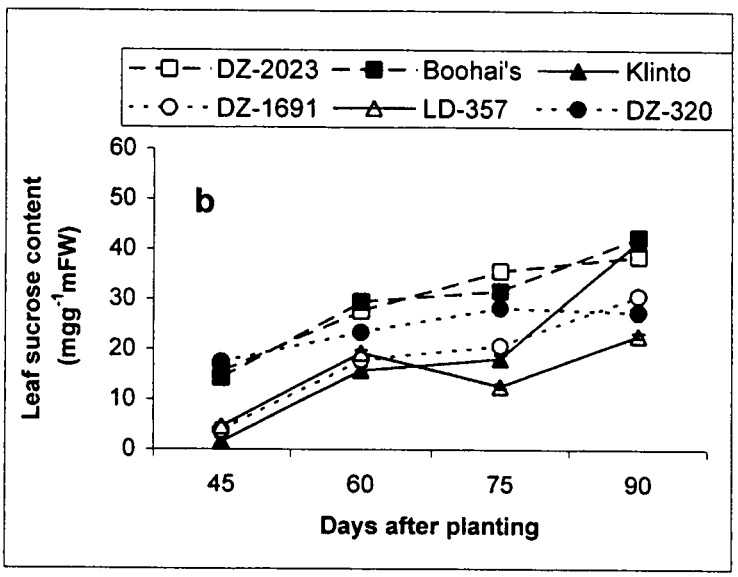


Fig.8.2. Sucrose content of leaf determined over different growth stages (time) for durum wheat genotypes grown under control (a) and stress (b) moisture levels. Values are means of three replications with (±SE).

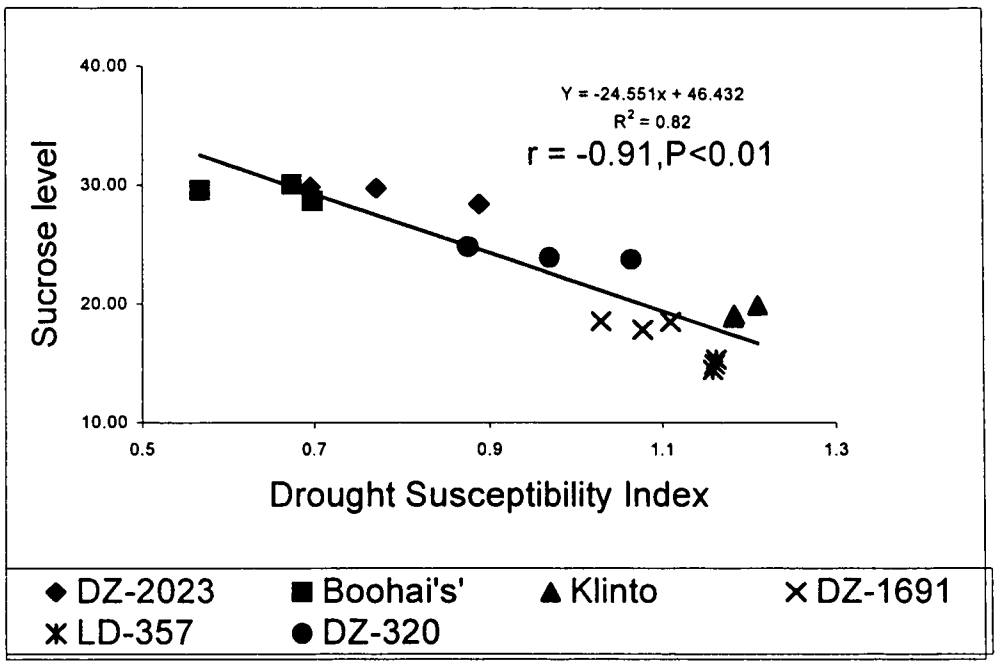


Fig.8.3. Relationships between drought susceptibility index and sucrose level of leaf for durum wheat genotypes grown under moisture deficit stress conditions.

Drought tolerant genotypes showed increasingly high allocation of sucrose to spike thereby maintaining the levels in their leaves to the higher level after anthesis. Drought sensitive genotypes, on the other hand continued to build up the sucrose pool in their stems while the level in their leaves was yet the lowest. Negative but significant correlation ($r = -0.61$; $P<0.05$) between level of sucrose in the spike and 'S' values implicated that drought induced accumulation of sucrose in the spike was also very much dependent on the capacity of genotypes to endure the stress.

8.5. Discussion

There have been contradictory reports regarding the effect of moisture stress on sugar accumulation in wheat. Some studies have reported that sugar content rose (Jones and Turner, 1981; Munns and Weir, 1981) while others have found that sugar content decreased (Hanson and Hitz, 1982) or remained unchanged (Morgan, 1992) during stress conditions. Results in this study showed an overall increase in the level of sugars under stress conditions. This was consistent with findings by other researchers (Kamil and Lösel, 1993; Al Hakim *et al.*, 1995). In agreement with Kerepesi and Galiba (2000), differences in the allocation and accumulation pattern of sucrose and glucose in plant organs were found. Huang and Jiang (2002) also noted that accumulation and allocation pattern of water-soluble carbohydrates under stress conditions have been different depending on species and plant organs. In agreement with Martin *et al.* (1993), Kerepesi *et al.* (1998) and Kerepesi and Galiba (2000), accumulation of sucrose and glucose in this study was found to correlate with the level of drought tolerance. Drought tolerant genotypes and moderately tolerant genotypes generally had a higher level of glucose and sucrose in their leaves and stems. The level of sucrose in the leaves of stress tolerant genotypes, however, was lower than the level in the control treatment (Fig 2 a&b), probably due to increased conversion of sucrose to glucose and fructose, which might serve as osmotic in maintaining leaf turgor. As a result the level of glucose at early stages was higher in tolerant and moderately tolerant types than in susceptible types. Kamil and Lösel (1995) have pointed out that glucose is the main osmotic factor in wheat representing 85.5% of the total increase in sugars of young plants undergoing moderate water stress. In drought susceptible genotypes, the rate of accumulation of sucrose and glucose was very slow while drought tolerant genotypes had fast initial accumulation in agreement with Kerepesi and Galiba (2000). This may be attributed to severe effects of stress on the rate of photosynthesis and carbon accumulation and allocation pattern. Drought stress has been found to reduce the rate of photosynthesis in many plant species (Faria *et al.*, 1998; Hashem *et al.*, 1998; Pankovic *et al.*, 1999). A decrease in photosynthesis induced by long

term-severe water stress has been attributed to both stomatal and non-stomatal limitations (Huang and Jiang, 2002). Initially, the rate of accumulation of glucose and sucrose in drought tolerant genotypes was higher in the stem than in the leaves, while the level of sucrose in the leaves was at its lowest level. This suggests that large quantities of the manufactured sucrose in the leaf is first mobilised to the stem where it is metabolised to glucose and other monosaccharides. As a result, the level of glucose in the stem was higher than any of the organs between 60-75 DAP (stem elongation-anthesis growth stages). The translocation of assimilates during this growth period is likely to be driven by high demands in the actively growing stem during this period. Level of sucrose tended to increase in the leaves after anthesis as the allocation of sucrose declined in the stem, probably due to decreased translocation of sucrose to the older stem and/or remobilisation of sucrose to the growing spikes (Davidson and Chevalier, 1992; Kiniry, 1993). Occurrence of drought during grainfilling period could also result in reduced accumulation of non-structural carbohydrates in the stem reserves (Davidson and Chevalier, 1992; Frederick and Camberato, 1995). The level of sucrose in the spike of drought tolerant genotypes was high at almost all growth stages, whereas the level of glucose was higher for susceptible genotypes than it was for tolerant genotypes. This could be due to the fact that drought tolerant genotypes were able to metabolise sucrose to glucose which eventually is converted to starch in the developing grain. In susceptible types, however, inefficiency in the conversion of glucose to starch could contribute to the higher level of glucose in their spikes. Wang *et al.* (1996), reported that drought stress can inhibit conversion of glucose to starch. Drought tolerant genotypes had a relatively higher rate of sucrose accumulation in their leaves and stem during critical growth stages, stem elongation (45 DAP), (60 DAP) heading and (75 DAP) after anthesis. This was in agreement with Frederick *et al.* (1990), Davidson and Chevalier (1992) and Kiniry (1993). This probably allowed them to allocate a substantial amount of glucose for maintenance of the growth of leaves so that increased photosynthesis would ensure the availability of assimilate for the growth of spike as stress continued.

Moreover, a substantial pre-anthesis accumulation of carbon could be ensured as a result of favourable growth of the stem because of allocation of a higher proportion of sucrose during the period between stem-elongation to anthesis. Rapid decline in the level of glucose and sucrose in the stem of drought tolerant genotypes after anthesis could be attributed to the increased allocation of sucrose from the leaves and stems to the spikes for subsequent conversion of glucose to starch for grainfilling.

8.6. Conclusions

These findings provided additional evidence on the differential responses of drought tolerant and susceptible genotypes in in the rate of accumulation of carbohydrates. Clear relationships between drought susceptibility indices and rate of carbohydrate accumulation among the various organs suggest the importance of carbohydrate as a potential trait to be used for screening of drought tolerant genotypes. In this study, level of sucrose and glucose of the stem showed strong correlation with the level of drought tolerance, and could be employed as a useful marker for selecting drought tolerant genotypes.

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CHAPTER 9

DNA polymorphism in relation to drought tolerance in durum wheat (*Triticum turgidum* L. var. *durum*).

9.1. Abstract

The aim of this study was to examine the genetic differences among genotypes with differing responses to drought stress and their progenies obtained from all possible cross combinations, and to asses the relationships between molecular markers and phenotypic traits associated with drought tolerance. DNA for AFLP analyses was extracted from 10 randomly selected F_2 plants from 15 different diallel crosses. DNA was analysed using bulk segregant analysis (BSA). Equal concentration of DNA from each of the 10 plants was bulked to form a sample for each cross combination. A total of six AFLP primer combinations were tested. Moderate to high levels of polymorphism were obtained with the primer combinations evaluated. There is considerable genetic diversity among the different progeny crosses based on Euclidean genetic distance estimates. The correlation coefficient for pair wise genetic distance estimates based on AFLP data, yield and yield components as well as morpho-physiological traits was significant and positive. Cluster analysis showed agreement between the grouping of genotypes on the basis of AFLP data and agro-morphological data. A number of AFLP fragments were found to be significantly correlated with various traits evaluated under stress conditions and were shown to affect a number of traits which are strongly correlated to the index for drought

susceptibility. Strong correlation between some of these fragments among themselves led to the conclusion that some of the fragments could represent inter or intra genetic loci with possible linkage to traits associated with drought tolerance. These features could be exploited for the identification of QTL associated with drought tolerance, and for use in marker assisted selection in the improvement of drought tolerance in durum wheat.

Key words: *AFLP, cluster analysis, correlation, morpho-physiological traits*

9.2. Introduction

The complex nature of drought stress has made improvement in drought tolerance through breeding a difficult task. Poor definition of the target environment (Fukai *et al.*, 1995) and difficulties in determining meaningful selection criteria contributing to improved yield under stress (Ludlow and Muchow, 1990) are the most common reasons for the slow rate of success in developing drought tolerant cultivars. Additional constraints are that field trials to evaluate drought tolerance are complex and difficult to manage (Courtois *et al.*, 2000). Field experiments rely on natural drought occurrence with little or no control of drought conditions and risk of rainfall at inappropriate periods. Drought tolerance is complex and involves the interaction of a number of individual traits that contribute to various resistance mechanisms (Levitt, 1980; Blum, 1988). Under appropriate conditions, selections are made of genotypes with the least possible yield reduction under water deficit stress (Courtois *et al.*, 2000).

However, these selections are greatly influenced by prevailing environmental conditions. The use of molecular markers can contribute considerably to the improvement of selection efficiency for drought tolerance (Quarrie *et al.*, 1997; Sari-Gorla *et al.*, 1999; Nachit *et al.*, 2000). Information on the use of molecular markers to study drought related traits has started to accumulate in various crops such as rice (Champoux *et al.*, 1995; Price *et al.*,

1997; Yadav *et al.*, 1997; Cooper *et al.*, 1999; Mackill *et al.*, 1999;), common bean (Schneider *et al.*, 1997), maize (Ribaut *et al.*, 1997; Sari-Gorla *et al.*, 1999) and wheat (Quarrie *et al.*, 1997; Nachit *et al.*, 2000).

The detection of polymorphic markers among genotypes in drought tolerance is a critical step in the identification of morpho-physiological traits related to drought tolerance (Champoux *et al.*, 1995; Price *et al.*, 1997; Cooper *et al.*, 1999). Efforts to demonstrate relationships between traits related to drought tolerance and productivity in water limiting environments is considered essential towards the improvement of drought tolerance and hence productivity under these conditions (Ludlow and Muchow, 1990; Blum, 1996; Gupta, 1997; Saini and Westgate, 2000). It has been suggested that studies to examine the genetic relationship among genotypes with different levels of drought tolerance, and productivity under dryland growing conditions is a prior step to the identification of QTL linked to drought tolerance and subsequent application of marker assisted selection (MAS) (Nachit *et al.,* 2000). The objectives of this study were: i) to examine genetic distances among genotypes with differing responses to drought stress and their progenies obtained from all possible cross combinations, and ii) asses the relationships between molecular markers and traits associated with drought tolerance.

9.3. Materials and Methods

Plant materials

Six durum wheat genotypes differing in their responses to moisture deficit stress (Solomon *et al.*, 2003) were crossed in a half diallel cross to generate an F_1 population. Parents and their F_1 progeny were evaluated for their yield and yield components response to moisture stress (Table 9.2.) (Solomon and Labuschagne, 2003) and various morpho-physiological traits (Table 9.2) (Chapter 6). Twenty plants per cross were grown under glasshouse conditions for DNA extraction.

DNA extraction

Ten plants were randomly selected to represent each cross. DNA was extracted from each plant separately according to the modified monocot method (Edwards *et al.*, 1991). Young leaves were collected and ground to a fine powder in a liquid nitrogen using a mortar and pestle. Extraction buffer, 15 ml (1M Tris-HCl pH 8; 0.25 M EDTA and 20% SDS) and 1 ml Cetyl triethyl ammonium bromide (CTAB) was added to the homogenate, vortexed and incubated at 65 ^{0}C for 60 minutes. Chloroform-isoamyl alcohol (24:1v/v) (10ml) was added to the homogenate followed by centrifugation for 15 minutes at 10,000 rpm. Chloroform extraction was repeated until the supernatant layer was visibly free of all debris. The supernatant was retained and the DNA precipitated over-night in 100% ethanol. The DNA precipitate was washed twice in 70% ethanol and dissolved in 250 µl sterile distilled water. The concentration was determined spectrophotometrically at 260 nm, and the quality of the DNA was further verified by agarose gel electrophoresis.

AFLP analysis

DNA was analysed using bulk segregant analysis (BSA) (Michlemore *et al.*, 1991). Equal concentration of DNA from the ten plants was bulked to form a sample for each cross combination.

Restriction digestion and ligation of adapters

A 25 µl reaction was set up with 5 µl of genomic DNA (250 ng), 5x reaction buffer (50 mM Tris-HCL(pH 7.5), 50 mM Mg-acetate and 250 mM K-acetate), 2.5 µl of DNA from tomato (control) (100 ng/µl), 2µl of *EcoRI/MseI* (1.25 units/µl each in 10 mM Tris-HCL (pH=7.4), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA, 50% (v/v) glycerol and 0.1% Triton) and sterile distilled water (15.5 µl). The mixture was incubated for 2 hrs at 37 °C. The restriction *enoducleases* were inactivated by incubating the reaction at 70°C for 15 minutes. The digested fragments were then ligated in a 25 µl the reaction containing 24 µl adater/ligation solution (*EcoRI/MseI* adapters, 0.4 mM ATP, 10 mM Tris-HCL

(pH 7.5), 10 mM Mg-acetate and 50 mM K-acetate) and 1 µl T4 DNA ligase (1 unit/µl in 10 mM Tris-HCL (pH 7.5), 1 mM DTT, 50 mM KCL, 50% glycerol (v/v)). The ligation product was diluted 1:10 in TE (10 mM Tr-s-HCL (pH 8.0), 0.1mM EDTA) buffer

Pre-selective amplification

Pre-selective reactions were performed in 51 µl containing 5 µl of diluted ligation product (1:10 in TE), 40 µl of pre-amp primer mix (T4 polynucleotide kinase (10 units/µl in 50 mM Tris-HCl (PH=7.6), 5x kinase buffer (350 mM Tris- HCl (pH 7.6), 50 mM $MgCL_2$, 500 mM KCl, 5 mM 2-mercatoethanol)), 5 µl of 10x PCR buffer (100 mM Tris-HCl (pH 8.3), 15 mM $MgCL_2$, 500 mM KCl) and 1U of Ampli-Taq DNA polymerase (GibcoBRL). PCR amplification consisted 20 cycles at 94°C for 30s, 56°C for 60s and 72°C for 60s. Pre-selective amplification products were diluted (1:50) in TE buffer (10 mM Tris-HCL (pH=8.0) and 0.1 mM EDTA).

Selective amplification

Selective PCR was performed in 20 µl PCR reaction containing 5µl of the diluted pre-selective reaction product (1:50), 4.5 µl of the Mse+3 primers (6.7 ng/µl, dNTPs), 1 µl of Eco+3 primers (27.8 ng/µl) (labelled with FAM and NED)) (Table 9.1), 2 µl of 10x PCR buffer (100 mM Tris-HCl (pH=8.3), 15 mM $MgCl_2$ and 500 mM KCl) and 5U of Ampli Taq DNA polymerase. Reactions were performed on an ABI 2700 Perklin-Elmer thermal cycler at the following conditions: 12 cycles at 94°C for 30 s, 65°C for 30s and 72°C for 60s, during which the annealing temperature was lowered by 0.7°C per cycle followed by 23 cycles at 94°C for 30s, 56 °C for 30s and 72°C for 60s.

A total of six selective primer combinations were used (Table 9.1).

After selective PCR, 5 µl of the selective amplification product was added to 24 µl formamide and 1 µl of Rox standard size marker, denatured for 10 minutes at 94°C and quickly cooled on ice. The AFLP fragments were resolved on an automatic ABI 310 capillary sequencer (PE Biosystems).

Table 9.1. List of adapter and primer sequences used

Mse-adapter	*EcoR1 adapter*
5'-GACGATGAGTCCTGAG-3'	5'-CTCGTAGACTGCGTACC-3'
3'-TACTCAGGACTCAT-5'	3'-CATCTGACGCATGGTTAA-5'
Mse-primers (5'-GATGAGTCCTGAGTAA-3')	*EcoR1*-primers (5'-GATGCGTACCAATTC-3')
Mse+CAG	*Eco*+ACA/FAM
	Eco+AAC/NED
Mse+CTA	
Mse+CTT	

Data analysis

AFLP fragments were coded as 1 for presence and 0 for absence. Distance matrices were compiled for all pairs of genotypes from binary data based on both unique and shared bands (AFLP) and variable intervals (for yield, yield related traits and morpho-physiological traits) (Table 9.2.) (Chapter 4 and 5) using the Euclidean distance method (Kaufman and Rousseeuw, 1990). The Euclidean distance is the square roots of the sum of squares of distances between the multidimensional space values of the variables for any two genotypes. Cluster analysis was performed on the genetic distance matrices generated by the Euclidean distance method to reveal the pattern of genetic relationships among genotypes using unweighted pair group method with arithmetic averages (UPGMA). Spearman rank correlation was computed between the genetic distance obtained from both AFLP and agro-morphological

Table 9.2. Mean values for yield, yield components and different morpho-physiolgical traits evaluated under moisture stress conditions.

Entry	Genotype	RGR	NAR	SLA	LWR	LAR	TDM	HI	WUE_{TDM}	T_{TDM}	T_G	WUE_G	Spike#	Spklt#	Kernel#	Kernelwt	Yield	S
1	DZ-2023	40.46	5.21	46.72	0.27	16.51	2.75	0.41	2.33	4.73	2.02	0.96	1.63	11.83	13.43	52.21	1.14	0.79
2	Boohai'S'	42.02	6.11	45.11	0.25	18.71	2.77	0.49	1.79	4.62	2.14	0.83	2.25	10.35	11.29	50.32	1.37	0.65
3	Klinto	24.67	3.52	24.93	0.31	10.77	3.12	0.13	1.65	3.02	0.40	0.22	1.50	11.15	9.96	27.93	0.42	1.19
4	DZ-1691	23.01	3.98	25.13	0.30	8.70	2.08	0.25	1.26	3.02	0.75	0.32	2.33	10.50	5.65	40.35	0.52	1.07
5	LD-357	27.25	5.20	25.70	0.27	7.88	2.10	0.11	1.11	2.60	0.29	0.12	1.15	8.78	6.29	31.66	0.23	1.16
6	DZ-320	26.42	4.87	25.86	0.31	8.46	2.03	0.23	1.14	1.81	0.44	0.26	1.58	12.23	9.59	31.50	0.46	0.97
7	1x2	37.20	6.65	27.39	0.30	11.36	3.27	0.41	2.19	4.96	2.02	0.89	2.75	10.61	11.12	43.81	1.33	0.72
8	1x3	45.29	20.95	24.14	0.28	7.93	1.87	0.45	1.58	3.24	1.44	0.71	1.00	11.44	18.50	45.17	0.84	0.99
9	1x4	39.60	11.50	28.00	0.29	11.62	2.61	0.37	1.85	3.74	1.37	0.68	2.83	12.48	9.11	30.81	0.96	1.07
10	1x5	34.56	5.37	25.72	0.30	10.18	2.53	0.36	2.05	4.42	1.60	0.74	3.84	10.00	5.88	40.44	0.92	0.91
11	1x6	39.28	6.44	29.32	0.29	12.28	2.64	0.47	1.61	3.25	1.51	0.75	1.25	13.67	21.75	46.43	1.23	0.86
12	2x3	40.94	10.92	20.79	0.34	11.81	1.70	0.37	1.61	4.18	1.55	0.60	1.89	9.72	10.40	43.81	0.63	1.04
13	2x4	34.23	5.11	35.23	0.30	16.47	2.25	0.44	1.65	4.46	1.96	0.73	2.44	10.50	12.58	40.22	0.99	1.02
14	2x5	29.56	6.04	23.63	0.26	8.13	2.27	0.31	1.94	6.21	1.90	0.60	3.33	11.00	6.33	33.69	0.70	0.79
15	2x6	38.18	5.57	32.36	0.29	12.54	2.14	0.41	1.35	4.18	1.70	0.54	1.72	11.27	10.94	56.95	0.87	1.02
16	3x4	35.79	7.23	25.86	0.29	15.72	1.34	0.32	0.79	2.53	0.80	0.25	1.83	10.87	10.56	22.32	0.42	1.21
17	3x5	32.62	9.08	19.46	0.29	10.04	1.95	0.19	1.09	3.38	0.63	0.20	1.33	10.44	5.25	52.12	0.36	1.18
18	3x6	27.78	3.58	34.10	0.31	12.13	2.55	0.26	1.60	3.00	0.79	0.42	1.89	10.08	12.63	22.69	0.67	1.01
19	4x5	32.10	8.92	16.81	0.37	10.15	1.77	0.22	1.48	4.52	0.91	0.32	2.00	10.14	4.67	45.22	0.39	0.90
20	4x6	27.43	3.47	46.11	0.29	11.89	1.96	0.25	1.49	3.66	0.90	0.37	1.75	8.75	10.54	35.39	0.48	1.10
21	5x6	28.83	3.67	33.15	0.30	10.95	1.99	0.21	1.47	4.67	1.11	0.41	2.25	12.66	6.05	24.69	0.43	1.13
Mean		33.68	6.83	29.31	0.296	11.63	2.27	0.32	1.54	3.82	1.25	0.52	2.03	10.88	10.12	38.94	0.73	0.99
SE±		2.25	0.89	3.43	0.011	1.44	0.17	0.03	0.13	0.55	0.17	0.04	0.31	0.65	1.44	2.80	0.08	0.05
$LSD_{0.05}$		3.80	1.50	5.78	0.019	2.42	0.28	0.05	0.21	0.92	0.29	0.07	0.52	1.09	2.42	4.72	0.13	0.09

RGR = relative growth rate, NAR = net assimilation rate, SLA = specific leaf area, LWR = leaf weight ratio, LAR = leaf area ratio, TDM = total dry matter production per plant, HI = harvest index, WUE_{TDM} = water use efficiency for total dry matter production per plant, T_{TDM} = transpiration efficiency for total dry matter production per plant, T_G = transpiration efficiency for grain yield per plant, WUE_G = water use efficiency for grain production per plant, Spike# = number of spike per plant, Spklt# = number of spikeletes per spike, Kernel# = number of kernels per spike, Kernelwt = individual kernel weight, Yield = grain yield per plant and S = drought susceptibility index

traits to compare the differences between the AFLP and the agromorphological clustering. Spearman rank correlation analysis was also carried out between AFLP fragments and mean values of the various yield, yield components and morpho-physiological traits evaluated under stress conditions. All analyses were carried out using the NCSS computer package (Hintze, 2000).

9.4. Results

A total of 465 fragments, varying in size from 60 to 538 bp, were scored for all six primer combinations. Primer combination *Mse+CTT* and *Eco+ACC* produced a total of 111 fragments ranging in size from 60 to 474 bp of which 78% were polymorphic. The total number of fragments generated by primer combination, *Mse+CTT* and *Eco+AAC* was 57 of which 61% were polymorphic. Primer combination *Mse+CTA* and *Eco+ACA* generated 110 fragments ranging in size from 62 to 538 bp with 64% polymorphisms. Primer combination *Mse+CTA* and *Eco+AAC* gave a total of 61 fragments in a size range of 62 to 359 bp of which 59% were polymorphic. Evaluation of the primer combination *Mse+CAG* and *Eco+ACA* and *Mse+CAG* and *Eco+AAC* resulted in a total of 73 and 53 fragments, respectively, at 71% and 62% polymorphism, respectively.

Estimates of genetic distance

Euclidean genetic distance estimates for 210 pair wise comparisons based on AFLP analysis were found to be normally distributed (Fig. 9.1). Genetic distances based on AFLP data was found to range from 0.420 between the cross of DZ-1691 x Boohai'S' and Boohai'S' x DZ-320 to 0.784 between DZ-2023 and LD-357 (Table 9.3), with a mean value of 0.633 ± 0.005. Euclidean genetic distance estimates from yield, yield related traits, and various morpho-physiological traits ranged from 0.171 between DZ-320 and DZ-1691 to 0.668 between Booha'S' and LD-357 (Table 9.3) with a mean of 0.367 ± 0.007. Correlation analysis based on the genetic distance values for 210 pair-wise

comparisons of both AFLP data and yield, yield components, and morpho-physiological traits showed significant and positive association ($r=0.51$, $P<0.05$).

Cluster analysis

The dendrogram from UPGMA cluster analysis based on AFLP markers revealed two major clusters (Fig.9.2) at 0.63 cut-off level. The phenon level chosen provided good resolution among clusters as shown by the fairly high co-phonetic correlation value (0.76). Cluster I was found to contain 10 genotypes with genetic distance estimates ranging from 0.51 between LD-357 and Klinto x LD-357 to 0.78 between DZ-2023 and LD-357. This cluster was further divided into two subclusters. The upper most subcluster contained the moderately tolerant genotype, DZ-320 and its crosses with Klinto (susceptible parent), DZ-1691 (moderately tolerant parent), LD-357 (susceptible parent). The Euclidean genetic distance in this subcluster ranged from 0.54 between Klinto x DZ-320 and DZ-320 to 0.73 between Klinto and DZ-320. The susceptible parents, LD-357, Klinto and progenies obtained from crosses of Klinto x DZ-1691, LD-357 x DZ-1691 and LD-357 x Klinto were grouped in the second subcluster.

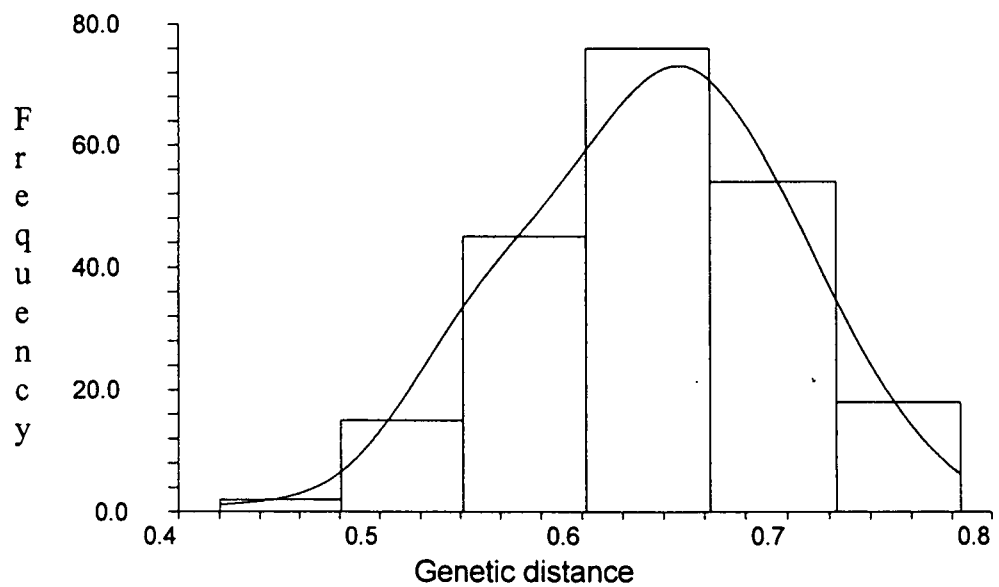

Fig. 9.1. Frequency distribution of 210 pair wise genetic distance calculated from AFLP data.

Table 9.3. Euclidean genetic distance estimates for 210 pair wise comparisons for durum wheat genotypes based on AFLP analysis (above diagonal matrix) and yield, yield components and different morpho-physiological traits evaluated under moisture stress conditions (below diagonal matrix).

entry	DZ-2023	Boohai'S'	Klinto	DZ-1691	LD-357	DZ-320	1x2	1x3	1x4	1x5	1x6	2x3	2x4	2x5	2x6	3x4	3x5	3x6	4x5	4x6	5x6
1 DZ-2023		0.63	0.72	0.73	0.78	0.68	0.49	0.51	0.49	0.52	0.54	0.63	0.63	0.69	0.63	0.67	0.73	0.7	0.72	0.7	0.66
2 Boohai'S'	0.18		0.74	0.76	0.77	0.66	0.52	0.55	0.59	0.65	0.64	0.46	0.53	0.62	0.52	0.68	0.73	0.67	0.69	0.71	0.7
3 Klinto	0.56	0.62		0.67	0.6	0.73	0.71	0.67	0.69	0.65	0.68	0.66	0.69	0.66	0.73	0.55	0.55	0.6	0.61	0.64	0.61
4 DZ-1691	0.53	0.56	0.23		0.66	0.73	0.7	0.73	0.65	0.67	0.67	0.7	0.67	0.69	0.7	0.64	0.63	0.62	0.6	0.56	0.65
5 LD-357	0.64	0.67	0.24	0.22		0.73	0.73	0.75	0.76	0.69	0.7	0.73	0.74	0.6	0.77	0.67	0.51	0.67	0.56	0.66	0.58
6 DZ-320	0.56	0.60	0.22	0.17	0.24		0.7	0.59	0.6	0.72	0.57	0.69	0.61	0.69	0.58	0.63	0.7	0.54	0.61	0.59	0.6
7 1x2	0.26	0.28	0.51	0.46	0.60	0.51		0.54	0.56	0.55	0.55	0.53	0.58	0.62	0.58	0.65	0.69	0.7	0.69	0.64	0.66
8 1x3	0.43	0.47	0.52	0.46	0.52	0.44	0.42		0.53	0.59	0.55	0.57	0.59	0.65	0.58	0.62	0.68	0.6	0.65	0.66	0.67
9 1x4	0.35	0.39	0.37	0.34	0.46	0.36	0.28	0.32		0.54	0.53	0.6	0.51	0.68	0.53	0.63	0.71	0.58	0.64	0.63	0.66
10 1x5	0.37	0.38	0.42	0.32	0.48	0.41	0.22	0.43	0.22		0.53	0.62	0.63	0.63	0.63	0.65	0.65	0.64	0.66	0.63	0.69
11 1x6	0.29	0.35	0.49	0.46	0.58	0.43	0.31	0.30	0.30	0.40		0.63	0.6	0.63	0.6	0.62	0.64	0.6	0.62	0.57	0.64
12 2x3	0.41	0.44	0.41	0.32	0.42	0.36	0.35	0.28	0.26	0.28	0.36		0.57	0.59	0.57	0.65	0.67	0.65	0.69	0.68	0.68
13 2x4	0.25	0.27	0.44	0.37	0.50	0.43	0.26	0.38	0.24	0.25	0.29	0.26		0.63	0.42	0.66	0.7	0.59	0.62	0.63	0.64
14 2x5	0.42	0.43	0.43	0.34	0.47	0.42	0.29	0.45	0.29	0.20	0.44	0.34	0.32		0.62	0.64	0.65	0.64	0.64	0.66	0.66
15 2x6	0.30	0.33	0.42	0.32	0.45	0.37	0.31	0.31	0.27	0.29	0.26	0.22	0.19	0.34		0.68	0.72	0.62	0.66	0.67	0.68
16 3x4	0.56	0.57	0.35	0.30	0.32	0.28	0.56	0.44	0.36	0.45	0.47	0.33	0.37	0.47	0.37		0.61	0.57	0.62	0.57	0.65
17 3x5	0.56	0.59	0.29	0.20	0.22	0.25	0.52	0.42	0.38	0.41	0.48	0.30	0.43	0.42	0.32	0.30		0.62	0.55	0.58	0.55
18 3x6	0.45	0.48	0.20	0.22	0.29	0.22	0.40	0.44	0.29	0.32	0.39	0.32	0.30	0.36	0.33	0.28	0.33		0.55	0.57	0.58
19 4x5	0.54	0.57	0.34	0.24	0.34	0.29	0.45	0.44	0.37	0.34	0.49	0.23	0.40	0.36	0.34	0.36	0.24	0.33		0.58	0.55
20 4x6	0.45	0.48	0.29	0.24	0.27	0.29	0.46	0.46	0.37	0.36	0.46	0.33	0.31	0.39	0.31	0.30	0.31	0.19	0.35		0.55
21 5x6	0.48	0.53	0.25	0.22	0.32	0.24	0.45	0.47	0.28	0.33	0.44	0.33	0.33	0.31	0.33	0.28	0.30	0.22	0.31	0.26	

The Euclidean genetic distance in this subcluster was found to range from 0.51 between Klinto x LD-357 and LD-357 to 0.78 between DZ-2023 and LD-357. Further bifurcation in this subcluster produced two groups; the group containing LD-357, LD-357 x Klinto and LD-357 x DZ-1691 and another one with DZ-1691 x Klinto.

Cluster II, was composed of 11 genotypes. The genetic distance spanned from 0.42 between Boohai'S' x DZ-1691 and Boohai'S' x DZ-320 to 0.77 between DZ-320 x Boohai'S' and LD-357. Two subclusters were identified. The tolerant parental line, Boohai'S' along with its progenies derived from the crosses with the rest of the parental lines, except for Boohai'S' x LD-357, were located in the same subcluster. The genetic distance in this subcluster vary from 0.420 between Boohai'S' x DZ-1691 and Boohai'S' x DZ-320 to 0.77 between the cross of Boohai'S' x DZ-320 and LD-357. The subcluster was further grouped into two major groups. The parental line, Boohai'S' and its progenies with DZ-2023 and Klinto were grouped together, whereas its progenies with DZ-320, DZ-1691 and LD-357 were grouped together in another group. The other major subcluster was made up of the parental line, DZ-2023 and its progenies derived from crosses of all the other parents, except for DZ-2023 x Boohai'S'. The genetic distance estimates in this subcluster vary from 0.49 between DZ-1691 x DZ-2023 and DZ-2023 to 0.76 between DZ-2023 x DZ-1691 and LD-357. Further bifurcation in this subcluster led to the formation of the group with DZ-2023 and its progenies obtained from crosses with both Klinto and DZ-1691, and another group with its progenies resulted from the cross of LD-357 and DZ-320. The parental line, DZ-1691 (moderately tolerant) appeared to be distinct from all genotypes in cluster I while cross of Boohai'S' x LD-357 appeared to be distinct from the rest in cluster II (Fig. 9.2).

Cluster analysis based on yield, yield components, total biomass yield, HI, water use efficiency (WUE) based on grain yield (WUE_G), based on total dry matter (WUE_{TDM}), transpiration efficiency both on the basis of grain yield (T_G) and dry matter production (T_{TDM}), relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio

(LWR) gave two major clusters denoted by I and II (Fig. 9.3). The Euclidean genetic distance estimates for the first cluster (I) ranged from 0.17 between DZ-1691 and DZ-320 to 0.67 between Booha'S' and LD-357. (Table 9.3). Further bifurcation of this cluster showed four clear groupings in which the first group encompassed two genotypes, DZ-1691 x LD-357 and Klinto x Booha'S'. The second group also contained three genotypes; the susceptible parental line, LD-537 and its cross with the other susceptible line, Klinto and DZ-1691 x Klinto were grouped together. DZ-320 x DZ-1691, Klinto x DZ-320 and DZ-320 x LD-357 were grouped together. The susceptible parental line, Klinto and moderately tolerant types DZ-1691 and DZ-320 were grouped together, however, further grouping resulted in a subgroups consisting of DZ-320 and DZ-1691 and Klinto alone.

Cluster II was composed of 10 genotypes placed into two subclusters. DZ-2023 x DZ-320 and DZ-2023 x Klinto were found in the first subcluster. The second subcluster was composed of eight genotypes, the two tolerant parental lines, DZ-2023 and Boohai'S', crosses of Boohai'S' x with DZ-1691, LD-357 and DZ-320 as well as crosses of DZ-2023 with Boohai'S', DZ-1691 and LD-357. The genetic distance estimates in this subcluster ranged from 0.18 between Boohai'S' x DZ-2023 to 0.64 between LD-357 and DZ-2023 (Table 9.3). Three distinct groups were identified under this subcluster. The parental lines, DZ-2023 and Boohai'S' were grouped together. Crosses of DZ-2023 with Boohai'S' and LD-357 as well as cross of Boohai'S' with LD-357 were grouped together with tight linkage between crosses of LD-357 with both DZ-2023 and Boohai'S'. Crosses of DZ-1691 with DZ-2023 and Boohai'S' and DZ-320 x Boohai'S' were placed in separate groups. Further bifurcation in this group resulted in tight linkage between Boohai'S' x DZ-320 and Booha'S' x DZ-1691.

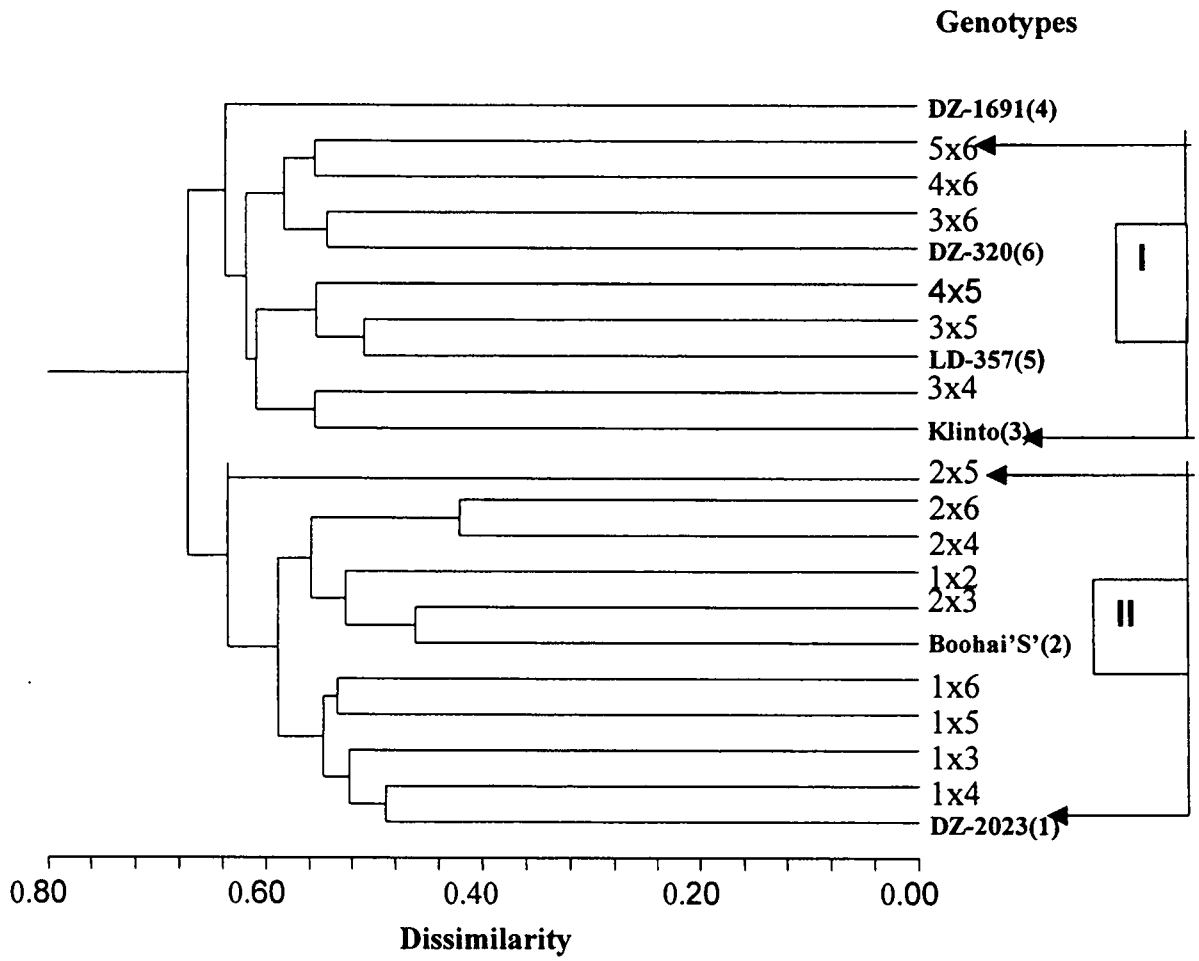

Fig. 9.2. Dendrogram depicting genetic relationships based on AFLP analysis, among durum wheat genotypes differing in drought tolerance and their progenies produced from all possible combinations. The number in brackets denotes parental line.

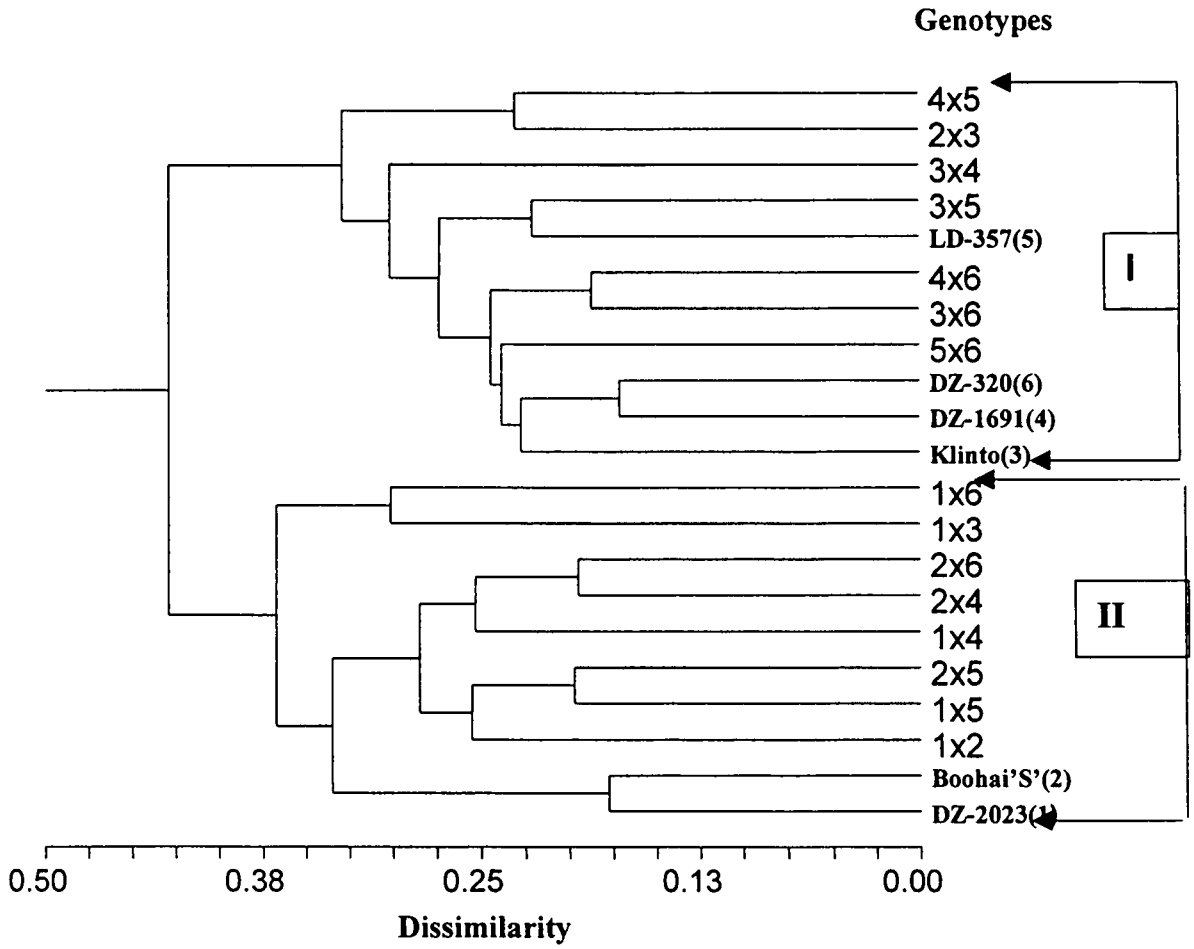

Fig. 9.3. Dendrogram depicting genetic relationships based on grain yield, yield components, and different morpho-physiological traits evaluated under stress conditions among durum wheat genotypes differing in their responses to moisture stress. The number in the brackets denotes parental lines.

Association of DNA fragments with yield, yield components, and morpho-physiological traits

Out of a total of 465 fragments obtained from the six primer combinations (Table 9.1), on average, approximately 65% of the fragments were polymorphic. However, only 11 fragments were strongly related to yield, yield related traits and/or morpho-physiological traits evaluated under moisture deficit stress conditions using simple correlation analysis (Table 9.4). AFLP-fragment with the size of 71 bp was significantly correlated (r= 0.49 and 0.50, $P<0.05$) with kernel number per spike and kernel weight, respectively. AFLP 71 bp fragment was highly and positively correlated with yield (r=0.85) and HI (r=0.85) ($P<0.01$). It had also a significant and positive correlation with NAR (r=0.47); $P<0.05$) and RGR (r=0.79; $P<0.01$). Its correlation with WUE_{TDM} and WUE_G was also highly significant. Its correlation with drought susceptibility index (S) was negative and significant (r=-0.63; P=0.01). Strong correlation between fragment 160 bp with the most important yield components, kernel number per spike and kernel weight resulted in strong correlation between grain yield and this AFLP fragment (Table 9.4). This was concomitantly expressed in its strong negative correlation (r=-0.59, $P<0.01$) with drought susceptibility index (S). This fragment was strongly correlated (r= 0.76; 0.74; 0.68; 0.74; $P<0.01$) with RGR, HI, T_G and WUE_G, respectively. Its correlation with WUE_{TDM} was also positive and significant (r=0.47). Positive and significant correlation (r=0.75, $P<0.01$) was also noted between the presence of band 71 bp and 160 bp (Table 9.4). Regardless of the absence of any significant correlation between fragment 220 bp and any of the yield components, the 220 bp fragment was significantly (r=0.61, $P<0.01$) correlated to grain yield. Consequently, its correlation with S was also significantly high and negative (r=-0.66). It also had a positive and significant correlation with HI (r=0.45) and WUE_G and T_G r=0.60 and 0.50, respectively. However, the 220 bp fragment had no significant correlation with RGR or with its components, except for SLA (r=0.47; $P<0.05$).

Table 9.4 Spearman rank correlation coefficients among AFLP fragments (denoted by 'X' and band length in base pair), yield, yield components and various morpho-physiological traits evaluated under stress conditions.

	X160	X220	X223	X266	X272	X276	X295	X331	X386	X398	Spike#	Spklt#	Kernel#	Kernel wt wt	Yield	S	RGR	NAR	SLA	LWR	LAR	TDM	HI	WUE_{TDM}	T_{TDM}	T_G	WUE_G
X71	0.75*	0.36	0.91**	0.83**	0.59*	0.34	0.59*	0.63*	0.55*	0.36	0.30	0.23	0.49*	0.50*	0.85**	-0.63**	0.79**	0.47*	0.19	-0.35	0.33	0.41	0.85**	0.72**	0.57*	0.87**	0.87**
X160		0.21	0.68**	0.91**	0.44	0.25	0.44	0.62*	0.39	0.44	-0.06	0.10	0.52*	0.68**	0.68**	-0.59**	0.76**	0.39	0.16	-0.22	0.32	0.29	0.74**	0.47*	0.36	0.68**	0.74**
X220			0.42	0.26	0.74**	0.58*	0.21	0.48*	0.44	0.48*	0.42	0.30	0.24	0.20	0.61**	-0.66**	0.13	-0.15	0.47*	-0.07	0.15	0.31	0.45*	0.41	0.42	0.50*	0.60**
X223				0.75**	0.65**	0.37	0.65**	0.51*	0.48*	0.19	0.38	0.21	0.37	0.51*	0.83**	-0.57*	0.64**	0.38	0.13	-0.31	0.24	0.38	0.80**	0.63**	0.48*	0.78**	0.80**
X266					0.48*	0.28	0.48*	0.68*	0.31	0.48*	0.06	0.22	0.46*	0.57*	0.75**	-0.53*	0.84**	0.52*	0.19	-0.26	0.33	0.37	0.76**	0.56*	0.35	0.67**	0.77**
X272						0.58*	0.21	0.71*	0.44	0.48*	0.39	0.24	0.42	0.15	0.74**	-0.54*	0.31	-0.11	0.51*	-0.08	0.26	0.30	0.61**	0.49*	0.36	0.59**	0.71**
X276							0.20	0.41	0.25	0.20	0.43	0.32	0.38	-0.11	0.51*	-0.40	0.21	-0.11	0.32	0.27	0.40	0.19	0.48*	0.46*	0.27	0.46*	0.51*
X295								0.02	0.21	-0.05	0.30	-0.11	0.31	0.26	0.52*	-0.46*	0.52*	0.44*	0.01	-0.23	0.22	0.17	0.60**	0.34	0.21	0.52*	0.52*
X331									0.41	0.71**	0.11	0.30	0.45*	0.32	0.66**	-0.40	0.57*	0.11	0.53*	-0.09	0.39	0.19	0.64**	0.41	0.36	0.60**	0.69**
X386										0.21	0.23	0.41	0.42	0.37	0.63**	-0.53*	0.34	-0.05	0.50*	-0.37	0.47*	0.49*	0.54*	0.44	0.69**	0.75**	0.62**
X398											0.04	0.08	0.30	0.44*	0.44*	-0.42	0.50*	0.22	0.36	0.17	0.35	0.04	0.43	0.25	0.33	0.42	0.52*
Spike#												-0.13	-0.28	-0.18	0.37	-0.29	0.01	-0.01	0.10	0.16	0.14	0.23	0.16	0.49*	0.57*	0.47*	0.37
Spklt#													0.27	-0.01	0.27	-0.13	0.20	0.12	0.16	-0.14	0.07	0.24	0.25	0.10	0.05	0.13	0.23
Kernel#														0.18	0.64**	-0.37	0.53*	0.04	0.58*	-0.27	0.59**	0.36	0.74**	0.34	0.00	0.46*	0.59**
Kernel wt wtwtwtwt															0.41	-0.51*	0.54*	0.41	-0.02	-0.28	0.18	0.10	0.48*	0.19	0.42	0.52*	0.45*
Yield																-0.73**	0.65**	0.19	0.54*	-0.30	0.54*	0.63**	0.90**	0.74**	0.50*	0.85**	0.95**
S																	-0.45*	-0.21	-0.21	0.17	-0.18	-0.47*	-0.62**	-0.65**	-0.57*	-0.72**	-0.78**
RGR																		0.74**	0.15	-0.37	0.46*	0.08	0.82**	0.41	0.39	0.71**	0.71**
NAR																			-0.45*	-0.20	-0.06	-0.24	0.41	0.07	0.17	0.30	0.25
SLA																				-0.27	0.73**	0.44	0.41	0.27	0.17	0.38	0.46*
LWR																					-0.10	-0.19	-0.34	-0.11	-0.18	-0.32	-0.25
LAR																						0.30	0.58*	0.29	0.25	0.53*	0.48*
TDM																							0.32	0.72**	0.30	0.38	0.53*
HI																								0.52*	0.35	0.82**	0.86**
WUE_{TDM}																									0.66**	0.72**	0.81**
T_{TDM}																										0.81**	0.64**
T_G																											0.90**

There was significant correlation between AFLP fragment 220 and 71 and 160 bp fragments. The 223 bp fragment was positively and significantly correlated with kernel weight, grain yield, RGR, HI, and all components of water use efficiency. Its presence was also positively and significantly correlated with 71 and 160 bp fragments (Table 9.4). There was significant and positive correlation between fragment 266 bp and kernel number per spike (r=0.46), kernel weight (r=0.57) and grain yield (r=0.75, P<0.01). As a result, the correlation between S and 266 bp band was negative and significant (r=-0.53). There was significantly high correlation between this band and HI (r=0.76) as well as WUE_G and T_G (Table 9.4). It had no significant correlation with any of the components of RGR, except for NAR (r=0.52; P<0.05). Its correlation with RGR itself, however, was significantly high (r=0.84). Presence of the 266 bp fragment was significantly and positively correlated with the presence of 71,160 and 223 bp fragments. None of the correlations between 272 bp fragment and yield components were significant. However, the correlation between the 272 bp fragment and grain yield was significantly high (r=0.74;P<0.01). A significant, but negative correlation existed between the 272 bp band and S. The correlation between the 272 fragment and all components of water use and transpiration efficiency was significant and positive except for the correlation with T_{TDM}. The correlation between HI and the 272 fragment was also positive and significant. Nevertheless, none of growth related traits were significantly correlated with this band, except for SLA (Table 9.4). The fragment was positively correlated with 71, 220, 223 and 266 bp fragments (r=0.59, 0.74, 0.65 and 0.48, respectively). Fragment 276 bp was positively correlated with grain yield but did not correlate with S or any of the yield components. It had, however, a positive and significant correlation with HI, WUE_G, WUE_{TDM} and T_G. It had significant correlation neither with RGR nor with any of the components of RGR. Its presence was positively correlated only with the 71 and 272 bp fragments. The AFLP fragment, 295 bp had positive and significant correlation with grain yield, RGR, NAR, HI, WUE_G and T_G. It also had significant but negative correlation with S (Table 9.4). The presence of this band was found to be significantly and positively correlated with 71, 223 and 266 bp fragments. The 331 bp AFLP fragment was significantly and positively correlated with kernel number per spike (r=0.45) and grain yield (r=0.66). Its correlation with S, however, was insignificant. None of its

association with components of RGR was significant, except for SLA though the correlation with RGR itself is positive and significant. It had, however, positive and significantly high correlation with WUE_G and T_G as well as HI (Table 9.4). Its presence was significantly and positively correlated with all AFLP fragments, except for the 276 and 295 bp fragments. The presence of the 386 bp fragment was positively and significantly correlated with grain yield ($r=0.63$; $P<0.01$), but not to other yield components. This fragment was significantly but negatively correlated with S. It also had positive and significant correlation with TDM, HI, T_{TDM}, WUE_G and T_G (Table 9.4). Its correlation with SLA and LAR was positive and significant (r= 0.50 and 0.47, respectively). It had, however, no significant correlation with any of the other AFLP fragments found to be associated with the various traits, except for the 71 and 223 bp fragment. AFLP fragment 398 was correlated significantly to kernel weight and yield, but its correlation to S was insignificant. It had no significant correlation with any of the components of RGR, but its correlation with RGR was significant and positive. It was also not correlated with TDM or HI as well as any of the water use efficiency measures, except for WUE_G . The presence of this fragment was positively correlated with AFLP fragments 220, 226, 272 and 331.

9.5. Discussion

The application of DNA-based technology in the improvement of drought tolerance can improve the precision and efficiency of breeding programs (Falconer and Mackey, 1996). Nevertheless, MAS for development of drought tolerant varieties in many crops, including wheat is far from routine use in breeding programs. The main reasons for this are problems in establishing selection criteria which can lead to improved yield under moisture limiting environments (Ludlow and Muchow, 1990), a lack of a clear understanding of the relationships between morpho-physiological traits with grain yield and adaptation in dry land environments (Blum, 1996; Saini and Westgate, 2000), poor definition of the target environment (Fukai *et al.*, 1995), limited information on the genetics and inheritance of those traits (Ludlow and Muchow, 1990; Gupta, 1997) and the complex inheritance patterns of drought tolerance

(Courtois *et al.*, 2000). Identification of QTL linked to drought tolerance or traits associated with drought tolerance requires the identification of traits related to drought tolerance as well as adequate genetic variability for the trait (Koebner and Snape, 1999).

In this study, the high level polymorphism between genotypes confirms the usefulness of the AFLP markers in the study of genetic relationships (Salamini *et al.*, 1997; Barrett and Kidwell, 1998; Dommini *et al.*, 2000). The normal distribution of genetic distances based on AFLP analysis also implies the presence of sufficient genetic divergence within the genotypes evaluated (Manifesto *et al.*, 2001). Parental lines used to generate progeny lines were genetically distinct as revealed by the cluster analysis (Fig 9.2). As a result, a wide range of genetic diversity was obtained in progeny of pairwise crosses.

The mean genetic distance obtained in this study closely related to earlier reports based on AFLP studies in spring wheat x winter wheat crosses (Barret and Kidwell, 1998) and German and Australian winter grown cultivars (Bohn *et al.*, 1999). Significant and positive correlation between genetic distances for all pair wise comparisons based on AFLP data and yield, yield components, and other morpho-physiological data showed differences in the performance of genotypes due to genetic differences. A similar study in rice showed that difference in performance among genotypes differing in their response to moisture stress was also due to their genetic difference (Mathew *et al.*, 2000). Nachit *et al.* (2000) demonstrated that differences in adaptation and productivity in dry land environments in durum wheat is strongly correlated with genetic variability based on AFLP data.

The results of cluster analysis on the basis of the AFLP and other agro-morphological features evaluated under stress conditions were similar. However, clustering of genotypes based on yield, yield related and other traits was not identical with the AFLP data. This is probably due to the confounding effects of the various traits examined as well as the inconsistency of genotype rankings with respect to the different traits. Expression of drought tolerance in various genotypes has been noted due to complex physiological mechanisms that are related to different morpho-physiological traits (Levitt, 1980; Blum, 1988). The relevance of any of these traits to the adaptation and productivity under moisture limiting environments varies depending on the genotype and the

target environment (Ludlow and Muchow, 1990; Blum, 1996). Thus, genotypes could cluster in the same or different groups depending their response to the traits examined regardless of how similar or distinct they might be genetically, since two genotypes could have the same genotype but a different phenotype or *vice versa (*Kearsey and Farquhar, 1998*)*. For instance, the AFLP data in this study showed that the susceptible and moderately tolerant parental lines, LD-357, Klinto, DZ-1691 and DZ-320 were genetically distinct. However, the dendrogram constructed from the agro-morphological traits showed that they belong to the same major cluster.

The AFLP fragments identified to be associated with yield, yield related traits and other morpho-physiological characters were shown to affect more than one character (Table 9.4). Moreover, the presence of bands with strong association with those traits was also significantly correlated with other bands with similar effects (Table 9.4). This suggests that the identified fragments represent genes or flanking DNA with pleiotropic effects that are associated with drought tolerance. Strong associations among bands affecting the same or different traits also imply that these AFLP fragments could be linked genetic loci. Thus, bands that had strong correlation to grain yield, also had strong correlations with HI, RGR, WUE_G and T_G. These traits are strongly and positively correlated to yield under stress conditions (Ludlow and Muchow, 1990; Gupta, 1997; Richards *et al.*, 2002). It was also observed that bands which were strongly correlated with grain yield were also strongly but negatively correlated with drought susceptibility index (S), since S quantifies the proportion of yield reduction under stress conditions compared to the non stress yield in relation to the overall yield performance of all genotypes under stress to non stress conditions (Fischer and Maurer, 1978). Thus, genetic factors that affect yield positively under stress should result in low values of S. The fragments which were found to be strongly affiliated with drought tolerance either directly or indirectly via other traits with strong influence on performance under stress could be potential DNA markers representing inter or intra allelic loci with possible linkage to drought tolerance.

9.6.Conclusions

Future research, however, is needed to investigate the linkage, segregation and inheritance pattern of traits with strong correlation with AFLP fragments with the purpose of identifying and locating QTLs; thereby developing appropriate mapping populations and to identify traits amenable to screening for their use in the development of QTL linked to drought tolerance and MAS.

9.7. References

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CHAPTER 10

Summary

In order to identify yield components associated with drought tolerance in durum wheat and evaluate the performace of Ethiopian durum wheat genotypes, 26 durum wheat genotypes, from different agro-ecologies of Ethiopia were evaluated under simulated moisture stress conditions. Stress caused dramatic reductions in grain yield and harvest index. Yield was significantly correlated primarily with number of kernels per spike and 100 kernel weight. Further decomposition of simple correlation coefficients into direct and indirect effects showed that number of kernels per spike and 100 kernel weight had the largest direct effects on grain yield, under both stressed and non-stressed conditions.

The expression of drought tolerance in the F_1 generation obtained from all possible combinations among genotypes differing in their responses to moisture deficit stress was studied. Analysis of variance revealed significantly high variability among genotypes for yield, yield components and drought tolerance measurements due to the effects of treatments. Drought tolerance was expressed in the crosses involving tolerant parents. The diallel analysis showed that mean squares for both GCA and SCA were significantly high at both moisture regimes. GCA:SCA ratios indicated predominance of additive gene action for all characteristics positively correlated with grain yield under stress or negatively correlated with drought susceptibility index.

Differences in water use and transpiration efficiencies and interrelationships among water use and transpiration efficiencies and associated traits were investigated among durum wheat genotypes with differing responses to moisture stress. Significantly high genotypic variability in the amount of cumulative water used before (ETba) and after (ETpa) anthesis was observed.

Susceptible genotypes used higher amounts of water before anthesis and lower amounts after anthesis. In contrast, tolerant genotypes used a higher proportion of water during the post-anthesis period. Significantly high variability among the genotypes was observed for various measures of water use and transpiration efficiencies, total dry matter and harvest index. Ranking of cultivars for water use efficiency based on grain yield (WUE_G) and transpiration efficiency based on grain yield, was consistent with ranking of cultivars for drought susceptibility indices. Drought susceptibility index was significantly but negatively correlated with harvest index, WUE_G and grain yield. However, it was positively and significantly correlated with the ETba:ETpa ratio. A high positive correlation of WUE_G with harvest index and grain yield with harvest index was found. Differences in flag leaf water potential were significant only for genotypes under stress treatments. Water potential declined with age under both treatment conditions, however, the fall was faster in stress sensitive types than in tolerant types in more advanced growth stages.

Inheritance of water use and transpiration efficiencies was studied in a hybrid population obtained from six parents, selected for their different responses to moisture stress. GCA and SCA effects were significant at both the moisture levels. The interactions of GCA and SCA with moisture levels were also highly significant. Analysis of the genetic components of variation demonstrated that WUE and T were under the control of additive and dominance type of genes. Narrow sense heritability estimates for water use and transpiration efficiencies based on grain yield (WUE_G) were higher at the moisture stress level. Measures of water use and transpiration efficiencies showed significantly high and positive genotypic and phenotypic correlations among them as well as with grain yield and harvest index.

The impact of the effect of moisture stress on growth and phenological development was examined among durum wheat genotypes differing in their tolerance to moisture stress. Drought stress was found to delay major growth stages and shorten the grain filling period. Drought tolerant genotypes had fast early growth, whereas susceptible ones had slow RGR initially. Variation in RGR

was associated with NAR and LAR. Differences in hybrid performance were due to significant GCA and SCA effects. Interactions of GCA and SCA with moisture level were also highly significant. Moderate to high levels of broad sense heritability estimates were found for most of the traits. Significantly high genetic and phenotypic correlations between NAR and RGR, and LAR and RGR were found. The genetic and phenotypic correlations of grain yield with total dry matter, harvest index, RGR and LAR were significant.

The effect of moisture stress on the content of water soluble carbohydrate (sucrose and D-glucose) was investigated in durum wheat (*Triticum turgidum* L. var. *durum)* above ground organs to assess and characterise the responses of genotypes with differing responses to drought stress. In all organs examined, drought tolerant genotypes accumulated more glucose and sucrose, particularly, at an early age. Stress caused an overall increase in the level of these carbohydrates, but the levels were highest in the stems compared to other organs. Level of drought (drought susceptibility index, 'S') was strongly related with the level of carbohydrates in the various plant organs

Genetic differences among genotypes with differing responses to drought stress and their progenies obtained from all possible cross combinations were assessed using AFLP markers. Moderate to high level polymorphisms were obtained with the primer combinations evaluated. Euclidean genetic distance estimates showed that there was considerable genetic diversity among the materials studied. The correlation coefficient for pair wise genetic distance estimates based on AFLP data and yield, yield components, and morpho-physiological traits was significant and positive. Cluster analysis showed that grouping of genotypes on the basis of AFLP data and agro-morphological data fairly agreed. A number of AFLP fragments were significantly correlated with the various traits evaluated under stress conditions. AFLP fragments were observed to affect a number of traits that were strongly correlated with drought susceptibility index.

Opsomming

Om opbrengs komponente te bepaal wat geassosieër word met droogte toleransie in durum koring en om die prestasie van Etiopiese durum koring genotipes te bepaal, is 26 durum koring genotipes van verskillende agro-ekologiese gebiede van Etiopië geëvalueer onder gesimuleerde vog stremmings toestande. Stremming het dramatiese afnames in graan opbrengs en oes indeks veroorsaak. Opbrengs was betekenisvol gekorreleer hoofsaaklik met aantal korrels per aar en 100 korrel massa. Verdere ontleding van eenvoudige korrelasie koefissiënte in direkte en indirekte effekte het getoon dat korrels per aar en 100 korrel massa die grootste direkte effekte het op graan opbrengs onder beide stremmings en nie-stremmings toestande.

Die uitdrukking van droogte toleransie in die F1 generasie verkry van alle moontlike kombinasies tussen genotipes wat verskil in hulle respons op vog stremming is bestudeer. Variansie analise het betekenisvolle variasie tussen genotipes vir opbrengs, opbrengs komponente en droogte toleransiemetings getoon a.g.v. die effek van die behandelings. Droogte toleransie is uitgedruk in die kruisings met tolerante ouers. Die dialleel analise het aangetoon dat gemiddelde kwadrate vir beide GCA en SCA betekenisvol hoog was by beide vog vlakke. GCA:SCA verhoudings het predominansie van additiewe geenaksie aangetoon vir alle eienskape positief gekorreleer met graan opbrengs onder stremming of negatief gekorreleer met droogte vatbaarheids indeks.

Verskille in water verbruik en transpirasie effektiwiteit en inter verwantskappe tussen water verbruik en transpirasie effektiwiteit en geassosieerde eienskappe is ondersoek in durum genotypes met verskillende reaksies op vog stremming. Betekenisvolle genotipiese variasie in die hoeveelheid kumulatiewe water gebruik voor (Etba) en na (Etpa) antese is gesien. Vatbare genotipes het groter hoeveelhede water gebruik voor antese en kleiner hoeveelhede na antese. In kontras, het tolerante genotipes 'n hoër proporsie water gebruik in die post-antese tyd. Betekenisvolle hoë variasie

tussen genotipes is gesien vir verskillende metings van water gebruik en transpirasie effektiwiteit, totale droë material en oes indeks. Rangordes van cultivars vir water verbruik effektiwiteit gebasseer op graan opbrengs (WUE_G) en transpirasie effektiwiteit gebasseer op graan opbrengs was in orreenstemming met rangordes van cultivars vir droogte vatbaarheidsindekse. Droogte vatbaarheids indeks was betekenisvol maar negatief gekorreleer met oes indeks, WUE_G en graan opbrengs. Dit was egter positief en betekenisvol gekorreleer met die Etba:Etpa verhouding. 'n Hoë positiewe korrelasie van WUE_G met oes indeks en graan opbrengs met oes indeksis is gevind. Verskille in vlagblaar water potensiaal was betekenisvol slegs vir genotipes onder vog stremming. Water potensiaal het afgeneem met ouderdom by beide behandelings vlakke, maar die afname was vinniger in stremmings sensitiewe tipes as in tolerante genotipes in meer gevorderde groei stadia.

Oorerflikheid van water verbruik en transpirasie effektiwiteit is bestudeer in 'n baster populasie verkry vanaf ses ouers, geselekteer vir verkskillende reaksies op vog stremming. GCA en SCA effekte was betekenisvol by beide vog vlakke. Die interaksie van GCA en SCA met vog vlakke was ook hoogs betekenisvol. Analise van genetiese komponente van variasie het getoon dat WUE en T onder beheer van additiewe en dominante gene was. Nou-sin oorerflikheids bepalings vir water verbruik en transpirasie effektiwiteit gebasseer op graan opbrengs (WUE_G) was hoër by die vog stremmings vlak. Metings van water verbruik en transpirasie effektiwiteit het betekenisvolle hoë en positiewe genotipiese en fenotipiese korrelasies getoon tussen hulle en met graan opbrengs en oes indeks.

Die impak van die effek van vog stremming op groei en fenologiese ontwikkeling is ondersoek in durum genotipes wat verskil t.o.v. toleransie vir vog stremming. Droogte stremming het die hoof groei fases vertraag en het die graanvul periode verkort. Droogte tolerante genotipes het vinnige vroeë groei, en vatbares het lae RGR getoon aanvanklik. Variasie in RGR is geassosieer met NAR en LAR. Verskille in baster prestasie was a.g.v. betekenisvolle GCA en SCA effekte. Interaksies van GCA en SCA met vog vlak was ook hoogs betekenisvol. Gemiddeld tot hoë vlakke van breë sin oorerflikheid is bereken vir meeste eienskappe. Betekenisvolle hoë genetiese en fenotipiese korrelasies tussen NAR en RGR, en LAR en RGR is gevind.

Die genetiese en fenotipiese korrelasies van graan opbrengs met totale droë material, oes indeks, RGR en LAR was betekenisvol.

Die effek van vogstremming op die inhoud van water oplosbare koolhidrate (sukrose en D-glukose) is ondersoek in durum koring (*Triticum turgidum var durum L)* bogrondse organe om die reaksie van genotipes te bepaal en te karakteriseer wat verskil t.o.v. hulle reaksie op vog stremming. In alle organe wat ondersoek is, het droogte tolerante genotipes meer glukose en sukrose geakkumuleer, veral op 'n vroeë groeistadium. Stremming het 'n algehele toename in die vlak van hierdie koolhidrate veroorsaak, maar die vlakke was die hoogste in die stamme in vergelyking met ander organe. Die vlakke van droogte (droogte vatbaarheids indeks 'S') was sterk gekorreleer met die vlak van koolhidrate in die verskillende plant organe.

Genetiese verskille tussen genotipes wat verskil t.o.v. hulle reaksie op vog stremming en die nageslagte wat verkry is van alle moontlike kruisings kombinasies is geëvalueer met AFLP merkers. Gemiddeld tot hoë vlakke van polimorfismes is gekry met die priemstuk kombinasies wat gebruik is. Euklidiese genetiese afstands bepalings het getoon dat daar groot hoeveelhede genetiese diversiteit is binne die getoetsde materiaal. Die korrelasie koeffisiënt vir paarsgewyse genetiese afstand bepalings gebasseer op AFLP data en opbrengs, opbrengs komponente en morfofisiologiese eienskappe was betekenisvol en positief. Tros analise het getoon dat groepering van genotipes op die basis van AFLP data en agro-morfologiese data redelik ooreenstem. 'n Aantal AFLP fragmente is gevind wat betekenisvol gekorreleer is met verskillende eienskappe wat geëvalueer is onder stremmings toestande. AFLP fragmente het 'n aantal eienskappe geaffekteer wat sterk gekorreleer is met droogte vatbaarheids indeks.