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ORIGINAL ARTICLE

Evaluation of Physiological Responses of Durum Wheat Landraces (Triticum Durum) to Terminal Drought Stress

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Ali Nori, Mostafa Ahmadizadeh, Hossein Shahbazi and Saeed Aharizad: Evaluation of Physiological Responses of Durum Wheat Landraces (Triticum Durum) to Terminal Drought Stress

ABSTRACT

In order to investigate the effect of drought stress on Chlorophyll fluorescence, Chlorophyll content and cell membrane stability of 25 durum wheat landraces,. An Experiment was conducted at the greenhouse of agricultural research station of Islamic Azad University, Ardabil, Iran in 2009. Plants were subjected to non-stress and dry conditions in complete randomized block design with 3 replications. The results showed that there were significant differences among genotypes, in all of the traits under study. The mean comparisons showed that Genotypes number 15 and 21 had the highest membrane stability under 20% and 30% PEG induced osmotic stress respectively. All traits were affected by water stress significantly ($p<0.01$).When drought stress continued, mean values of Chlorophyll content and Chlorophyll fluorescence parameters increased in all three measurements (7, 14 and 21 days after stress). Initial fluorescence (F0) in stress and non-stress conditions had a positive correlation with the CMS under 30% PEG. Based on the results of this experiment, it was inferred that damage to photo-system II increases as the result of damage to the cell membrane. A positive and significant correlation was observed between chlorophyll content with Fv/Fm parameter in non-stress condition.

Key words: Durum wheat, chlorophyll fluorescence, electrolyte leakage, PEG6000, genetic diversity.

Introduction

Drought stress is one of the most important environmental factors which limit plant photosynthesis. It has shown that photosynthesis reduction in such condition is associated with malfunction of biochemical reactions [21]. Thus, for crop production in semiarid areas, proper monitoring of water plant is essential for the development of appropriate, sustainable, irrigation programs [10]. For this reason screening for improved plant to drought tolerance, is a rather difficult and time-consuming process which seems to be a promise for breeding programs of many agricultural crops. Lack of appropriate physiological traits comprised in the breeding programs is the reason why plant breeders have not included more analytical approaches into the selection process. Any desirable physiological traits should be rapidly and easily measured and more efficiently evaluated than parameters of final yield [27,30].

Chlorophyll a and b present in leaves of higher plants are the main pigments of photosynthesis in the chloroplasts and have important functions in the absorption and exploitation of light energy, thereby influence photosynthetic efficiency [22].

Chlorophyll content is positively associated with photosynthetic rate which increases biomass

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production and grain yield. Significant relationships between chlorophyll content and yield and yield components facilitate selection of high yielding genotypes [23]. Photosystem II (PSII) is highly sensitive to environmental inhibiting factors and water stress will damage its reaction centers severely. The chemical reaction of PSII is also affected strictly by water stress [21]. In recent years, the technique of chlorophyll fluorescence has become ubiquitous in plant physiological studies. No investigation into the photosynthetic or stress performance of plants under field conditions seems complete without some fluorescence data [25]. A promising approach is the use of chlorophyll fluorescence, a technique that can provide large amounts of data with a minimum of expertise and time and without injury to the plants. Chlorophyll fluorescence works on the principle that photosynthesis is one of the core functions in the physiology of plants. The functional state of photosynthesis has been considered an ideal physiological activity to monitor the health and vitality of plants [24]. Chlorophyll fluorescence techniques are often used to detect environmental, chemical and biological stress in plant tissue [25]. One of the most important parameters in rapid fluorescence kinetics is variable Fluorescence (Fv), i.e., the difference between maximal and minimal fluorescence (Fm-F0). The variable to maximum fluorescence ratio (Fv/Fm) is an indicative of potential or maximum quantum yield of PS II [5]. The declining slope of Fv/Fm is a good indicator to evaluate photo-inhibition of plants exposed to environmental stresses such as drought and heat, accompanied by high irradiance [2,13].

According to Paknejad *et al.,* [19,20] drought stress reduces the variable (Fv) and initial (F0) fluorescence parameters and quantum yield (Fv/Fm). Under dry conditions chlorophyll fluorescence was considered as a useful tool for screening and breeding of wheat cultivars [9,11,12].

Vazan [29] reported that drought stress reduces variable fluorescence (FV), initiative fluorescence (F0) and quantum yield (FV, FM).

Electrolyte leakage tests have been widely used to assess the level of plant tolerance to various stresses. These tests determine the degree of cell membrane injury caused by stress based on electrolyte leakage from the cells. The technique is relatively simple, repeatable and rapid and requires inexpensive equipment, can be used on plant material from a variety of cultural systems and it is suitable for the analysis of large numbers of samples [16,3,1].

According to the previous studies, there is a relation between various physiological responses of crop plants to drought and their tolerance mechanisms [26,14,15].

The objective of this study was to evaluate the applicability of the parameter(s) derived from the degree of cell membrane stability, chlorophyll content and fluorescence kinetics to evaluate drought stress response of durum wheat landraces and distinguish drought tolerance among the tested durum wheat landraces.

Material and methods

Experiments were undertaken on 20 durum wheat (Triticum durum Desf.) landraces along with five controls (Korifla, Chakmak, Zardak, Haurani-1 and Omrabi-5). They were grown under irrigated and drought conditions, base on randomized complete block design with three replications. The experiment was carried out in the greenhouse of agricultural research station of Islamic Azad University, Ardabil branch, Iran (Northwest of Iran).

Leaf Chlorophyll Content and Chlorophyll Fluorescence:

Chlorophyll content index was measured in the middle of flag leaf, in three times with one week intervals. Measurements were done at 10 o'clock in morning by portable chlorophyll-meter CCM model made by Opti-scince. Measurements of chlorophyll fluorescence were performed both on control and stressed plants at the beginning of water stress period. Intact flag leaves of durum wheat plants were adapted to darkness for 15 min using light-with-holding clips. Chlorophyll a fluorescence was measured by a portable fluorimeter (OS30-p made by Opti-Scince Company). It was represented by F0, Fm, Fv and Fv/Fm (photosynthetic yield or quantum yield). Where, F0=initial fluorescence, Fm=maximum fluorescence, Fv= Relative variable fluorescence (Fm-Fo). Temperature=28?c. Time range=10µs3. This measurement was done from 9-11a.m in three times with one week intervals.

Electrolyte Leakage Measurement for the Estimation of Cell Membrane Stability:

To measure cell membrane stability, Five leaves per genotype with uniform size and age were collected, immediately weighed and cut into segments (cut in 1 cm segments), Segments originating from the same leaf were put into 20 ml of deionised water in a test tube and washed slowly using a rotary shaker (100 rpm) at room temperature to remove solutes from both leaf surfaces and damaged cells due to cutting and then exposed either to 0% (control) or to 20% and 30% PEG 6000 for 15 h in the darkness. Electrolyte leakage was then measured before (ECi) and after (ECf) 4 h of rehydration and ultimately after autoclaving (ECt).

Cell membrane injuries were expressed as an index of injury calculated as Id= [(Rs-Rc)/(1-Rc)] $\times 100$, where Rs and Rc represent (ECfαECi)/(ECtαECi) to control or PEG-treated

tissues, respectively [4].

Results and discussion

The results of analysis of variance showed that there is a significant difference in 1% probability level between (20% and 30% PEG6000) inductive stress (Table 1). The interaction between genotype and condition was also significant in this experiment. Mean comparison of genotypes in 20% PEG induced stress, showed that Genotype 15 had the lowest electrolyte leakage and therefore highest membrane stability in osmotic stress at 20% PEG. In 30% PEG, Genotype 21 had the least amount of cell membrane leakage (Figure 1). Water stress can increase reactive oxygen species synthesis (ROS) that produce Proteins, membrane lipids and photosynthetic pigments degradation with a loss of cell membrane stability [17,6]. Tolerance drought strategy could be associated to integrity cell membrane preservation and its rapid reparation [18]. Ahmadizadeh *et al.*, [1] evaluated effects of different levels osmotic stress with PEG6000 on 37 durum wheat landraces from Northwest Iran and the Azerbaijan Republic, observed significant different between levels of PEG and reported the drought tolerant genotypes, had high stability membrane and conduction electric in drought stress. Bajji *et al.,* [4] also studied three cultivars of durum wheat that got similar results which confirms the results of this research.

Chlorophyll Content of Flag Leaf:

After exertion, under stress and normal conditions, analysis of variance for flag leaf chlorophyll content have been measured in 3 times with 7 days intervals (Table 2). There was a significant difference between stress and non-stress conditions in all 3 measurements (7, 14 and 21 days) from leaf chlorophyll content point of view. But in separated conditions (stress and non-stress) no significant difference was observed in chlorophyll content in 3 measurements, however, the studied genotypes had a significant difference in chlorophyll content in all of 3 measurements and also in both conditions. This difference was in 1% probability level. In drought stress condition, chlorophyll content reduced in two last measurements, (days 14 and 21) comparing with the onset of stress (Table 3). However in this condition, no significant difference was observed among these three measurements that were similar to the results obtained by Casterilo and Calcargo [7] and Casterilo and Verojili [8].

Tas and Tas [28] pointed out that chlorophyll content decrease with ripeness of seed in stress condition, which confirms the results of this experiment. In non-stress condition, in the first time, genotypes 3, 4 and 8 had the most chlorophyll content, and genotypes13 and 15 had the least. In the second measurement, genotype 12 had the most and genotypes 5, 18 and 15 had the least. In the third measurement, genotypes 4 and 7 had the most and genotypes 25, 10 and 18 had the least chlorophyll content. In drought condition, in the first measurement genotypes 12, 9, 4, and 15 had the most and genotypes 20, 22, and 8 had the least chlorophyll content respectively. In the second measurement, genotypes 12, 9, 4, and 15 had the most and genotypes 20, 22, and 10 had the least amount. In the third measurement, genotypes 12, 3 and 4 had the most and genotypes 20, 22 and 10 had the least chlorophyll content (Table 4).

Flag Leaf Chlorophyll Fluorescence:

Analysis of variance for chlorophyll fluorescence parameters which have been measured 3 times after stress exertion in 7 days intervals are presented in table 2. The results of this experiment showed that there are significant differences among the environments (stress and normal) in all of the chlorophyll fluorescence (Fm/Fv, F0 and Fm) parameters, in all 3 measurements. But no significant difference was observed in separated condition (stress and non-stress) in 3 measurements from all of parameters. The result Araus *et al.,* [2] is conforms. A significant difference was observed among the genotypes from all three chlorophyll fluorescence parameters in all three chlorophyll fluorescence parameters in all three measurements that it is an indicator of genetic diversity among the genotypes. The effect of measurement time was significant for all of 3 parameters in 1% probability level. Amounts of these parameters reduced by time passage and getting close to ripeness. It confirms the results of experiment Tas and Tas [28]. Therefore long stress time has had an effect on fluorescence amount. It was observed that stress in measurement time has caused the increase of evaluated parameters amounts.

According to the Table 5, Following Results Was Obtained about F0 Parameter:

In the first measurement, genotype 20 had the most and genotype 5 had the least (however there was no significant difference among the genotypes) amount. In the second time, genotypes 19, 20 and 15 had the most amounts and genotypes 25, 22 and 23 genotypes had the least amount respectively.

In the third time, genotypes 7 and 6 had the most and genotypes 4 and 10 had the least amount respectively (they had no significant difference with other 21 genotypes).

According to the Table 6, Following Results Was Obtained about F0 Parameter:

In the first time genotypes 24, 19 and 17 had the most and genotypes 18, 2 and 16 had the least amounts. In the second time, genotypes 20, 19 and 15 had the least amounts. In the third time, genotypes 6 and 24 had the most and genotype 16 had the least amount (it had no significant difference with other 22 genotypes).

According to the Table 5, Following Results Was Obtained about Fm Parameter:

In the first time, genotypes 4, 2 and 1 had the most and genotypes 17,16 and 11 had the least amounts. In the second time, genotypes 8, 20 and 18 had respectively the most and genotypes 22, 24 and 23 had respectively the least amounts. In the third time, genotypes 7,6 and 19 had the most and genotypes 11,13 and 1 had the least amounts.

According to the Table 6, Following Results Was Obtained about Fm Parameter:

In the first time, genotypes 24,19 and 10 had the most and genotypes 18,6 and 17 had the least amounts.

In the second time, genotypes 8,5 and 10 respectively had the most amounts and genotypes 24, 2 and 22 had respectively the least amount. In the third time, genotypes 15,5 and 24 had respectively the most and genotypes 2, 17 and 16 had respectively the least amounts.

According to the Table 5, Following Results Was Obtained about Fv/Fm Parameter:

In the first measurement, genotypes 4, 1 and 5 had the most and genotypes 23, 25 and 22 had the least amount. In the second measurement, genotypes 8, 12, 23, 25, 2, 10 and 13 had respectively the most and genotypes 15, 20, 22 and 18 had the least amount. In the third measurement, genotypes 5, 17 and 10 had respectively the most and genotypes 6, 1 and 13 had respectively the least amount.

According to the Table 6, Following Results Was Obtained about Fv/Fm Parameter:

In the first measurement genotypes 5, 8, 4, 1, 2 and 24 had the most and genotypes 18, 17, 16, 14, and 25 had the least amounts. In the second measurement, genotypes 23,12,10,7 and 5 had respectively the most and genotypes 17,1 and 19 had respectively the least amounts. In the third measurement, genotypes 18,9 and 15 had respectively the most and genotypes 2,13 and 6 had respectively the least amount.

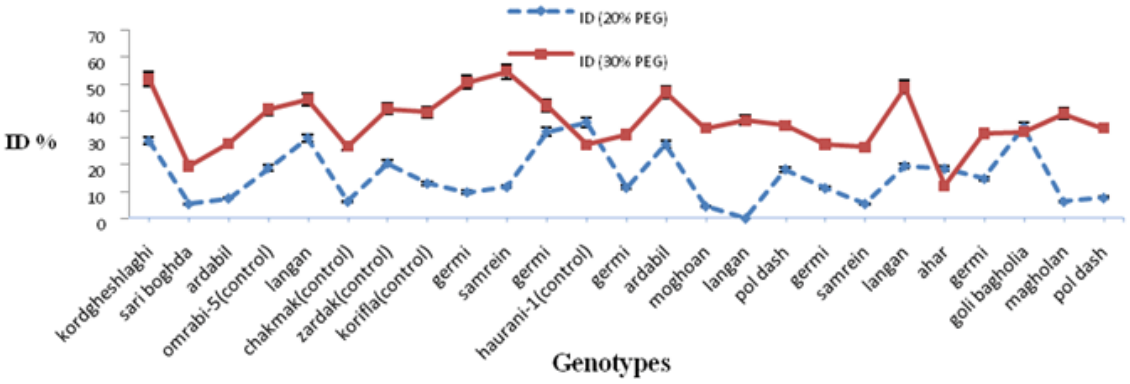

Fig. 1: Index of damage (ID %) durum wheat genotypes in two level osmotic stresses (PEG6000).

Table 1: Mean squares of index of damage (ID) in two level osmotic stresses (PEG6000).

S.O.V	df	MS
Rep	2	1704.38**
Condition	1	14016.9**
Genotype	24	387.74*
C × G	24	222.59*
Error	98	300.67

** and * significant 1%, 5% level of probability, respectively.

Table 2: Combine analysis of variance (factorial split plot in time) for the flag leaf chlorophyll content (CCI) and chlorophyll fluorescence parameters.

S.O.V	df	Mean Squares			
		CCI	F0	Fm	Fv/Fm
Replication (R)	2	14.791NS	3.882 NS	57.087 NS	88.242 NS
Condition (C)	1	18027.46**	778.809**	158296.89**	32359.68**
Genotype (G)	24	2014.53**	309.882**	9658.89**	1171.86**
C × G	24	1151.72**	110.614**	6344.62**	624.77**
Error 1	98	20.799	41.361	335.019	119.015
Time (T)	2	286.655**	14798.67**	402654.85**	5817.209**
T × C	2	9.196 NS	24.109 NS	2788.749 NS	672.08 NS
T × G	48	42.097**	273.88**	5069.627**	1670.8**
T × C × G	48	50.889**	49.831*	2452.071**	698.74**
T × R	4	42.097 NS	41.489 NS	66.663 NS	402.536*
Error 2	196	15.124	30.617	352.038	146.724

**,* and Ns, significant at 1%, 5% level of probability and non-significant, respectively

Table 3: Mean comparison of normal and stress conditions in three measurements (day 7, 14 and 21) for the flag leaf chlorophyll content (CCI) and chlorophyll fluorescence parameters.

	Mean ± S.E.M											
	CCI			F0			Fm			Fv/Fm		
condition	7 day	14 day	21 day	7 day	14 day	21 day	7 day	14 day	21 day	7 day	14 day	21 day
Normal	29.37±1.82	26.57±1.6	26.74±1.81	87.8±0.79	83.2±1.05	68.1±1.06	391.37±4.8	359. 9±4.3	288.4±4.7	774.5±3.3	766.6±2.o	767.4±2.0
Total		27.5636±1.026			79.74±0.79			346.5±3.93			769.55±1.475	
Stress	41.5±1.32	39.7±1.4	39.2±1.4	89.8±1.06	85.5±1.3	71.6±0.8	433.5±5.6	387.4±5.7	331.2±4.8	795.3±3.3	779.09±1.5	785.07±2.2
Total		40.22±0.809			82.37±0.81			384.09±4.17			786.51±1.51	

Table 4: Mean comparison of genotypes in three measurements (day 7, 14 and 21) for the flag leaf chlorophyll content (CCI) in normal and stress conditions.

		Normal condition			Stress condition		
No.	Landraces	7 day	14 day	21 day	7 day	14 day	21 day
1	kordgheshlaghi	41.016 ABCDE	34.416 BCDEFG	43.283 ABC	31.063 EF	31.083 GHI	25.5 IJK
2	Sari boghda	34.016 DEF	27.3 DEFGH	30.916 CDE	35.316 DEF	31.016 GHI	33.266 EFGHIJ
3	Ardabil	52.083 A	40.666 BC	42.566 ABC	53.666 ABC	50.7 ABCD	54.65 AB
4	Omrabi-5 (Contro)	49.783 AB	44.966 B	52.75 A	56.566 AB	54.333 ABC	54.533 AB
5	Langan	26.983 FG	21.583 GHIJ	20.55 EFG	38.6 CDEF	34.4 FGH	29.183 GHIJ
6	Chakmak (Control)	31.933 EF	35.566 BCDEF	29.35 CDE	51.766 ABC	42.166 CDEFG	46.217 ABCDE
7	Zardak (Control)	43.683 ABCDE	44.45 B	47.616 AB	39.616 CDEF	40.766 DEFG	45.966 BCDE
8	korifla (Control)	48.533 AB	38.65 BCDE	38.666 ABCD	27.783 FG	31.333 GHI	30.15 FGHIJ
9	Germi	40.666 ABCDE	39.983 BCD	42.416 ABC	56.683 AB	57.65 AB	48.816 ABCD
10	Samrein	6.066 IJ	11.483 JKL	6.016 H	33.366 DEF	29.216 GHI	24.383 JK
11	Germi	34.816 DEF	27.9 CDEFGH	14.616 FGH	39.133 CDEF	33.543 FGH	40.05 BCDEFGH
12	Haurani-1 (Control)	47.733 ABC	59.633 A	48.666 AB	59.2 A	59.3 A	60.366 A
13	Germi	1.883 J	10.683 JKL	10.683 GH	39.216 CDEF	39.583 DEFG	39.55 CDEFGHI
14	Ardabil	32.433 EF	19.883 HIJK	20.983 EFG	41.983 BCDEF	45.533 BCDEF	44.016 BCDEF
15	Moghoan	3.183 J	8.05 KL	9.6 GH	56.55 AB	54.216 ABC	51.083 ABCD
16	Langan	18.766 GH	25.4 FGH	25.783 DEF	44 ABCDE	48.266 ABCDE	43.166 BCDEFG
17	Pol dash	10.166 HIJ	10.9 JKL	3.75 H	33.283 DEF	25.416 HI	27.3 HIJ
18	Germi	14.833 HI	4.866 L	8.316 GH	47.8 ABCD	49.116 ABCD	42.333 BCDEFG
19	Samrein	8.366 HIJ	15.5 HIJKL	13.333 FGH	30.583 EF	29.1 GHI	25.283 IJK
20	Langan	44.316 ABCD	39.566 BCD	41.1 ABC	15 G	10.466 J	12.816 K
21	Ahar	26.416 FG	24.5 FGHI	30.7 CDE	47.3 ABCD	49.85 ABCD	43.45 BCDEFG
22	Germi	27.466 FG	12.583 IJK	12.85 FGH	27.033 FG	20.316 IJ	23.716 JK
23	Goli Bagholia	36.433 CDEF	26.1 EFGH	31.733 CDE	44.083 ABCDE	41.566 CDEFG	45.933 BCDE
24	Magholan	40.05 BCDE	34.483 BCDEFG	37.15 BCD	48.85 ABCD	49.95 ABCD	53.633 ABC
25	Pol dash	12.7 HIJ	5.266 L	5.15 H	41.55 BCDEF	35.466 EFGH	36.95 DEFGHIJ

Values with the same superscript letters are no significantly different at $P < 0.05$.

Table 5: Mean comparison of genotypes in three measurements (day 7, 14 and 21) for chlorophyll fluorescence parameters in normal condition.

		F0			Fm			Fv/Fm		
No.	Landraces	7 day	14 day	21 day	7 day	14 day	21 day	7 day	14 day	21 day
1	kordgheshlaghi	82.33 A	84.6 ABCDEFG	65.6 7B	442.6 ABC	385 AB	252.3 J	814.6 AB	776.6 ABCD	739.3 GH
2	sari boghda	87.66 A	81.6 ABCDEFG	71.6 B	452.3 AB	374.6 AB	299 DEFG	802 ABC	777 ABCD	756.3 DEFGH
3	ardabil	83.33 A	80.6 ABCDEFG	65.3 B	425.3 ABCD	386.3 AB	270.6 GHIJ	803.3 ABC	776.6 ABCD	758.3 DEFG
4	omrabi-5(contro)	87.66 A	87 ABCDEF	61 B	458 A	386 AB	264.3 IJ	817.6 A	774 ABCDEF	748.3 FGH
5	langan	80 A	84.6 ABCDEFG	64.3 B	379 ABCD	377 AB	268.6 HIJ	812.3 ABC	768.3 ABCDEFG	804.3 A
6	chakmak(control)	87 A	84 ABCDEFG	91.6 A	417.3 ABCD	362 ABCD	343.6 B	786 ABCD	768 ABCDEFG	736.3 H
7	zardak(control)	86 A	88.3 ABCD	93.6 A	417 ABCD	387.6 AB	391/6 A	793.3 ABC	774.3 ABCDEF	758.3 DEFG
8	korifla(control)	85.6 A	85 ABCDEFG	70.3 B	433.3 ABCD	410.3 A	293.6 DEFGH	786.6 ABCD	789.6 A	767 BCDEF
9	germi	87.3 A	84 ABCDEFG	62.3 B	464.6 ABCD	359.6 ABCD	255.3 J	760 ABCDE	763.6 BCDEFG	761 DEF
10	samrein	90.3 A	87 ABCDEF	61 B	392 ABCD	373 ABC	276.3 GHIJ	764.6 ABCDE	759 CDEFG	785 BC
11	germi	83 A	80.3 ABCDEFG	67.3 B	348 BCD	310.6 DE	167 K	770.6 ABCDE	776.3 ABCDE	785 BC
12	haurani-1(control)	83 A	81.6 ABCDEFG	65.6 B	410.6 ABCD	384 AB	313.3 CD	796.3 ABC	786.33 AB	782.6 BC
13	germi	85 A	89.3 ABC	64 B	381.6 ABCD	384.6 AB	252.3 J	785 ABCD	776.6 ABCD	739.3 GH
14	ardabil	94 A	88 ABCDE	65 B	409 ABCD	356.3 ABCD	293.3 DEFGH	769.3 ABCDE	755.3 DEFG	770.6 BCDE
15	moghoan	92 A	95.6 AB	63.6 B	366.6 ABCD	317.33DE	284 FGHI	755 BCDE	707.3 H	775 BCDE
16	langan	83 A	82 ABCDEFG	63.3 B	345.3 CD	366.3 ABCD	282.6 FGHI	755.3 BCDE	771.6 ABCDEF	783.6 BC
17	pol dash	81.3 A	79 BCDEFG	64.6 B	329 D	313.3 DE	258.3 IJ	758 ABCDE	753.3 EFG	790 AB
18	germi	94.6 A	92.3 AB	65.6 B	389.3 ABCD	401 A	311 CDEF	769 ABCDE	753 FG	755.3 EFGH
19	samrein	92.3 A	97 A	66.3 B	404.6 ABCD	386.3 AB	337.6 BC	767 ABCDE	773 ABCDEF	766.3 CDEF
20	langan	97.3 A	97 A	73.6 B	363.3 ABCD	405.6 A	315.6 CD	774 ABCDE	748.6 G	764.6 CDEF
21	ahar	89.3 A	72.3 CDEFG	68.3 B	376.3 ABCD	335.6 BCDE	284.3 EFGHI	759.6 ABCDE	765 BCDEFG	757.3 DEFG
22	germi	86.6 A	69.3 FG	68 B	350 BCD	285.3 E	273.6 GHIJ	752 CDE	753 FG	763.6 CDEF
23	goli bagholia	93.3 A	70.3 EFG	70.3 B	377.6 ABCD	319 CDE	312.6 CDE	723 E	779 ABC	777.6 BCD
24	magholan	94.6 A	70.6 DEFG	64 B	376 ABCD	292.3 E	315.3 CD	761.3 ABCDE	761.3 CDEFG	775 BCDE
25	pol dash	90.3 A	68.3 G	66 B	375 ABCD	338 BCDE	294.3 DEFGH	726.6 DE	778.6 ABCD	784.6 BC

Values with the same superscript letters are no significantly different at $P < 0.05$.

Table 6: Mean comparison of genotypes in three measurements (day 7, 14 and 21) for chlorophyll fluorescence parameters in stress condition.

		F0			Fm			Fv/Fm		
No.	Landraces	7 day	14 day	21 day	7 day	14 day	21 day	7 day	14 day	21 day
1	kordgheshlaghi	94 ABC	87 ABCD	65.3 BC	447.3 BC	384 FGH	304.3 DEF	815 BC	763.6 GH	770.3 BC
2	sari boghda	77 CD	66.3 DE	69.3 BC	408.6 BCD	243.3 J	245.6 G	811 BC	774.3 CDEFGH	747.3 C
3	ardabil	84 BCD	88 ABCD	73 BC	439.3 BCD	378.6 FGH	380.3 AB	804 BCD	777.6 BCDEFGH	796.6 AB
4	omrabi-5(contro)	88.3 ABCD	91.6 ABC	76.3 BC	444 BC	431.6 ABC	357.3 ABC	815 BC	787 ABCDEF	795.6 AB
5	langan	81.6 BCD	88.3 ABCD	70.3 BC	392.3 BCD	441.6 AB	387 AB	893 A	792 ABCD	785.6 ABC
6	chakmak(control)	81.6 BCD	87.6 ABCD	94 A	465.6 B	396.6 DEFG	382.3 AB	791.6 BCD	772.3 DEFGH	766.6 BC
7	zardak(control)	92.6 ABC	86.6 ABCD	78.6 ABC	446.3 BC	415 BCDE	326.6 CDEF	801 BCD	792.3 ABCD	773 BC
8	korifla(control)	88.3 ABCD	91 ABC	70 BC	415.3 BCD	447.6 A	326.6 CDEF	827.3 B	779.3 BCDEFGH	790.3 ABC
9	germi	95.3 ABC	92.3 ABC	69.6 BC	463.3 B	414.6 BCDE	326.6 CDEF	793.6 BCD	771 DEFGH	808.6 AB
10	samrein	94.6 ABC	91.6 ABC	68.3 BC	468.6 B	428.3 ABC	342.6 BCDE	800 BCD	795.3 ABC	789 ABC
11	germi	84 BCD	85.6 ABCD	65 BC	393.3 BCD	390.6 EFG	288.6 F	782 BCDE	780.3 BCDEFG	780.6 ABC
12	haurani-1(control)	84 BCD	84 ABCDE	69.6 BC	430.3 BCD	414 BCDE	301.3 EF	802.6 BCD	799 AB	768 BC
13	germi	91.3 ABCD	83 ABCDE	71.6 BC	448 BC	373.6 GH	292.3 F	785.6 BCDE	770.6 DEFGH	766.3 BC
14	ardabil	95.3 ABC	90.6 ABC	69.6 BC	432.3 BCD	403.3 CDEF	322.3 CDEF	778.6 CDE	768 EFGH	791 ABC
15	moghoan	91 ABCD	96 AB	74.6 BC	452 BC	412.3 CDE	401.6 A	788.6 BCD	767.6 EFGH	802.6 AB
16	langan	78.6 CD	83 ABCDE	61 C	360 DE	384.3 FGH	286 FG	772.6 CDE	783.6 BCDEFG	799.6 AB
17	pol dash	96.6 ABC	94.3 AB	70 BC	377 CDE	388.6 EFG	281 FG	759.3 DE	756.6 H	769 BC
18	germi	72 D	92.6 ABC	71.3 BC	305.6 E	417 BCDE	361.6 ABC	741.3 E	770 DEFGH	823 A
19	samrein	101 AB	97.6 A	68.3 BC	469.6 B	413 CDE	295 F	784.6 BCDE	766.3 FGH	773 BC
20	langan	96 ABC	99.6 A	73.6 BC	464.6 B	424.3 ABCD	348.6 BCD	787 BCDE	769.6 DEFGH	784.3 ABC
21	ahar	91.3 ABCD	74 BCDE	71.6 BC	445 BC	333.3 I	347.6 BCD	784.6 BCDE	778 BCDEFGH	793.3 ABC
22	germi	95.3 ABC	62.6 E	70 BC	445.6 BC	266.3 J	318 CDEF	782 BCDE	790 ABCDE	781.3 ABC
23	goli bagholia	93.3 ABC	67.3 DE	73.6 BC	449.3 BC	394.3 EFG	378.6 AB	792 BCD	806.3 A	796.67AB
24	magholan	105.6 A	88.3 ABCD	79.3 AB	545 A	258.6 H	384.6 AB	808.6 BC	779.3 BCDEFGH	799.3 AB
25	pol dash	94 ABC	69.6 CDE	66.6 BC	429.6 BCD	331 I	294 F	778.6 CDE	786.6 ABCDEF	775 BC

Values with the same superscript letters are no significantly different at $P < 0.05$.

Table 7: Correlation between physiological traits in two conditions.

	Condition	ID (30% PEG)	ID (20% PEG)	F_0 *(Normal)*	F_v/F_m *(Normal)*	F_0 (Stress)	F_v/F_m (Stress)	(CCI) (Normal)
ID	20% PEG6000	0/27						
F_0	Normal	0/06	-0/21					
F_v/F_m	Normal	0/14	0/42 *	0-/22				
F_0	Stress	0/38	-0/23	0/48 *	0-/19			
F_v/F_m	Stress	0/30	0/28	-0/22	0/47*	-0/05		
Chlorophyll Content (CCI)	Normal	0/13	0/32	0/09	0/48*	0/11	0/38	
Chlorophyll Content (CCI)	Stress	-0/26	-0/02	-0/16	-0/003	-0/007	0/21	0/28

*Significant at 5% level of probability

Correlations:

Correlation coefficients of physiological traits measured in draught stress and normal conditions are presented in table 7. F0 in stress and non-stress conditions had a positive correlation with the damage to the membrane under 30% PEG. It can be inferred that as the damage to cell membrane increases the amount of damage to photo-system II increases. But the correlation with the damage at 20% PEG was negative. The correlation was positive and significant between F0 in stress condition with F0 parameter in normal condition (0.48*), (Table 7). The positive stress condition is an indicator of changes in the same direction with this parameter in both conditions. A positive and significant correlation in possibility level of 5% was observed between chlorophyll content with Fv/Fm parameter in normal condition (0.48). This correlation observed between chlorophyll content in normal condition; and Fv/Fm parameter in stress condition had a positive and significant correlation with Fv/Fm parameters in normal condition. A positive and significant correlation was observed between Fv/Fm in normal condition with damage to the cell membrane under 20% inductive stress. This relation does not seem logical and it can be inferred that Fv/Fm index does not seem useful to evaluate cytoplasm cell membrane stability.

Conclusion:

Present results showed that fluorescence parameters, Chlorophyll content and cell membrane stability as a non-invasive and efficient method are very advantageous to use in plant screening for stress tolerance. The main problem, especially in selection for improved drought tolerance, is the lack of reliable and sufficiently sensitive parameters of selection. There is a need for more detailed research on existing drought resistant native plants and the use of these genotypes as sources of germoplasm in breeding resistance against environmental stress.

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