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EVALUATION OF DROUGHT TOLERANT WHEAT GENOTYPES USING MORPHO-PHYSIOLOGICAL INDICES AS SCREENING TOOLS

S.M. MUJTABA[*], SUMMIYA FAISAL, MUHAMMAD ALI KHAN, M.U. SHIRAZI AND M.A. KHAN

Plant Physiology Division, Nuclear Institute of Agriculture (NIA),Tandojam, Sindh, Pakistan
[*]*Corresponding author's email:* mujtaba_nia@yahoo.com

Abstract

As water is the major limiting factor for agricultural crop production in arid and semi-arid areas, hence, twenty six wheat genotypes were screened under terminal drought stress for assessing their desiccation tolerance potentials. Experiment was conducted in completely randomized design (CRD) with three replicates and two irrigation treatments (control and terminal drought). Water scarcity had significantly reduced growth and yield contributing attributes while six genotypes performed relatively better under drought stress. Highest germination potentials (62.66%) were observed in MAS-20/2014 at -1.0 MPa osmotic stress under control conditions. However, genotype MAS-3/2014 exhibited maximum grain yield (23.32 g plant^{-1}) while MAS-20/2014 showed minimum reduction (15.70%) in grain yield under drought stress. Physiological studies highlighted that better yielding genotypes exhibited relatively less reduction in chlorophyll contents (5.93%) in MAS-20/2014, nitrate reductase activity (6.70%)in MAS-11/2014 and osmotic potentials (0.769 MPa) in MAS-3/2014, while more relative increase in proline accumulation (83.35%) in MAS-20/2014 , glycine-betaine (92.43%) in MAS-3/2014, total soluble sugars (36.65%) in MAS-23/2014 and potassium contents (3.66%) in MAS-6/2014 were analyzed under drought. These findings illustrated that wheat genotypes MAS-2/2014, MAS-3/2014, MAS-8/2014, MAS-12/2014, MAS-18/2014 and MAS-20/2014 exhibited better tolerance under drought conditions making them suitable for enhancing the productivity of rain fed and arid areas.

Key words: Chlorophyll, Germination, Grain yield, Osmo-protectants, Nitrate reductase.

Introduction

Drought is a natural hazard which intensifies the water scarcity and brings significantly adverse impacts on global economy. Global warming, irregular and insufficient patterns of rainfalls and un-judicious use of water resources are the leading causes of soil water deficit (Rosegrant & Cline, 2003). It is a worldwide problem, which has confined the quality and productivity of crops. Out of 1474 million ha cultivated land of world, 86% area comes under rain fed cultivation (Kumar, 2005).

Wheat is a staple food of about 35% population of world. There is an elevation in demand for wheat production due to exponentially increasing human population. Under extreme climatic conditions, current rate of wheat production is not sufficient to fulfill food demands of the world due to limited irrigation resources and low ground water table (Moaveni, 2011). Furthermore, rapid increase in population growth, urbanization, industrialization and agricultural development has increased country's water requirement. Pakistan has diverse climatic conditions and two-third of the land area lies in semi-arid and arid climate regions (Chaudhry&Rasul, 2004). Experts have also predicted extreme water scarcity in coming decades in Pakistan (Anon., 2005).

Plants have developed specific adaptive mechanisms to survive and acclimatize under moisture deficit conditions (Price *et al*., 2002). Under semi-arid regions, water is limiting factor for seed germination, as seed water potential reduces under extreme water deficiencies in soil. Similarly, plant growth, photosynthesis and dry matter production is also reduced which ultimately affects the gain yield. In wheat, germination, tillering and reproductive stages are considered as most sensitive traits to drought stress (Passioura, 2007). Nouri-Ganbalani *et al*. (2009) documented that about 17 to 70% grain yield of wheat was declined under reduced soil water conditions. Katerji *et al.* (2009) also reported that imposition of drought stress during ear formation and flowering stages had caused 37% and 18% decline in grain and straw yields.

Plants respond varyingly under environmental stresses. The main strategy adapted by plants under drought conditions, may involve accumulation of compatible organic and inorganic solutes (Dijksterhuis & De Vries, 2006). Proline and betaine, as low molecular weight amino acids, enable the plants to survive under low osmotic potentials (Jaleel *et al*., 2007). However, Vendruscolo *et al*. (2007) also found that proline is engaged in tolerance mechanisms against oxidative stress.

In order to utilize the uncultivated lands of arid and semi-arid regions of the country, there is a dire need to develop and identify the drought tolerant wheat genotypes. Furthermore development of drought tolerant wheat genotypes will be fruitful for future breeding programme in the country.

Keeping these considerations in view, this study was designed to evaluate the drought tolerance of various wheat genotypes developed by the breeders of Nuclear Institute of Agriculture, Tandojam, using growth, yield and physico-chemical responses of the genotypes under drought conditions.

Materials and Methods

Experiment was conducted in the cemented tanks (tank size =3 m^2 and 1 m depth) filled with sandy clay loam soil, in wire netted pot house (controlled conditions) at Nuclear Institute of Agriculture (NIA), Tandojam during the year 2014-15. Twenty six wheat genotypes were obtained from Plant Breeding and Genetics Division, NIA, Tandojam. Layout of the experiment was in completely randomized design (CRD) with two treatments, i.e. control (normal irrigations) and terminal drought (only soaking dose).Each treatment was replicated thrice. Sowing was done on 10th November, 2014. The plant to plant and row to row spacing was maintained as 10 and 20cm, respectively. Nitrogen was applied in two split doses in the form of urea@120kg N ha^{-1} while phosphorus was applied in the form of DAP at the rate of 70Kg P_2O_5 ha^{-1} as a basal dose. Seed germination potentials were also estimated under controlled conditions by germinating seeds on filter paper in Petri dishes under -0.5, -0.75 and -1.0 MPa osmotic stresses using polyethylene glycol (PEG-6000).

Yield related traits: Agronomical parameters, such as, plant height, number of tillers, productive tillers, spike length, grain yield and 1000 grain weight were recorded at the time of crop maturity following the standard procedures.

Physiological analysis: Various osmoprotectants, such as, proline, glycine-betaine (GB), total soluble sugars (TSS) and potassium(K^+) contents were analyzed using the methods of Bates *et al.* (1973), Grieve & Gratan's (1983), Razi *et al.* (1985) and Ansari & Flowers's (1986), respectively. Nitrate reductase activity (NRA), total chlorophyll contents and osmotic potentials in the fresh flag leaves were determined following the standard procedures as described by Jordon's (1984), Lichtenthaler *et al.* (1987) and Ludlow (1987), respectively.

Statistical analysis: The data was statistically analyzed using analysis of variance (ANOVA) and least significance difference (LSD) is presented at 5% probability level in Duncan's Multiple Range Test (DMRT) using Statistix-8.1 software (Steel *et al.*, 1997).

Results

Drought exerted negative effects on growth and development of wheat genotypes thus ultimately reducing the grain yield. Seed germination potentials were reduced with increase in PEG induced osmotic stress. Maximum germination was shown by MAS-20/2014 (62.66%) with minimum percent reduction over control (37.34%) followed by MAS-24/2014, MAS-5/2014, MAS-22/2014, MAS-9/2014and MAS-15/2014 at -1.0 MPa osmotic stress. Whereas, minimum germination was observed in MAS-13/2014, MAS-23/2014and MAS-21/2014 wheat genotypes at -1.0 MPa (Table 1).

Table 1. Germination percentage (%) of wheat genotypes under different levels of PEG induced osmotic stress

Wheat genotypes	Control	-0.5 MPa		-0.75 MPa		-1.0 MPa	
		-0.5 MPa	Relative decrease (%)	-0.75 MPa	Relative decrease (%)	-1.0 MPa	Relative decrease (%)
MAS-1/2014	100	83.22	16.8	63.22	36.8	53.23	46.77
MAS-2/2014	98.33	79.32	19.3	60.21	38.8	49.11	50.06
MAS-3/2014	95	77.62	18.3	70.11	26.2	40.66	57.20
MAS-4/2014	96.66	80.31	16.9	72.33	25.2	51.72	46.49
MAS-5/2014	100	84.25	15.8	75.27	24.7	62.29	37.71
MAS-6/2014	97.66	71.66	26.6	60.22	38.3	47.33	51.54
MAS-7/2014	99.72	66.33	33.5	55.66	44.2	39.77	60.12
MAS-8/2014	100	65.77	34.2	56.39	43.6	44.29	55.71
MAS-9/2014	100	73.33	26.7	64.22	35.8	61.19	38.81
MAS-10/2014	98.66	70.44	28.6	59.61	39.6	43.17	56.24
MAS-11/2014	99.33	59.19	40.4	51.33	48.3	37.00	62.75
MAS-12/2014	98.61	60.00	39.2	55.22	44.0	43.21	56.18
MAS-13/2014	96.66	59.22	38.7	52.00	46.2	33.22	65.63
MAS-14/2014	97.77	61.31	37.3	57.66	41.0	44.00	55.00
MAS-15/2014	100	76.29	23.7	69.39	30.6	62.00	38.00
MAS-16/2014	100	67.33	32.7	55.00	45.0	39.00	61.00
MAS-17/2014	99	71.00	28.3	60.33	39.1	42.11	57.46
MAS-18/2014	100	75.22	24.8	61.00	39.0	45.00	55.00
MAS-19/2014	97.33	69.44	28.7	59.77	38.6	44.17	54.62
MAS-20/2014	100	76.29	23.7	70.00	30.0	62.66	37.34
MAS-21/2014	100	73.44	26.6	62.49	37.5	39.88	60.12
MAS-22/2014	100	77.00	23.0	66.00	34.0	61.39	38.61
MAS-23/2014	98	69.33	29.3	59.00	39.8	37.00	62.24
MAS-24/2014	100	78.66	21.3	68.27	31.7	62.22	37.78
Khirman	99	86.00	13.1	72.66	26.6	62.00	37.37
Chakwal-86	98	85.00	13.3	75.00	23.5	64.00	34.69

LSD = 18.135

Plant height was reduced under water stress in all genotypes, however, genotypes MAS-7/2014, MAS-9/2014, MAS-10/2014, MAS-11/2014 and MAS-14/2014 maintained comparatively better growth (Table 2). However, highest reduction in plant height was observed in MAS-21/2014 (40.98%) under terminal drought stress. Tillering capacity and productivity of plants were decreased in the genotype MAS-18/2014 along with check varieties Khirman and Chakwal-86 which produced maximum tillers while no sterility was observed in MAS-10/2014 under water scarce conditions. Similarly, genotype MAS-11/2014 followed by MAS-10/2014 exhibited maximum spikes while genotype MAS-22/2014 followed by MAS-19/2014 andMAS-15/2014 produced minimum spikes under terminal drought stress. Momentous decrease in spike length was observed in all wheat genotypes under drought while minimum reduction was observed in MAS-9/2014 (11.60%) followed by MAS-10/2014 (11.87%). Drought significantly reduced plant biomass in all genotypes as compared to control.

Decrease in grain yield was also estimated under extreme water shortage due to decrease in growth and yield related parameters. Ten genotypes exhibited relatively less reduction (<20%) in yield and yield contributing components under terminal drought. Highest grain weight was produced by MAS-9/2014 (23.32g), MAS-3/2014 (23.29g) and MAS-11/2014 (23.18g) under control while MAS-18/2014 (19.17g) and MAS-3/2014(19.11g) genotypes had maximum grain weight under drought. However, lowest grain weight was recorded in the genotype MAS-6/2014 (Table 2). Similarly, 1000-grain weight was also reduced while; genotype MAS-20/2014 showed minimum reduction in grain yield under drought stress over control, i.e. 15.70% (Table 3).

Reduced soil moisture also perturbed the physiological mechanisms of wheat genotypes. Accumulation of proline and glycine betaine (GB) was enhanced in tolerant genotypes under drought conditions. Maximum increase (83.35%) in proline was estimated in MAS-20/2014 while minimum rise (18.95-20.61 µmolg^{-1}.F.wt) was noted in MAS-19/2014 (Fig. 1).Similarly, highest accumulation of glycine-betaine was found in MAS-3/2014 with 92.42% increase over control while MAS-18/2014 showed lowest GB contents under osmotic stress (Fig. 2). Total soluble sugars (TSS) were also increased under extreme soil water deficit with maximum accumulation in MAS-22/2014 (1.616 m mol g^{-1}F.wt) (Fig. 3).

Photosynthetic pigments were reduced under inadequate moisture condition. Better chlorophyll contents were analyzed in MAS-20/2014 (with minimum decrease of 5.93%) followed by MAS-1/2014 (12.13%), MAS-18/2014(19.06%) and MAS-2/2014 (21.15%)(Fig. 4).Same decreasing trend in nitrate reductase activity was determined under drought, however, MAS-11/2014, MAS-8/2014, MAS-16/2014and MAS-2/2014 showed better NR-activities (Fig. 5).Osmotic potentials of wheat genotypes were generally decreased (more –ve values) under drought stress while MAS-3/2014 genotype maintained minimum OP, i.e., -0.769 MPa followed by MAS-13/2014 (-0.972 MPa) (Fig. 6). Increased in potassium uptake was found in all genotypes under reduced soil moisture. Highest potassium accumulation of 3.66%, 3.37% and 3.35% was observed in MAS-6/2014, MAS-1/2014 and MAS-3/2014, respectively while lowest K^+ contents (2.55%) were assessed in MAS-8/2014 under water deficit soil conditions (Fig. 7).

Discussion

Plant growth is one of the major processes affected under reduced soil water contents (Chaves *et al*., 2009) which causes decrease in grain yield. All wheat genotypes showed diversity in their ability to survive under moisture stress. Drought stress inhibited plant growth by decrease in biomass. Reduce soil water contents at reproductive stage had caused significant decrease in plant height, tillers, number of spikes plant^{-1}, spike length and biomass in all genotypes. Decrease in these agronomic traits is linked with the reduction in activity of meristematic tissues which are responsible for elongation as well as inefficiency of photosynthetic tissues under insufficient availability of water (Siddique *et al*., 1999).

The growth stage at which plant is exposed to water scarcity is more critical for yield than intensity of water deficit (Dhanda *et al*., 2004; Aranjuelo *et al*., 2011). Under terminal drought stress, soil moisture reduces towards crop maturity. Plant height, number of spikes, spike length, grains and thousand grain weights were decreased significantly in various genotypes under drought stress (Mirbahar *et al*., 2009). Tolerant genotypes showed better yields due to higher translocation of photosynthates towards grains at the time of grain filling under terminal drought (Inagaki *et al*., 2007).

Another important mechanism of protection against water stress is accumulation of osmolytes such as carbohydrates, amino acids, amides and inorganic solutes (Kuznetsov & Shevyakova, 1999). Abrupt increase in the accumulation of proline, glycine-betaine, soluble sugars and potassium contents was observed under drought stress, which also raised osmotic potentials of plant cell (Nayyar & Walia, 2003). These osmolytes also facilitate in osmotic adjustment. There is a strong positive correlation between increased content of intracellular proline and plant’s ability to survive under high salinity and water constraints (Chaves & Oliveira, 2004). Under water deficit conditions, proline synthesis provides reserves of organic nitrogen that are mobilized during plant recovery after resumption of normal supply of water. A strong positive correlation was exhibited among grain yield and proline accumulation under drought stress (Andarab, 2013).

Drought stress exhibited adverse effects on photosynthetic pigments by disintegrating the chloroplast. In this experiment,total chlorophyll contents underwent reduction in many wheat genotypes under extreme water scarcity. The main reason behind disrupted photosynthetic activity was stomatal closure which reduced the concentration of cellular CO_2 (Rahbarian *et al*., 2011). Photosystem-II is more susceptible to soil water deficit than photosystem-I of chloroplast (Duraes *et al*., 2001). Less reduction in total chlorophyll contents, NRA and osmotic potentials occurred in drought tolerant genotypes. Paknejad *et al*. (2007) also documented the better chlorophyll pigments in high yielding tolerant varieties. Tayeb (2006) reported that decrease in chlorophyll contents was faster in drought sensitive genotypes as compared to drought tolerant genotypes. Similar reducing trends in terms of chlorophyll pigments were observed in under tested sensitive wheat genotypes while least reduction occurred in drought tolerant MAS-20/2014 genotype.

Table 2. Effect of terminal drought stress on plant height, number of tillers, spikes and spike length of wheat genotypes.

Genotypes	Plant height (cm)			No. of tillers per plant			No. of spikes per plant			Spike length per plant (cm)		
	Control	Drought	Relative decrease (%)	Control	Drought	Relative decrease (%)	Control	Drought	Relative decrease (%)	Control	Drought	Relative decrease (%)
MAS-1/2014	73.67	59.67	19.00	10	8	20.00	9	7	22.22	14.27	12.17	14.72
MAS-2/2014	74.67	60.33	19.20	12	10	16.67	10	9	10.00	13.38	10.66	20.33
MAS-3/2014	94.33	75.65	19.81	11	9	18.18	10	7	30.00	13.44	10.88	19.05
MAS-4/2014	88.33	68.33	22.64	9	5	44.44	5	4	20.00	11.82	9.19	22.25
MAS-5/2014	91.67	71.33	22.18	9	7	22.22	6	4	33.33	12.27	8.69	29.18
MAS-6/2014	79.00	58.67	25.74	11	7	36.36	9	7	22.22	12.39	9.22	25.59
MAS-7/2014	79.00	66.33	16.03	10	6	40.00	9	5	44.44	12.49	9.17	26.58
MAS-8/2014	111.3	75.67	32.04	11	7	36.36	9	5	44.44	11.66	8.33	28.56
MAS-9/2014	90.67	74.33	18.02	13	11	15.38	12	10	16.67	13.79	12.19	11.60
MAS-10/2014	76.33	62.12	18.62	14	12	14.29	13	12	7.69	14.83	13.07	11.87
MAS-11/2014	75.33	61.59	18.24	15	13	13.33	14	13	7.14	14.88	12.37	16.87
MAS-12/2014	83.33	67.35	19.18	10	8	20.00	9	7	22.22	13.69	11.73	14.32
MAS-13/2014	77.33	62.33	19.40	11	8	27.27	8	6	25.00	11.92	9.21	22.73
MAS-14/2014	86.67	70.67	18.46	11	8	27.27	8	6	25.00	12.73	8.06	36.68
MAS-15/2014	73.00	57.00	21.92	7	4	42.86	6	3	50.00	10.85	8.21	24.33
MAS-16/2014	80.00	65.19	18.51	13	11	15.38	11	8	27.27	14.79	12.21	17.44
MAS-17/2014	82.67	64.00	22.58	9	7	22.22	5	4	20.00	12.71	10.03	21.09
MAS-18/2014	90.67	73.31	19.14	9	8	11.11	7	6	14.29	13.97	11.67	16.46
MAS-19/2014	73.33	59.33	19.09	8	5	37.50	7	3	57.14	11.77	9.13	22.43
MAS-20/2014	93.67	75.33	19.58	12	10	16.67	10	7	30.00	14.59	12.27	15.90
MAS-21/2014	101.67	60.00	40.98	10	6	40.00	7	4	42.86	11.55	8.31	28.05
MAS-22/2014	79.67	61.33	23.01	8	4	50.00	5	2	60.00	10.78	7.56	29.87
MAS-23/2014	77.00	50.33	34.63	9	6	33.33	9	6	33.33	11.68	9.19	21.32
MAS-24/2014	81.00	60.00	25.93	9	5	44.44	9	5	44.44	12.39	9.07	26.80
Khirman	84.00	65.67	21.83	12	10	16.67	11	10	9.09	14.61	12.12	17.04
Chakwal-86	82.00	64.00	21.95	13	11	15.38	11	10	9.09	13.79	11.29	18.13
LSD ($p \leq 0.05$)	**16.058**			**3.4713**			**4.2387**			**2.6646**		

Table 3. Effect of terminal drought stress on straw weights, grain yield and 1000-grain weights of wheat genotypes.

Genotypes	Straw weight per plant (g)			Grain weight per plant (g)			1000-Grain weight (g)		
	Control	Drought	Relative decrease (%)	Control	Drought	Relative decrease (%)	Control	Drought	Relative decrease (%)
MAS-1/2014	19.30	7.34	61.97	22.39	18.89	15.63	48.08	39.77	17.28
MAS-2/2014	24.13	8.16	66.19	21.33	17.78	16.64	47.96	38.83	19.03
MAS-3/2014	28.87	9.73	66.30	23.29	19.11	17.95	47.63	37.79	20.66
MAS-4/2014	22.54	7.59	66.33	18.66	13.27	28.89	43.22	30.33	29.82
MAS-5/2014	33.01	10.69	67.62	19.59	14.17	27.67	43.87	29.75	32.18
MAS-6/2014	21.75	8.15	62.52	17.73	10.29	41.96	42.84	28.85	32.65
MAS-7/2014	33.67	10.76	68.04	20.19	15.23	24.57	44.04	31.65	28.14
MAS-8/2014	30.51	9.55	68.70	17.69	11.09	37.31	45.54	34.39	24.49
MAS-9/2014	29.01	9.44	67.46	23.32	18.69	19.85	48.92	40.21	17.80
MAS-10/2014	30.58	10.53	65.57	22.27	17.84	19.89	48.81	39.87	18.31
MAS-11/2014	33.64	11.19	66.74	23.18	18.58	19.84	48.63	40.65	16.41
MAS-12/2014	19.68	7.10	63.92	20.25	16.73	17.38	49.50	40.69	17.79
MAS-13/2014	40.04	13.43	66.46	17.58	11.22	36.18	44.86	33.27	25.84
MAS-14/2014	30.98	10.63	65.69	20.79	15.17	27.03	45.60	34.40	24.56
MAS-15/2014	12.25	5.11	58.30	19.76	13.11	33.65	44.87	30.29	32.49
MAS-16/2014	36.43	13.19	63.80	20.23	16.39	18.98	47.74	38.62	19.11
MAS-17/2014	21.99	8.12	63.07	18.74	13.39	28.55	45.78	33.56	26.69
MAS-18/2014	38.11	13.17	65.45	22.28	19.17	13.96	46.81	37.73	19.39
MAS-19/2014	15.48	6.19	60.02	18.65	12.57	32.60	45.25	29.67	34.43
MAS-20/2014	18.11	7.21	60.19	22.29	18.33	17.77	45.78	38.59	15.70
MAS-21/2014	30.15	11.22	62.78	19.37	14.66	24.32	44.50	31.55	29.10
MAS-22/2014	26.98	9.21	65.86	18.67	12.75	31.71	45.02	32.22	28.43
MAS-23/2014	29.21	10.11	65.39	19.67	13.83	29.69	44.72	29.37	34.33
MAS-24/2014	35.14	12.69	63.89	20.37	14.79	27.39	45.44	30.23	33.47
Khirman	27.01	9.12	66.24	21.37	17.21	19.47	45.98	37.88	17.62
Chakwal-86	25.21	9.32	63.03	19.27	16.13	16.29	45.40	37.29	17.87
LSD ($p \leq 0.05$)		**22.694**			**6.1807**			**7.7630**	

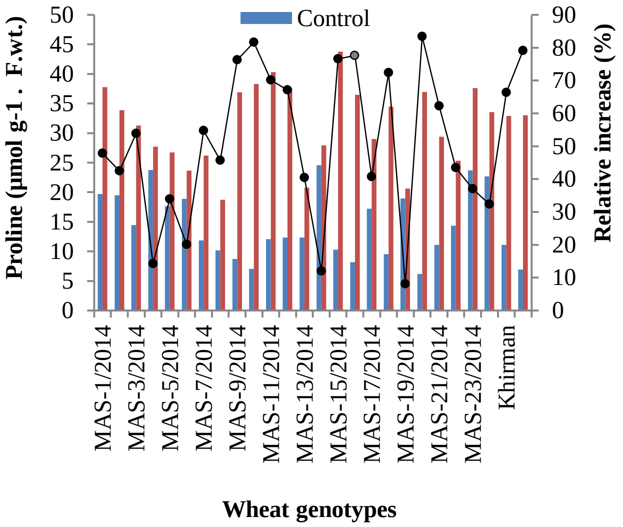

Fig. 1. Impact of terminal drought stress on proline accumulation in wheat genotypes.

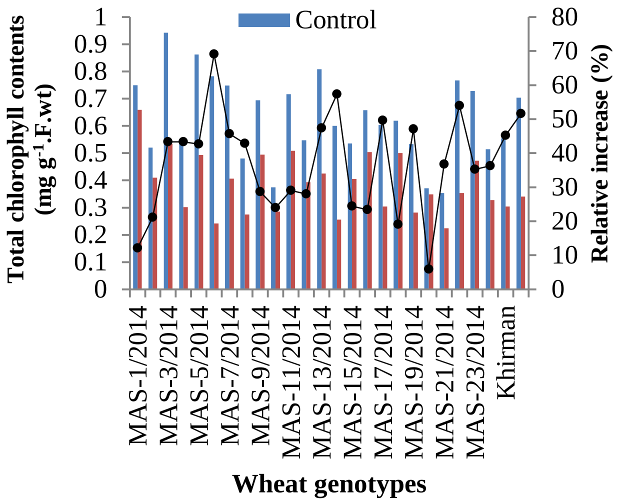

Fig. 4. Impact of terminal drought stress on chlorophyll pigments in flag leaves of wheat plants.

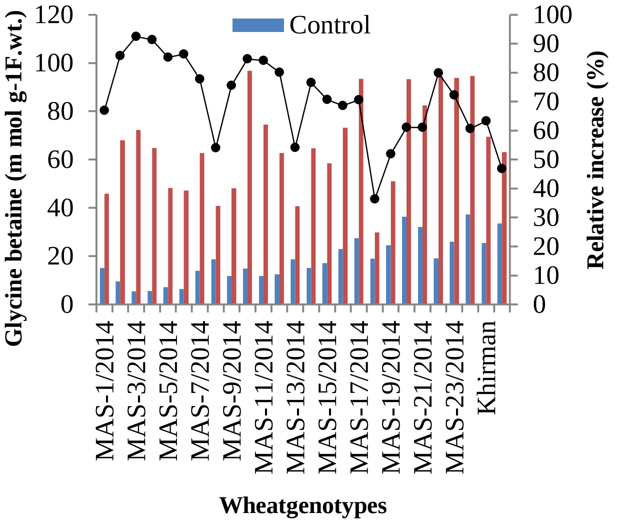

Fig. 2. Impact of terminal drought stress on glycine-betaine accumulation in wheat genotypes.

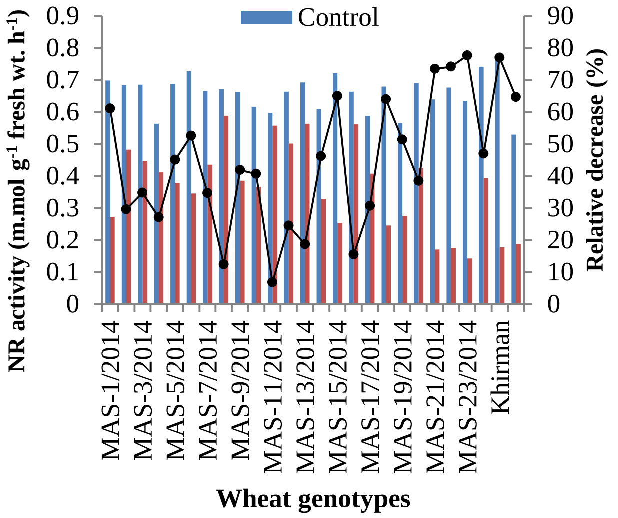

Fig. 5. Impact of terminal drought stress on nitrate reductase enzymatic activities of wheat crop.

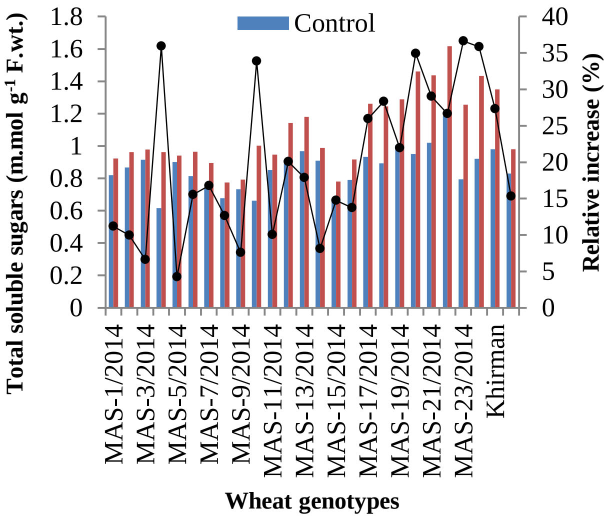

Fig. 3. Impact of terminal drought stress on amelioration of soluble sugars in wheat crop.

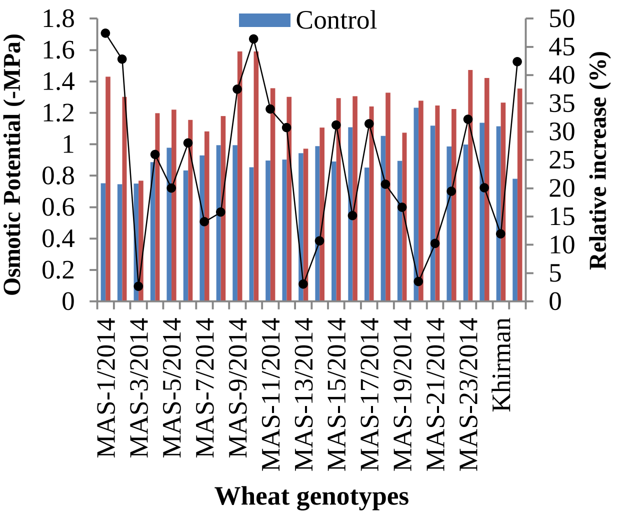

Fig. 6. Impact of terminal drought stress on osmotic potentials of wheat crop.

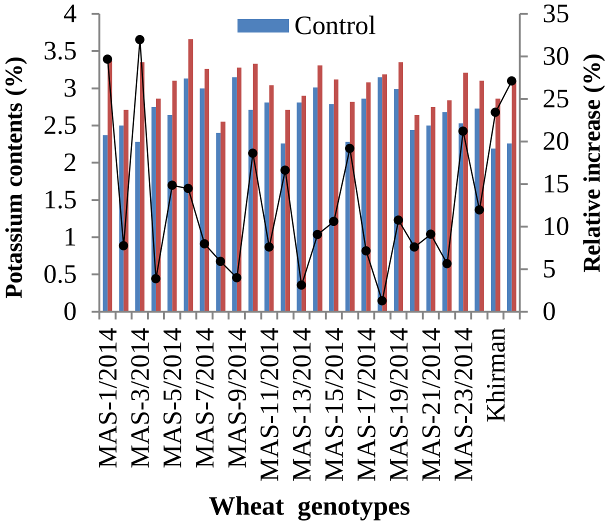

Fig. 7. Impact of terminal drought stress on uptake of potassium ions.

Conclusion

On the basis of relative decrease (<20%) in different growth variables and increase in concentrations of osmoprotectants, genotypes MAS-2/2014, MAS-3/2014, MAS-8/2014, MAS-10/2014, MAS-12/2014,MAS-16/2014, MAS-18/2014and MAS-20/2014were categorized as tolerant to water stress while MAS-01/2014, MAS-07/14, MAS-09/2014 and MAS-21/2014 genotypes were under the category of moderately tolerant.

References

Andarab, S.S. 2013. Study of correlation among yield and yield components affecting traits on bread wheat under drought stress and non-stress conditions. *Annals Biol. Res*., 4:286-289.

Anonymous. 2005. *Pakistan country water resources assistance strategy –Water economy: Running dry*. South Asia Region, Agriculture and Rural Development Unit Report No. 34081-PK.Washington, DC.

Ansari, R. and T.J. Flowers. 1986. Leaf to leaf distribution of ions in some monocotyledonous plants grown under saline conditions. In. *Prospects for Biosaline Research* (Ed.): Ahmed and A. San-Pietro. University of Karachi, Pakistan. pp. 167-180.

Aranjuelo, L., G.Molero, G. Erice, Jean, C. Avice and S. Nogue. 2011. Plant physiology and proteomicsreveals the leaf response to drought in alfalfa (*Medicago sativa* L.). *J. Exp. Bot.*, 62(1): 111-123.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205-207.

Chaudhry, Q.Z. and G. Rasul. 2004. Agroclimatic classification of Pakistan. *Sci. Vision.*, 9(1-2 & 3-4): 59.

Chaves, M.M. and M.M. Oliveira. 2004. Mechanisms underlying plant resilience to water deficits: Prospects for water saving agriculture. *J. Exp. Bot.*, 55: 2365-2384.

Chaves, M.M., J. Flexas and C. Pinheiro. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.*, 103: 551-560.

Dhanda, S., G.S. Sethi and R.K. Behl. 2004. Indices of drought tolerance in wheat genotypes at early stages of plant growth. *J. Agron. Crop Sci*., 190(1): 6-12.

Dijksterhuis, J. and R.P. De Vries. 2006. Compatible solutes and fungal development. *Biochem. J*., 399: e3-e5.

Duraes, F.O.M., C.R. Casela and A.C. Oliveira. 2001. Chlorophyll fluorescence technique as a potential tool to help in the screening for resistance to foliar diseases in maize. *Brazilian Phytopathol*., 32: 1-6.

Grieve, C.M. and S.R. Gratan. 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant Soil*., 70: 303-307.

Inagaki, M.N., J. Valkoun and M.M. Nachit. 2007. Effect of soil water deficit on grain yield in synthetic bread wheat derivatives. *Cereal Res. Comm*., 35: 1603-1608.

Jaleel, C.A., R. Gopi, B. Sankar, P. Manivannan, A. Kishorkumar and R. Sridharan. 2007. Studies on germination, seedling vigor, lipid peroxidation and proline metabolism in *Catharanthusroseus*seedlings under salt stress. *South Afr. J. Bot*., 73(2): 190-195.

Jordon, J.S. 1984. Optimization of the *Invivo* assay conditions for nitratereductase in barley (*Hordeum vulgare* L. cv. Irgi).*J. Sci. Food*, 35: 725-730.

Katerji, N., M. Mastrorilli, J.W. Hoornc, F.Z. Lahmerd, A. Hamdyd and T. Oweise. 2009. Durum wheat and barley productivity in saline–drought environments. *Euro. J. Agron*., 31(1): 1-9.

Kumar, D. 2005. Breeding for drought resistance. In: (Eds.): Ashraf, P.J.C. and J. Harris. *Abiotic Stresses*. The Howarth Press, New York, pp. 145-175.

Kuznetsov, V.L.V. and N.I. Shevyakova. 1999. Proline under stress: Biological role, metabolism, and regulation. *Russ. J. Plant Physiol.*, 46: 274-288.

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic membranes. *Methods Enzymol*., 148: 350-382.

Ludlow, M.M. 1987. Defining root water status in the most meaningful way to relate to physiological processes. In: *Measurement of soil and plant water status*. (Eds.): Hanks, R.J., R.W. Brown. Logan, UT, USA: Utah Agricultural Experiment Station, U.S.A., pp. 47-53.

Mirbahar, A.A., G.S. Markhand, A.R. Mahar, S.A. Abro and N.A. Kanhar. 2009. Effect of water stress on yield and yield components of wheat (*Triticum aestivum* L.) varieties. *Pak. J. Bot*., 41(3): 1303-1310.

Moaveni, P. 2011. Effect of water deficit stress on some physiological traits of wheat (*Triticum aestivum* L.). *Agric. Sci. Res. J*., 1(1): 64-68.

Nayyar, H. and D.P. Walia. 2003. Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and abscisic acid. *Bio. Plant*., 46: 275-279.

Nouri-Ganbalani, A., G. Nouri-Ganbalani and D. Hassanpanah. 2009. Effects of drought stress condition on the yield and yield components of advanced wheat genotypes in Ardabil, Iran. *J. Food Agric. Environ*., 7: 228-234.

Paknejad, F., M. Nasri, H.R.T. Moghadam, H. Zahedi and M.J. Alahrnadi. 2007. Effects of drought stress on chlorophyll fluorescence parameters, chlorophyll content and grain yield of wheat cultivars. *J. Biol. Sci*., 7: 841-847.

Passioura, J.B. 2007. The drought environment: physical, biological and agricultural perspectives. *J. Exp. Bot.*, 58(2): 113-117.

Price, A.H., J.E. Cairns, P. Horton, H.G. Jones and H. Griffiths. 2002. Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTL approach: Progress and new opportunities to integrate stomatal and mesophyll responses. *J. Exp. Bot*., 53: 989-1004.

Rahbarian, R., R. Khavari-Nejad, A. Ganjeali, A. Bagheri and F. Najafi. 2011. Drought stress effects on photosynthesis, chlorophyll fluorescence and water relations in tolerant and susceptible chickpea (*Cicer arietinum* L.) genotypes. *Acta Biol. Cracov. Bot*., 53:47-55.

Riazi, A., K. Matruda and A. Aslam. 1985. Water stress induce changes in concentration of proline and other solutes in growing regions. *J. Exp. Bot*., 36(172): 1716-1725.

Rosegrant, M.W. and S.A. Cline. 2003. Global food security: Challenges and policies. *Sci*., 302: 1917-1919.

Siddique, M.R.B, A. Hamid and M.S. Islam. 1999. Drought stress effect on photosynthetic rate and leaf gas exchange of wheat. *Bot. Bull. Academ. Sin*., 40(20): 141-145.

Steel, R.G.D., J.H. Torrie and D.A. Deekey. 1997. *Principles and procedures of statistics: A biometrical approach*. (3rd ed.) McGraw Hill Book, New York.

Tayeb, M.A. 2006. Differential response of two *Vicia faba* cultivars to drought: growth, pigments, lipid, peroxidation, organic solutes, catalase, and peroxidase activity. *ActaAgron. Hung.*, 54:25-37.

Vendruscolo, A.C.G., I. Schuster, M. Pileggi, C.A. Scapim, H.B.C.Molinari, C.J. Marurand L.G.C. Vieira. 2007. Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J. Plant. Physiol*., 164(10): 1367-1376.

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