African Journal of Biotechnology Vol. 7 (14), pp. 2341-2352, 18 July, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

*Full Length Research Paper*

# Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes

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Accepted 16 June, 2008

**With a view to understanding the traits which can be used as a quick criteria for drought tolerance, field and laboratory experiments were used to evaluate nine wheat (*Triticum aestivum* L.) genotypes; seven local varieties with two introduced genotypes from International Center for Agricultural Research in the Dry Areas (ICARDA). The field experiment was grown under two water regimes (stress and non stress treatments). The stress treatment induced by withholding irrigation after emergence and giving two supplementary irrigations, one after 60 days post-sowing and the other after 90 days post-sowing and non stress (well-watered). Combined analysis of variance over two seasons showed highly significant differences among wheat genotypes in all the studied traits and water stress decreased them significantly. The superior genotypes 1,2 and 6 which gave higher relative water content (RWC) accumulated more free proline (Pro) and had lower drought susceptibility index (*S)* values, whereas genotypes 3, 4 and 9 had the lowest RWC, Pro accumulation and had the highest *S* values. Indicating that accumulated Pro acts as a compatible solute regulating and reducing water loss from the cell during episodes of water deficit. High RWC and Pro over-accumulation were recognized as beneficial drought tolerance indicators and may be used as selection criteria in wheat breeding program. Effects of drought stress in laboratory experiment were induced by polyethylene glycol (PEG) (0, 15 and 25%, with three replicates) and applied on germination of wheat genotypes seeds. The PEG induced a drop in the shoot, root biomass and coleoptiles length which was the greatest in genotypes 3, 4 and 9, while the decrease in genotypes 1, 2 and 6 was little under the various levels from PEG. The variability of leaf-proteins was analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). It is concluded that leaf protein profiles could be useful marker in the studies of genetic variation and classification of adapted cultivars under control and stress conditions.**

**Key words:** Wheat, drought tolerance, proline, relative water content, polyethylene glycol, protein electrophoresis.

## INTRODUCTION

Drought is a worldwide problem, constraining global crop production seriously and recent global climate change has made this situation more serious (Pan et al., 2002). Drought is a complex physical-chemical process, in which many biological macromolecules and small molecules are involved, such as nucleic acids (DNA, RNA, microRNA), proteins, carbohydrates, lipids, hormones, ions, free radicals and mineral elements. Drought is connected with almost all aspects of biology. Currently, drought study has been one of the main directions in global plant biology and biological breeding.

The impacts of drought condition on grain development and yield of crops depend on their severity and the stage of plant growth during which they occur. Seedling emergence is one stage of growth that is sensitive to water deficit. Therefore, seed germination, vigor and coleoptiles length are prerequisites for the success of stand establishment of crop plants. Under semiarid regions, low moisture is limiting factor during germination.

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The rate and degree of seedling establishment are extremely important factors in determine both yield and time of maturity (Rauf et al., 2007). Some researches demonstrated the importance of coleoptiles length (protective sheath that covers the shoot during emergence) in achieving optimum fall and establishment (Dilday et al., 1990), particularly when seed is planted deep to reach moisture in dry soils. Consequently, there is need to improve the genetic tolerance of crops at the seedlings stages.

Selection for drought tolerance at early stage of seedlings is most frequently carried out by including chemical drought induced molecules like poly ethylene glycol (PEG 6000) in the medium. Lagerwerff et al. (1961) indicated that PEG can be used to modify the osmotic potential of nutrient solution culture and thus induce plant water deficit in a relatively controlled manner, appropriate to experimental protocols (Money, 1989; Zhu et al., 1997). Polyethylene glycol molecules with a $M_r$ ≥6000 (PEG6000) are inert, non-ionic and virtually impermeable chains that have frequently been used to induce water stress without causing physiological damage and maintain uniform water potential through out experiment periods (Lu and Neumann, 1998). Molecular of PEG 6000 are small enough to influence the osmotic potential but large enough to not be absorbed by plant and not expected to penetrate intact plant tissues rapidly (Carpita et al., 1979). Because PEG does not enter the apoplast, water is withdrawn from the cell. Therefore, PEG solution mimic dry soil more closely than solutions of low $M_r$ Osmotica, which infiltrate the cell wall with solutes (Veslues et al., 1998).

Wheat (*Triticum aestivum* L.) is a staple food for more than 35% of the world population and it is also the first grain crop in Egypt. Improvement of grain yield in wheat has traditionally relied on direct selection for this trait (Braun et al., 1992). Development of stress tolerant varieties is an objective of many breeding programs, but success has been limited by adequate screening techniques, and the lack of genotypes that show clear differences in response to well defined environmental stresses. Therefore, wheat breeders are always looking for means and sources of genetic improvement for grain yield and other agronomic traits. The adoption of new technologies such as molecular markers may help in achieving some of the goals to increase food production. To achieve this goal, modern plant breeding teams are endeavoring to integrate new plant biotechnology methods with traditional breeding techniques based on classical genetics.

Plant breeding efforts to improve drought tolerance would be aided by the identification of biochemical markers associated with improved field performance under drought condition. In fact, stress tolerance in plants has long been accepted as a mutagenic trait dependent on the coordinated expression of certain genes and the silencing of others (Romo et al., 2001). The expression of these genes is influenced by multifarious environmental factors that the products of these gene including proteins and transcription factors, can directly protect cells from dehydration and regulate gene expression and signal transduction in phosphoinositide metabolism (Shinozaki and Yamaguchi, 1997; Foolad, 2004).

Changes in their expression can be detected by studying the protein pattern of expression. Recently, the numbers of available molecular markers recognized for use in define which genes are regulatory and which are primary gene products positively contributing to stress tolerance includes isozyme, total protein, seed protein, RAPDs, AFLPs and microstellites. DeVienne et al. (1999) have been studied drought tolerance by using larg-scale 2-D gel electrophoresis; they quantify protein spot intensities and these mapped as protein quantity loci in maize. Therefore, the utility of molecular markers for example protein patterns, is in their lineage to genes of economic important and can be examined for possible association to important traits such as disease resistance and drought resistance (Torkpo et al., 2006).

However, electrophoresis markers could provide an indirect method for genome probing by exposing structural variation in protein banding patterns (Cooke, 1984). It could be useful for identification, characterization and to screen the variability present among population, produced through either *in vivo* or *in vitro* method and to select the desirable genotypes under control or stress conditions of particular genotypes (Payne et al., 1981; Akpabio, 1988; Liioh, 1990).

The present study was carried out in attempt to (I) find a speed and ease technique for screening wheat genotypes for drought tolerance, (ii) quantify associations between traits and yield responses to drought, (iii) investigate the physiological bases of any associations between traits and yield responses to drought, (iv) ascertain whether genotypes which treated with polyethylene glycol treatment evokes qualitatively similar effects as those under water stress, and (v) employ gel electrophoresis of protein in the leaves of nine varieties of wheat to evaluate the genetic variability under drought conditions.

## MATERIALS AND METHODS

### Plant materials

Nine bread wheat genotypes (*T. aestivum L.*), in Table 1 seven local varieties Giza 168, Giza 163 , Sahel 1, Gemmeza 7, Gemmeza 9, Sakha 69, Giza 167 with two introduced genotypes from ICARDA (Rufom-5 and Kavco-8) were used in two experiments, field and laboratory experiments to find a reproducible, fast and easy technique for screening wheat genotypes for drought tolerance

### The field experiments

The experiments were conducted at the Experimental Farm, Faculty of Agriculture Suez Canal University, Ismailia, Egypt during 2005/2006 and 2006/2007 seasons. Irrigation water was supplied by sprinklers to provide two water regimes during plant growth.

Drought was created in this rain-free environment by withholding irrigation after 30 days from sowing and giving two supplementary irrigations, one after 60 days post-sowing and the other after 90 days post-sowing. Control treatment was well watered throughout the growing period as needed to minimize water shortage until 10 days prior to maturity. Water application was monitored via a water meter and the Control treatment (well-watered) received 420 mm, while the drought experiment (severe stress) received 140 mm. The experimental plot consists of 6 rows, 3 m long with 5 cm row to row. All cultural practices were carried out as recommended for wheat production in this area.

**Crop measurements**

Date of heading was recorded on all sub-plots in each experiment as the date when 50% of shoots had reached this stage. For agroomic traits analysis, twenty guarded plants were randomly selected from each plot for each genotype. Total tillers and effective tillers per plant were counted. At physiological maturity, plant height was measured from the soil surface to the top of the spike on the main shoot. Spike length (cm), 1000 kernel weight (g), bological yield/$m^2$ (g), grain yield/$m^2$ (g), harvest index (%) and drought susceptibility index for each genotype were determined.

Drought susceptibility index (S) provides a measure of stress resistance based on minimization of yield loss under stress as compared to optimum conditions. It was used to characterize relative stress tolerance of all genotypes according to (Fischer and Maurer, 1978) from the following formula:

$$S = \frac{1 - Y_d / Y_p}{D}$$

Where $Y_d$ = mean grain yield in stress environment, $Y_p$ = mean grain yield in non stress environment, D = environment stress intensity = 1 - (mean $Y_d$ of all genotypes / mean $Y_p$ of all genotypes)

**Relative water content (RWC)**

Relative water content was determined according to Schonfeld et al. (1988), where fresh weight for twenty discs from the youngest fully expanded leaf *were* determined within 2 h after excision. Turgid weight was obtained after soaking the discs for 16 to 18 h in distilled water. After soaking, discs were quickly and carefully blotted dry with tissue paper prior to determine of turgid weight. Dry weight was obtained after drying the discs sample for 72 h at 70°C. Relative water content was calculated from the following equation:

RWC = [(fresh weight – dry weight)/(turgid weight- dry weight)] x 100

**Proline determination**

Proline was determined in fully expanded leaves according to Pesci and Beffagna (1984). The samples (50 mg fresh weight) were extracted with 10 ml of sulphosalicylic acid solution for 1 h at room temperature and filtered on Whatman fiber glass paper. A part of extract was added to 4 ml ninhydrin reactive and 4 ml of acetic and incubated in boiling water for 1 h. After fast cooling in ice, the samples were added to 5 ml of toluene and strongly shaken. The toluene phase, containing the colored complex was used to measure the absorbance at 515 nm versus toluene. From obtained absorbance values it has been calculated the proline amount of each sample by means of a calibration curve, made by starting from known amount of proline.

**Laboratory experiment**

The nine wheat genotypes were used to study the effect of low moisture stress by using polyethylene glycol (PEG) 6000. Solution was prepared according to weight by volume i.e. 0(distilled water, control), 150 g (15 %) and 250 g (25 %) PEG was dissolved in 850 and 750 ml of distilled water, respectively. Seeds were placed on the moist germination papers to provide appropriate moisture stress for seed germination.

**Seedlings data**

Data were recorded at three different moisture levels on germination period to determine the survival percentage after two weeks, root length, shoot length, fresh weight of shoot, and fresh weight of root. Coleoptiles length measured as a length of protective sheath that covers the shoot during emergence.

**Protein electrophoresis**

The leaf was ground thoroughly in a pre-chilled mortar and leaf was manually ground to a fine powder under liquid $N_2$ and mixed in a buffer 1.0 M Tris-HCl buffer, pH 8.0, containing 250 mM NaCl, 25 mM EDTA, 0.5 % (w/v) SDS 10 mM β-mercaptoethanol. After centrifugation at 13,000 rpm for 10 min, the supernatants were collected and considered as leaf protein extracts. Protein concentration was determined by absorbance at 595 nm (Bradford, 1976). A standard curve was prepared with bovine serum albumin. The supernatants were fractionated by 10% SDS-PAGE (Laemmili, 1970); running and staining were standard procedures. Electrophorogram for each variety were scored

**Statistical analysis**

A spilt plot design with three replicates was used in the field experiments, where water regimes were in main plots, and wheat genotypes in sub plots. For individual experiments, analyses of variance (ANOVA) were carried out for physiological traits and grain yield using the software Genstat version 6.1 (Lawes Agricultural Trust, Roth Amsted Experimental Station). Replications were regarded as random effects, while irrigation treatments and genotypes were fixed effects. For ANOVAs across years, Bartlett's test ($p$ = 0.05) was used to test the homogeneity of variances, and years were regarded as random effects according to Steel et al. (1997). Treatment means were compared using the least significant difference of the means of Fisher, calculated from standard errors of the difference of the means using appropriate degrees of freedom, when the ANOVA indicated significant differences. To confirm the relative importance of the various characters, a set of genetic parameters (genotypic and phenotypic coefficient of variability, heritability in broad sense and genotypic correlation between grain yield and the other traits) were calculated according to Hallauer and Miranda (1988).

## RESULTS AND DISCUSSION

General effects of genotype, treatments and year. Table 2 summarized the mean square of each factor and their interactions for all traits evaluated in the study. Considering the main factors, all traits showed statistically significant variations with the exception of RWC for the year factor. Genotype (G) x water (W) treatment interac-

**Table 1.** Name, pedigree and origin for wheat genotypes under investigation.

| Entry | Name | Pedigree | Origin |
|---|---|---|---|
| 1 | Sahel 1 | NS 732/PIMA//VEERY "S" | EGYPT |
| 2 | Giza 168 | MIL/BUC/seriCM93046-8M-OY-OM-2Y-OB | EGYPT |
| 3 | Giza 163 | T.aestivum/Bon//Cno/7c CM33009-F-15M-4Y-2M-1M-1M-1Y-0M | EGYPT |
| 4 | Gemmeiza 3 | Bb/7c2//4504Kal315 sk8/4/Rrv/ww15/3/Bj"S"//on3/Bon.Gm4024-1Gm-13Gm-oGm | EGYPT |
| 5 | Gemmieza 9 | ALD"S"/HUAC"S"//CM74A.630/SX | EGYPT |
| 6 | Rufom -5 | ICD 85-0988- 6AB- TR- 3AB- OTR | Mex/ Syr |
| 7 | Kavco -8 | ICW 85- 0012- 300L-300AP-300L-OAP | Syr/ LEB |
| 8 | Sakha 69 | Inia/R1//7C/Yr "S"Cm430-25-65-35-0S | EGYPT |
| 9 | Giza 167 | AU/UP301//511/SX/3/Pew"S"/4/Mai"S"Mai | EGYPT |

**Table 2.** Significance of mean squares due to different sources of variation for evaluating 9 wheat genotypes.

| Characters | df | Genotype(G) | Water regime(W) | Seasons (S) | G x W | G x S | W x S | G x W x S |
|---|---|---|---|---|---|---|---|---|
| | | 8 | 1 | 1 | 8 | 1 | 1 | 8 |
| Days to 50 % heading (days) | | 310.5** | 101.8** | 49.7** | 120.2** | 51.3** | 46.3** | 6.18 ns |
| Plant height (cm) | | 371.3** | 369.8** | 182.3** | 298.7** | 143.3** | 82.3** | 60.8** |
| Number of effective tillers | | 125.7** | 148.2** | 116.9** | 38.3** | 24.7** | 18.2 ns | 22.2** |
| Spike length (cm) | | 82.9** | 56.5** | 75.2** | 79.5** | 93.4** | 4.7 ns | 53.6** |
| 1000 kernel weight (g) | | 108.6** | 87.7** | 37.7** | 48.7** | 165.5** | 79.8** | 75.1** |
| Biological yield/ $m^2$ (g) | | 699.6** | 601.7** | 105.6** | 161.4** | 232.8** | 121.3** | 291.7** |
| Grain yield/ $m^2$ (g) | | 512.6** | 427.6** | 195.9** | 158.2** | 224.2** | 112.3** | 284.7** |
| Harvest index (%) | | 115.6** | 94.9** | 110.2** | 46.7** | 39.8** | 43.0** | 59.6** |
| Relative water content (%) | | 46.8** | 41.7** | 36.4** | 19.1 ns | 23.4 ns | 21.3 ns | 16.8 ns |
| Proline content | | 125.6** | 69.4** | 75.5** | 18.6 ns | 24.1 ns | 26.1 ns | 13.9 ns |
| Drought susceptibility index | | 79.8** | 88.3** | 80.8** | 51.1** | 60.6** | 79.1** | 67.5** |

tions were also detected for eight traits, indicating variable performance of genotypes in different growing conditions. The non-significant G x W, G x S, W x S and G x W x S interactions for RWC and Pro content may indicate that these traits can be described as constitutive traits. A character is said to be constitutive when its expression in environment independent, i. e. differences between genotypes are relatively constant in a range of environments. Constitutive character is not expected to show high GE interaction; therefore constitutive characters can be of advantage in some environments as a tool for selection to drought under optimal conditions. The presence of high GE interaction complicates breeding work because it makes it difficult to predict how genotypes selected under a given set of conditions will perform in a different set of conditions (Ceccarelli and Grando, 1991).

## Grain yield and its attributes response to drought

To utilize any local or introduced genotypes effectively in breeding for drought tolerance, it is necessary to characterize and evaluate these genotypes for desirable traits. Subjecting wheat genotypes to water stress decreased remarkably all the measured traits Table 3. Compared to well-watered genotypes, drought caused reductions in days to 50% heading, plant height, number of effective tillers, spike length, 1000-kernel weight, biological and grain yield as well as harvest index by 4.78, 14.7, 36.3, 23.7, 16.4, 32.9, 43.2 and 12.7%, respectively. However, genotypes which flowered and matured earlier may have been favored by partial escape from drought and have an ability to complete their life before dehydrated by high summer temperatures. Whereas, the decrease in plant height in all genotypes in response to drought stress may be due to decrease in relative turgidity and dehydration of protoplasm which is associated with a loss of turgor and reduced expansion of cell and cell division (Arnon, 1972a). Grain yield generally depends on spike length, number of effective tillers and 1000-kernel weight.

In this present study, overall mean these traits of the genotypes were influenced by drought stress. The decrease in1000-kernel weight may be due to disturbed nutrient uptake efficiency and photosynthetic translocation within the plant (Iqbal et al., 1999) that produced shriveled kernels due to hastened maturity. This is possible due to the shortage of moistures which forces plant to

**Table 3.** Mean values of various traits of wheat genotypes under two water regimes.

| Genotype | Control (well water treatment) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | HD (days) | PH (cm) | No.ET | SL (cm) | 1000 kw (g) | BY (g) | GY (g) | HI (%) |
| 1 | 99.0 | 72.6 | 2.2 | 13.9 | 42.9 | 934.8 | 553.9 | 59.2 |
| 2 | 102.2 | 76.0 | 1.6 | 12.8 | 39.7 | 860.6 | 469.4 | 54.5 |
| 3 | 97.3 | 79.9 | 1.1 | 12.1 | 31.0 | 643.4 | 363.7 | 56.5 |
| 4 | 100.8 | 82.0 | 1.3 | 10.5 | 36.3 | 745.5 | 348.1 | 46.6 |
| 5 | 101.1 | 80.6 | 3.1 | 11.8 | 38.2 | 845.9 | 410.2 | 48.4 |
| 6 | 100.1 | 80.7 | 2.7 | 13.7 | 40.4 | 799.5 | 471.4 | 58.9 |
| 7 | 100.8 | 80.8 | 2.5 | 11.3 | 39.0 | 900.8 | 434.0 | 48.1 |
| 8 | 105.3 | 70.8 | 3.2 | 14.4 | 42.2 | 870.8 | 417.2 | 47.9 |
| 9 | 100.4 | 75.9 | 2.1 | 13.2 | 37.6 | 965.8 | 461.2 | 47.7 |
| Overall mean | 100.77 | 77.7 | 2.2 | 12.6 | 38.5 | 840.7 | 436.5 | 51.9 |
| LSD | 1.23 | 4.66 | 0.28 | 0.98 | 2.25 | 26.9 | 21.7 | 1.36 |
| **Genotype** | **Drought treatment** | | | | | | | |
| 1 | 94.3 | 59.2 | 1.8 | 11.2 | 35.4 | 820.8 | 458.6 | 55.8 |
| 2 | 97.3 | 66.3 | 1.2 | 9.8 | 33.2 | 670.8 | 290.4 | 43.2 |
| 3 | 92.9 | 65.8 | 1.0 | 8.8 | 25.9 | 525.5 | 199.2 | 37.9 |
| 4 | 96.0 | 72.2 | 1.1 | 8.4 | 30.3 | 475.8 | 200.3 | 42.0 |
| 5 | 96.3 | 70.3 | 2.0 | 9.9 | 31.9 | 480.5 | 220.6 | 45.9 |
| 6 | 96.2 | 69.5 | 1.9 | 10.8 | 34.6 | 720.0 | 385 | 53.4 |
| 7 | 95.3 | 68.7 | 1.2 | 8.2 | 32.0 | 429.7 | 180.3 | 41.9 |
| 8 | 100.3 | 70.0 | 1.1 | 10.1 | 35.3 | 482.3 | 111.9 | 23.2 |
| 9 | 95.0 | 54.4 | 1.3 | 9.5 | 31.4 | 471.3 | 181.5 | 38.5 |
| Overall mean | 95.9 | 66.2 | 1.4 | 9.6 | 32.2 | 564.0 | 247.5 | 42.4 |
| RD% | 4.78 | 14.7 | 36.3 | 23.7 | 16.4 | 32.9 | 43.2 | 12.7 |
| LSD | 1.23 | 4.66 | 0.28 | 0.98 | 2.25 | 26.9 | 21.7 | 2.55 |

HD = heading date, PH = plant height, No .ET = number of effective tillers, SL = spike length, BY = biological yield, GY = grain yield, HI = harvest index, RD% **=** the relative percentage of decrease.

complete its grain formation in relatively lesser time (Riaz and Chowdhrv, 2003b).

Under drought conditions the availability of current assimilates for extending seed filling will often be severely reduced. In such circumstances, a genotype that can mobilize reserves of carbohydrates in the stem will be able to maintain better seed filling. It is worthy to note that genotypes 1, 2 and 6, which we believe have resistant to water deficit, had a feature of developmental plasticity. Developmental plasticity is defined as the ability of plant to produce flowers with minimum of vegetative structure, and this enables them to produce seed on a limited supply of water. This coupled with the ability to produce an abundance of vegetative growth, flowers and seeds under abundant of water (Quarrie et al., 1999).

Harvest index, as long as the source of assimilates and their supply to ears was 51.9% for non-stress and 42.4% for stress treatments. Austin (1994) suggested that high harvest index may be due to improved resistance to drought by making the plants much shorter along with enhancing the supply of nutrient substances to young kernels.

## Drought susceptibility index and drought tolerance

The drought susceptibility index (*S)* is independent of yield potential and drought intensity, and is potentially useful for comparisons of drought susceptibility of genotypes between drought levels and experiments, since larger values of *S* indicate greater drought susceptibility. The results indicated that *S* ranged from 0.35 to 1.51, where the wheat genotypes 1, 2 and 6 expressed lower *S*. The genotypes 3, 4, 5, 7 and 9 had higher *S* values (Figure 1).

The drought susceptibility index was negatively and significantly associated with grain yield under stress conditions. The genotypic correlation between grain yield and S values was negatively and significantly (-0.77), indicating selection for this character under stress environment might result in decrease susceptibility to stress.

## Relative water content and drought tolerance

Sinclair and Ludlow (1985) proposed that RWC was better measure for plant's water status than thermodyna-

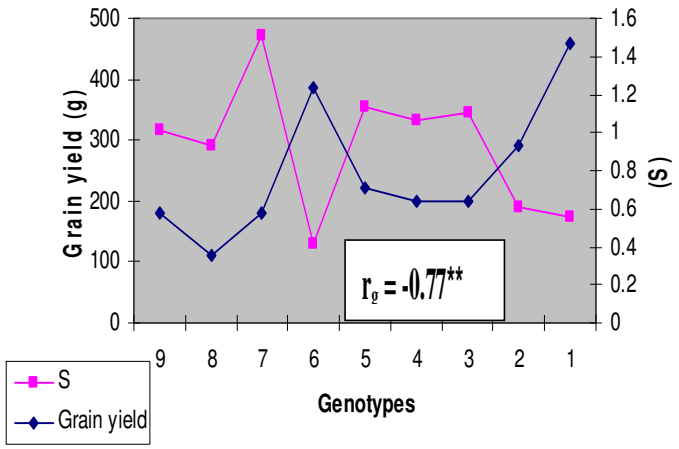


**Figure 1.** Effect of drought susceptibility in index (S) on yield response to drought.

mic state variable (water potential, turgor potential and solute potential). In this present study, RWC was determined to give indication on the plant water status under drought condition. RWC decreased with water stress in all the tested genotypes. Similar observations have been repotted in Common Bean (Korir et al., 2006). Genotypes 1, 2 and 6 had higher RWC content while genotypes 3, 4 and 9 had lower RWC under water stress (Figure 2). This genotypic variation in RWC may be attributed to differences in the ability of the variation to absorb more water from the soil and or the ability to control water loss through the stomata's. These findings are in agreement with those reported by Sinclair and Ludlow (1985). It may also be due to differences in the ability of the tested genotypes to accumulate and adjust osmotically to maintain tissue turgor and hence physiological activities. Varietals differences in RWC may also be a result of varieties maximizing on soil water reserves by fully extracting water in the existing rooting zone and or extending rooting depth to increase water reserve for crops (Schonfeld et al., 1988; Siddique et al., 2000). At the cellular level, plants attempts to alleviate the damaging effects of stress by altering their metabolism to cope with stress.

The genotypic correlation between gain yield and RWC underwater stress was positively and significantly (0.84). Therefore these genotypes which maintained higher RWC under stress conditions is believed to be more droughts tolerant and gave higher yielding than others. Similarly, Bennet et al. (1987) and Schonfeld et al. (1988) found that superior performance of drought tolerant soybean, maize and wheat under water stress environment is attributing to osmoregulation when stress set in. These cultivars difference in RWC could be used to select high yielding genotypes that maintain cell turgor under water stress environment to give high relative yield. Moreover, it can be concluded that these genotypes which tend to increase their RWC acquire their tolerance from great solute accumulation and metabolites, hence, osmotic adjustment occurred with higher RWC (Ritchie et al., 1990). Thus, these results recognized RWC as a beneficial drought tolerance indicator and may be used as selection criteria in wheat breeding program.

## Proline content and drought tolerance

In view of fact that the accumulation of Pro is tightly controlled by genes and cDNA encoding osmolyte biosynthesis and only achieved when the rate of synthesis prevails over that degradation, probably because too much Pro is toxic to cell plant (Yokota et al. 2006). In present work, the sharp increased in Pro content might theoretically, attribute to the genes for synthesis and degradation of Pro which are up-regulated strongly under drought stress. It might be an adaptation to the purpose of which is to overcome the stress condition and it could supply energy for growth and survival and thereby help the plant to tolerate stress (Sankar et al., 2007).

Pro content increased by 13 folds in genotype 1 and by 12 folds in genotypes 6 due to water stress (Figure 3). Although, these statements suggest that Pro is not directly involved in the drought resistance and is not essential for improved resistance. But where Pro increase does occur it improves resistance from a quantitative point of view in these three genotypes, at the least in the cellular level, which corroborated with those reported in sugarcane (Errabll et al., 2006). These genotypes which had high Pro content might increase ability to synthesize osmotic regulators (Pro) for protection from the damage of soil water deficits. Furthermore, Pro may play a role as an enzyme-stabilizing agent and has the ability to mediate osmotic adjustment, stabilized sub-cellular structure and scavenge free radicals (Hassanein, 2004).

Grain yield correlated positively and significantly (r=0.81**) with Pro accumulation under water stress. It is also observed that genotypes 1, 2 and 6 which gave high RWC, accumulated more free Pro and had a lower *S* values. Indicating that accumulated pro might act as a compatible solute regulating and reducing water loss from the cell during episodes of water deficit. Because Pro has hydrophilic property, it might replace water molecules around nucleic acid, protein and membranes during water shortages. It might also prevent interaction between destabilize ions and cellular components by replacing the water molecules around these components, thereby protecting against destabilization during drought (Yokota et al., 2006).

However these genotypes which have pro over-accumulation, clearly demonstrated that selection for pro could be used as a biochemical marker for increased stress tolerance in conventional crop breeding program and could lead to development of varieties and eventually to plants with heritable stress resistance. In addition to Shivkumar et al. (1998) and Silverira et al. (2003) who showed that pro accumulation was indeed a heritable trait

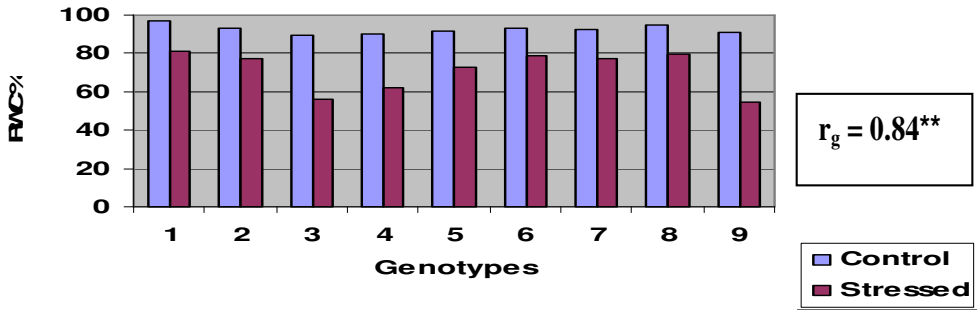


**Figure 2.** Relative water content comparison of nine wheat genotypes under two water regimes.

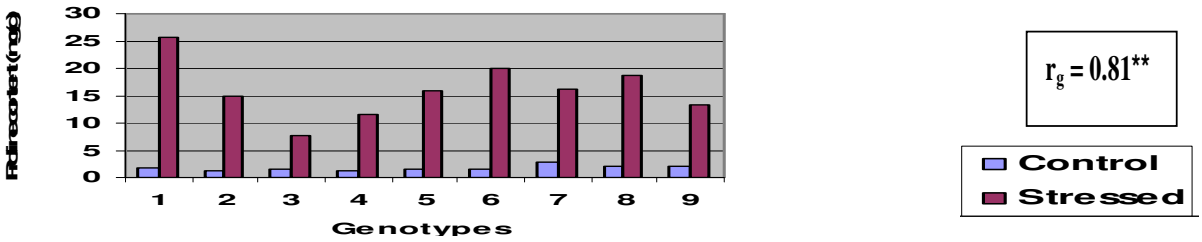


**Figure 3.** Proline content comparison of nine wheat genotypes under two water regimes.

and they concluded that selection for high Pro had been effective and played an important role in rehydration of protoplasm and osmotic adjustment are hypothesize to enhance drought tolerance in plants.

## Comparison of drought tolerance among wheat genotypes

As screening technique, the survival ability of the nine wheat genotypes to tolerate chemical desiccation by PEG during the growth of seedling is exhibited in Figure 4. Genotypes differed in survival days (SD) (an index of drought tolerance) at seedling stage. Genotypes 3, 4 and 9 dried first with about 8.2 and 3.7 days of average survival days (SD) after applying 15 and 25% PEG, respectively. Hence, their drought tolerance appeared to be the poorest under drought stress. Genotypes 1, 2 and 6 survived longer than the other genotypes (about 13.1 and 7.6 days after applying the same concentration from PEG. Genotypes 5 and 7 were intermediate with survival times of 10.7 and 5.2 days.

Seedling development under laboratory conditions have been accepted as suitable growth stages for testing the drought tolerance in wheat. It could be speculated that the presence of increased concentrations of PEG during the growth of seedling inhibits the developmental traits and survival of wheat seedling (Table 4). Whereas shoot length was always decreased by exposure to all the stress levels tested, there was an increase in root length associated with 25% PEG treatment for genotypes 1, 2 and 6. This reflects an adaptive response involving an increase in root length to reach deeper water. Similar observation was reported by Leila (2007) for Pearl millet. However, the reduction in the shoot length and the root length may be due to an impediment of cell division and elongation leading to kind of tuberization. This tuberization and the lignifications of the root system allow the plant to enter a slow-down state, while waiting for the conditions to become favorable again (Fraser et al., 1990).

The development of the root system in response to water deficit suggests that the expression of certain genes controlling root formation is stimulated by drought conditions (Badiane et al., 2004). In addition to dominant alleles controlled the length of roots and the feature could be easily incorporated in breeding for drought resistance (Vijendradas, 2005). The tested genotypes varied significantly in their reaction to PEG seedling shoot, root length and weight but the differences decreased with the further plantlet development.

The PEG induced a drop in the shoot and root biomass which was the greatest in genotypes 3, 4 and 9 while the decrease in genotypes 1, 2 and 6 biomass was greatest under the various levels from PEG. The reduction in shoot weight was attributed to lower number and development of smaller leaves with increased PEG of the growth media.

Seedling establishment under water stress can be improved by selecting for long coleoptiles. Genotype 6 showed the longest coleoptiles under both PEG treatments. It might be that the seed has a very limited source

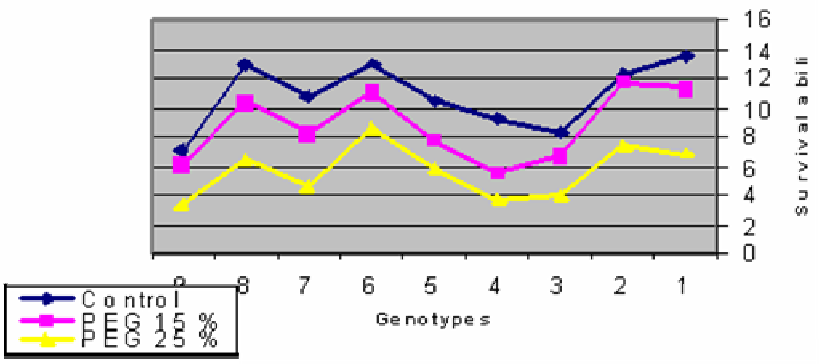


**Figure 4.** The survival ability days of wheat genotypes under two concentrations from PEG.

**Table 4.** Effect of polyethylene glycol treatments on wheat seedling traits.

| | PEG( Control) | | | | | PEG (15 %) | | | | | PEG (25 %) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | SL Cm | RL Cm | SW (g) | RW (g) | CL Cm | SL Cm | RL Cm | SW (g) | RW (g) | CL Cm | SL Cm | RL Cm | SW (g) | RW (g) | CL Cm |
| **1** | 9.3 | 11.5 | 1.66 | 1.16 | 4.72 | 7.2 | 6.2 | 0.86 | 1.03 | 4.52 | 6.2 | 7.0 | 0.33 | 0.28 | 3.86 |
| **2** | 7.5 | 9.7 | 0.95 | 0.98 | 4.9 | 6.5 | 4.1 | 0.78 | 0.72 | 2.92 | 4.3 | 6.0 | 0.95 | 0.14 | 1.92 |
| **3** | 6.8 | 8.0 | 0.81 | 0.84 | 2.72 | 3.1 | 3.2 | 0.73 | 0.62 | 2.08 | 2.3 | 5.1 | 2.17 | 0.21 | 1.69 |
| **4** | 8.8 | 5.0 | 0.37 | 0.39 | 2.52 | 4.2 | 2.7 | 0.23 | 0.28 | 1.17 | 3.5 | 0.2 | 0.166 | 0.05 | 1.05 |
| **5** | 10.5 | 4.5 | 0.49 | 0.45 | 3.2 | 4.1 | 1.2 | 0.13 | 0.45 | 1.82 | 2.5 | 2.0 | 0.136 | 0.07 | 1.12 |
| **6** | 10.0 | 6.0 | 0.52 | 0.37 | 5.0 | 4.9 | 6.9 | 0.11 | 0.24 | 3.2 | 3.7 | 5.3 | 0.13 | 0.16 | 2.83 |
| **7** | 7.4 | 7.5 | 0.92 | 0.73 | 4.96 | 6.3 | 1.9 | 0.67 | 0.61 | 2.20 | 5.1 | 1.3 | 0.09 | 0.124 | 1.83 |
| **8** | 8.5 | 4.4 | 0.59 | 0.52 | 3.4 | 4.3 | 1.0 | 0.16 | 0.36 | 1.50 | 2.2 | 0.4 | 0.14 | 0.143 | 0.66 |
| **9** | 7.5 | 11.0 | 1.03 | 0.93 | 4.0 | 6.3 | 4.7 | 0.76 | 0.75 | 2.26 | 3.1 | 0.8 | 0.06 | 0.126 | 1.93 |
| **LSD** | 0.38 | 2.13 | 0.16 | 0.09 | 0.18 | 1.62 | 1.2 | 0.08 | 0.03 | 0.16 | 3.01 | 1.14 | 0.09 | 0.11 | 0.08 |

SL = Shoot length, RL = Root length, S/R = Shoot/Root length ratio, SW = Shoot weight, RW = Root weight, CL = Coleoptile length.

of energy for metabolic life cycle. The quicker the seed forms vegetative parts, the earlier they start photosynthesis to provide energy for growth and root formation at later stages. This would allow more protection for seedling growth under moisture stress.

It is worthy to mention that traits observed in PEG experiments confirmed the observations obtained from field experiments, where genotypes which had the highest yielding in field experiments had the highest vigor ratting in PEG experiments and vice versa. This technique would appear to be suitable for screening large populations to improve drought tolerance prior to yield testing.

## Effect of PEG on the protein patterns using SDS-PAGE

In an attempt to understand the molecular basis of drought tolerance, proteomics using SDS-PAGE was analyzed to identify protein patterns involved in drought stress response in the nine Wheat genotypes. Detection of proteins which levels are altered by PEG stress was done by comparing patterns from control and PEG-treated plants. Proteins were extracted from ten-day-old wheat seedlings, which were treated with 15 and 25% PEG and were separated by SDS-PAGE. A set of control plants was grown without added PEG under the same condition as the stressed plants. However, protein bands detected ranged from 12 to 124 kDa (Figure 5). The newly synthesis protein bands were observed at molecular weight 40 kDa in Refum-5 cultivar under treatment at 25% PEG, only. Consequently, this band can be considered as a molecular marker to characterize drought tolerance and interpreted as an adaptive band to drought stress; this newly synthesized protein might indicate that PEG stress induced a stress related gene to produce this drought inducible protein. Water deficit alters plant gene expression and leads to specific gene, producing an increase of their transcripts and thus an increase of corresponding proteins (Ingram and Bartels, 1996).

Kavoco-8, Sakha 69 and Giza 167 cultivars exhibited higher intensity in the appearance of bands under

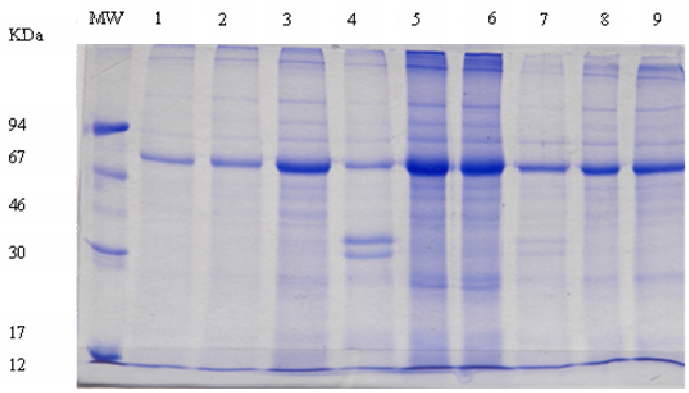


**Figure 5a.** Protein Profile on SDS-PAGE of Wheat genotypes under PEG stress. Lanes 1 to 9: Sahel 1: 1 = control; 2 = 15% PEG; 3 = 25% PEG; Giza 168: 4 = control; 5 = 15% PEG; 6 = 25% PEG; Giza 163: 7 = control; 8 = 15% PEG; 9 = 25% PEG.

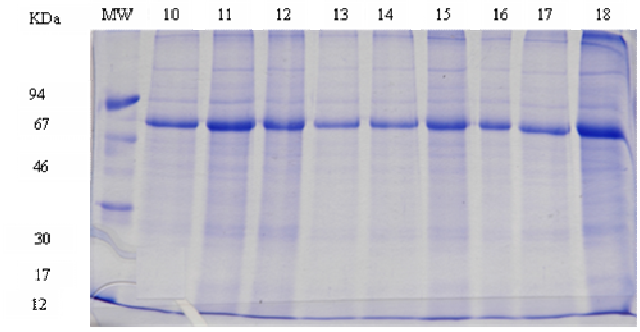


**Figure 5b.** Protein Profile on SDS-PAGE of Wheat genotypes under PEG stress. Lanes 10 to 18: Gimmeiza 3: 10 = control; 11 = 15% PEG; 12 = 25% PEG; Gemmeiza 9: 13 = control; 14 = 15% PEG; 15 = 25% PEG; Ruform-5: 16 = control; 17 = 15% PEG; 18 = 25% PEG.

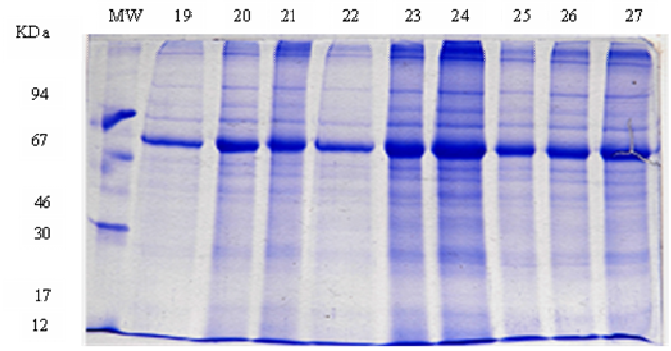


**Figure 5c.** Protein Profile on SDS-PAGE of Wheat genotypes under PEG stress. Lanes 19 to 27: Kavco-8: 19 = control; 20 = 15% PEG; 21 = 25% PEG; Sakha 69: 22 = control; 23 = 15% PEG; 24 = 25% PEG; Giza167: 25 = control; 26 = 15% PEG; 27 = 25% PEG.

drought whereas were faint in control plants. These faint bands may be intact proteins or degradation products (Close et al., 1993) considering that the band intensity is directly related to protein concentration in the wheat seedlings (Diana et al., 2002). Various investigators suggested that the low protein concentration is attributed to the decrease rate of protein synthesis, the increase activities of hydrolyzing enzymes, the decreased availability of amino acids or the denaturizing of the enzymes involved in amino acids and protein synthesis (Dubey 1994; Dubey and Rani, 1990). Riccardi et al. (1998) reported that water deficit induced the expression of proteins not specifically related to this stress, but rather to reactions against cell damage.

Higher plant exposed to drought conditions exhibit a characteristic set of cellular and metabolic response, including a decrease or increase in the synthesis of protein (Elizabeth Bray, 1988; Elumalai et al., 2000). Also, it was found that 35 and 37 kDa protein bands were more abundant in treated plants than in controlled plants. Furthermore, the enhanced expression of these proteins, which also existed in the control plants, were specifically increased and clearly observed in plants grown under drought condition. It could be the drought-induced genes encoded proteins that are supposed to play an important role in water stress response. They confer desiccation tolerance, protect cellular structure or are involved in the signal transduction pathway that leads to gene induction under these conditions (Romo et a., 2001). These results are in agreement with findings of Ericson and Alfinito (1984) who stated the two different protein bands with MW of 32 and 20 kD in the stressed tobacco plants. However, these results revealed that the expression of these proteins was genetically regulated, depending on the PEG concentration well as the genetic differences of Wheat genotypes. Protein profiles could be useful markers in the studies of genetic diversity and classification of adapted cultivars, thereby improving the efficiency of wheat breeding programs (Mohd et al., 2007).

## Genetic behavior of plant characteristics under water stress

The phenotypic and genotypic coefficient of variability (PCV and GCV) and heritability in broad sense for the studied traits were calculated (Table 5). The PCV was higher under stress conditions than non stress conditions. Under stress conditions, small differences in PCV and GCV were observed for plant height (18.9 and 17.1%, respectively) and number of effective tillers (9.4 and 8.3%, respectively) which may be due to genetic nature of these traits.

Since biological yield and grain yield per plant are highly variable characters and easily influenced by the environmental factors, there was great discrepancy between PCV and GCV for these traits under stress conditions. PCV (56.4 and 47.7%, respectively) for biological yield and grain yield were higher than GCV (43.2 and 30.6% respectively) displaying a great influence of

**Table 5.** Estimation of some genetic parameters for the studied traits under well-watered and severe water stress treatments.

| Selection criterion | Well watered treatment | | | | Severe stress treatment | | | |
|---|---|---|---|---|---|---|---|---|
| | PCV (%) | GCV (%) | $h^2$ | $r_g$ | PCV (%) | GCV (%) | $h^2$ | $r_g$ |
| **I- Field experiment** | | | | | | | | |
| Heading date | 12.1 | 11.6 | 91.5 | 0.41 | 19.5 | 15.8 | 80.7 | 0.54 |
| Plant height | 21.4 | 9.5 | 60.2 | 0.38 | 18.9 | 17.1 | 73.1 | 0.31 |
| No. of ET | 14.8 | 10.1 | 68.3 | 0.68 | 9.4 | 8.3 | 64.5 | 0.59 |
| Spike length | 18.9 | 4.6 | 59.6 | 0.66 | 38.6 | 27.5 | 61.2 | 0.47 |
| 1000-kernel weight | 16.7 | 12.3 | 76.1 | 0.41 | 38.9 | 26.2 | 68.0 | 0.53 |
| Biological yield | 37.8 | 14.2 | 50.0 | 0.65 | 56.4 | 43.1 | 43.2 | 0.41 |
| Grain yield | 25.7 | 12.4 | 56.3 | - | 47.7 | 39.8 | 30.6 | - |
| Drought susceptibility index | - | - | - | - | 11.8 | 8.9 | 85.6 | 0.77 |
| Relative water content | 10.2 | 9.4 | 92.7 | 0.44 | 12.6 | 10.1 | 90.8 | 0.84 |
| proline content | 13.4 | 11.3 | 30.1 | 0.18 | 17.5 | 13.9 | 69.5 | 0.81 |
| **II- Laboratory experiment** | | | | | | | | |
| Survival ability | 19.5 | 13.2 | 0.76 | 0.72 | 20.9 | 14.8 | 71.1 | 0.78 |
| Shoot seedling length | 35.0 | 28.6 | 47.1 | 0.25 | 42.4 | 34.5 | 51.2 | 0.45 |
| Root seedling length | 15.2 | 10.8 | 58.9 | 0.51 | 18.4 | 13.3 | 53.4. | 0.59 |
| Shoot seedling weight | 27.5 | 18.6 | 37.8 | 0.27 | 29.9 | 26.8 | 36.9 | 0.30 |
| Root seedling weight | 5.1 | 4.8 | 41.9 | 0.34 | 5.7 | 4.35 | 40.8 | 0.41 |
| Coleoptiles length | 11.6 | 10.7 | 77.0 | 0.23 | 18.2 | 16.9 | 70.8 | 0.68 |

other than genetic factors on both characters. In the PEG experiment, shoot seedling length showed the highest PCV and GCV followed by shoot seedling weight under control and 25% PEG. The substantially greater phenoltypic variance indicated a strong masking effect of the environment which may make genetic improvement through selection of drought tolerant genotypes problematic. Reduction in genetic variability under stress, which has been reported, suggests rigorous and careful selection of drought tolerant genotypes.

Concerning the heritability values, it is clear that, heritability estimates decreased under stress conditions than non-stress conditions. Heritability values ranged from 50.00 for biological yield to 92.7% for relative water content under non-stress conditions. Whereas, it ranged from 30.6% for grain yield to 90.8% for relative water content under stress conditions. However, broad sense heritability for grain yield was very low (30%). This finding is contrary with the results of Lu et al. (1991) and Riaz and Chowdhrv (2003a) who observed high heritability estimates for grain yield. Thus, heritability is not a constant value and depends on the method of estimation and the procedures used by the breeder which influences its magnitude and genetic improvement obtain through selection. Furthermore, environment may also interact with the genotypic constitution to influence heritability. Therefore our results discussed here be relevant only to the genotypes under our study and the environment condition existing at the experimental site.

Heritability for RWC was much higher than that for yield or any of the yield components in wheat. The high heritability for RWC suggests that phenotypic selection for RWC in wheat may be more efficient for drought tolerance. Chaudhary et al. (1989) showed that osmotic adjustment and RWC, both behave as simple inherited characters. The heritability values in a broad-sense may be useful as first approximations but not as definitive values for the improvement of drought tolerance in wheat. Genotypic variance components were large and signifycantly different from zero ($P$<0.05) for all seedling characteristics. The genetic correlation for survival ability and other seedling characteristics varied depending on the character and the environments

Generally, the results of field and laboratory experiments indicated that genotypes of wheat differed with respect to their water status. The tolerant genotypes had high levels of Pro accumulation, RWC, root length, coleoptiles length and grain yield, indicating its better ability to osmoregulate and enhance survival ability compared with susceptible genotypes. It is more amenable to select for survival ability, Pro accumulation, RWC and coleoptiles length in drought stressed environments which are quick and easy procedure than yield or yield components. Dilday et al. (1990) reported the importance of survival ability and coleoptiles length as indicator for drought tolerance.

## Conclusion

Two primary schools of thought have influenced plant breeders who target their germplasm to drought-prone areas. The first of these philosophies state that high input responsiveness and inherently high yielding potential,

combined with stress-adaptive traits will improve performance in drought-affected environments (Van Ginkel et al., 1998; Rajaram and Van Ginkel, 2001; Betran et al., 2003). The breeders who advocate selection in favorable environments follow this philosophy. Producers, therefore, prefer cultivars that produce high yields when water is not so limiting but suffer minimum loss during droughty seasons (Nasir Ud-Din et al*.,* 1992).

The second is the belief that progress in yield and adaptation in drought-affected environments can be achieved only by selecting under the prevailing conditions found in target environments (Ceccarelli and Grando, 1991; Rathjen, 1994). The theoretical framework to this issue has been provided by Falconer (1989) who wrote, "Yield in low and high yielding environments can be considered as separate traits which are not necessarily maximized by identical sets of alleles". Van Ginkel et al. (1998) showed that the traits suitable for a given environment with its own weather conditions may be unsuitable (or even harmful) in another environment. The weakness of this approach is that input responsiveness, so important in the wetter, admittedly less frequent but much more productive years cannot be easily maintained in the germplasm. Several researchers have concluded that selection will be most effective when the experiments are done under both favorable and stress conditions (Fischer and Maurer 1978; Nasir Ud-Din et al., 1992; Pauk et al., 1995) Rajaram and Van Ginkel (2001) showed that selection in alternating drought and non-drought environments at the International Maize and Wheat Improvement Center (CIMMYT) has resulted in a significant progress in the development of wheat germplasm adapted to dry areas globally.

Generally, our results firstly clearly showed that different wheat genotypes differently responded to water stress at different stages in terms of physiological mechanisms. Genotypes with the highest productivity under well watered treatment suffered less under water stress and gave minimum yield loss during droughty. This may be due to the best combined of yield along with the physiological factors. These results led to the proposals (a) that Pro accumulation might provide a reliable laboratory screening test for drought resistance in cereal breeding program, and (b) that cultivar which believed to be more drought resistant usually maintained higher leaf RWC under stress.

The confined seedlings environment of laboratory experiment would not reflect accurately the phenotype of shoot and root growth under field conditions. Nevertheless, if genes can be identified that control the expression of traits that enhance the performance of these genotypes in this test environment, then it might possible to incorporate these traits into other genetic backgrounds for crop improvement. The results of SDS-PAGE analysis could be reveled two different genetic mechanisms that drought stress resulted in over expression of some genes and/or *de novo* induction of gene expression. As shown in the present study, this technique can be used on large scale to include the largely out-breeding species. It is concluded that leaf protein profiles could be useful markers in the studies of genetic variation and classification of adapted cultivars, thereby improving the efficiency of wheat breeding programs in cultivar development.

Currently, sustainable development is the key point. To take advantage of full use of crop physiological potential for high production and safe food with better quality, the study of physiological and biochemical aspect of stress tolerance have broad future for solution to grain issue on the globe. Based on this research, the combination of molecular biology and plant physiology is the key of mechanism of drought tolerance. Thus, further work is required to identify and manipulated the genes controlling the physiological and molecular traits and to gear our research to the right direction of drought tolerance.

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