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Screening of Wheat Genotypes (*Triticum durum* L.) in Response to Drought Stress by Some Physiological and Biochemical Indices

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ABSTRACT

Among several factors known, drought remains the most important factor affecting wheat yield worldwide. Under the present study, total eleven wheat genotypes were screened to study the physiological and biochemical characters for drought tolerance. Pot experiment for two seasons was carried out with a view to understand the traits which help to find out the drought tolerant wheat genotypes quickly. Stress treatment was given on 60, 70 and 80 days after post-sowing and non stress (well watered) as control. Analysis of variance showed significant results for all traits studied. Stress significantly affected the 50% maturity and decreased the plant height, spike length and grain yield. Compared with drought susceptibly index (DSI), drought tolerance efficiency (DTE %) was found to be increased in all genotypes under stress conditions. Genotypes for membrane stability index (MSI %), proline and total soluble sugar content were also analyzed. Results showed that, increased MSI, proline, sucrose and glucose helped all genotypes to stand better under stress than control ones. Physiological characters are the yield stability parameters and could be useful for evaluating drought tolerance wheat genotypes while a biochemical character plays a role in osmotic adjustment including stabilization of cell membrane under stress conditions.

Key words: *Triticum durum L., Drought, Grain yield, Membrane stability index, Proline content.*

INTRODUCTION

Wheat production in the arid region is often limited by less moisture conditions. Visible symptoms of plant exposure to drought are leaf wilting, a decrease in plant height, spike length and ultimately reduction in grain yield. Drought stress at the grain filling period dramatically reduces grain yield[1]. Breeding for drought resistance is complicated by the lack of fast, reproducible screening techniques and the inability to routinely create defined and repeatable water stress conditions when a large amount of genotypes can be evaluated efficiently[2].

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Achieving a genetic increase in yield under these environments has been recognized to be a difficult challenge for plant breeders[3]. Thus, drought indices which provide a measure of drought based on yield loss under drought conditions in comparison to normal conditions have been used for screening drought-tolerant genotypes[4]. These indices are either based on drought resistance or susceptibility of genotypes[5]. Drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress[6].

Drought tolerance efficiency and DSI are the yield stability indices which based on how much reduction was realized under drought stress. The genotypes with high DTE and low DSI were evaluated as drought resistant and genotypes with low DTE and high DSI were considered as drought susceptible. Some researchers found that the cultivars which had the lowest DSI values were drought resistant than the cultivars with the highest DSI values[7].Stress susceptibility index (SSI) of the cultivar, which can be used to identify genotypes that produce high yield under both stress and non-stress conditions[8]. MSI significantly decreases under water stress, also shows a more decline in susceptible genotypes. A decrease in MSI reflected the extent of lipid peroxidation caused by reactive oxygen species[9]. It has been reported that drought stress tolerant and intermediate tolerant genotypes were superior to susceptible ones in maintaining membrane stability.

Osmotic adjustment is a well-known mechanism by which plants tolerate drought. Compatible solutes are produced at higher levels when plants experience osmotic stress as a means to facilitate osmotic adjustment[10-11]. These compounds accumulate in high amounts mainly in cytoplasm of stressed cells and behave as osmoprotectants of membrane and protein integrity[12]. High accumulation of proline[13] and sugars[14] under stress is a characteristic feature of most plants. The osmotic adjustment allowed the maintenance of turgor pressure for cell elongation and several metabolic functions, although the complex relationships between turgor maintenance, growth and osmotic adjustment were also experimented on the basis of stress-induced modifications of cell wall properties[15]. Proline seems to have diverse roles under osmotic stress conditions, such as stabilization of proteins, membranes and subcellular structures, and protecting cellular functions by scavenging reactive oxygen species[16].

Osmotic adjustment is a mechanism to maintain water relations under osmotic stress. It involves the accumulation of a range of osmotically active molecules including soluble sugars, sugar alcohols, proline, organic acids etc[17]. Various authors point to the role of soluble sugars in the protection against stresses. Mobilization of storage reserves in the endosperm of cereal seeds is tightly regulated and has a primary pivotal role in the interactions among sugar, ABA and gibberellin pathways responsible for the response to drought[18]. A central role of sugars depend not only on direct involvement in the synthesis of other compounds, production of energy but also on stabilization of membranes[19], action as regulators of gene expression[20] and signal molecules. So, analysis of proline and soluble sugar content could be a very good criterion for selecting tolerant genotypes under drought stress condition. The present study was undertaken to assess the selection criteria for identifying drought tolerance, so that suitable *durum* wheat genotypes can be recommended for cultivation in the arid and drought prone areas.

MATERIALS AND METHODS:

Plant Material

Seeds of 11 wheat genotypes (*Triticum durum* L.), five susceptible (HI-8498, GW-1139, MPO-1215, PDW-291 and RAJ-1555) and six resistant (A-28, Arnej-206, Arnej- 9-30-1, Gujarat wheat-1, AR-07-30 and AR-07-33) differing in their degree of susceptibility to drought were studied for various physiological and biochemical approaches. Selected wheat genotypes were procured from “Main Wheat Research Station” Vijapur, Sardarkrishinagar, Dantiwada Agricultural University and “Regional and Wheat Research Station”, Arnej, Anand Agricultural University.

Experiment and drought stress conditions

For natural drought induction, the pot trial was conducted for two *rabi* seasons during the years 2010-2011 to 2011-2012 in polyhouse. Total eleven genotypes of *durum* wheat were sown in the sterilized plastic pots (*28* x *25cm dia*.).In each pot, six seeds were sown and normal agronomical practices were followed. Water stress treatment was given at three different stages on 60, 70 and 80 days after sowing (DAS). For control, pots were irrigated regularly according to the requirement of plants. For physiological observations plants were grown till maturity. Leaf samples for biochemical analysis were collected after taking the physiological observations on 60, 70 and 80 days after drought treatment.

Days of 50% flowering and days for 50 % maturity

Observations about days of 50% flowering and days for maturity were recorded [21].

Plant height, spike length and grain yield

Plant height, spike length and total grain yield were measured[22]. Four plants were randomly chosen from each pot to measure these parameters.

Drought tolerance efficiency (%) and Drought susceptibility index (DSI)

Drought tolerance efficiency (DTE) and DSI for grain yield were calculated[23].

$$DTE\ (\%) = \frac{Yield\ under\ stress\ condition}{Yield\ under\ control\ condition} X100$$

$$DSI = (1 - \frac{yd}{yp})/D$$

Where *yd* – yield under stress condition (gm), *yp* – yield under control condition (gm) and *D* = 1- mean yield of all genotypes under drought stress/ mean yield of all genotypes under control condition.

Membrane stability index

Membrane stability index (MSI) was determined [24].

$$MSI = 1 - \frac{C1}{C2}$$

Where $C_1$and C_2 are the samples at 40^{O}C and 100^{O}C for 30 and 10minutes respectively (Electric conductivity).

Proline

Proline content of wheat genotypes was determined[25].

Proline (mg/g of tissue) = Sample O.D x Graph factor x Dilution factor/Weight of tissue (mg)

Total soluble sugar content

Total soluble sugars (sucrose and glucose) from the wheat genotypes were determined by phenol-sulphuric acid method [26] with some modifications.

Total soluble sugar (mg/g F.W.) = Sample O.D x Graph factor x Dilution factor/ Weight of the tissue (mg)

Statistical analysis

Statistical analysis was done by factorial completely randomized design (F-CRD) with two factors, genotypes and treatment having three replications for 60, 70 and 80 DAS separately. Simple correlation coefficient analysis was done by using SPSS software between all parameters by using means of both control and treatments together for all traits studied.

RESULTS AND DISCUSSION

Days of 50% flowering and days for maturity

All genotypes showed the differences in their days of 50% flowering and days for maturity (Fig.1). The results obtained were similar for days to 50% flowering and 50% maturity for all the genotypes under control as well as under stress condition. Under stress conditions, susceptible genotype HI-8498 and PDW-291 required 58 and 52 days for 50% flowering while tolerant genotypes AR-07-33 and AR-07-30 to 61 and 60 day for 50% flowering. Susceptible genotypes HI-8498, GW-1139, MPO-1215 and tolerant genotypes A-28, Arnej-206 and Arnej-9-30-1 showed the lowest days for 50% maturity, suggesting that, these genotypes showed the escape mechanism of drought tolerance and can be suitable for dry land agriculture. Above results are in agreement with the results [27], in which they studied the genetics of drought tolerance in wheat (*Triticum durum* Desf.).

Plant height, spike length and grain yield

Drastic reduction in plant height, spike length and grain yield of all wheat genotypes were observed under stress condition than control condition (Fig. 2).

Plant height and spike length traits are directly related to the yield and its components. Susceptible genotypes GW-1139 (52.7 cm) and HI-8498 (6.17 cm) showed the highest plant height and spike length among all the genotypes under stress condition and could be used in water deficit condition for better yield. Among all the susceptible and tolerant genotypes, HI-8498 (4.42 g m^{-2}) and AR-07-30 (3.38 g m^{-2}) exhibited significantly higher grain yield under stress condition than the other genotypes and were found to be stable and drought tolerant genotypes.

Drought tolerance efficiency (DTE %) and drought susceptibility index (DSI)

Significant differences were observed in DTE and DSI in all the genotypes under stress condition. The DTE in susceptible genotypes was ranged from 66.10 to 86.12% while in tolerant genotypes; it was ranged from 71.00 to 82.07 %. DSI ranged from 0.86 to 1.96 in the susceptible genotypes and 0.80 to 1.62 in tolerant genotypes (Fig. 3). More DSI was found in susceptible genotype RAJ-1555 (1.96) whereas low in PDW-291 (0.86). Tolerant genotype A-28 had the highest DSI (1.62) and Arnej-206 had the lowest (0.80).

It was concluded that, susceptible genotypes HI-8498, PDW-291 and tolerant genotype Arnej-206 had the highest DTE and lowest DSI among all the genotypes. Drought tolerance and stability of the genotypes were characterized using drought susceptibility index (S)[8].Findings of this study showed that, the breeders should choose the indices on the basis of stress severity in the target environment and DTE and DSI are suggested as useful indicators for *durum* wheat breeding under drought stress condition.

Membrane stability index (MSI %)

Membrane stability is a widely used criterion to assess crop drought tolerance. Tolerant genotypes A-28 and GW-1 showed the highest MSI (34.41, 30.33 and 36.99 %) under stress condition at three stages, showing the less electrolyte leakage and cell membrane damage (Fig.4). Data also showed that, the significant increase in the membrane stability index (MSI) was observed in all the genotypes under stress condition at three stages. Cell membrane is one of the first targets of plant stresses[28] and membrane stability is a widely used criterion to assess crop drought tolerance.

Proline content

Proline plays an important role in water stress tolerance mechanism(s) in plants due to its ability in opposing oxidative stress and considered this as the most important strategy in plants to overcome water deficit effects. Significant differences in proline content were observed in all the genotypes under both conditions.

Tolerant genotypes A-28 (28.44 µg g^{-1} FW) and Arnej-206 (16.3 and 28.50 µg g^{-1} FW) showed the highest proline content under stress conditions than other genotypes (Fig.4). Among all the susceptible genotypes, Raj-1555 (22.16, 22.2 and 19.31 µg g^{-1} FW) showed the highest proline content and gives the better performance under stress conditions at the three stages. Proline plays an important role in water stress tolerance mechanism(s) in plants due to its ability in opposing oxidative stress and this is considered as the most important strategy in plants to overcome water deficit effects[29].

Total soluble sugar content (Sucrose and glucose)

Overall sucrose content in all genotypes under both control and stress conditions at three stages was ranged from 1.67 to 6.06mg g^{-1} and 6.77 to 14.07 mg g^{-1} respectively (Fig.5). Results indicated that there was more increase in sucrose content in all the tolerant wheat genotypes under stress conditions at three stages than the susceptible genotypes. While glucose content in all the genotypes under control condition at three stages was ranged from 3.81 to 16.06 mg g^{-1} FW while under stress conditions; it was ranged from 15.60 to 33.52 mg g^{-1} FW respectively .Glucose content was higher in tolerant genotype AR-07-33 (16.51 mg g^{-1} FW) while tolerant genotypes A-

28 contained lower glucose (3.81 mg g^{-1} FW) under control conditions at 60 DAS. There were significant differences observed among all the genotypes under both conditions. Results indicated that, the tolerant wheat genotypes showed the highest glucose content under control condition at the three stages as compared with the susceptible ones. More increase of glucose content was observed in all tolerant wheat genotypes under stress conditions at the three stages than susceptible genotypes.

Total soluble sugar is one amongst the most important cyto-solutes and accumulates in higher plants during the adaptation to various abiotic stresses especially during drought stress. Among all the tolerant genotypes, genotype AR-07-33 showed the highest sucrose content under stress condition at 60 and 70 DAS, while, in all the susceptible genotypes, HI-8498 showed better performance under both control as well stress conditions at 60 and 80 DAS. A significant increase in glucose content was observed in all the six tolerant wheat genotypes (A-28, Arnej-206, Arnej-9-30-1, GW-1, AR-07-30 and AR-07-33) under stress conditions at 70 DAS. Among susceptible genotypes, better performance was shown in the genotype RAJ-1555 under stress conditions at 60 and 80 DAS. Results indicated that, the tolerant genotypes showed the highest sucrose and glucose content and better performance under both conditions at three stages than susceptible genotypes could be used for assessing the drought tolerance mechanism. Soluble sugar content plays a very important role in carbohydrate metabolism and has a close relationship with photosynthesis and production[30]. The level of sugar content is a sign of the supply ability of grains to use assimilates [31]. Observed the increase in soluble sugars in all the five wheat cultivars under PEG induced drought stress[32].

Correlation between physiological and biochemical parameters

Correlation coefficient study showed the significant correlation of days to 50% maturity with days of 50% flowering, while plant height had significant correlation with 50% maturity. Highly significant correlation was found between spike length and plant height while grain yield was significantly correlated with plant height and spike length and non-significantly correlated with 50% flowering, 50% maturity (Table 1). Table 2 showed the combined correlation study under drought stress at 60, 70 and 80 days for total soluble sugars, proline content and membrane stability index. Results indicated that all biochemical parameters are highly significant with each other.

Table 1: Correlation study of traits related to drought tolerance

	50% flowering	50% maturity	Plant height	Spike length	Grain yield
50% flowering	1.000				
50% maturity	0.428*	1.000			
Plant height	0.288	0.417*	1.000		
Spike length	-0.064	0.074	0.691**	1.000	
Grain yield	0.101	0.163	0.455*	0.446*	1.000

Note: n= 66,*Significant at 0.05 = 0.404, **Significant at 0.01 = 0.515

Table 2: Combined correlation study under drought stress at 60, 70 and 80 days

	Sucrose	Glucose	Proline	MSI
Sucrose	1.000			
Glucose	0.806**	1.000		
Proline	0.928**	0.761**	1.000	
MSI	0.671**	0.432**	0.738**	1.000

Note: n= 66, *Significant at 0.05 = 0.242, **Significant at 0.01 = 0.315

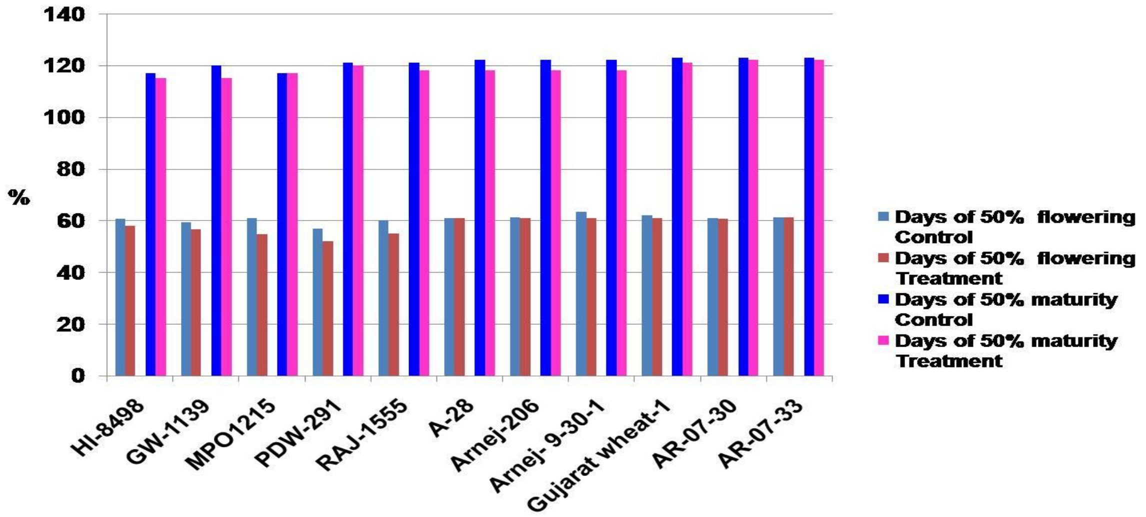

Fig. 1: Days of 50% flowering and days of 50% maturity of wheat genotypes under control and stress conditions (60 DAS)

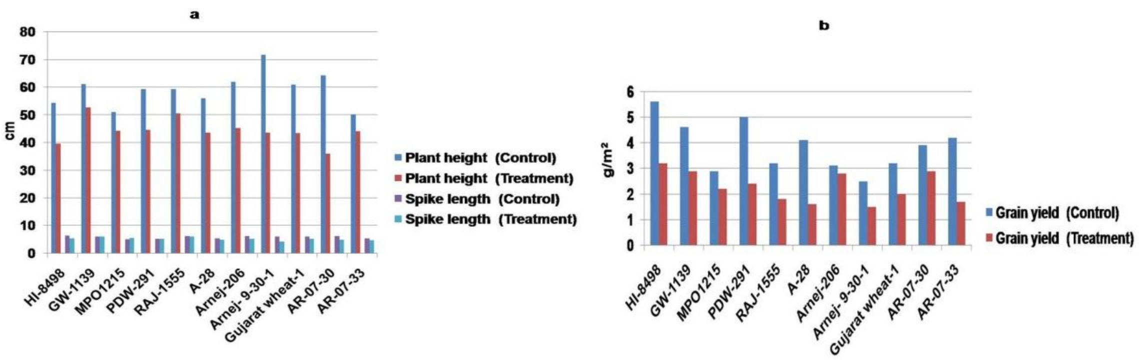

Fig. 2: a) Plant height and spike length. b) Grain yield of wheat genotypes under control and stress conditions

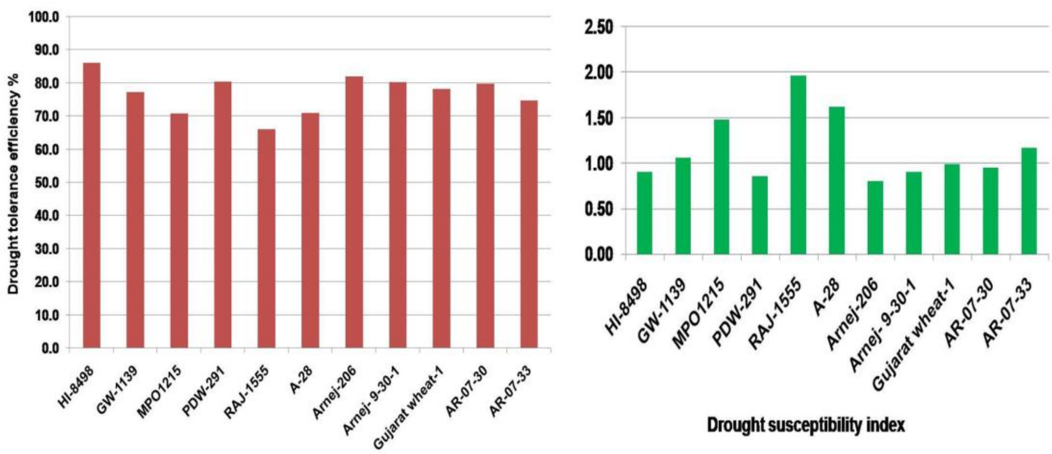

Fig. 3: Drought tolerance efficiency (%) and Drought susceptibility index of wheat genotypes under stress conditions

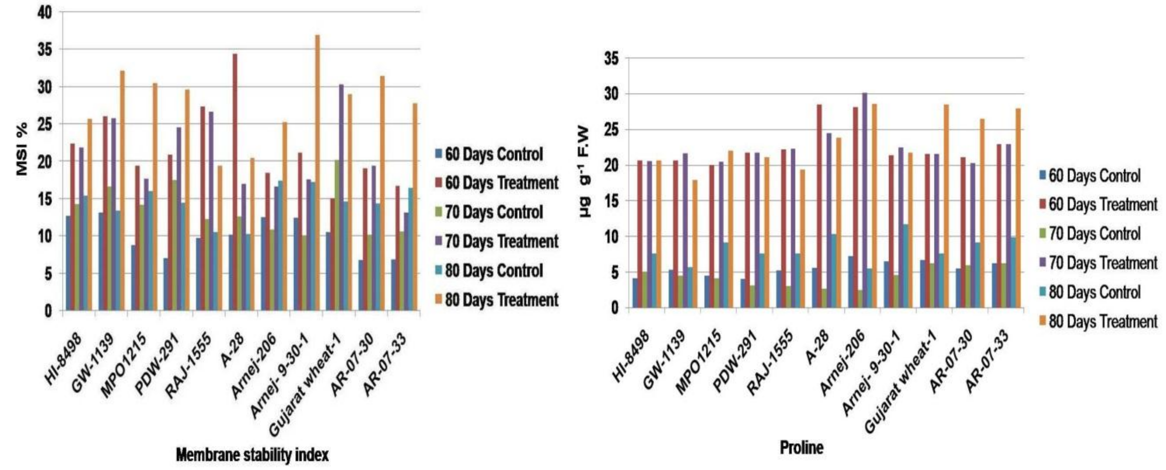

Fig. 4: Membrane stability index and proline content in leaves of the wheat genotypes under control and stress (at 60, 70 and 80 days) conditions

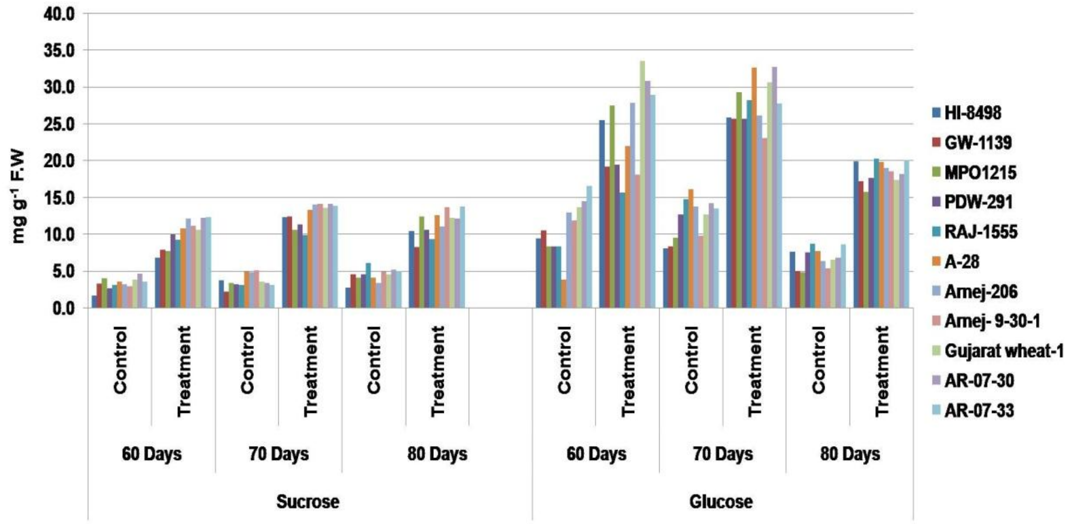

Fig. 5: Total soluble sugar (sucrose and glucose) content in leaves of the wheat genotypes under control and stress (at 60, 70 and 80 days) conditions

CONCLUSION

If the strategy of breeding program is to improve yield under stress condition, it may be possible to explain local adaptation to increase stress tolerant from selection conducted directly in that environment[33].The findings of this study showed that the eleven wheat genotypes gave significant results for all the above indices under stress condition. Grain yield, high DTE, low DSI, high MSI, proline content and total soluble sugar are suggested as useful indicators for *durum* wheat. Therefore, genotypes which showed higher amount of these indices were identified as the most tolerant genotypes. They showed considerable potential to improve drought tolerance in *durum* wheat through breeding programs.

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