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Research Article

Physiological and biochemical responses of hexaploid wheat cultivars to drought stress

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Abstract

Wheat is the most important cereal crop; it is staple diet for more than one third of the world population. Wheat production is influenced by several harsh environmental stresses that adversely affect its growth, metabolism and yield. Among them, Drought stress is the main factor limiting the productivity of wheat crop worldwide. Plants experience drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high. These two conditions often coincide under arid and semi-arid climates. Eighty percent area of Pakistan comes under arid and semi-arid region, so wheat productivity is adversely reduced in these areas due to lack of rain fall. Wheat yield under drought stress can be improved by several methods like conventional breeding, genetic transformation and marker assisted selection. For applying any of these methods, a close scans of morphological and physio-biochemical parameters are necessary. In the present study with 92 genotypes, a decrease of 35.57% for relative water content and 23.08% was observed for chlorophyll content in the stressed conditions. And an increase in metabolite level such as sugar (28.86%), SOD (4.53%) and canopy temperature (20.38%) was observed in moisture stress. Various genotypes have been identified which can be used further in plant breeding systems.

Keywords: Wheat; Drought; Genotypes; Physio-biochemical; Metabolites

Introduction

Meteorological drought (Discontinuity in precipitation) joined with higher evapotranspiration leads to agricultural drought [1]. Drought has been a major focus of research for several decades as due to climate change the number of drought periods will increase in the future [2]. With global warming, drought will occur more frequently and will be expected to affect crop production more severely [3]. Drought effects plants at cellular, physiological, metabolic and molecular levels and have serious impact on various developmental processes, such as seedling emergence and growth, root and shoot development and later leaf development [4-6]. Drought response studies are challenged by the complexity of the trait caused by the environmental interactions. Drought tolerance can be estimated by the mode, timing, and severity of the dehydration stress and its occurrence with other abiotic and biotic stress factors [7, 8]. To counter

adverse effects of different environmental stresses, plant have evolved special mechanisms and undergone a serial of physiological changes, but the "cross-talk of stresses" and "cross-tolerance to stresses" have not been extensively explored [9].

Crop genotypes having potential tolerance for environmental stresses is a common approach for improvement of crop productivity [10]. Drought tolerance is now considered by both plant breeders and biotechnologists to be a valid breeding target. Therefore some drought stress indices or selection criteria 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 [11-13].

Wheat is the major staple food in Pakistan, so it is cultivated and merchandized on commercial scale. Pakistan is among ten major wheat-producing countries of the world in terms of area under wheat cultivation, total productivity and per acre yield, It is recorded that Wheat currently contributes 75 percent of Pakistan's daily caloric diet with per capita wheat consumption of around 124 kg per year, one of the highest in the world [14, 15]. Majority of area in Pakistan falls into arid-semi-arid regions, depending upon the climatic conditions. About 88% of total geographic area (79.6 mha) is covered by arid lands. The crop production in these areas is mainly dependent on the short and unpredictable rainfall [16, 17]. That's why, among different issues, water scarcity pose a serious threat to wheat production when less or no precipitation occurs in the year especially during winter [18].

Keeping in view the importance of wheat crop in Pakistan and problem of low yield in drought conditions as compared to developed countries it becomes imperative to take an immediate action to develop such varieties which can perform well under drought stress. So this study is planned for molecular and physiological evaluation of wheat cultivars for drought tolerance.

Materials and methods

Diverse germplasm of bread wheat was used for the study of genetic diversity and for screening drought tolerant genotypes using agronomic, morphological, physiological and molecular traits. The germplasm used in the study was comprised of 92 candidate lines. NARC-09, NARC-2011, Chakwal-95, Inqalab 98 was cultivated as control checks. Pedigree for selected genotypes is represented in Table S1.

Field experiments were carried out in year 2013-2014 and 2014-2015 at National agricultural research centre (NARC), Islamabad, Pakistan. Seeds were obtained from Wheat wide crosses, NARC, and were sown under control conditions in the tunnel and fully irrigated conditions in field following Randomized complete block design. Rain out plot shelters were covered with green sheets and plastic sheets to block water coming through precipitation. Tunnel was lined with 3 feet high brick wall underground to stop water seepage from surrounding areas. The seeds were sown in 1 meter rows having 3 replications. Non-stress plots were irrigated until maturity at sowing time, tillering, booting and post anthesis stage [19]. Various physiological and biochemical attributes like chlorophyll content [20], Canopy temperature [21] Relative water content [22], Electrolyte leakage [23] Proline content [24], Soluble carbohydrate content [25] and Superoxide dismutase activity [26, 27] were recorded.

Table 1. Pedigree of genotypes used in the study

Sr. No	Nursery Name	Pedigree
1	**SAWSN -11**	HIDDAB
2		KLEIN CACIQUE
3		BERKAT
4		W.15.92/4/Pastor//HXL7573/2*BAU/3WBLL1
6		QG4.37A/4/MILAN/KAUZ/PRINIA/3/BAV92/5/…
7		ONIX/4/MILAN/KAUZ/PRINIA/3/BAV92
8		ACHTAR/4/MILAN/KAUZ/PRINIA/3/BAV92
9		CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/…
10		CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/…
11		CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/…
12		MILAN/KAUZ/PRINIA/3/BAV92/4/ATTILA/BAV92//…
13		GK ARON//AGSECO7846//2180/4/2*MILAN/KAUZ//…
14		SW89-5124*2/FASAN/3/ALTAR84/AE.SQ//2*OPATA
15		SOKOLL/ROLF07
16		SOKOLL/FRTL/2*PIFED
17		BAV92/SERI
18		MILAN/KAUZ/PRINIA/3/BAV92/4/WBLL1*2/KUKUNA
19		BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/…
20		BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/…
21		HUANIL//2*WBLL1*2/KUKUNA
22		FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP/KAUZ/5/…
23		ONIX/ROLFO7
24		BARCENAS S 2002/4/MILAN/KAUZ/PRINIA/3/BAV92
25		CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/…
26		GK ARON//AGSECO7846//2180/4/2*MILAN/KAUZ//…
27		SW89-5142*2/FASAN/3/ALTAR 84/AE.SQ//2*OPATA
28		CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA
29		CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA
30		D67.2/PARANA 66.270// AE.SQUAROSSA(320)/3/…
31		PASTOR*2/BAV92/5/FRET2/KUKUNA/FRET2
32		GOUBARA-1/2*SOKOLL
33		PSN/BOW//MILAN/3/2*PARUS/PASTOR
34		FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP/KAUZ/5/…
35	**SAWSN – 12****	GK ARON//AGSECO7846//2180/4/2*MILAN/KAUZ//…
36		SKUAZ/BAV92//SOKOLL
37		SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92/5/…

38		VORB/3/T. DICOCCON PI94625/AE.SQUARROSA (32…
39		vORB/3/T. DICOCCON PI94625/AE.SQUARROSA (32…….
40		CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE. SQUARROSA (20……)
41	**SYNTHATIC D- GENOME SELECTION**	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (882)/6/ATTILA/10/
42		URES/JUN//KAUZ/3/ALTAR 84/AE. SQ//2*OPATA
43		CROC_1/AE.SQUARROSA (205)//KAUZ/3/ENEIDA/4/FINSI
44		FILIN/IRENA/5/CNDO/R143//ENTE/MEXI-2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/6/…
45		CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/OCI/5/
46		FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/6/BERKUT
47		FILIN/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/6/BERKUT
48		CROC_1/AE.SQUARROSA (205)//KAUZ/3/2*PJN/BOW//OPATA
49	**7HTWSN****	SAUL/3/ACHTAR*3//KANZ/KS85-8-4/4/SAUL
50		KACHU#1/3/C80.1/3*BATAVIA//2*WBLL1/4/KACHU
51		SAUL#1/KACHU
52		SAUL#1/KACHU
53		D67.2/PARANA 66.270// AE.SQUAROSSA(320)/3/…
54		VORB/3/T. DICOCCON PI94625/AE.SQUARROSA (372)
55		CNDO/R143//ENTE/MEXI-2/3/AEGILOPS SQUARROSA
56		D67.2/PARANA 66.270// AE.SQUAROSSA(320)/3/…
57		T. DICOCCON PI225332/ AE.SQUARROSA (895)//…
58		D67.2/PARANA 66.270// AE.SQUAROSSA(320)/3/…
59	**4CSiSA-SB****	d67.2/PARANA 66.270// AE.SQUAROSSA(320)/3/Cunningham/4/…
60		MUU/4/BAV92//IRENA/KAUZ/3/HUITES/5/BAV92//IRENA/KAUZ/3/…
61		PBW343*2/KHVAKI*2/3/ACHTAR*3//KANZ/KS85-8-5
62		CHIR3/4/SIREN//ALTAR84/AE.SQUARROSA (205)/3/3*BUC/5/…
63		VORB/3/T. Dicoccon PI94625/ AE. SQUARROSA (372)//3*PASTOR
64		MURGA/KRONSTAD F2004
65		CHIRYA. 3
66		Kachu#1/4/croc_1/ AE. SQUARROSA (205)// KAUZ/ 3/
67		CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/…
68		VORB/4/D67.2//PARANA 66.270//AE. SQUARROSA (320)/3/…
69	**HISTORICAL SET***	OPATA/RAYON//KAUZ
70		WL-711/CROW'S
71		TD-1

72		BLUEBIRD/GALLO/3/GABOTO/SIETE-CERROS-66//BLUEBIRD/CIANO-67
73		CHAKWAL-97
74		Oasis/Skauz//4*BC/3/2*Pastor
75		KVZ//BUHO//KALBB
76		MUNIA/SHTO//AMSEL
77		DWL-5023/SUNBIRD,MEX//SUNBIRD,MEX
78		ATTILA/3/HUI/CARC//CHEN/CHTO/4/ATTILA
79		CHILERO/2*STAR/4/BOBWHITE//BUCKBUCK/PAVON-76/3/2*VEERY-10
80		F1n/ACS//ANA
81		KAUZ/PASTOR
82		INQALAB 91*2/TUKURU
83	**RF (NUWYT)***	MILAN/S87230//BABAX
84		SOKOLL/3/PASTOR//HXL7573/2*BAU
85		GA-2002/Chakwal-50
86		PASTOR/MILAN//MILAN/kauz
87		PFAU/SERIIB//AMAD/3/WAXWING
88		TC870344/GUI//TEMPORALERAM87/AGR/3/2*WVLL1
89		W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1
90		9L039
91	RFVOBT022*	RFVOBT022*
92	Turaco/FCT 73*	Turaco/FCT 73*

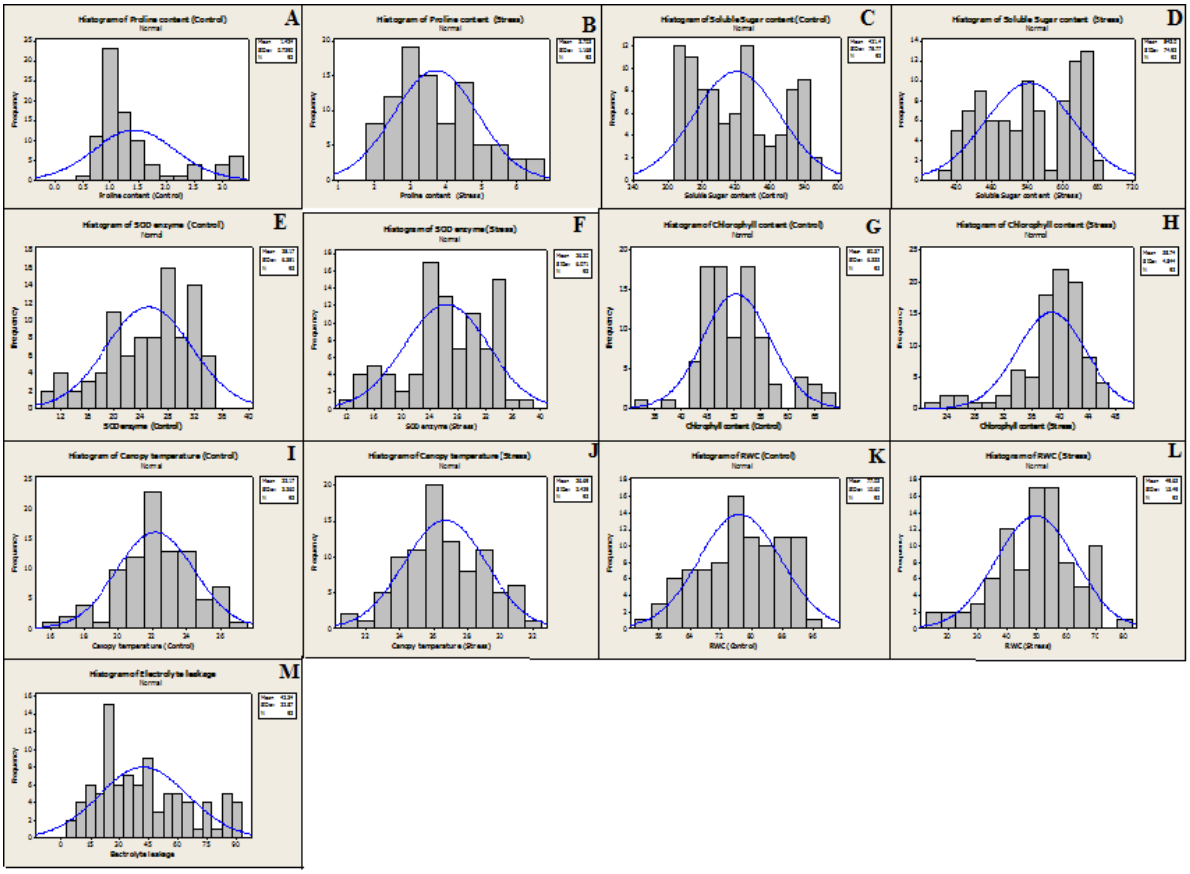

Figure 1. Frequency distribution of biochemical and physiological parameters. (A). Proline content (Control) (B). proline content (Stress) (C). Soluble carbohydrate content (Control) (D). Soluble carbohydrate content (Stress) (E). Super oxide dismutase enzyme (control) (F). Super oxide dismutase enzyme (Stress). (G). Chlorophyll content (Control). (H). Chlorophyll content (Stress) (I). Canopy temperature (Control). (J). Canopy temperature (Stress). (K). Relative water content (Control) (L). Relative water content (Stress) (M). Electrolyte leakage

Results

Drought stress tolerance is a very complicated phenomenon involving numerous metabolic adjustments. Analyses of metabolic pathways provide important supplementing evidence for better understanding of stress mechanisms [28]. Drought tolerance can be mediated by biochemical compounds such as amino acids like proline, organic acids and sugars by acting as compatible solutes to maintain cellular functions [29, 30].

Highly significant difference was observed for treatments, genotypes and their interactions for all the physiological-biochemical parameters at $p<0.001$. However, for some parameters, no significant difference was observed between the two years of observation and the interactions. All the results of analysis of variance are depicted in Table 1. Descriptive statistics for all the treatments is depicted in Table 2. Overall, the drought stress had an inhibitory effect on chlorophyll and relative water content, but the production of sugar, proline, SOD was increased. Canopy temperature and electrolyte leakage also increased in drought stress treatment. A decrease of 35.57% for relative water content and 23.08% was observed for chlorophyll content in the stressed conditions. Other metabolites increased in

stressed conditions, such as sugar (28.86%), SOD (4.53%) and 20.38% increase for canopy temperature was observed in drought stress.

Table 2. Analysis of variance for physiological and biochemical parameters

		Proline content			**Sugar content**			**Superoxide dismutase enzyme**		
Source	DF	Adj MS	F	P	Adj MS	F	P	Adj MS	F	P
Genotypes	91	3.3369	370.68	0	21776	723.71	0	158.58	389.22	0
Year	1	0.0342	3.8	0.044	101360	3368.57	0	103.84	254.86	0
Treatments	1	446.0944	49554.42	0	1623690	53961.47	0	207.18	508.51	0
genotypes*year	91	0.2927	32.51	0	518	17.2	0	0.54	1.33	0.086
genotypes*treatments	91	0.005	0.56	0.997	39	1.31	0.101	1.67	4.09	0
year*treatments	1	0.5441	60.44	0	11600	385.5	0	12.4	30.44	0
Error	91	0.009			30			0.41		
		Chlorophyll content			**Canopy temperature**			**Relative water content**		
Source		Adj MS	F	P	Adj MS	F	P	Adj MS	F	P
Genotypes	91	115.69	262.11	0	21478	48.04	0	549.8	224.57	0
Year	1	3.8	8.61	0.004	1400451	3132.22	0	198.4	81.05	0
Treatments	1	12787.91	28971.33	0	516305	1154.76	0	65952.7	26938.95	0
genotypes*year	91	9.27	21	0	0.454	1.02	0.47	2.7	1.08	0.352
genotypes*treatments	91	0.15	0.33	1	544637	1218.12	0	20.4	8.33	0
year*treatments	1	2.42	5.49	0.021	0.315	0.7	0.952	35.9	14.68	0
Error	91	0.44			0.447			2.4		
		Electrolyte leakage								
Source		Adj MS	F	P						
Genotypes	91	1008.2	609.4	0						
Year	1	44.28	26.77	0						
Error	91	1.65								

Table 3. Descriptive statistics for biochemical and physiological parameters

Variable	**Minimum**	**Maximum**	**Mean**	**SE Mean**	**Median**	**StDev**	**Variance**	**CoefVar**
Proline content								
Irrigated	0.46	3.37	1.42	0.07	1.13	0.72	0.53	51.18
Stress	1.84	6.74	3.7	0.12	3.49	1.16	1.35	31.45
Sugar content								
Irrigated	311.11	557.3	421.39	7.9	418.01	75.77	5740.76	17.98
Stress	408.33	658	543.01	7.81	544.31	74.92	5612.97	13.8
Superoxide dismutase enzyme								
Irrigated	9.66	34.16	25.16	0.66	26.22	6.35	40.33	25.23
Stress	12.16	37.83	26.3	0.63	26.41	6.07	36.86	23.08
Chlorophyll								
Irrigated	33.4	68.1	50.37	0.66	49.26	6.33	40.1	12.57
Stress	21.73	46.23	38.74	0.5	39.9	4.84	23.46	12.5
Canopy temperature								
Irrigated	15.67	27	22.17	0.23	22	2.26	5.1	10.19
Stress	21	32.33	26.69	0.25	26	2.42	5.9	9.1
Relative water content								
Irrigated	53.74	94.55	77.03	1.11	77.53	10.6	112.35	13.76
Stress	14.88	81.66	49.63	1.41	50.53	13.49	182.09	27.19
Electrolyte leakage								
	5.93	91.2	42.34	2.38	38.39	22.87	523.14	54.02

Soluble carbohydrate content

Carbohydrate changes are of particular importance on account of their direct relationships with physiological processes such as ABA signaling, photosynthesis, translocation and respiration. [31]. As a result of drought stress, amylase activity increases, soluble sugar content increases by decomposing starch [32]. In the present experiment, changes in soluble carbohydrate content during water stress and recovery were examined in leaves of 92 varieties of bread wheat and there was significant difference $P<0.01$ was observed among genotypes in irrigated and stress conditions. Interactions between genotypes and treatments have shown no significant difference in observations as depicted in anova results. The mean values for controlled treatment were ranged from 311.11 to 557.30 µg/g. An increase in sugar content was observed when water stress was applied. The readings ranged from 408.33-543.01 in both years. Maximum suger content value in stressed conditions was observed in AG-024 which was 658 µg/g. These results were in accordance with [33]. who observed that the soluble carbohydrate concentration in well-watered wheat plants was lower than those of stressed plants.

Proline content

Proline (content) is closely related to stress tolerance especially under soil water deficits [34]. Many reports from crops and other plants have proved this relationship [35, 36]. There was significant difference $P<0.005$ observed between genotypes for proline content in year 2013-2014 and 2014-2015. However interaction between genotypes and treatments did not show any significant difference. The mean value for irrigated and stress treatments were 1.42 and 3.7 respectively. Maximum value for proline content in irrigated conditions was 3.48 and in stress condition an increase upto 6.74 was noticed in both years. Minimum values for proline content was recorded in AG-091 which was 0.62 (control) and 1.7 (Stress). These results are in accordance with [37]. who stated that proline content increases under water stress

Super oxide dismutase enzyme

The major reactive oxygen species (ROS) scavenging mechanisms include superoxide dismutase (SOD), enzymes and metabolites from the ascorbate-glutathione cycle, and catalase (CAT). They are located throughout the different compartments of the plant cell. SOD is the front-line enzyme in ROS attack since it rapidly scavenges superoxide, one of the first ROS to be produced [38, 39]. Significant differences were observed for the values of SOD enzymes among genotypes, treatments and years. For both years the SOD values ranged from 9.50 to 12.16 in controlled conditions and from 33.34 to 37.83 in stressed conditions. Significant increase was observed for the genotypes with stress treatment. Minimum SOD values were observed for AG-091(12.16 and 10.23) and maximum (34.55 and 35.58) for AG-028 in stressed conditions. These results were in contrast to [40]. Who observed that Superoxide dismutase (SOD) activity was very low and showed non-significant increase under water-stress in tolerant genotypes. However the results were consistent with the findings of [41]. who speculated that superoxide dismutase (SOD) activity increased with the decrease in osmotic potential in drought.

Chlorophyll content

To check for delayed senescence of leaves, particularly flag leaves, portable chlorophyll meters such as the Minolta SPAD are extensively used, due to their speed and ease of use [42, 43]. In stressed vegetation, leaf chlorophyll shows decrease suggesting an overall drop in light absorption [44-46].

Chlorophyll content values were significant for all the genotypes. The interaction between genotypes and treatments did not

show much significant differences. Chlorophyll content values ranged from 33.40 to 68.10 in irrigated and were decreased to 21.73-46.17 in stressed conditions. The results proved the findings of [3]. who concluded that a reduction of the chlorophyll content under drought stress conditions occurs which may be due to a reduced leaf growth under drought stress conditions resulting in a reduced cell expansion leading to a relatively lower chlorophyll density in the leaves.

Canopy temperature

Canopy temperature can provide information on transpiration as the main contributor to reduced leaf temperature [47, 48] and it is taken as a relative measure of water flow associated with water extraction from the soil under water deficit [49]. Mean canopy temperature in irrigated conditions for both years was observed around 22.2 and in stressed treatment it was increased to 26.69 mean values. The reults were highly different for genotypes, treatments and years but no significant difference for the canopy temperature values was noticed for interactions of genotypes and treatments awith the years. Highest difference in canopy temperature was observed in AG-034 (31.67 in irrigated and 21 in stressed conditions) and minimum in AG-024 (16 in irrigated and 21 in stressed conditions).

Table 4. Drought tolerant lines based upon physio-biochemical attributes

Genotypes	Proline(µmol/g)	Sugar (µg/g)	SOD	CT
AG-010	6.12	651.29	33.31	23
AG-012	5.95	643.64	35.27	23.67
AG-017	6.28	655.56	29.54	26.67
AG-024	6.66	658	23.89	29
AG-051	6.03	644.45	35.98	25.33

Relative water content and electrolyte leakage

The results were highly significant for genotypes and treatments among the years for relative water content. However no significant difference was observed for interaction between genotypes and years. In year 2013-2015 relative water content ranged from 53.74-94.55 in irrigated and 14.88 to 81.66 in stressed conditions. Results obtained were highly significant among genotypes and treatments for both years. Under stressed conditions cell membranes are subject to changes often associated with the increase in the cell permeability [50]. In case of electrolyte leakage as a measure of cell membrane stability (CMS) there was normal distribution of trait. The genotype AG029 had highest electrolyte leakage of 91.2%. The electrolyte leakage progressively amplified with increasing drought severity. These results are in agreement with those of [33, 51, 52].

Conclusion

The present study was carried out to demonstrate that genetic variation can cause drought resistance in plants growing in dry and semi-dry regions of Pakistan and similar countries in the world. Our results revealed that different wheat cultivars can cope with drought stress by employing strategies such as the production of compatible solutes (reducing sugars, proline) and antioxidant enzymes. The cultivars given in Table 3 might be considered as a suitable candidate for cultivation in semi-arid regions of this country.

Authors' contributions

Research planning, undertaking and analysis: SK Chaudhari, Supervision of the whole research plan and write up: M Arshad and N Ilyas.

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