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Introduction of a new selection index for improvement of drought tolerance in common wheat (*Triticum aestivum* L.)

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ABSTRACT

In order to select drought tolerant wheat genotypes, an experiment was conducted in a randomized complete block design (RCBD) with three replications under two different rainfed and irrigated conditions during the growing season 2010-2011. Principal component analysis (PCA) showed that integrated selection index (ISI) was correlated with chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll total (Chl T), relative chlorophyll content (RCC), relative water content (RWC), proline concentration (PC) and excised leaf water retention (ELWR) indicating that these screening techniques can be useful for selecting drought tolerant genotypes. Screening drought tolerant genotypes using mean rank, standard deviation of ranks and rank sum discriminated genotypes (18), (11) and (15) as the most drought tolerant. Therefore, they are recommended to be used as parents for genetic analysis, gene mapping and improvement of drought tolerance in common wheat.

Key words: bread wheat, integrated selection index, biplot, ranking method

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INTRODUCTION

Plants are exposed to numerous stress factors during their lives, which is of a significant effect on the growth of plants. Biotic (pathogen, competition with other organisms) and abiotic (drought, salinity, radiation, high temperature or freezing etc.) stresses cause changes in normal physiological functions of all plants, including economically important cereals as well. All these stresses reduce biosynthetic capacity of plants and might cause some destructive damages on plants [1]. Drought stress has the highest percentage (26%) when the usable areas on the earth are classified in view of stress factors. It is followed by mineral stress with 20% part, cold and freezing stress with 15% part. Whole the other stresses get 29% part whereas only 10% area is not exposed to any stress factor [2].

Therefore, drought stress is one of the most widespread environmental stresses, which affect growing and productivity, it induces many physiological, biochemical and molecular responses on plants, so that plants are able to develop tolerance mechanisms which will provide to be adapted to limited environmental conditions [3]. Wheat (*Triticum aestivum*) is the world's widely adapted crop, providing one-third of the world population with more than half of their calories and nearly half of their protein. Wheat is mainly grown on rainfed lands and about 35% of the area of developing countries consists of semiarid environments in which available moisture constitutes a primary constraint on wheat production. Climatic variability in these marginal environments causes large annual fluctuations in yield. Selection of wheat genotypes with better adaptation to drought stress should increase the productivity of rainfed wheat [4]. Improvement of wheat productivity for this abiotic stress is therefore an important objective of plant breeding program. Most of cereal plants respond to water stress through a range of morpho-physiological adaptations or processes. However, these physiological attributes could be used as reliable indicators for the selection of genotypes/cultivars for drought tolerance [5, 6, 7].

However, the physiological basis of their stress tolerance is not well understood. An understanding of how plants respond to water deficits and in certain instances, are able to tolerate them should lead us eventually to ways of optimizing plant productivity in marginal environments [8]. In the frame of "physiological window" mild drought induces in plants regulation of water loss and uptake allowing maintenance of their leaf relative water content (RWC) within the limits where photosynthetic capacity and quantum yield show little or no change [9]. Water deficient was found to reduce the relative water content (RWC) in plant leaves. The high RWC and low excised leaf water loss (RWL) have been suggested as important indicators of water status [10, 11]. Rong_Hua et al. [12] concluded that chlorophyll content (SPAD) could be considered as a reliable indicator in screening barley genotypes for drought tolerance. Proline accumulates generally in response to drought stress and plays the role as an osmolyte for osmotic adjustment. Proline accumulation varies with the degree of plant drought tolerance. Therefore, proline could be used for the evaluation of plant drought tolerance or sensitivity [10]. Photosynthesis, which is the most significant process influence crop production, is also inhibited by drought stress. Studies have shown that the photosynthetic rate (Pn) of leaves of both C3 and C4 plants decreases as relative water content (RWC) and water potential (Ψ) decrease [13].

The objective of this study was to determine an effective and reliable selection index for screening drought tolerant genotypes of bread wheat.

MATERIALS AND METHODS

Twenty landraces of bread wheat (*Triticum aestivum* L.) listed in Table 1 were provided from Seed and Plant Improvement Institute of Karaj, Iran. They were assessed using a randomized complete block design with three replications under two irrigated and rainfed conditions during 2010-2011 growing season in the experimental field of College of Agriculture, Razi University, Kermanshah, Iran (47° 9′ N, 34° 21′ E and 1319 m above sea level). Mean precipitation in 2010–2011 was 509.50 mm. The soil of experimental field was clay loam with pH7.1. Sowing was done by hand in plots with three rows 2 m in length and 20 cm apart. The seeding rate was 400 seeds per m^2 for all plots. At the rainfed experiment, water stress was imposed after anthesis. Non-stressed plots were irrigated three times after anthesis, while stressed plots received no water. At harvest time, yield potential (Yp) and stress yield (Ys) were measured from 2 rows 1 m in length. The following physiologic and metabolic characters were also measured in the stress condition.

Relative chlorophyll content (RCC)
The chlorophyll content in the flag leaf was determined using a chlorophyll meter (SPAD-502, Japan). Five flag leaves of each genotype grown in rainfed condition were measured after anthesis stage. Three measurements in the middle of the flag leaf were made randomly for each plant, and the average sample was used for analysis.

Relative water content (RWC)
Relative water content was determined according to Turner [14], where fresh leaves were taken from each genotype and each replication after anthesis stage and weighed immediately to record fresh weight (FW). Then they were placed in distilled water for 4 h and weighed again to record turgid weight (TW). After that subjected to oven drying at 70°C for 24 h to record dry weight (DW). The RWC was calculated using the following equation:

$$RWC = ((FW - DW)/(TW - DW)) \times 100$$

Relative water loss (RWL)
Five young fully expanded leaves were sampled for each of three replications at anthesis stage. The leaf samples were weighed (FW), wilted for 4 hour at 35°C, reweighed (W4h), and oven dried for 24 h at 72°C to obtain dry weight (DW). The RWL was calculated using the following formula [15]:

$$RWL\ (\%) = [(FM - W4h)/(FW - DW)] \times 100$$

Excised leaf water retention (ELWR)
Excised leaf water retention was determined according to Farshadfar et al [16], where the youngest leaves before anthesis stage were collected and weighed (FW), left for 4 h, then wilted at 20°C and reweighed (W4h). ELWR was calculated using the following formula:

$$ELWR\ (\%) = [1 - ((FW - W4h)/FW))] \times 100$$

Proline concentration (PC)
The PC was determined according to the method of Bates et al. [17]. Plant material (0.5 g) after anthesis stage was grinded with 10 ml of 3% sulfosalicylic acid. The homogenate was filtered and 1 ml of glacial acetic acid and 1 ml acid ninhydrin reagent were added to a 1 ml of filtrate. Then the mixture was shaken by hand and incubated in boiling water bath for 1 h. After that, it was transferred to ice bath and warmed to room temperature. 2 ml toluene was added to the mixture and the upper toluene layer was measured at 520 nm using UV spectrophotometer.

Chlorophyll a, b and total (Chl a, Chl b, Chl T)
Chlorophylls *a* and *b* were measured by the method described by Horii et al. [18] with a slight modification after anthesis stage. 3 ml of 99.5% methanol was added to the leaf tissue (50 mg) and incubated in dark for 2 h. Samples were homogenized and centrifuged at 10000 rpm for 10 min. Absorbance of the samples at 650 nm and 665 nm was measured by the UV spectrophotometer. Absolute methanol (99.5%) was used as a blank. Chl *a*, Chl *b* and Chl T content were calculated using following equations:

Chlorophyll a (µg/mL) = $16.5\times A_{665} - 8.3 \times A_{650}$

Chlorophyll b (µg/mL) = $33.8 \times A_{650} - 12.5 \times A_{665}$

Total chlorophyll (µg/mL) = $25.8 \times A_{650} + 4.0 \times A_{665}$

Integrated selection index (ISI)
Based on factor analysis of physiological traits under water deficit and the following three formulas, ISI was calculated:
(1) $S_{ij}= (X_{ij} - \mu_j)/ \sigma_j$
(2) $MP_{ij} = (S_{ij}d + S_{ij}w)/2$
(3) $ISI_i = b_1MP_{i1} + b_2MP_{i2} +\ldots+ b_jMP_{ij}$

where S_{ij} = standardized physiological value of trait j (j = 1 to 10, i.e. RWC, PC, RWL, ELWR, RCC, Chl a, Chl b, Chl T, Yp and Ys) in genotype i under irrigated and drought conditions, X_{ij} = physiological value of genotype i on trait j, μ_j = mean value of trait j in all genotypes, σ_j = the standard deviation of trait j, MP_{ij} = the mean productivity of trait j on genotype i, b_j the weight value of trait j, bj was populated from the average contribution to factor 1 and ISI = integrated selection index.

Formula (1) standardizes the value of different traits to the same unit of measure; formula (2) evaluates the appearance of genotypes for each trait; and formula (3) integrates the appearance of genotypes for all traits. When defining weight values for each trait, average contribution of factor 1 to 10 major traits related to drought resistance at irrigated and rainfed conditions in the factor analysis were considered as b_j and trait had negative functions in the final result (Table 2). Using physiological data of irrigated and rainfed conditions, the formerly proposed selection index related to drought resistance was calculated.

Statistical analysis
Analysis of variance, mean comparison using Duncan's multiple range test (DMRT), correlation analysis between mean of the characters measured and principal component analysis (PCA), based on the rank correlation matrix were performed by MSTAT-C, SPSS ver. 16 and STATISTICA ver. 8. Standard deviation of ranks (SDR) was measured as:

$$S_i^2 = \frac{\sum_{j=1}^{m}(R_{ij} - \overline{R}_{i.})^2}{l-1}$$

where R_{ij} is the rank of *in vivo* drought tolerance indicator and $\overline{R}_{i.}$ is the mean rank across all *in vivo* drought tolerance indicators for the ith genotype and SDR= $(S^2_i)^{0.5}$.

Rank sum (RS)= Rank mean ($\overline{R}$) + Standard deviation of rank (SDR) [19].

RESULTS AND DISCUSSION

The results of analysis of variance (ANOVA) showed significant differences for all the characters investigated in the rainfed condition (**Table 3**). The results revealed that water stress decreased yield of all genotypes significantly.

Table 1. Genotype codes

Genotype	Code	Genotype	Code
WC-47560	1	WC-4860	11
WC-4506	2	WC-47620	12
WC-47632	3	WC-4992	13
WC-47574	4	WC-4973	14
WC-47481	5	WC-47374	15
WC-47407	6	WC-47358	16
WC-4827	7	WC-4573	17
Azar 2	8	WC-47536	18
WC-47392	9	WC-47572	19
WC-4978	10	WC-4953S	20

Table 2. Contribution of factors 1 to 9 of major traits related to drought tolerance under rainfed and irrigated conditions.

Trait	rainfed	irrigated
Grain yield (Y)	-0.213	0.453
RCC	0.431	0.132
ELWR	0.927	-0.403
RWC	0.092	0.590
RWL	-0.959	0.057
Chl a	0.176	-0.808
Chl b	-0.034	0.864
Chl T	0.172	-0.017
PC	-0.200	0.419

Table 3. Analysis of variance for physiological traits

		Mean squares									
		grain yield		RWC		PC		RWL		ELWR	
S.O.V.	df	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated
Replication	2	46591*	19442*	462533	70476	0.034	0.026	1203	1994	928	6860
Genotype	19	16743*	33976*	231266.8**	15251*	0.019*	0.043	601.9*	107.97	464.3*	19.6
Error	38	4581334	581992.7	539365	238285	0.533	0.983	7118	3808	4333	810.8

** and **: Significant at 1% and 5% level of probability respectively; S.O.V: Source of variation, d.f: Degree of freedom.*

Table 3 continued

		Mean squares							
		RCC		Chl a		Chl b		Chl T	
S.O.V.	df	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated
Replication	2	46.62	17.7	6.5	0.082	3.3	0.215	3.24	0.331
Genotype	19	26*	652.16	3.2*	1.943	0.5*	0.987	1.6*	1.248*
Error	38	594	94.699	55.17	60.58	17.28	23.2	1.3	18.64

Maximum decrease in yield was observed in genotypes 2 and 12. Nevertheless, the yield values were increased after drought stress in genotypes 4, 13 and 15 against other varieties in the same condition (**Table 4**).

Persistence in RWC content of cultivars in water stress conditions may serve as good indicator of drought tolerance. Genotypes no.20 and 11 had higher RWC content while genotypes no. 6, 10 and 2 displayed lower RWC under water stress (**Table 4**). Merah [20] reported that RWC % was an important indicator of water stress in leaves. RWC is closely related to cell volume, therefore it may more closely reflect the balance between water supply to the leaf and transpiration rate [21]. Sairam and Saxena [22] reported that relative water content (RWC) in leaves of wheat cultivars under irrigated and stress conditions showed a decreasing trend with age in all genotypes. The decrease of RWC in stressed plants might be associated with the decrease in plant vigour as was observed in many plant species [23, 24]. Relative water content had been identified as potential physiological marker for drought tolerance in many crop plants such as barley (*Hordium Vulgare* L.) [25], sunflower (*Halianthus annus* L.) [26], sugarcane (*Saccharum officinarum* L.) [27], durum wheat (*Triticum durum*) [20], wheat and its wild relatives (28). Genotypes no.4, 8 and 16 had higher RWL, while genotypes 15, 18 and 12 indicated lower RWL under water stress (Table 4). Assessment of excised leaf water loss (ELWL) is an important selection criterion for water stress tolerance in plants [29, 30]. This trait is moderately heritable [31] and can be easily estimated in a large population [32]. In our study, genotypes 8 and 4 displayed the lowest and genotypes no. 15, 18 and 12 the highest values for ELWL.

Chlorophyll maintenance is essential for photosynthesis under drought stress. Higher Chl content and lower percent decrease under stress in tolerant genotype of wheat have also been reported [33, 34]. Proline concentration is linked with plant anti drought under drought stress condition [35]. Under rainfed conditions, some of drought tolerant genotypes accumulated more proline in the flag leaf tissues when compared to drought sensitive genotypes. Genotypes no. 20, 4 and 18 had higher PC content while genotypes 10 and 2 showed lower PC under water stress (Table 4). The results exhibited that the highest amount of RCC was attributed to genotypes no. 18, 5 and 13. The highest Chl a, Chl b and Chl T belonged to genotypes no. 15, 11 and 15 respectively (**Table 4**).

Table 7: Ranks (R), ranks mean ($\overline{R}$) and standard deviation of ranks (SDR) of physiological indicators of drought tolerance

Genotype code	Ys	R	Yp	R	RWC%	R	RWL%	R	ELWR	R	RCC	R	PC	R
1	1.38	15	1.79	12	65.79	6	72.05	5	55.12	4	46.60	11	0.3336	8
2	0.90	20	1.88	10	46.80	18	74.44	6	50.65	9	43.26	19	0.1766	19
3	1.21	18	1.41	17	61.55	9	78.58	8	46.88	17	44.16	17	0.2263	13
4	1.88	5	1.61	15	56.54	12	98.54	20	40.49	19	43.93	18	0.694	2
5	1.52	13	1.60	16	71.37	4	80.23	10	48.35	14	51.64	2	0.4053	4
6	1.80	7	2.35	6	37.67	20	85.99	17	48.47	13	42.45	20	0.3463	7
7	1.96	4	2.51	4	62.78	8	84.30	15	52.78	7	49.88	5	0.186	18
8	1.67	11	2.80	2	71.89	3	92.90	19	35.24	20	46.00	12	0.206	16
9	1.88	6	2.16	8	49.65	14	79.18	9	47.74	15	48.03	7	0.1896	17
10	1.39	14	1.83	11	41.32	19	71.74	4	52.80	6	46.64	9	0.174	20
11	1.69	10	2.21	7	75.68	2	84.15	14	49.07	12	48.03	8	0.327	9
12	1.03	19	1.40	18	54.58	13	66.80	3	55.13	3	45.47	16	0.2313	11
13	2.04	2	1.79	13	59.58	10	78.47	7	50.66	8	51.56	3	0.23	12
14	1.98	3	2.57	3	47.77	17	85.59	16	52.91	5	45.67	14	0.317	10
15	1.60	12	0.94	20	64.86	7	56.06	1	65.54	1	46.64	10	0.351	6
16	1.22	17	1.34	19	48.36	14	89.31	18	43.16	18	45.55	15	0.3676	5
17	1.32	16	1.73	14	48.34	16	83.60	12	49.31	11	49.97	4	0.2196	14
18	1.77	8	2.83	1	69.84	5	65.49	2	61.53	2	53.63	1	0.5036	3
19	1.70	9	1.91	9	57.13	11	83.96	13	47.10	16	45.71	13	0.2126	15
20	2.26	1	2.38	5	86.09	1	81.33	11	50.21	10	49.14	6	0.8726	1

Table 4 continued

Genotype code	Chl a	R	Chl b	R	Chl T	R	ISI	R	$\overline{R}$	SDR	RS
1	3.4	6	1.4	16	4.91	9	0.6594	4	8.72	4.26	12.98
2	2.7	11	1.9	4	4.6	10	-0.7102	17	13.00	5.74	18.74
3	3.3	7	1.2	18	4.5	12	-0.5499	15	13.72	4.17	17.89
4	3.0	10	1.5	11	4.5	13	-0.4781	13	12.54	5.57	18.11
5	2.7	12	1.4	17	4.2	15	0.0151	11	10.72	5.19	15.91
6	1.9	19	1.9	5	3.8	19	0.6404	5	12.54	6.57	19.11
7	2.5	15	1.5	12	4.0	16	0.3664	6	10.00	5.29	15.29
8	2.6	14	1.8	6	4.4	14	-0.7013	16	12.09	6.09	18.18
9	2.1	17	1.8	7	4.0	17	-0.5349	14	11.90	4.50	16.4
10	2.0	18	2.5	3	4.6	11	0.3535	7	11.09	6.00	17.09
11	2.7	13	3.1	1	5.8	2	1.8824	1	7.18	4.95	12.13
12	3.1	9	0.9	20	4.0	18	-1.1295	20	13.63	6.39	20.02
13	1.8	20	1.6	10	3.4	20	-0.7704	18	11.18	6.22	17.4
14	3.6	3	1.5	13	5.1	4	-0.0576	12	9.09	5.59	14.68
15	4.2	1	1.8	8	6.0	1	0.6699	3	6.36	6.00	12.36
16	3.6	4	1.5	14	5.2	3	-1.0519	19	13.27	6.23	19.5
17	2.3	16	2.8	2	5.1	5	0.325	8	10.72	5.17	15.89
18	3.2	8	1.8	9	5.1	6	1.7331	2	4.27	3.03	7.3
19	3.6	5	1.5	15	5.1	7	0.0815	10	11.18	3.54	14.72
20	3.86	2	1.2	19	5.1	8	0.0862	9	6.63	5.57	12.2

An integrated selection index for drought resistance was proposed and used to identify drought tolerant genotypes. In this index, ten traits including relative water content (RWC%), proline concentration (PC), relative water loss (RWL%), excised leaf water retention (ELWR), chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll total (Chl T), relative chlorophyll content (RCC) and grain yield under rainfed and irrigated conditions were chosen as the most relevant factors related to drought resistance, as determined by multivariate statistical analysis (factor analysis). In our study, genotypes no 12, 16 and13 displayed the lowest and genotypes no. 11, 18 and 15 the highest values for ISI.

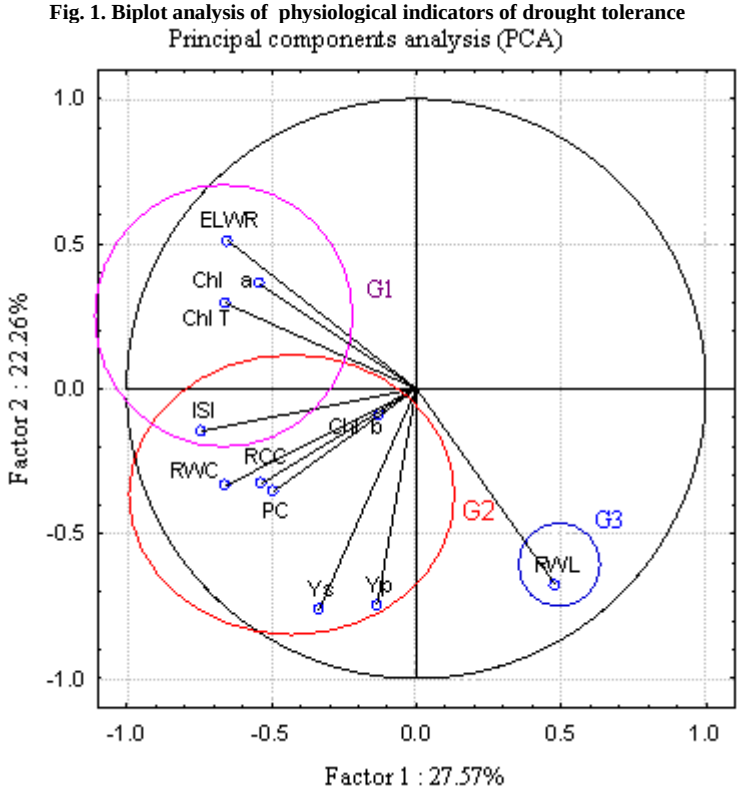
The integrated selection index was correlated with grain yield under rainfed and irrigated conditions, chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll total (Chl T), relative chlorophyll content (RCC), relative water content (RWC%), proline concentration (PC) and excised leaf water retention (ELWR) (**Fig. 1**).

Screening physiological indicators and drought tolerant genotypes

(i) Biplot analysis method

To better understand the relationships, similarities and dissimilarities among the physiological indicators of drought tolerance, principal component analysis (PCA), based on the rank correlation matrix was used. The main advantage of using PCA over cluster analysis is that each statistics can be assigned to one group only [36]. The relationships among different indices are graphically displayed in a biplot of PCA_1 and PCA_2 (**Fig. 1**). The PCA_1 and PCA_2 axes which justify 49.83% of total variation, mainly distinguish the indices in different groups. One interesting interpretation of biplot is that the cosine of the angle between the vectors of two indices approximates the correlation coefficient between them. The cosine of the angles does not precisely translate into correlation coefficients, since the biplot does not explain all of the variation in a dataset. Nevertheless, the angles are informative enough to allow a whole picture about the interrelationships among the *in vivo* indices [37]. ELWR, Chl a, Chl T and ISI we refer to group 1= G1 indices. The PCs axes separated RWC%, RCC, PC, Chl b, Ys, Yp and ISI in the second group (G2) and RWL in a single group (G3). As the cosine of the angle between the vectors of two indices approximates the correlation between them therefore, G1 indices were positively correlated (an acute angle), the same conclusion was obtained for the G2 indices, while G1 was negatively correlated with G3 indices (an obtuse angle).

Fig. 1. Biplot analysis of physiological indicators of drought tolerance



(ii) Ranking method

The estimates of indicators of drought tolerance (**Table 4**) indicated that the identification of drought-tolerant genotypes based on a single criterion was contradictory. For example, according to PC, the desirable drought-tolerant genotype was (20), while according to ELWR the desirable drought-tolerant genotype was no. (15). To have an overall judgement the following ranking method was used. To determine the most desirable drought tolerant genotype according to the all indices mean rank and standard deviation of ranks of all drought tolerance criteria were calculated and based on these two criteria the most desirable drought tolerant genotypes were identified. In consideration to all indices, genotypes (18), (11) and (15) showed the best mean rank and low standard deviation of ranks in stress condition, hence they were identified as the most drought tolerant genotypes which is in complete agreement with the results of our new index (ISI), while genotypes (12), (16) and (6) as the most sensitive.

Biplot analysis and ranking methods have been used for screening drought tolerant genotypes by Farshadfar and Elyasi in wheat [19], Farshadfar et al. in chickpea [38] and Farshadfar et al. [39] in bread wheat

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