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Physiological tests as predictive appreciation for drought tolerance in durum wheat (*Triticum durum* Desf.)

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Abstract. Genetic advances in grain yield under rainfed conditions have been achieved by empirical breeding methods. Progress is slowed, however, by large genotype x season and genotype x location interactions arising from unpredictable rainfall, which is a feature of dry environments. A good understanding of factors limiting and/or regulating yield now provides us with an opportunity to identify and then select for physiological traits that increase drought tolerance and yield under rainfed conditions. Applying different physiological tests to appreciate drought tolerance in seedlings of durum wheat varieties leads to faster selection methods. Five (5) tests (germination, chlorophyll fluorescence extinction, electrolyte leakage, water and osmotic potential) were undertaken to evaluate the level of tolerance to water stress for 190 wheat accessions. Chlorophyll fluorescence extinction measurement seems to be the most reliable test enabling the discrimination of varieties according to their drought tolerance.

Keywords: Drought tolerance evaluation, durum wheat and physiological tests

Abbreviations: PEG, Poly-Ethylene Glycol; Fv, variable fluorescence; Fm, maximal fluorescence; INRAT, Agriculture Research National Institute of Tunisia; Chl., Chlorophyll; IDu-WUE, European Union project IDu-WUE (ICA3-CT-2002-10028)

INTRODUCTION

Achieving genetic increases in yield under rainfed conditions has always been a difficult challenge for plant breeders. Water loss can lower leaf water potentials, leading to reduced turgor, stomatal conductance and photosynthesis and, ultimately, to reduced growth and lighter yields. Several physiological characters, which can contribute to continued growth under water stress, have been identified (Garcia del Moral et al., 2003; Sayar et al., 2005). For example, osmotic adjustment is considered

to be an adaptation to water stress, by which an increase in the solute content of cells can lead to maintenance of turgor and turgor-related process at low water potentials (Kumar & Elston, 1992). Among other criteria found in sensitive varieties of wheat is negative value of water potential in stressed compared to control plants (Benlaribi et al., 1990). Chlorophyll fluorescence was used for detecting plant tolerance to heat and water stresses (Havaux et al., 1988), whereas, electrolyte leakage is used to study the changes in membrane structure and permeability (Martin et al., 1987; Vasquez-Tello et al., 1990).

Because yield and drought resistance are controlled at separate genetic loci (Blum, 1983 ; Morgan, 1984), breeding should involve the identification of physiological traits responsible for drought resistance. The utility of a particular measurement depends upon the rapid assessment of the plant at a critical stage using large quantities of plant material. Some physiological screening tests are suitable for testing large numbers of plant genotypes (Sayar et al., 2005).

A physiological approach can complement empirical breeding and can enhance the rate of yield improvement in four ways.

First, it can identify important traits for which there is inadequate genetic variation in the breeder's population. Second, large seasonal variation in yield and subsequent genotype x environment interactions will slow genetic gain for yield. Specific targeting of physiological characters that limit yield and have a high heritability may be more effective than direct selection for yield (Sayar et al., 2007).

Third, physiological traits can sometimes be measured off-season, or in controlled conditions, so that several generations can be grown each year with selection. Fourth, selection for physiological traits, particularly in early generation, can be more cost effective. Yield trials are expensive to conduct and if the population can be culled in earlier generations by effective physiological criteria, then this will allow more high-yielding, adapted entries to be tested, and greater replication in yield trials to increase selection precision.

Since, in Tunisia, durum wheat genotypes are mostly grown under rainfed conditions, they inevitably suffer from drought stress during the reproductive period of growth when stored water becomes depleted. High-yielding durum wheat varieties from several countries are being introduced in Tunisia and there is, therefore, a need for the screening and development of drought tolerant cultivars. The complexity of the drought effects requires an understanding of the plant's responses at the physiological level. It is therefore important to have a reliable physiological test to discriminate among tolerant or sensitive varieties for a breeding program. The physiological and morphological responses of cereals to water stress have been studied extensively (Boyer & Westgate, 2004; Blum, 2005).

This paper explores opportunities to improve drought tolerance evaluation in water-limited environment by:

- Comparing five tests in order to determine the most convenient to use in breeding programs.
- Testifying to the possibility of early identification of drought tolerant genotypes among 190 wheat accessions.

MATERIALS AND METHODS

This study was carried out at Kef research station (INRAT) located in a semi-arid area (36º14'N 8º27'E) in western Tunisia. On the basis of preliminary results of a field trial, 190 durum wheat accessions were divided into five groups according to their drought tolerance based on yield criteria (Table1). Determining the most reliable test for the evaluation of water stress tolerance required use of two variety groups as a reference, a group of sensitive varieties (GR1) and another of tolerant ones (GR5). Germination was studied on media differing in osmotic potentials (0, -0.2, -0.4, -0.6 and -0.8 MPa) prepared by adding PEG 10000 to distilled water according to Van't Hoff's equation (Lang, 1967). The other tests were carried out on the last fully expanded leaf on the main tiller at tillering stage (decimal Zadocks stage Z3.0).

Table 1. List of used genotypes for each group (GR) according to their drought tolerance based on grain yield (kg ha^{-1}); n : genotype number per group.

GR1(n =38)		GR2 (n =40)		GR3 (n = 38)		GR4 (n =38)		GR 5 (n = 36)	
Genotype	Yield (Kg.ha-1)	Genotype	Yield (Kg.ha-1)	Genotype	Yield (Kg.ha-1)	Genotype	Yield (Kg.ha-1)	Genotype	Yield (Kg.ha-1)
SHABHA	500	CHABA/DERAA	3005	MESSAPIA	3640	ROQUENO	4175	CIMMYT-73	4745
HAURANI	635	HEIDER	3005	COLORADO	3650	ANGRE	4175	MIKI-1	4770
CAPEITI 8	735	BOLIDO	3015	KARIM	3680	SVEVO	4190	CIMMYT-136	4775
CAPPELLI	1090	FORTORE	3015	1804	3705	RADIOSO	4210	TUNSYR-1	4780
TRINAKRIA	1125	SEBATEL-1	3020	TENSIFT-1	3705	CIMMYT-36	4215	RAZZAK (TUN)	4805
OMLAHN-3	1335	APPULO	3055	TERBOL97-3	3715	ILLORA	4215	DUROI	4850
PLINIO	1350	SEBAH	3060	ADYT02-505	3755	Marsyr-1	4215	GEROMTEL-1	4850
OMRUF-2	1680	CIMMYT-41	3070	NORBA	3670	BLK2	4230	LAHN	4855
CIMMYT-260	1810	CIMMYT-266	3125	AZEGHAR-2	3680	GRAZIA	4235	TOMOUH	4895
ANTON	1965	Saada3/Dds//Mtl1	3130	Simeto	3680	MARJANA	4250	AW12/BIT	4910
DERAA	1965	OFANTO	3145	IRIDE	3685	CIMMYT-23	4255	H.MOUL/CHA 88	4915
NILE	2165	OMBAR	3185	OUASERL-1	3685	BICREDERAA-1	4260	CIMMYT-172	4940
YOUSEF-1	2170	COLOSSEO	3190	VITRON	3687	GIDARA-2	4265	KRF	4960
TELSET-5	2180	CIMMYT-78	3215	DUREX	3710	DUILIO	4280	MOHAWK	4965
MURLAGOST-1	2215	ZEINA 1	3217	JAWHAR	3715	LAGONIL-2	4280	ITALO	4995
BIC/3/CH1//G//S	2265	PRODURA	3218	JORDAN	3740	OUASLAHN-1	4280	REVA	5070
WADALMEZ-1	2385	CIMMYT-47	3219	TORREBIANCA	3750	GARGANO	4285	MRB17	5070
VALBELICE	2465	CIMMYT-67	3220	KHABUR-1	3775	CIMMYT-52	4295	AMMAR-1	5090
QUADRATO	2520	FLAMINIO	3225	Sebou	3780	STOJOCRI-3	4300	CHAM-1	5095
KRS/HAUCAN	2560	SAJUR	3230	GEZIRA-17	3785	MASSARA-1	4310	CIMMYT-222	5120
ICARDA-125	2570	ARIESOL	3230	OMRABI 5	3815	SULA	4325	KRONOS	5135
VALNOVA	2615	MEXICALI 75	3240	Gr/Boy	3815	ARCOBALENO	4330	BCRCH-1	5220
TAREK	2620	ANOUAR	3250	MARZAK	3825	CORTEZ	4330	SENADUR	5255
CHACAN	2625	FURAT-1	3255	LOUKOS-1	3835	CANNIZZO	4340	ICARDA-121	5270
APPIO	2630	ICARDA-78	3255	MERIDIANO	3845	CHABHA-88	4355	AINZEN-1	5360
JABATO	2635	1807	3265	MOULSABIL 2	3850	MAAMOURI-1	4355	GUEROU-1	5360
BOMBASI	2685	PIETRAFITTA	3275	BORLI	3855	AGHRASS-1	4365	CIMMYT-247	5385
BRACHOUA	2690	OMRABI 3	3275	CIMMYT-198	3860	WEST BRED TUR	4375	CICCIO	5390
ALDEANO	2695	ASTIGI	3300	CIMMYT-104	3915	QUABRACH-1	4400	Bigost-1	5460
CRESO	2735	BRAVADUR	3320	Inrat69	3916	ATLAST-1	4420	LAGOST 3	5475
OMSNIMA-1	2740	OTB-6	3340	YOUNES-1	3925	VARANO	4440	OURGH	5480
QUADALETE	2745	IXOS	3365	OMBIT-1	3935	QUA //ER /MA /3/U	4445	BOLENGA	5530
BRONTE	2750	Kabir1	3390	LIRA B 45	3945	WEST BRED 881	4445	BICRE	5530
PLATANI	2810	ARTENA	3405	AUS-1	3950	AWALI-1	4450	LESINA	5605
BRADANO	2815	1805	3405	AMEDAKUL-1	3955	RIL-USA	4470	MONGIBELLO	5620
OUASLOUK-1	2815	CIMMYT-108	3435	CLAUDIO	3965	ISLY	4485	ARCANGELO	5905
GALLARETA	2820	BOABDIL	3450	YASMINE	3965	OMGENIL-3	4485		
BOLO	2830	ORT-1	3465	CANYON	3970	ARISLAHN-5	4510		
		DON PEDRO	3485						
		DURCAL	3400						
Mean yield	2208.95		3234.23		3798.24		4322.24		5150.97
Ecartype	679.130		131.181		106.816		96.182		299.406

1. Physiological tests

1.1 Germination test

Thirty (30) seeds of each variety were first disinfected by immersion in a calcium hypochlorite solution, containing 5% active chlorine, for 5 min. The seeds were then washed three times with sterilised distilled water. Germination tests were carried out in sterilised Petri dishes [(150 x 15 mm) covered at the bottom with a cotton layer]. The

dishes were moistened with equal amounts of PEG solutions and kept moist by adding 3 ml of the same solution, daily. Germination was carried out in a dark growth chamber (Model MB-60B, Percival Manufacturing Company, USA) at 25°C ± 0.5°C and 80% ± 1% relative humidity. Seeds were considered germinated when the radicle (coleorhizae) was at least 1 mm long. The germination percentage was determined after 7 days.

1.2 Chlorophyll fluorescence

Chlorophyll fluorescence was measured using a portable fluorometer (Handy PEA). Chl. fluorescence was expressed as the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm). Each value is a mean of 10 measurements.

1.3 Electrolytes leakage

Electrical conductivity of the solution containing the electrolytes leaking from leaf segments was used to assess the degree of drought tolerance. Segments (0.5 cm long) were cut from young fully expanded leaves on the main tiller. Leaf tissue (200 mg) was shacked for 30 minutes in a test tube containing 4 ml of demineralised water. It was then rinsed 3 times in order to eliminate surface electrolytes (Blum & Ebercon, 1981). The segments were then placed on a dry filter paper in Petri dishes under dark conditions (Martin et al., 1987). After 2 hours the segments were placed in another set of tubes containing 15 ml of demineralised water and shacked for 30 min. The conductivity of the solution (Lt) was then determined using a conductimeter. Other readings were made after 4, 6, and 8 hours of stress. The highest specific conductivity (Lk) of each variety was obtained after shacking leaf segments previously autoclaved for 30 min at 120°C. This value is directly linked to the concentration of electrolytes in the leaf. The level of electrolyte leakage in a given sample was calculated as 100Lt/Lk.

1.4 Water potential

Seeds from the 190 varieties were grown in 12 x 13 cm-pots containing compost. Watering was halted after 4 weeks when plants reached tillering stage. Water potential measurements were made before and after withholding water by using a pressure bombe on the youngest fully expanded leaf.

1.5 Osmotic potential

Osmotic potential was measured on samples taken before and after the start of the stress treatment. Samples of leaf tissue (500 mg) from each plant were placed in Eppendorf tubes with 4 holes in the bottom and frozen at -30°C. After 12 hours, the samples were removed from the freezer, thawed, then centrifuged at 15000 xg for 15 min in other Eppendorf tubes. For each sample, a 200 µl- aliquot was placed in a capsule and its osmotic pressure was measured with a freezing point Osmometer (Knauer rang 400–1600).

Statistical analysis

An augmented design was applied with 4 checks; the analysis of variance was done using MSTAT statistical package (NISSEN, 1990).

RESULTS AND DISCUSSION

2.1 Germination tests under water stress condition

After 7 days of imbibition, germination rates of various varieties decreased as the concentration of PEG increased (Fig. 1). However, it was only 9% at 25% PEG for the sensitive varieties (GR1), 10% for GR2, 14% for GR3 and 15% for GR4, whereas the tolerant varieties (GR5) showed a rate of 28%. At 30% PEG, only some seeds of varieties in GR5 germinated whereas no germination was observed for the rest of the varieties. The statistical analysis showed a significant difference between varieties ($P<0.01$) and a highly significant difference ($P<0.001$) between the different PEG concentrations, whereas the interaction genotypes x treatment was not significant (Table 2).

The fifth group (drought tolerant varieties) grown on 25% PEG was significantly higher (Fisher test at 5%) than the other groups, which showed no difference.

Germination test under water stress induced by PEG solution allowed the discrimination among varieties for their drought tolerance. According to Levitt (1980), germination test represents a good quick and reliable method for evaluating a large number of varieties in a short period of time.

2.2 Chlorophyll fluorescence

The Fv/Fm ratio in plants grown in suitable conditions is around 0.8 and decreases towards 0 under stress conditions. The results obtained in this study showed a considerable difference among the varieties. Compared to their respective controls, tolerant cultivars in GR5 showed a decrease of 16% of the ratio Fv/Fm (Fig. 2), whereas the susceptible cultivars in GR1 showed a higher reduction (63%). For the rest of the varieties the decrease was 30%, 40% and 51% for GR4, GR3 and GR2, respectively. Theses differences among the tolerant and sensitive varieties indicate that a measurement of photosynthetic parameters such as Fv/Fm may be used as a rapid and reliable test for drought tolerance. Chl. fluorescence measurements allowed the discrimination among tolerant and sensitive varieties (Table 2). Under water stress conditions the tolerant varieties maintained a higher photosynthetic activity than the sensitive varieties. This technique was also used in breeding programs to select for cold tolerance in durum wheat (Bertin et al., 1992) and for salinity tolerance (Piri, 1991). These results emphasized the importance of measuring chlorophyll fluorescence to evaluate plant adaptation to abiotic stress. For drought tolerance, the results of this study have shown that maintaining photosynthetic activity in rapidly dehydrated leaves is a characteristic of tolerance and may be used as a good indicator of drought tolerance in wheat. Although the measurements made in this study concern only the dehydration in leaves and does not include other types of mechanisms, this technique

could be reliable to discriminate among tolerant and sensitive varieties. Havaux & Lannoye, 1985 showed that the high inhibition of photosynthesis under water stress may not be only the result of chlorophyll degradation and stomatal closure, but is also a result of changes in the function of the thylakoid membranes resulting in a decrease in quantum yield of the primary photochemical reactions in PSII leading to an alteration of energy distribution between the two photosystems in favor of PSI.

2.3 Electrolyte leakage

Our results show that, in all varieties, electrolyte leakage increased as the time of dehydration increased (Fig.3). A significant difference was found between varieties ($P<0.01$). The difference between the sensitive and the tolerant varieties was significant ($P<0.01$) at all sampling times (Table 2). The leakage reached 21 and 11%, after 2 hours, 44 and 31% after 4 hours, 81 and 59% after 6 hours, 88 and 70% after 8 hours for sensitive and the tolerant varieties, respectively. The GR4, GR2 and GR3 showed a significantly higher leakage than the tolerant varieties in GR5. The differences were not significant between GR4 and GR2. However the varieties of GR1 showed a significantly higher electrolyte leakage than those in GR2 and GR4 at 6 hours stress (Fig. 3).

The results from electrolyte leakage measurements showed that membrane integrity was conserved for tolerant compared to sensitive varieties. This shows the importance of this test in discriminating among tolerant and sensitive varieties. This is in agreement with the conclusion of Martin and al. (1987) that electrolyte leakage was correlated with drought tolerance in different species such as Quercus sp., *Cornus florida, Acer saccharum* and *Juglans nigra*. The same conclusion was reported by Vasquez tello and al. (1990) for two different species (*Paseolus* and *Vigna*). The leakage was due to damage to cell membranes which become more permeable (Senaratna & Kersie, 1983).

2.4 Water potential

Water potential was measured before and after withholding water for 15 days for each variety. The mean value of water potential varied between -0.29 and -0.30 MPa in control plants of the five groups and between -2.74 and -1.15 MPa after 15 days of stress (Fig. 4). Significant differences were noticed between the values of water potential before and after stress for all the varieties (Table 2). The value of water potential for sensitive varieties changed from -0.29 to -2.74 MPa and from -0.29 to -1.15 MPa for tolerant varieties (GR5) with significant differences between the two groups. The remaining three groups showed fewer negative values. We observed also for sensitive varieties higher negative values of water potential than the tolerant varieties. These results agree with those of Benlaribi et al. (1990) on wheat, which showed a correlation between maintaining higher values of leaf water potential and drought tolerance. Similar conclusions were reported under cold stress (Park & Tsunoda, 1983; Amsaa, 1991) and salinity stress (Hadda & Coudret, 1991).

Table 2. Combined analysis of variance showing the mean squares of the genotypes and treatment factors and the interaction genotype x treatment for the germination test (GT), water potential (ψH), osmotic potential (ψo), electrolyte leakage (EL) and chlorophyll fluorescence (Fv/Fm).

Source of variation	GT (%)	ψ_H (MPa)	ψo (MPa)	EL (%)	Fv/Fm (%)
Genotype	817,94**	0,74***	0.003**	576.90**	460.13***
Treatment	27046,55***	30,74***	0.580**	3195.51**	13104.3***
Treatment x Genotype.	171.68	0,65***	0.003*	36.60**	460.13***
Error	89.23	0.01	0	3.58	1.86
CV %	21.28	8.85	3.99	3.55	1.73

*, **, ***: Significant at P<0,05; 0,01 and 0,001 respectively

Table 3. The 5 Durum wheat groups classification for drought tolerance for different used tests	
Germination test	group5 > group4 =group3 = group2 > group1
Osmotic potential	group5 > group4 = group3 = group2 =group1
Water potential	group5 >group4 >group3 =group2 > group1
Electrolyte leakage	group5 > group4 = group3 = group2 > group1
Chlorophyll Fluorescence	group5 > group3 = group4 > group2 > group1

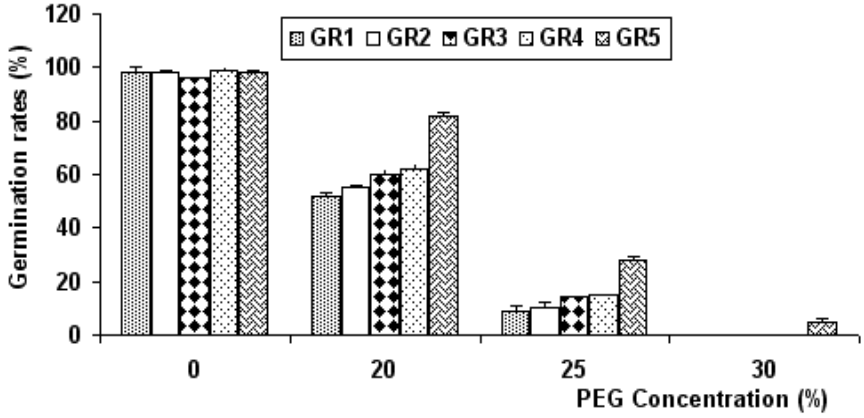

Fig. 1. Grains germination rates variation expressed in (%) after 7 days. Germination tested on media differing in osmotic potentials 0 MP (distilled water), -0.2 MPa (PEG 20%), -0.6 MPa (PEG 60%) and -0.8 MPa (PEG 80%). Germination tests were carried out in sterilised Petri dishes moistened with equal amounts of PEG solutions and kept moist by adding 3 ml of solution. Each value is a mean of 30 measurements. Error bars indicate the SE of the mean.

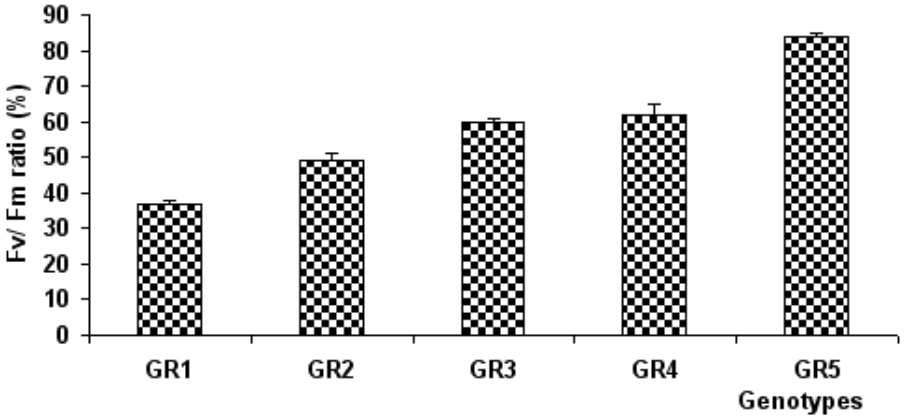

Fig. 2. Fv/Fm ratio (expressed as % of the control) variation 15 days after stopping irrigation for the five durum wheat groups. Measures are taken on the last fully expanded leaf of the main tiller for check (Full irrigation) and stressed plants. Each value is a mean of 10 measurements. Error bars indicate the SE of the mean.

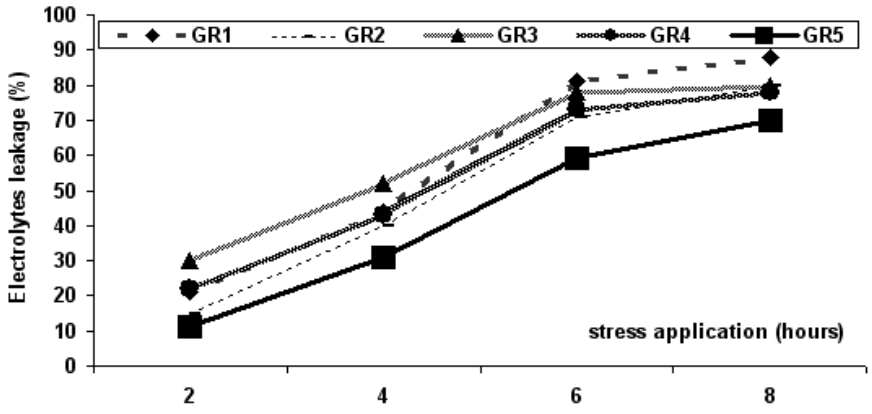

Fig. 3. Electrolyte leakage variation 15 days after stopping irrigation for the five durum wheat groups. Measures were taken on the last fully expanded leaf of the main tiller for check (Full irrigation) and stressed plants. Each value is a mean of 30 measurements.

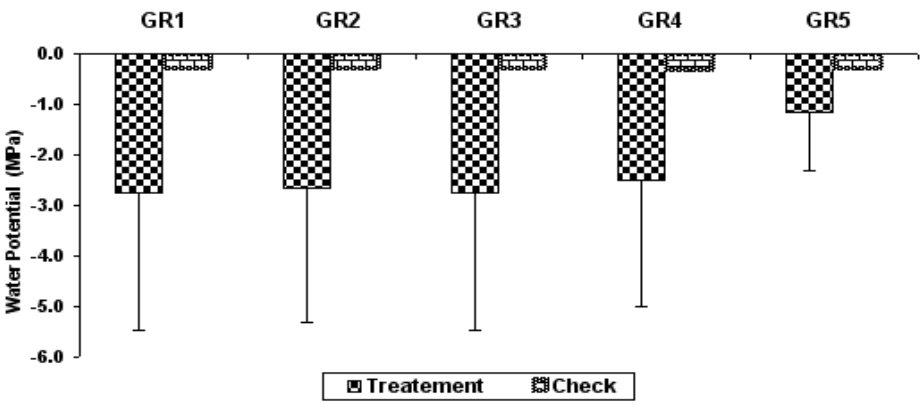

Fig. 4. Leaf water potential variation 15 days after stopping irrigation for the five *durum wheat* groups. Measures were taken on the last fully expanded leaf of the main tiller for check (Full irrigation) and stressed plants. Each value is a mean of 30 measurements. Error bars indicate the SE of the mean.

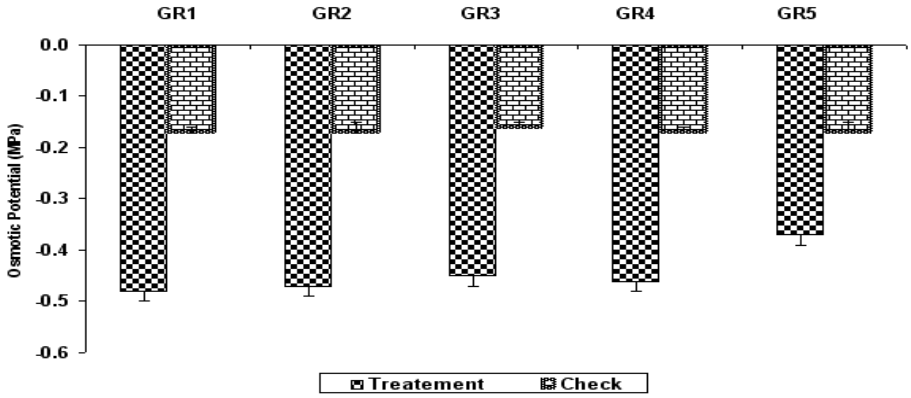

Fig. 5. Leaf osmotic potential variation 15 days after stopping irrigation for the five durum wheat groups. Measures were taken on the last fully expanded leaf of the main tiller for check (Full irrigation) and stressed plants. Each value is a mean of 30 measurements. Error bars indicate the SE of the mean.

2.5 Osmotic potential

Water stress resulted in the increase of osmotic potential in the cells of the 190 varieties (Fig.5). Osmotic potential was highest (-0.49 MPa) in the sensitive variety 15 days after withholding water. The value of osmotic potential of the tolerant varieties was nearly the same as for the control (unstressed).

The differences were highly significant among control and stressed plants in all varieties ($P < 0.01$). The differences between GR2, GR3 and GR4 were not significant

under stress conditions. Only the tolerant varieties showed less negative osmotic potentials (Fig. 5).

Osmotic pressure is an indication of solute concentration in the cell, but it does not necessarily indicate the occurrence of osmoregulation (Lorent, 1988). In fact, the increase in osmotic pressure often results from a passive accumulation of solutes resulting from tissue dehydration. For the tolerant variety, an active accumulation of solutes (osmoregulation) was occurring. This is the only adaptive and positive response beneficial to the plant under water stress conditions (Turner, 1986). Osmoregulation enables the plant to maintain high turgor pressure and as well to survive under stress conditions. Jones et al. (1981) reported that osmotic potential is considered an important selection criterion for rice. Osmotic adjustment protects also the photosynthetic apparatus against photo-inhibition (Downton, 1983) and hence confers dehydration tolerance (Flowers & Ludlow, 1986).

CONCLUSION

Under water stress conditions, varieties in GR5 were the most tolerant whereas those in GR1 were the most sensitive. Germination and osmotic potential tests under stress conditions could not distinguish among varieties and thus can be regarded as less accurate. The water potential test indicated a better tolerance for GR3 varieties whereas electrolyte leakage test showed no difference among GR2, GR3 and GR4 varieties. Chlorophyll fluorescence extinction measurement under water stress conditions seemed to be the most reliable test enabling the classification of the variety groups according to their drought tolerance. These results emphasized the importance of measuring chlorophyll fluorescence as an early test to evaluate drought tolerance in screening large numbers of cultivars for drought tolerance.

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