Research Journal of Agronomy 2 (2): 36-43, 2008
ISSN: 1815-9354

Application of Coleoptile Growth Response Method to Differentiate Osmoregulation Capability of Wheat (*Triticum aestivum L.*) Cultivars

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Abstract: Osmoregulation, which involves maintenance of cell turgor by accumulation of solutes in response to drought stress, is one of the mechanisms involved in drought tolerance. Several methods have been proposed to be used in order to differentiate wheat genotypes in terms of osmoregulation capability. Among this coleoptile growth under drought stress condition is a valuable method as cultivars comparison and hence selection could be done at early growth stages. In order to compare osmoregulation capability of wheat genotypes which are recommended for cultivation under different environmental condition in Iran, an experiment was conducted in the crop science laboratory of Shahid Bahonar university of Kerman. Seeds were germinated in dark long caps under normal and drought stress condition which was imposed by 20 and 30% polyethylene glycol (6000) solutions. Coleoptile growth was measured in nearest mm 48 hours after germinated seeds exposed to drought stress. Frequency distribution of coleoptile growth under stress condition showed a bimodal distribution to each a significant normal distribution was fitted. Suggesting that genotypes could be classified into two different groups the first distribution located at 8.93 mm and the second at 21.4 mm. Higher coleoptile growth in the first group was attributed to high osmoregulation capability, while lower growth was assigned to low osmoregulation capability. On the other hand, cultivars with high coleoptile growth had significantly higher grain yield compared to those with low coleoptile growth under drought stress condition. High osmoregulation cultivars were suggested to be used for cultivation under dry condition and in breeding programs to increase drought tolerance by.

Key words: Osmoregulation, coleoptile growth, drought stress, wheat

INTRODUCTION

During their growth, crop plants are exposed to many environmental stresses that reduce their growth and productivity. Among these, drought stress is the most important one which is caused mainly by low soil water content (Bohnert *et al*., 1995). It decreases as the period between irrigation or rainfall (drought severity) increases. Prolonged dry periods decreases soil water potential which in turn makes it more difficult for plants to absorb water from the soil (Hare *et al*., 1998). Plant physiological activities changes as the level of water stress they exposed to, is changes. Under such condition, maintenance of relatively high amount of water in shoot parts may increase plant performance (Cosgrove, 1986). Osmoregulation, which involves maintenance of cell turgor or volume by accumulation of solutes in response to increases in water stress, is a significant adaptation mechanism to dry conditions in many plant species (Hellebust, 1976; Sen Gupta and Berkowitz, 1987). It is considered as one of the mechanisms that help plants to maintain high turgor under dry condition (Morgan, 1980; Santakumari and Berkowitz, 1990; Wyn Jones *et al*., 1979). The capability of plant for osmotic adjustment determines the degree of turgor maintenance (Hsiao *et al*., 1976). The major advantage of osmoregulation is the maintenance of positive turgor as water deficits develops (Hsiao *et al*., 1976; Morgan, 1992).

Substantial differences had been shown to exist between wheat cultivars in the capacity of mature leaves to accumulate solutes in response to water stress (Morgan, 1980, 1983). F_2 lines derived from a cross between high and low osmoregulation lines showed 2 overlapping distribution in cell solute content indicating that a single recessive gene is responsible for high osmoregulation (Morgan, 1991). The gene is shown to be located on chromosome 7 of genome A (Morgan and Tan 1996; Morgan, 1980).

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Several experiments showed that in diverse wheat genetic background, differences in osmoregulation are positively associated with differences in grain yield (Hare *et al*., 1998; Morgan, 1983). Osmoregulation apparently causes plants to have higher evapotranspiration and harvest index (Morgan and Condon, 1986). It has been suggested that selection for osmoregulation in plant breeding programs for increasing yield under dry condition is worthwhile, as the grain yield increased by 50% in bread wheat lines with osmoregulation capability (Morgan, 1983; Morgan *et al*., 1991).

Genotypes are usually characterized for osmoregulation by measuring responses of osmotic potential and relative water content to changes in water potential of leaves of glasshouse or field-grown plants (Richards, 2004). This method, however, is difficult since the measurement should be done on many samples over a relatively long period of developing stress and needs the worker to be very skillful using the available technology. Sampling technique is also very important, because osmotic potential is affected by the time, the tissue and even by the instrument (Morgan, 1988), which is used for the sampling.

As a surrogate of direct measurement of osmoregulation, coleoptile growth under drought stress condition have been proposed to be measured in wheat (Morgan, 1988). This method is very simple because it only needs to measure coleoptile growth under drought stress in the first week of ontogeny.

Wheat is the major cereal crop in Iran. It is cultivated over a wide range of regions which are characterized by semi-arid climatic condition. Therefore, it usually exposed to drought stress during the growth. It is of major importance for farmers, therefore, to have more drought among them some are recommended for dry land cultivation. However, little is known regarding the mechanisms enable them to have higher yield under drought stress condition. The aim of this study was, therefore, to investigate the osmoregulation capabilities of Iranian wheat genotypes.

MATERIALS AND METHODS

Osmoregulation capability of 33 wheat cultivars was examined using coleoptile growth under 2 different levels of drought stress. Table 1 shows some plant features, responses to environmental stresses and origins of these cultivars. Since coleoptile and seminal root growth was intended to be continued for more than what is usually considered enough for germination tests and also to simulate the conditions similar to what is exists in the soil, dark long cups were used as growing media. There were 10 seeds in each cup, which were carefully weighted up to five decimal places using an analytical balance before germination and growth and placed in a marked location in the cup. Seeds were then soaked for 2 h in tap water, surface sterilized with 2% (v v^{-1}) commercial bleach solution for 3 min, rinsed with distilled water and placed on Watman paper in the cups. At first 12 mL of distilled water, which was enough for imbibitions was added. Initial length of coleoptiles and roots was measured 48 h later. Germinated seeds were exposed to 2 levels of drought stress imposed by 20 and 30% PEG solutions as well as control which was imposed by distilled water. Thirteen milliliter of 20 and 30% (w w^{-1}) Polyethylene Glycol 6000 (PEG) solutions were then

Table 1: Characteristics of wheat cultivars used in the experiment

Cultivar	1000 grain weight (g)	Response to environmental stresses	Yield (t ha^{-1})	Cultivar	1000 grain weight (g)	Response to environmental stresses	Yield (t ha^{-1})
Ghods	42	-	6	Omid	39	-	4
Navid	41	Semi-tolerant to cold stress	5	Azar2	46	Tolerant to drought and relatively tolerant to cold stress	4
Hirmand	37	Tolerant to salt and drought stress	5	Rowshan	32.5	Tolerant to salt and drought stress	4
Rasoul	36	-	4	Khazar	40	Susceptible to cold stress	4..3
Alvand	40	Relatively tolerant to salt and drought stress and tolerant to cold stress	6. 5	Toos	38	Tolerant to drought and cold stress	6..3
Alamot	40	-	4	Shahryar	40	-	2.7
Mahdavi	49	Tolerant to salt stress	7	Shiraz	38	Tolerant to salt stress	7.4
Zarin	39	Relatively tolerant to cold stress	6.4	Dez			
	42.5	Tolerant to terminal heat stress	6				
Darab2	37.5	-	5..9	Hamoun	44.5	Tolerant to drought and salt stress	6.6
Tajan	38	-	6..3	Pishtaz	30	Tolerant to terminal drought stress	4.7
Atrak	35.5	-	5.8	Saisoun	32	-	4
Niknejad	37	Tolerant to drought stress	6.7	Gascojen	39	-	4
Kavir	38	Tolerant to salt and terminal drought stress	6..3	Gaspard	32	-	5
Chamran	39	Tolerant to heat and drought stress	6..3	B.C Rowshan (winter)	45	-	4.5
Shiroud	38.5	-	6..5	B.C Rowshan (spiring)	38	-	3.5
Marvdasht	36	-	6.7	Falat	35	-	4.5
Sardary	39	Tolerant to cold stress	3.5				

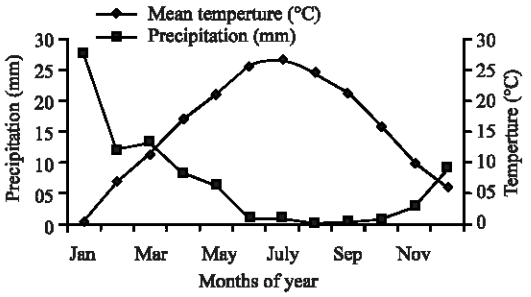

Fig. 1: Monthly means precipitation (mm) and temperature (°C) of Kerman city

added to the stress treatments while 13 mL of distilled water was added to control ones. Final coleoptile lengths were again measured 24 h after application of drought stress. All measurements were done with a ruler under a dissecting microscope (wild M8). Care was taken to avoid any damage to the growing parts. In each case growth was expressed as the difference between the initial and final measurements. The layout of the experiment was a factorial based on randomized complete block design with three replications. Experiment was done at a constant temperature of 22-23°C. Data were subjected to analysis of covariance, taking initial weight of the seeds as covariate.

In order to compare wheat cultivars yield under control and drought stress condition an experiment was also conducted in the field of agricultural research station of Kerman University. Monthly mean values of precipitation (mm) and temperature (°C) over a period of 50 years are given in Fig 1.

The field experiment was a split-plot based on randomized complete block design with three replication in which drought stress and control treatments were assigned to main plots and cultivars to the sup-plots. Needed amount of nitrogen and phosphorous fertilizers were calculated based on soil test and added to the soil before planting. In each sup-plot there were 3 rows which were 200 cm in length and distanced 20 cm from each other. All plots were irrigated after planting to assure a full crop establishment. In drought stressed main plots irrigation was stopped after stem elongation stage of growth. Plants were harvested from the middle rows. Grain yield and total shoot biomass were measured. Harvest index was expressed as the ratio of grain yield to total shoot biomass.

RESULTS

Initial seed weight was found to have significant effect on seedling early growth (Table 2). Cultivars average initial seed weight was compared on several

Table 2: Mean squares in the analysis of variance of wheat seedling characteristics germinated and grown under normal and drought stress condition

		Means	Squares
S.O.V	Degrees of freedom	Root growth	Coleoptile growth
Cultivar	32	927.07**	190.41**
Drought stress	2	14553.81**	6182.95**
Cul* D. stress	64	292.67**	32.06**
Seed weight	1	94.08*	40.15**
Error	195	24.75	6.95

*and**: Significant at 5% and 1% probability level, respectively

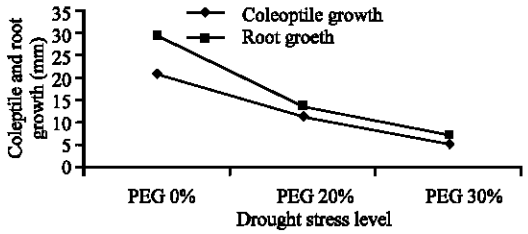

Fig. 2: Mean values of coleoptile and root growth (mm) of wheat cultivars under control, low and high drought stress condition

random samples using t-student test criterion. Results showed that there were significant differences among them (not shown). Therefore, analysis of covariance was performed to seek for the effects of initial seed weight as a covariate on coleoptile and root growth. Wherever the effect was significant adjusted data were used for further analysis. Results are shown in Table 2. As this table shows, both genotype and drought stress had significant effects on coleoptile and roots growth. Meanwhile, there was an interaction between genotype and drought stress. Generally seedling growth was inhibited by drought stress, in all wheat cultivars (Fig. 2).

Root growth was, on the average, inhibited more than coleoptile growth (Huang and Reddman, 1995). On the other hand, which coleoptile and root growth of cultivars under control condition were the same. However significant differences were found among them under low and high drought stress condition (Fig. 2).

The histogram of frequency distribution of the average values of coleoptile growth under low stress (PEG 20%) condition is shown in Fig. 3a. The bimodality of the frequency was clear even when class interval increased to 3 mm suggesting that genotypes could be classified into 2 different populations. To test the normality of the data, at first normal probability plot for the data was prepared using the method described by Bliss (1967). Both calculated Anderson-Darling statistic (0.918) and the p-value which was less than 0.005 indicated that one single normal distribution can not be fitted to the data

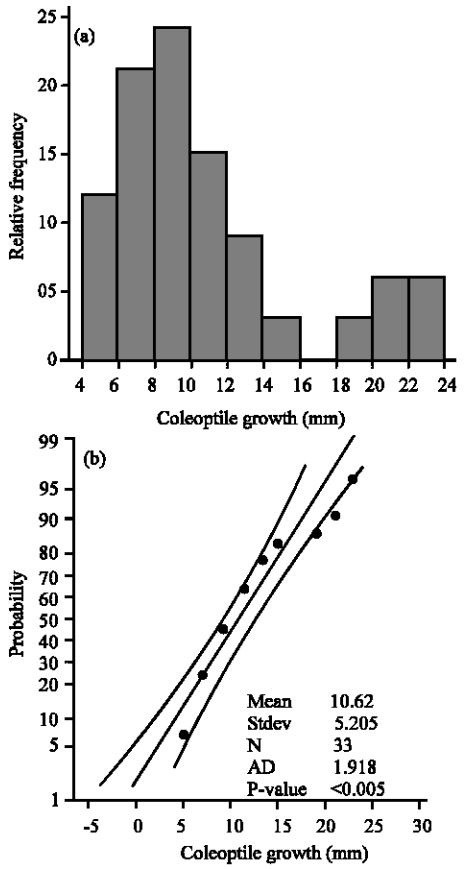

Fig. 3: (a) Histogram showing relative frequency distribution of the data regarding coleoptile growth of wheat genotypes germinated and grown under drought stress condition imposed by 20% PEG solution, (b)Anderson-Darling statistics and p-value showing that one single normal distribution can not be fitted to all data

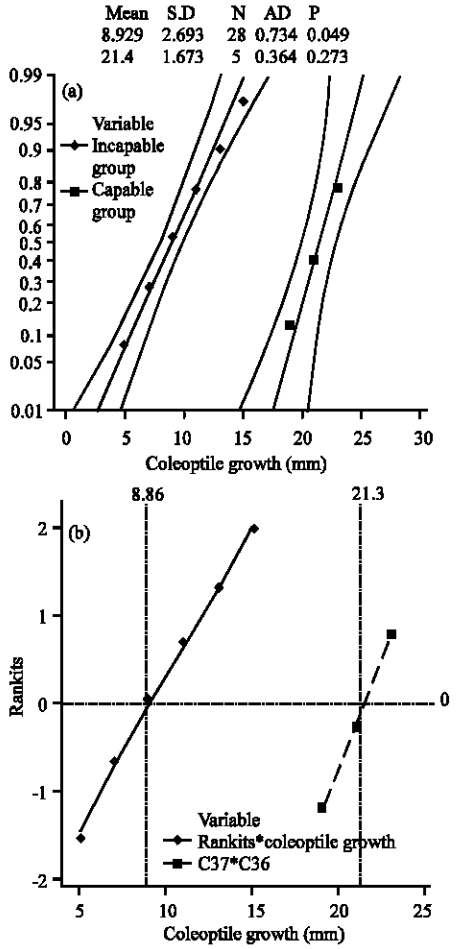

Fig. 4: (a) Normal probability plot of the data regarding coleoptile growth of wheat genotypes grown under drought stress imposed by 20% PEG solution. (b) Rankit test of normality of the data regarding wheat genotypes coleoptile growth germinated and grown under low drought stress condition imposed by 20% PEG solution

(Fig. 3b). The problem was therefore considered a case of mixed populations. To find which data points representing each population, at first the three points which were located almost outside the 95% confidence interval of predicted values were considered to belong to another population. Normal probability plots were again prepared for the 2 populations (Fig. 4a). A p-value of 0.049 which is very close to significance level and Anderson-Darling statistics of 0.734 were found for the first group. For the second group the values for the same quantities were 0.273 and 0.346, respectively suggesting that a normal distribution can significantly be fitted to the data points of the second group. Predicted parametric means and standard deviations of these distributions were found to be 8.93 and 2.69 mm for the first and 21.4 and 1.67 mm for the second distribution. Resulted plots indicated that there are 2 distinct normal distributions. Linear regression procedure in all cases was performed and the existence of the coefficients was statistically tested using ANOVA-procedure. And 95% confidence interval for the predicted data was also calculated and the lines were plotted.

Neither p-values nor Anderson-darling statistics were significant when another point was included into the second group of cultivars. Furthermore, minimum error sum of squares in the analysis of variance of linear regression was obtained only when the last there points was excluded from the total points, suggesting that the point of truncation is somewhere between 16 and 18 mm.

A Rankit test of normality was also performed to make sure that 2 significant normal distributions could be fitted

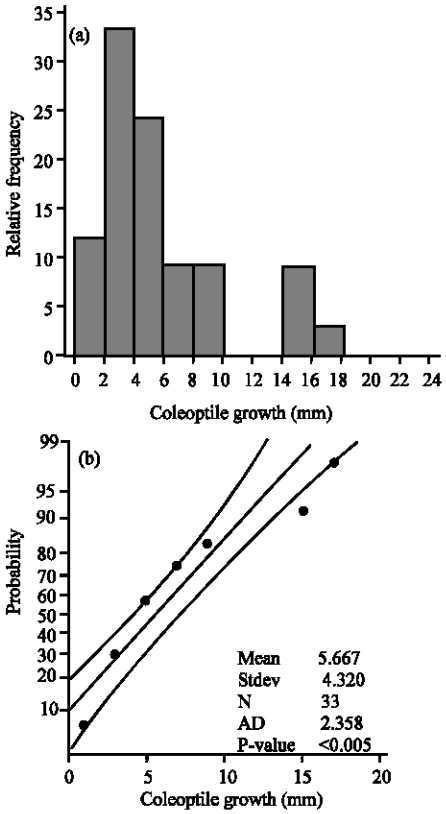

Fig. 5: (a) Histogram showing relative frequency distribution of the data regarding coleoptile growth of wheat genotypes germinated and grown under drought stress condition imposed by 30% PEG solution. (b) Normal probability plot of the data regarding coleoptile growth of wheat genotypes grown under drought stress impose by 30% PEG solutions

to the data (Fig. 4b). The distribution parametric means and standard deviations were again predicted graphically which were in accordance with what was obtained from normal probability plots.

The same procedures were applied to the data obtained from seedlings grown under high stress (30% PEG) condition. However, no significant distributions were fitted to them though frequency distribution of the data again showed a bimodal shape (Fig. 5 and 6).

A significantly normal distribution was fitted to the data for the second group of genotypes indicating that these genotypes could be separated significantly from the other. The data for the first group was not significantly fitted to a normal distribution. However, this group was considered as incapable for osmoregulation locating at 4.16 mm while the second is the capable group located at 15.5 mm.

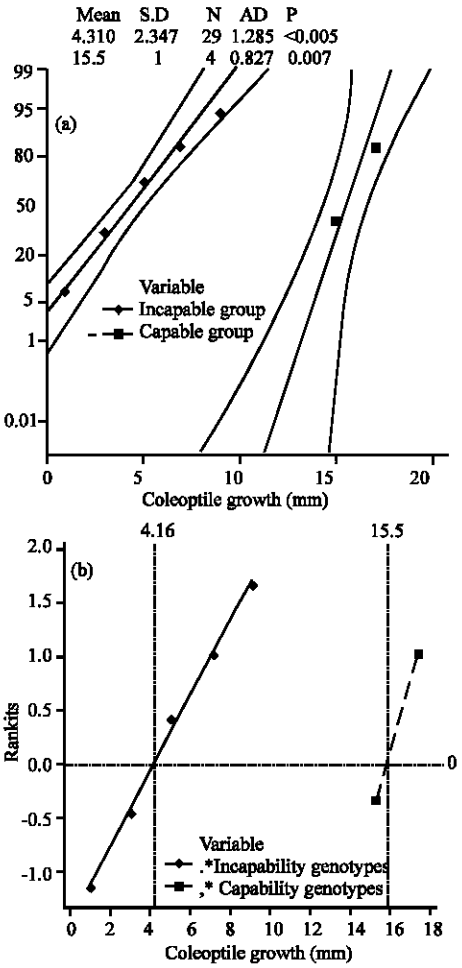

Fig. 6: (a) Normal probability plot of the data regarding coleoptile growth of wheat genotypes grown under drought stress imposed by 30% PEG solution. Anderson-Darling statistics and p-value for the incapable group are 0.827, 0.007, respectively. (b) Rankit test of normality of the data regarding wheat genotypes coleoptile growth germinated and grown under high drought stress condition imposed by 30% PEG solution

The same results were obtained in the case of root growth under 20% PEG solution. However, the data of root growth under 30% PEG solution could not make the 2 populations separated from each other, clearly.

Looking back to the cultivars included in each class interval, it was found that cultivars Alvand, Rowshan, Back Cross Rowshan (winter type), Dez and Kavir were included in the second and the other cultivars in the first distribution.

Significant differences were found between cultivar groups mean in terms of coleoptile and root growth

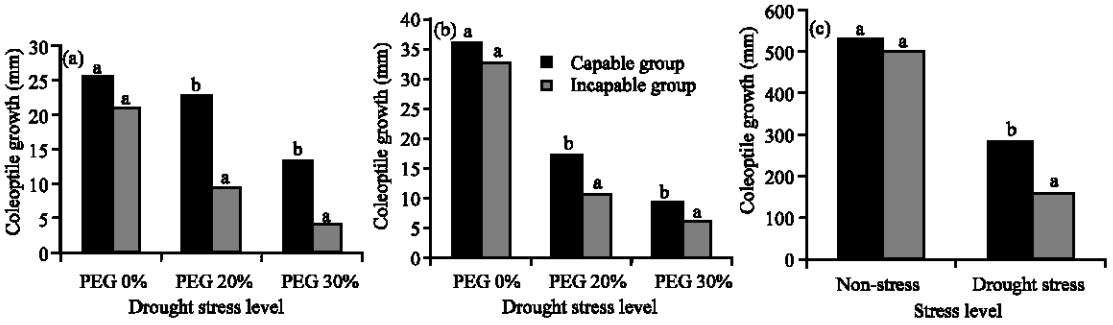

Fig. 7: Mean Coleoptile growth (mm) (a) and Root growth (mm) (b) of wheat cultivars classified as incapable and capable for osmoregulation were grown under drought stress level and Grain yield (g m^{-2})(c) of the same cultivar groups are also shown

Table 3: Pearson correlation coefficients between wheat seedling characteristics and yield of wheat cultivars grown under normal and drought stress condition

	Coleoptile growth (PEG0%)	Coleoptile growth (PEG20%)	Coleoptile growth (PEG30%)	Root growth (PEG20%)	Root growth (PEG30%)	Biologic yield (Control)	Biologic yield (stress)	Grain yield (control)
Coleoptile growth (PEG 20%)	0.61**	1						
Coleoptile growth (PEG 30%)	0.58**	0.82**	1					
Root growth (PEG 20%)	0.43ns	0.4*	0.53**	1				
Root growth (PEG 30%)	0.41ns	0.41*	0.56**	0.9**	1			
Biologic yield (control)	0.33ns	0.2ns	0.13ns	0.04ns	0.18ns	1		
Biologic yield (stress)	0.34*	0.28*	0.22*	0.21ns	0.12ns	0.12ns	1	
Grain yield (control)	0.33ns	0.39ns	0.39*	0.66**	0.03ns	0.17**	0.15*	1
Grain yield (stress)	0.43ns	0.37*	0.37*	0.29*	0.07*	0.06ns	0.76**	0.35*

*and**: Significant at 5% and 1%probability level, respectively. ns Non-significant

Table 4: ANOVA output for Grain yield, shoot biomass and HI in wheat cultivars as affected by water and drought stress

S.O.V.	Degrees of freedom	Grain yield	Shoot biomass	Harvest Index
Drought Stress	1	1468855.35**	5689757.53**	0.089*
Error(a)	2	3034.76	67501.48	0.003
Cultivar	32	9651.16**	73635.79*	0.01**
Cul* D.stress	2	4573.17**	86953.48**	0.007ns
Error(b)	128	5161.86	45976.07	0.008

*and**: Significant at 5% and 1% probability level, respectively. ns : Non-significant

showing that the second group has the ability to grow more than twice as that of the first group under drought stress condition (Fig. 7a,b).

Under high stress condition sample mean valves for coleoptile growth of first and second groups of cultivars were 10.5 and 3.29 mm, respectively. Under low stress condition the value were 16.96 and 8.21 mm.

Significant correlation coefficients were found between coleoptile growth under low and high drought stress condition and grain yield under both control and drought stress condition (Table 3). Mean grain yield of cultivars with high coleoptile growth was 1.8 times more than that of cultivars with low coleoptile growth (Fig. 7c). Drought stress had a significant effect on yield as well as on shoot biomass and harvest index (Table 4).

DISCUSSION

In this experiment, cultivars were classified into two groups based on the response of coleoptile growth under drought stress. As Ray *et al.* (1972) pointed out plant cells and tissues can grow in accordance with water uptake. For water uptake to takes place in turn, turgor pressure is necessary though should be accompanied by cell wall stress relaxation which could be achieved by lowering the counter pressure exerted by cell wall on protoplast as Schopfer (2006) described. To keep the torgur pressure high under drought stress condition, solutes should accumulate in cells in accordance with growth. Solute deposition is mainly occurring in growing cells (Kutschera, 1991) because it is needed to drive the uptake of water necessary for cell expansion (Silk *et al.*, 1986).

Changes in osmotic potential in responses to the changes in water potential was not measured in this experiment as the contamination of seedling parts with PEG solutions, was considered as a barrier against proper sampling of the tissues. However, Morgan's (1988) conclusion that cultivars with high osmoregulation capability, maintaining higher coleoptile growth under

drought stress condition, compare to low osmoregulation capability and other reports of genotypic differences in elongation of the shoots and most of stressed seedlings (Helmenck and Pfeifer, 1954; Morgan, 1988; Younis *et al*., 1963) which lend support to those differences in osmoregulation in expanding as well as expanded tissues we suggest that maintaining high growth under drought stress could be attributed to the ability of keeping high cell turgor pressure under such condition. In other word osmoregulation may exist in cultivars with high coleoptile growth while cultivars with low coleoptile growth may be considered as those with low osmoregulation capability. Coleoptile growth was on the average 9 mm higher in osmoregulation group compare to incapable ones under drought stress condition, though there was no difference between the two groups under control condition.

Grain yield data under drought stress condition were correlated significantly with coleoptile growth (0.37*) under the same condition. Cultivars which were classified as osmoregulation capable yielded 57.7% more than those which were incapable. It seems that selecting osmoregulation capable cultivars for cultivation under dry condition may increase grain yield considerably.

Another interpretation of the results may consider the lower growth of both coleoptile and of roots the second groups of cultivars. This may attributed to increasing levels of ABA as a growth inhibitor in response to environmental stresses such as drought. It has been shown that different organs respond differently to the increase of ABA concentrations. For example, roots are shown to be less sensitive to ABA compared to the shoots. Results of this study showed that roots growth decreased more than that of coleoptile under drought stress in all cultivars. This is in accordance with (Huang and Reddman, 1995) and may suggest that roots and coleoptile tissues were somehow not exposed to a drought stress level, which otherwise could induce increase in ABA concentration. Therefore it is unlikely that reduced growth to be attributed to increase in ABA concentration.

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