Chromosomal localization of the genes controlling quantitative indicators of drought toleranc in wheat substitution lines

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ABSTRACT

To locate the genes controlling drought resistance and screening quantitative indices of drought tolerance, wheat substitution lines of Cheyenne (Donor) into the genetic background of Chinese Spring (Recipient) were tested in a complete randomized block design with three replications under two different water regimes (irrigated and rain-fed). The results of analysis of variance exhibited significant differences between the lines for yield potential (Y_p), mean productivity (MP), stress tolerance index (STI), geometric mean productivity (GMP), abiotic tolerance index (ATI), stress yield (Ys), tolerance index (TOL), yield index (YI) and stress susceptibility percentage index (SSPI) indicating the presence of genetic variation and possible chromosomal localization of the genes controlling drought tolerance. Screening drought tolerance indicators using correlation analysis displayed that the most suitable drought tolerance criteria for screening substitution lines were MP, GMP and STI. Mean comparison revealed that most of the genes controlling yield and drought tolerance are located on chromosomes 2A, 2B, 4B, 5B, 7B and 1D. Principal component analysis justified 96.10% of total variation in the data matrix. Ward's hierarchical clustering of genotypes and biplot analysis of the first two principal components showed that most of t 2A, 2B, 4B, 5B, 7B and 1D.

Key words: wheat substitution lines, screening techniques, drought tolerance, chromosomal localization
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INTRODUCTION

Drought stress currently limits wheat productivity in most of semi-arid regions and, as a result of climate change, will increasingly affect wheat production globally. Therefore, improving drought resistance is a major objective in plant breeding programs for rain-fed agriculture in semi-arid regions [1, 2]. The most widely used criteria for selecting high yield performance are mean yield, mean productivity (average yield performance under stress and non stress conditions) and relative yield performance in drought-stressed and more favourable environments. Relative yield (yield of an individual genotype under drought relative to that of the highest yielding genotype in the population) could be used to assess the yield potential of a genotype under water stress conditions [3]. The best criteria for finding drought tolerant genotypes in semi-arid regions are yield stability and comparison of yield in stress and non-stress conditions, because, dispersion of rainfall is unsuitable, in these regions [4]. Genotypes can be categorized into four groups based on their performance in stress and non-stress environments: genotypes express uniform superiority in both stress and non-stress environments (Group A); genotypes perform favorably only in non-stress environments (Group B); genotypes yield relatively higher only in stress environments (Group C); and genotypes perform poorly in both stress and non-stress environments (Group D). The optimal selection criterion should distinguish Group A from the other three groups [5]. Several drought resistance indices were proposed based on genotypes performance in stress (Y_s) and non-stress (Y_p) conditions. Rosielle and Hamblin [6] defined tolerance (TOL) index as the difference in yield between the non-stress and stress conditions and mean productivity (MP) index as the yield mean of stress and non-stress environments. Fischer and Maurer (1978) proposed a stress

susceptibility (SSI) index as relative yield performance in stress and non-stress conditions. Fernandez [5] defined stress tolerance index (STI), which can be used to identify genotypes high performance high in stress and non stress conditions. The geometric mean productivity index (GMP) introduced by Fernandez [5], which often used by breeders interested in relative performance, since drought stress can vary in severity in field environment over years [7]. Gavuzzi et al. [8] defined yield index (YI), by genotype yield on average yield of stress condition. Other yield based estimates of drought tolerance are drought resistance index (DI) and yield stability index (YSI), which introduced by Lan [9] and Bouslama & Schapaugh [10], respectively. In recent years several drought resistance indices were suggested based on genotypes performance in stress (Y_s) and non-stress (Y_p) conditions, consisted of abiotic tolerance index (ATI), stress susceptibility percentage index (SSPI) and stress non-stress production index (SNPI) [11].

On the other hand, knowledge about the chromosomal location of QTLs controlling drought tolerance is the most important step in breeding programs for genetic improvement of drought resistance through gene transfer. Thus, the success of any selection based on classical and/or molecular methods for developing drought tolerant varieties depends on our information about QTLs controlling drought tolerance. Drought tolerance is a complex trait, expression of which depends on action and interaction of different morphological, physiological and biochemical characters. Identification of the genes responsible for morphological and physiological traits related to drought, their location on chromosomes and their inheritance pattern have been reported [12, 2].

Genetic materials such as alien additions, substitutions, translocations, deletions, monosomes, ditelosomes, and nullisomes are valuable genetic resources for both plant breeding and basic research [13]. Substitution lines are justified for many purposes: 1) to study the location of the individual chromosomes or genes and determine their effect in genotypes with different genetic backgrounds, 2) to improve the agronomic value of cultivated wheat varieties by incorporating a character and 3) to study the hybrid vigor [14].

The objectives of the present investigation were locating QTLs controlling drought tolerance in wheat and screening quantitative indicators of drought tolerance.

MATERIALS AND METHODS

To locate QTLs controlling quantitative indicators of drought tolerance, 23 genotypes consists of 21 substitution line series of *Cheyenne* (as donor) into the genetic background of *Chinese Spring* (as recipient) and their parents were kindly provided from the gene bank of the Agricultural Research Institute of the Hungarian Academy of Sciences.

The experiment was conducted in a randomized complete block design with three replications under two different environments (irrigated and rainfed) at the experimental farm of College of Agriculture, Razi University, Kermanshah, Iran (47° 20´ N latitude, 34° 20´ E longitude and 1351.6 m altitude). Climate in the region is classified as semiarid with mean annual rainfall of 378 mm. Minimum and maximum temperature at the research station were -27°C and 44°C, respectively. Each replication consisted of 23 genotypes with 1.5 m length and 0.5 m wide and the distance between two plots was 30 cm. Single seeds were planted in two rows with 25 cm distance; in other word, harvest area was 0.75m^2 per plot.

Calculation of drought tolerance indices

Drought tolerance indices were calculated based on grain yield per plot for stress (Ys), non-stress (Yp) and total mean of grain yield for stress ($\overline{Y}_s$) and non-stress ($\overline{Y}_P$) conditions as follows:

1- Stress susceptibility index (SSI) [15]:

$$SSI = \frac{1 - (Y_S / Y_P)}{1 - (\overline{Y}_S / \overline{Y}_P)}$$

2- Tolerance (TOL) and mean productivity (MP) [6]:

$$\text{TOL} = Y_P - Y_S \qquad MP = \frac{Y_S + Y_P}{2}$$

3- Stress tolerance index (STI) and geometric mean productivity (GMP) [5]:

$$\text{STI} = \frac{Y_S \times Y_P}{\overline{Y}_P{}^2} \qquad GMP = \sqrt{(Y_S \times Y_P)}$$

4- Yield index (YI) [8]:

$$YI = \frac{Y_S}{\overline{Y}_S}$$

5- Yield stability index (YSI) [10]:

$$YSI = \frac{Y_S}{Y_P}$$

6- Drought resistance index (DI) [9]:

$$DI = Ys \times (Ys/Yp)/\overline{Y}_S$$

7- Abiotic tolerance index (ATI), stress susceptibility percentage index (SSPI) and stress non-stress production index (SNPI) [11]

$$ATI = [(Yp-Ys) / (\overline{Y}_P / \overline{Y}_S)] \times [\sqrt{Yp \times Ys}]$$

$$SSPI = [Yp-Ys /2(\overline{Y}_P)] \times 100$$

$$SNPI = [\sqrt[3]{(Yp+Ys)} / (Yp - Ys)] \times [\sqrt[3]{Yp \times Ys \times Ys}]$$

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.

RESULTS AND DISCUSSION

Interrelationship among indices with grain yield
To determine the most desirable drought resistance criteria, Spearman's rank correlation between yield under stress and non-stress conditions and indices of drought resistance were calculated (**Table 1**). The results indicated that MP, STI and GMP had a significant (P<0.01) positive correlation with yield under non-stress condition, while TOL, ATI and SSPI showed a significant (P<0.01) negative correlation. The indices SSI, MP, STI, GMP, YI, YSI, DI and SNPI revealed a significant (P<0.01) positive correlation with yield under stress condition, therefore only three indices MP, STI and GMP exhibited positive significant correlation with yield under both stress and nonstress conditions. Some researchers believe in selection based on only favorable condition [16], and/or only stress condition [8], but others have chosen a mid-point and believe in selection based on both favorable and stress conditions [5, 17]. Farshadfar et al. [18] believe that most suitable indices for selection of drought resistance cultivars, is an indicator which has a relatively high correlation with grain yield in both conditions. Fernandez [5] reported that MP fails to distinguish between group A and group B, but according to our results STI, MP and GMP displayed significant correlation with each other and with Ys and Yp (**Table 1**; **Fig. 2**), hence they are expected to discriminate group A from group B and group C. Accordingly, STI, GMP and MP discriminate drought tolerant genotypes with high grain yield under both stress and nonstress conditions (group A). The results of this investigation were in close agreement with the findings of Fernandez [5], Farshadfar et al. [18], Talebi et al. [19] and Nouri, et al. [20] .

Table 1. Spearman's rank correlation between drought resistance indices and yield of stress and non-stress conditions

Indices	Y_P	Y_S	SSI	TOL	MP	STI	GMP	YI	YSI	DI	ATI	SSPI
Y_S	.377											
SSI	-.148	.799**										
TOL	-.679**	.341	.777**									
MP	.860**	.720**	.265	-.308								
STI	.674**	.876**	.507*	-.038	.944**							
GMP	.684**	.875**	.503*	-.043	.946**	.995**						
YI	.377	1.000**	.799**	.341	.720**	.876**	.875**					
YSI	-.148	.799**	1.000**	.777**	.265	.507*	.503*	.799**				
DI	-.023	.882**	.974**	.695**	.386	.615**	.611**	.882**	.974**			
ATI	-.945**	-.136	.385	.830**	-.737**	-.517*	-.523*	-.136	.385	.279		
SSPI	-.679**	.341	.777**	1.000**	-.308	-.038	-.043	.341	.777**	.695**	.830**	
SNPI	-.280	.630**	.916**	.811**	.106	.325	.315	.630**	.916**	.889**	.502*	.811**

*,** *Significant at 5% and 1% level of probability, respectively*

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Table 2. Mean squares for yield of stress and non-stress conditions and drought resistance indices

Source of Variation	DF	Mean of Square						
		Yp	**Ys**	**SSI**	**TOL**	**MP**	**STI**	**GMP**
Replication	2	177.11^{ns}	276.68^{ns}	0.07^{ns}	4.60^{ns}	0.22^{ns}	0.01^{ns}	0.39^{ns}
Genotype	22	817.98^{**}	541.01^{*}	0.06^{ns}	4.47^{*}	1.34^{**}	0.04^{**}	1.65^{**}
Error	44	284.61	245.21	0.04	2.48	0.40	0.01	0.57
Coefficient of Variation (%)		13.96	23.25	19.39	22.19	6.56	14.65	8.02
		Mean of Square						
		YI	**YSI**	**DI**	**ATI**	**SSPI**	**SNPI**	
Replication	2	0.02^{ns}	0.01^{ns}	0.05^{ns}	230.10^{ns}	1.90^{ns}	4.57^{ns}	
Genotype	22	0.04^{*}	0.02^{ns}	0.06^{ns}	293.01^{**}	1.85^{*}	2.48^{ns}	
Error	44	0.02	0.01	0.03	118.33	1.03	1.57	
Coefficient of Variation (%)		13.16	14.91	24.65	21.92	22.19	37.66	

*; ** *significant at the 5% and 1% probability levels, respectively, ns; non significant*

Assessment of drought tolerant genotypes

The results of analysis of variance for Yp, MP, STI, GMP, ATI Ys, TOL, YI, and SSPI indicated significant differences between drought tolerance criteria (**Table 2**) indicating the presence of genotypic variation for indices and yield which can be due to substituted chromosomes from donor parent (*Cheyenne*) into the genetic background of recipient parent (*Chinese Spring*). Farshadfar et al. [2] significant differences for drought resistance indices reported in bread wheat. The indices SSI, YSI, DI and SNPI didn't show significant variation among genotypes.

The results of mean comparison by LSD procedure at 5% and 1% probability levels and ranks of genotypes for indices is given in Table 3. The results indicated that the identification of drought-resistance genotypes based on a single index was contradictory in comparison with other indices, therefore genotype selection was done considering correlation (**Table 1**) and grouping of indices regarding biplot analysis (**Fig. 2**). The genotypes 5D, 3A, 2A, 2B, 4A and *Chinese Spring* had the highest drought resistance based on SSI, TOL, DI, YSI, ATI, SSPI and SNPI, and the genotypes 1D, 4B, 5B, 2A, 7B, 3B and 2D exhibited the most drought resistance based on GMP, STI and MP. The genotypes 1D, 6B, 2D, 5B and 3B revealed the highest yield in non stress condition, while the highest yield in stress condition were observed for 1D, 4B, 2A, 2B and *Chinese Spring*.

Ward's hierarchical cluster analysis

Ward's hierarchical clustering for grouping genotypes based on ranks of drought resistance indices and yield of stress and non-stress conditions (**Fig. 1**), confirmed the results of mean comparison, consequently based on clustering classification four distinctive groups were identified. The first group consisted of genotypes 2A, 2B, 4B, 5B, 7B and 1D which had a desirable resistance to drought based on GMP, STI, MP and YI and the highest yield for both stress and nonstress conditions. The second group included genotypes 3A, 4A, 5D and *Chinese Spring*, which had a desirable resistance to drought based on SSI, TOL, YSI, DI, ATI, SSPI and SNPI with moderate yield in both environments. The lowest group for measured indices and grain yield in two environments was the third group, which consisted of 5A, 6A, 3D, 7D and *Cheyenne*. The fourth group displayed a moderate value for indices and grain yield.

Biplot analysis

To better understand the relationships, similarities and dissimilarities among drought tolerance indices and assessment of drought tolerant genotypes,, 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 [21].

Principal component analysis for two way table of genotypes ranks for drought resistance indices and grain yield in two conditions showed that the first component explained 56.60% of the variation in the data matrix and indicated a high correlation between Ys with all indices (except ATI) thus, the first component can be named as stress-resistant component and it separates the stress-resistant genotypes from stress-susceptible genotypes [5]. The second component explained 39.50% of total variability and revealed a high positive correlation between Y_p with MP, GMP and STI; therefore, the second component can be named as the yield potential component which separates the high yielder from the low yielder genotypes [5]. Biplot for the first two components were properly explained and confirmed the results of genotypes grouping based on cluster analysis and relationship among drought resistance attributes with Ys and Yp (**Fig. 2**), thus the genotypes 2A, 2B, 7B, 5B, 1D and 4B had the most desirable performance for yield and drought resistance (group 1). The genotypes 4A, 3A, 5D and *Chinese Spring* displayed a

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desirable resistance to drought, but didn't have a desirable production of grain yield. On the other hand, relationship among GMP, MP, STI and YI with Ys and Yp were properly illustrated, considering the angles and the direction between vectors of these attributes. The relationships among ATI, TOL, SSPI, SNPI, YSI, SSI and DI with Ys were also observed by biplot.

Table 3. Mean comparison based on yield of stress and non-stress conditions, drought resistance indices and genotypes ranks for indices.

Genotype	Yp	Ys	SSI	TOL	MP	STI	GMP
1A	126.08(10)	68.43(12)	0.96(9)	57.65(12)	97.26(11)	0.59(11)	92.09(11)
2A	120.31(14)	81.18(4)	0.72(4)	39.13(5)	100.74(8)	0.67(4)	98.72(4)
3A	105.45(17)	73.74(9)	0.68(3)	31.71(3)	89.60(16)	0.54(15)	87.87(16)
4A	101.34(20)	63.66(17)	0.82(6)	37.68(4)	82.50(19)	0.44(18)	79.52(18)
5A	102.79(19)	38.85(23)	1.41(22)	63.94(18)	70.82(23)	0.28(23)	62.56(23)
6A	115.73(15)	49.44(21)	1.24(21)	66.29(19)	82.58(18)	0.38(19)	74.69(19)
7A	130.26 (7)	72.16(10)	1.01(12)	58.09(13)	101.21(7)	0.64(9)	96.68(8)
1B	127.03 (9)	67.80(13)	1.03(13)	59.23(14)	97.42(10)	0.58(12)	91.60(12)
2B	121.26(13)	79.39(5)	0.76(5)	41.87(6)	100.33(9)	0.66(6)	97.92(6)
3B	138.31 (5)	67.32(14)	1.17(19)	70.99(22)	102.82(5)	0.65(7)	96.12(9)
4B	136.50 (6)	82.02(3)	0.88(8)	54.48(10)	109.26(2)	0.77(2)	105.55(2)
5B	138.45 (4)	76.65(8)	1.00(11)	61.80(16)	107.55(3)	0.73(3)	102.92(3)
6B	141.70 (2)	51.87(18)	1.43(23)	89.83(23)	96.79(12)	0.51(17)	85.70(17)
7B	124.60(11)	78.24(6)	0.83(7)	46.35(8)	101.42(6)	0.67(5)	98.64(5)
1D	153.96 (1)	87.32(1)	0.97(10)	66.65(20)	120.64(1)	0.92(1)	115.77(1)
2D	139.21 (3)	69.57(11)	1.06(14)	69.64(21)	104.39(4)	0.64(8)	96.70(7)
3D	92.73 (23)	50.70(20)	1.09(16)	42.03(7)	71.72(22)	0.34(21)	65.80(22)
4D	127.99 (8)	64.53(15)	1.12(18)	63.46(17)	96.26(13)	0.58(13)	90.34(13)
5D	101.12(21)	78.02(7)	0.53(2)	23.10(2)	89.57(17)	0.55(14)	88.54(14)
6D	123.55(12)	64.02(16)	1.07(15)	59.53(15)	93.79(15)	0.54(16)	88.33(15)
7D	104.61(18)	48.12(22)	1.20(20)	56.49(11)	76.37(20)	0.34(22)	69.02(21)
Spring Chinese	105.93(16)	85.09(2)	0.42(1)	20.84(1)	95.51(14)	0.62(10)	94.81(10)
Cheyenne	100.67(22)	51.20(19)	1.11(17)	49.47(9)	75.93(21)	0.35(20)	71.62(20)
Lsd (%5)	27.781	25.786	0.309	2.592	1.045	0.179	1.242
Lsd (%1)	37.126	34.460	0.413	3.464	1.397	0.240	1.659

Genotype	YI	YSI	DI	ATI	SSPI	SNPI
1A	1.02(12)	0.58(9)	0.59(13)	3149(16)	23.85(12)	10.42(12)
2A	1.21(4)	0.68(4)	0.82(3)	2204(9)	16.19(5)	15.14(5)
3A	1.09(9)	0.70(3)	0.79(4)	1504(4)	13.12(3)	18.70(4)
4A	0.95(17)	0.64(6)	0.64(9)	1596(5)	15.59(4)	21.74(3)
5A	0.58(23)	0.37(22)	0.23(23)	2186(8)	26.46(18)	4.60(23)
6A	0.73(21)	0.45(21)	0.35(21)	2783(12)	27.43(19)	6.50(21)
7A	1.07(10)	0.55(12)	0.61(11)	3087(15)	24.04(13)	9.32(14)
1B	1.01(13)	0.54(13)	0.59(12)	2897(14)	24.51(14)	11.44(10)
2B	1.18(5)	0.66(5)	0.78(5)	2303(10)	17.32(6)	14.65(6)
3B	1.00(14)	0.48(19)	0.50(18)	3762(21)	29.37(22)	7.43(19)
4B	1.22(3)	0.61(8)	0.75(6)	3240(18)	22.54(10)	11.65(9)
5B	1.14(8)	0.56(11)	0.63(10)	3580(19)	25.57(16)	9.30(15)
6B	0.77(18)	0.37(23)	0.28(22)	4304(22)	37.17(23)	4.67(22)
7B	1.16(6)	0.63(7)	0.73(8)	2584(11)	19.18(8)	12.02(8)
1D	1.30(1)	0.57(10)	0.74(7)	4312(23)	27.57(20)	10.33(13)
2D	1.03(11)	0.53(14)	0.59(14)	3695(20)	28.81(21)	10.77(11)
3D	0.75(20)	0.52(16)	0.51(16)	1240(3)	17.39(7)	13.73(7)
4D	0.96(15)	0.50(18)	0.51(17)	3173(17)	26.26(17)	8.04(17)
5D	1.16(7)	0.77(2)	0.92(2)	1066(1)	9.56(2)	39.20(1)
6D	0.95(16)	0.53(15)	0.52(15)	2885(13)	24.63(15)	8.81(16)
7D	0.71(22)	0.47(20)	0.39(20)	2020(7)	23.37(11)	7.85(18)
Spring Chinese	1.26(2)	0.81(1)	1.03(1)	1161(2)	8.62(1)	34.41(2)
Cheyenne	1.02(12)	0.58(9)	0.59(13)	3149(16)	23.85(12)	10.42(12)
Lsd (%5)	0.215	0.182	0.304	17.913	1.667	2.061
Lsd (%1)	0.288	0.244	0.406	23.938	2.228	2.754

Fig. 1. Dendrogeram of Ward's hierarchical clustering of genotypes ranks based on drought resistance indices and yield of stress and non-stress conditions
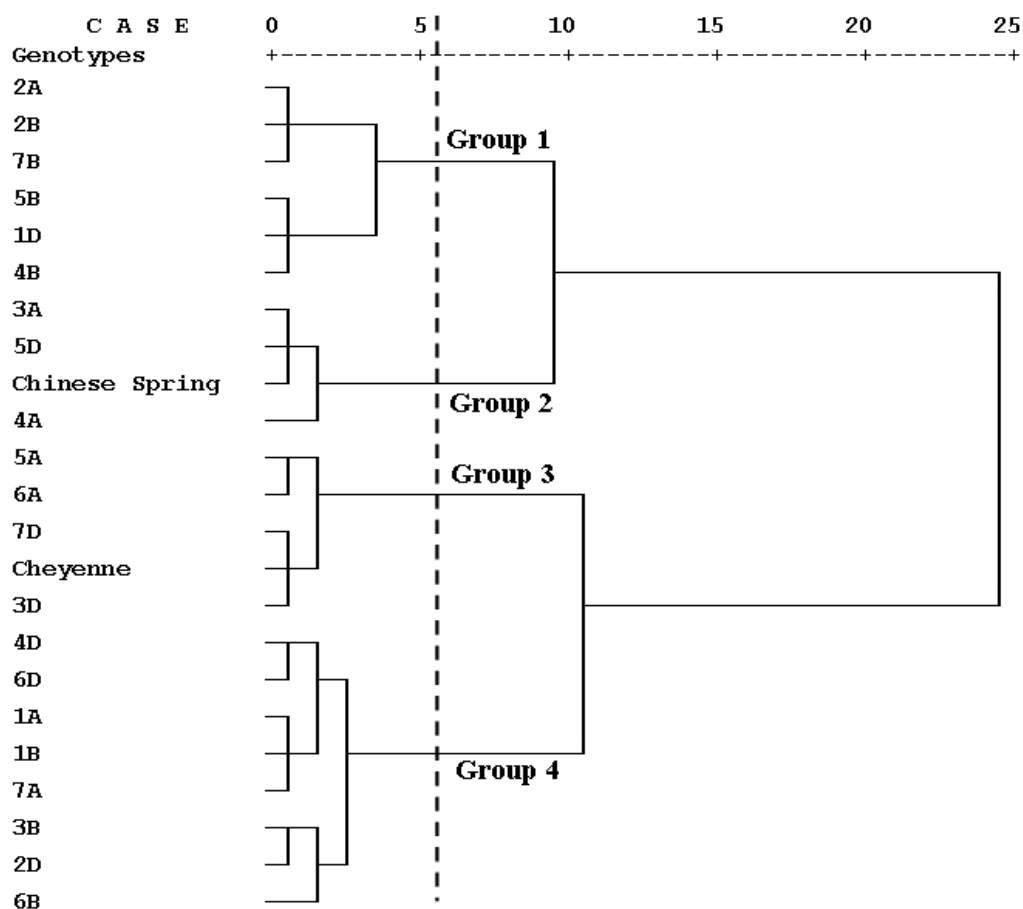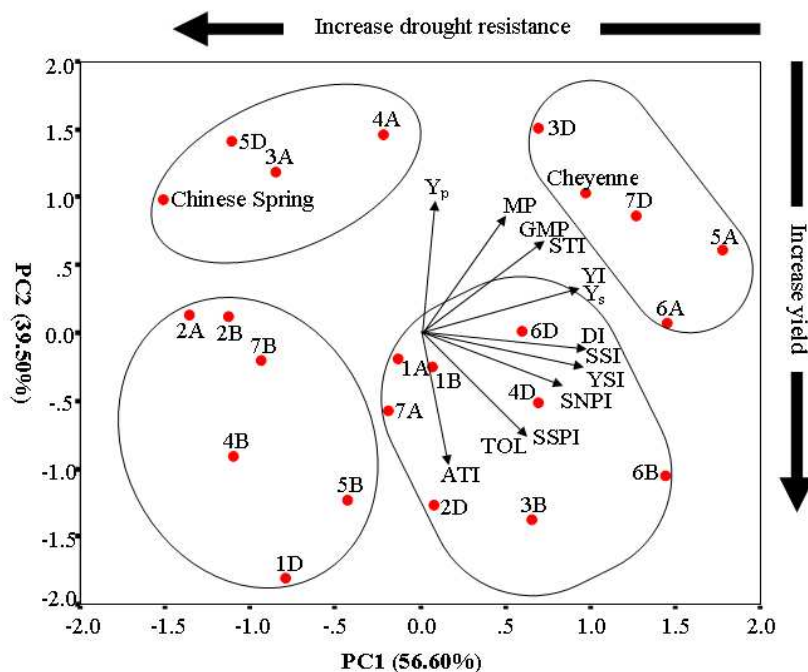

Fig. 2. Biplot of drought resistance indices in substitution lines based on two first components and grouping of genotypes

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CONCLUSION

The overall judgement is that GMP, MP and STI are desirable indices for selection drought resistant genotypes with high grain yield in both stress and nonstress conditions. Accordingly, genotype responses for these attributes are similar, thus one of them can be used for genotypes selection. On the other hand, we obtained possibility of chromosomal localization of the genes controlling drought resistance, because genetic variation for the drought indicators can be due to substituted chromosomes from donor parent into the genetic background of recipient parent. In addition, the results showed that most of the genes controlling quantitative criteria of drought resistance are distributed in genome A (chromosome 2A), genome B (chromosome 2B, 4B, 5B and 7B) and genome D (chromosome 1D).

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