



Chromosomal localization of the genes controlling callus induction and *in vitro* drought tolerance criteria in wheat-barley disomic addition lines using mature embryo culture

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ABSTRACT

In order to detect quantitative trait loci (QTLs) involved in the genetic of callus induction and in vitro predictors of drought tolerance and screening in vitro indicators of drought tolerance, wheat-barley disomic addition lines were used in a completely randomized design (CRD) with five and three replications for callus induction and drought experiments at the Agricultural College of Razi University, Kermanshah, Iran during 2010-2011. Highly significant differences ($P < 0.01$) were observed among the genotypes for callus primary diameter (CPD), callus primary fresh weight (CPFW), callus growth rate (CGR), callus relative fresh weight growth (CRFWG), callus relative growth rate (CRGR), callus induction percentage (CIP) and in vitro tolerance (INTOL) indicating the presence of genetic variability, different responses of genotypes to callus induction and possible chromosomal localization of callus induction and in vitro indicators of drought tolerance using mature embryos. Mean comparison of the traits measured in callus induction showed that disomic addition line 7H had the highest amount of CPD, CPFW, CRFWG, CRGR and CIP, accordingly most of the QTLs controlling callus induction characteristics are located on chromosome 7H with the highest efficiency of added chromosome (EAC) and positive effect for improvement of wheat and barley tissue culture traits. Screening drought tolerant genotypes and in vitro indicators of drought tolerance using mean rank, standard deviation of ranks and biplot analysis, discriminated genotype 4H with maximum EAC as the chromosome carry QTLs monitoring drought tolerance in barley. Therefore it is recommended to be used as parents for genetic analysis, gene mapping and improvement of drought tolerance in common cereals using chromosome engineering.

Key words: Disomic addition lines, gene location, *in vitro* drought indices, mature embryo.

INTRODUCTION

Numerous studies have been conducted on the genetic control of plant tissue culture responses [38]. The majority of QTL analyses of TCR (tissue culture response) traits were conducted with monocots such as rice, barley and maize, probably because of economical importance of these plant species [8]. In wheat there have been several attempts to define the location and nature of

specific loci that may influence the tissue culture response. Such studies are particularly appropriate in wheat since not only are there marked varietal differences in culturability [31], but it is also possible to investigate the influence of specific chromosomes and chromosome arms through the use of chromosome substitution and translocation lines [29,33]. In interpreting the results of such experiments, it is often difficult to differentiate between the effects due to the presence of alien genetic material or the absence of previously existing material.

A more meaningful analysis is possible using addition lines where a single defined alien chromosome is present in a wheat background [27]. Wheat and barley are two important cereals worldwide. Wheat (*Triticum aestivum* L.) is the world's most widely adapted crop, supplying one-third of the world population with more than half of their calories and nearly half of their protein [32]. Barley (*Hordeum vulgare* L.) is one of the world's major cereal crops ranking fourth behind wheat, rice and maize in terms of agronomic importance [37]. The hybridization of wheat and barley makes it possible to transfer useful characters such as earliness, tolerance to drought, soil salinity and various nutrition quality parameters from barley into wheat [19].

Plant cell and tissue culture has been a useful tool to study stress tolerance mechanisms under *in vitro* conditions [16]. Whole-plant drought tolerance undoubtedly is a very complicated interaction of genotypes, environmental factors and varied mechanisms at the plant level; however, if a significant association can be found between whole-plant response to drought stress and a cellular-level response, then this information would be most useful in selection of genotypes for drought-tolerance. Smith et al. [28] reported a correlation between responses to drought in the field and responses to drought in culture.

A number of useful wheat variants has been developed through tissue culture for drought tolerance [24,15] and salt tolerance [11, 23].

Polyethylene glycol (PEG) has long been used at *in vitro* culture to reduce water potential of nutrient solutions and to stimulate water stress without the risk of being taken up by the plants [26]. However, immature embryos are the most frequently used as explants for the tissue culture, although it has many disadvantages. For example, the growth stage of immature embryo appropriate for isolation is strictly limited, suitable embryo size for tissue culture varies with varieties and environmental conditions and growth of donor plant and immature embryo isolation are all time-consuming, expensive and laborious. Alternatively, the use of mature embryo is easy to handle and available at any time [34].

The objectives of the present research were (i) to locate the genes controlling callus induction criteria (ii) *in vitro* indices of drought tolerance and (iii) screening *in vitro* indicators of drought tolerance using mature embryo culture of wheat-barley disomic addition lines.

MATERIALS AND METHODS

Plant genetic materials

The plant materials consisted of 9 genotypes including 7 Disomic Addition Lines (DALs) of barley (*Hordeum vulgare* L., $2n = 2x = 14$, HH, cv. Betzes) (H = donor) in the genetic background of bread wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD, cv. Chinese spring = CS) along with two donor (barley, cv. Betzes) and recipient (bread wheat, cv. CS) parents. The DALs were named as 1H to 7H indicating addition of chromosomes 1H to 7H into the genome of CS, respectively. The seeds were kindly provided by Dr. M. Tahir, ICARDA, Syria. The *in vitro* experiments were conducted as follows:

(i) Callus induction

Mature seeds were surface-sterilized for 5 min in 70% ethanol and kept in 5% sodium hypochlorite for 10-15 minutes. Then seeds were rinsed five or six times with sterile distilled water and, after straining the water, the embryos were isolated from seeds. The culture medium for callus induction stage was MS medium (Murashige and Skoog, 1962) containing 2 mg / l of 2,4-dichlorophenoxyacetic acid and was supplemented with 30 g/l sucrose and 8 g/l agar. pH was adjusted to 5.8. Ten embryos per genotype were cultured per petri dish (with the scutellum up). The cultures were kept in darkness at 25° C for four weeks.

(ii) Subculture

The Subculture medium was the same as the callus induction medium. After establishment, calli were subcultured at 2 weeks intervals until enough callus material was obtained to initiate the drought stress stage.

(iii) In vitro experiment of drought tolerance

PEG 6000 was added to MS medium to concentrations of 20% (w/v) before the pH was adjusted to 5.8. The calli were transferred onto drought stress medium. The control calli were transferred onto on PEG-free medium.

Characters measured in callus induction stage

A completely randomized design (CRD) with five replications was carried out. After 7 days of embryo culture, callus primary diameter (CPD) and callus primary fresh weight (CPFW) were measured and after 28 days of embryo culture callus growth rate (CGR), callus relative fresh weight growth (CRFWG), callus relative growth rate (CRGR) and callus induction percentage (CIP) were measured as follows [2] :

(i) CPD was evaluated by measuring mean callus diameter (mm) after 7 days of embryo culture as:

$d = (a \times b)^{1/2}$ where d, a and b are diameter, length and width of callus.

(ii) CPFW was evaluated by measuring fresh weight of callus 7 days after callus induction.

(iii) CRFWG was calculated by the formula of [12] as:

$$CRFWG = [(W_2 - W_1) / W_1]$$

where W_1 = fresh weight after 7 days of embryo culture and W_2 = final fresh weight after four weeks of embryo culture.

(iv) CGR was evaluated by measuring mean callus diameter (mm) [22], after 7, 14, 21 and 28 days of callus induction. Calculation of CGR per replication was as:

$$CGR_1 = \frac{d_7}{7}, CGR_2 = \frac{d_{14} - d_7}{7}, CGR_3 = \frac{d_{21} - d_{14}}{7} \text{ and } CGR_4 = \frac{d_{28} - d_{21}}{7}$$

$$CGR_{rep} = \frac{CGR_1 + CGR_2 + CGR_3 + CGR_4}{4}$$

CGR for each genotype was the mean of five CGR_{rep} .

(v) CRGR was calculated by the formula of AL-Khayri and AL-Bahrany [13] as:

$$CRGR = (\ln W_2 - \ln W_1) / \text{Number of days}$$

where, W_1 = fresh weight of the callus 7 days after embryo culture and W_2 = fresh weight of callus 28 days after embryo culture and the number of days was 21.

(vi) CIP was calculated when the embryos formed the callus.

Drought experiment

A completely randomized design (CRD) with three replications was carried out. Before transferring to drought medium, fresh weight and diameter (0 day) of calli were measured and after 16 days of transferring onto PEG-medium the traits CGR, CRFWG, CRGR, relative water content (RWC), callus growth index (CGI), reduction percentage (RP), relative tolerance and *in vitro* tolerance (INTOL) were calculated as follows:

In this stage CGR, RFWG and RGR were calculated the same as in callus induction stage with some differences as:

(i) CGR was evaluated by measuring mean callus diameter (mm) after 0, 4, 8, 12 and 16 days of PEG-medium. CGR per replication was calculated as:

$$CGR_1 = \text{diameter (0 day)}, \quad CGR_2 = \frac{d_4 - d_0}{4}, \quad CGR_3 = \frac{d_8 - d_4}{4}, \quad CGR_4 = \frac{d_{12} - d_8}{4}, \quad CGR_5 = \frac{d_{16} - d_{12}}{4}$$

$$CGR_{rep} = \frac{CGR_1 + CGR_2 + CGR_3 + CGR_4 + CGR_5}{5}$$

CGR for each genotype was the mean of three CGR_{rep} .

(ii) Callus relative growth rate was the same as callus stage only number of days was 16.

(iii) Relative water content (RWC) was measured by the formula of Abdelsamad [1] as:

$$RWC = [(W_2 - W_1) / W_2] \times 100$$

where W_1 is the dry weight after 16 days in PEG-medium and W_2 the fresh weight after 16 days in PEG-medium.

(iv) *In vitro* tolerance (IT): was calculated [13] as:

$$IT = RGR_{\text{treatment}} / RGR_{\text{control}}$$

(v) Callus growth index (CGI) or increasing value of callus fresh weight was calculated [1] as:

$$RFGW_{\text{stress}} = (W_1 - W_0) / W_0 \quad \text{and} \quad RFGW_{\text{control}} = (W_1 - W_0) / W_0$$

$$CGI = \frac{RFGW_{\text{stress}} - RFGW_{\text{control}}}{2}$$

where W_0 is the weight of callus before treatment and W_1 the final weight of callus after 16 days of treatment and control for $RFGW_{\text{stress}}$ and $RFGW_{\text{control}}$, respectively.

(vi) Percentage of relative tolerance (Rt%): Rt% was calculated [1] as:

$$Rt\% = [a/b] \times 100$$

where a = fresh weight under stress after 16 days and b = fresh weight after 16 days under control

(vii) Reduction percentage (R%): R% was calculated [1] as:

$$R\% = (a-b) \times 100 \text{ ([1]Abdelsamad, 2007)}$$

where a = fresh weight under stress after 16 days and b = fresh weight after 16 days under control.

(viii) Efficiency of added chromosomes (EAC): EAC was calculated [6, 4] for both experiments as:

$$ACE\% = \frac{Y_{DA} - Y_{CS}}{Y_{CS}} \times 100$$

where Y_{DA} = character of disomic addition lines and Y_{CS} = character of recipient parent (CS).

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, rank mean and standard deviation of ranks were performed by the softwares STATISTICA, MSTAT-C and SPSS ver. 16.

RESULTS AND DISCUSSION

Callus induction stage

Highly significant differences ($P < 0.01$) were observed among the genotypes for CPFW, CGR, CRFWG, CRGR and CIP (Table 1) indicating the presence of genetic variability, different responses of genotypes to callus induction and possible localization of the genes controlling callus induction characteristics in barley at *in vitro* level using mature embryos of wheat-barley disomic addition lines.

Capacity of plant tissue is genetically controlled and specific for each genotype. Genotype effects on callusing ability from wheat and barley mature embryo cultures were reported in durum wheat [35,25] and bread wheat [30,14].

Table 1. Analysis of variance for callus induction and drought tolerance criteria using mature embryos of disomic addition lines

S.O.V	df	Mean squares					
Callus stage		CPD _{mm}	CPFW _{gr}	CGR _{mm}	CRFWG _{gr}	CRGR _{gr}	CIP _%
Genotypes	8	3.147**	0.1**	0.007**	0.791**	0.0005**	0.066**
Error	36	0.886	0.002	0.002	0.005	0.000006	0.011
Drought stage							
S.O.V	df	Mean squares					
		CGR _{mm}	RFWG _{gr}	RGR _{gr}	RWC _%	INTOL	-
Genotypes	8	0.322**	1.24 ^{ns}	0.002*	78.437 ^{ns}	7.494**	-
Error	18	0.047	0.547	0.001	34.844	1.422	-

*, ** significant at 0.05 and 1% level of probability; ns: non-significant

Mean comparison of traits in callus induction

Mean comparison of the traits measured in callus induction (Table 2) showed that disomic addition line 7H had the highest amount of CPD, CPFW, CRFWG, CRGR and CIP. Maximum amount of CGR was attributed to addition line 5H with no significant difference with 7H, accordingly most of the QTLs controlling callus induction characteristics are located on chromosome 7H, hence chromosomes 7H is suitable for improving wheat and barley tissue culture traits through intergeneric crossing.

The CIM analysis detected two loci on chromosome 2H and one locus on 5H controlling CGR [40]. QTLs monitoring CGR in immature embryo culture of barley have already been mapped on chromosomes 1H, 2H and 5H in the Harrington (HA) × TR306 (TR) cross [9], and on chromosomes 2H and 3H in the Steptoe (ST) × Morex (MO) cross [39]. Immature embryo culture suggested that CGR is a polygenic trait and the effect of chromosome on this trait depend on the time of life cycle of plant (mature and immature embryos). Genetic studies of tissue-culture traits, such as callus growth, will make it possible to transfer genes controlling desirable tissue-culture traits into recalcitrant cultivars or species.

The 100 percent of embryos in 2H, 4H, 5H, 6H, 7H and recipient produced callus after 28 days. Özgen *et al* [20] reported that correlation between callus induction frequency and culture efficiency ($r=0.888$, $P<0.01$) in mature embryo culture indicated that culture efficiency tended to increase with increasing callus induction rate. In plant tissue culture, a desirable genotype is expected to possess high callus induction. However, numerous studies have shown the absence of such a relationship between callus induction and plant regeneration capacity and thus, the independence of these characters from each other [1]. On the contrary, Birsin *et al.* [21] suggested that genotypes with high callus induction also caused an increase in the number of plants transferred to soil.

Table 2. Mean comparison of callus induction traits using mature embryos of disomic addition lines

Genotype*	CPD		CPFW		CGR		CRFWG		CRGR		CIP	
1H	3.385	bc	0.017	b	0.181	bcd	1.293	c	0.039	b	80	a
2H	3.973	bc	0.013	c	0.157	cd	1.001	d	0.033	c	100	a
3H	3.958	bc	0.017	b	0.197	abc	0.378	g	0.014	e	98	a
4H	4.421	ab	0.015	b	0.188	bcd	0.896	e	0.038	b	100	a
5H	4.582	ab	0.017	b	0.259	a	0.988	d	0.033	c	100	a
6H	4.525	ab	0.015	b	0.205	abc	1.401	b	0.041	ab	100	a
7H	5.383	a	0.028	a	0.234	ab	1.566	a	0.043	a	100	a
Ch. s	3.273	bc	0.007	d	0.131	d	0.685	f	0.025	d	100	a
Betzes	2.803	c	0.012	c	0.187	bcd	1.509	a	0.043	a	51.61	b

* Means followed by the same letter are not significantly different at 0.05 probability level.

Efficiency of added chromosomes (EAC)

The efficiency of added chromosomes (Table 3) indicated that chromosomes 7H had the highest efficiency with positive effect for improvement of CPD, CPFW, CRFWG and CRGR. The highest efficiency for improvement of CGR belonged to chromosome 5H. Chromosomes 1H and 3H revealed negative effect for CIP and 3H also exhibited negative effect for CRFWG and CRGR, therefore transfer of chromosomes 1H and 3H is not suitable for improvement of callus induction criteria.

Table 3. The EAC of callus induction criteria in disomic addition lines using mature embryo culture

genotypes	CPD	CPFW	CGR	CRFWG	CRGR	CIP
1H	3.42	142.85	38.16	88.75	56	-20
2H	21.38	85.71	19.84	46.13	32	0
3H	20.92	142.85	50.38	-44.81	-44	-2
4H	35.07	114.28	43.51	30.80	52	0
5H	39.99	142.85	97.70	44.23	32	0
6H	38.25	114.28	56.48	104.52	64	0
7H	64.46	300	78.62	128.61	72	0

Drought tolerance experiment

Some of the tissue culture traits were influenced by the genotype in mature embryo culture at drought stress experiment. Significant differences were observed among the addition lines for CGR, RGR and INTOL (Table 1) indicating possible chromosomal localization of the genes controlling *in vitro* drought tolerance indices.

Mean comparison between the genotypes (Table 4) showed that maximum RFWG, RGR, RWC and INTOL belonged to chromosome 4H, accordingly most of the QTLs controlling drought tolerance criteria in barley are located on chromosome 4H. Maximum CGR and RWC was related to chromosome 5H but as the amount of INTOL was negative for chromosome 5H, therefore this chromosome is not desirable for improvement of drought tolerance. Farshadfar et al. [4, 7] showed that the genes controlling salt and drought tolerance are also located on chromosome 4H and 5H. Molnar et al. [19] reported that the genes located on chromosome 4H of barley were able to increase water use efficiency in wheat substitution lines.

Table 4. Mean comparison of *in vitro* drought tolerance criteria using mature embryos of wheat-barley disomic addition lines

Genotype*	CGR	RFWG	RGR	RWC	INTOL
1H	1.191 bc	0.059 b	0.001 ab	79.999 a	0.031 ab
2H	0.856 c	0.333 b	0.01 ab	70.532 ab	0.0009 ab
3H	1.088 c	-0.169 b	-0.012 b	68.914 ab	-1.559 b
4H	1.204 bc	1.735 a	0.053 a	75.331 a	0.943 a
5H	1.712 a	-0.186 b	-0.014 b	77.133 a	-0.924 ab
6H	1.169 bc	-0.017 b	-0.005 ab	69.738 ab	-0.231 ab
7H	1.571 ab	-0.444 b	-0.039 b	69.486 ab	-4.390 c
Ch. s	0.950 c	-0.238 b	-0.019 b	62.856 b	-0.590 ab
Betzes	1.767 a	0.058 b	0.003 ab	73.058 ab	0.572 ab

*Means followed by the same letters are not significantly different at 0.05 probability level

Efficiency of added chromosomes (EAC)

The efficiency of added chromosomes (Table 5) indicated that chromosome 4H had the highest efficiency with positive effect for improvement of RFWG, RGR, INTOL, CGI and R%, hence it is suitable for improvement of drought tolerance. Addition lines 3H, 5H and 7H showed the lowest efficiency with negative effect for INTOL.

Table 5. The EAC of traits under study of DAL of mature embryo in drought stress

genotypes	CGR	RFWG	RGR	RWC	INTOL	CGI	Rt %	R %
1H	25.36	124.78	105.26	27.27	105.25	76.293	96.578	57.142
2H	-9.89	239.91	152.63	12.21	100.15	15.086	147.983	114.285
3H	14.52	28.99	36.84	9.63	-164.23	-130.603	179.651	157.142
4H	26.73	828.99	378.94	19.84	259.83	566.379	159.703	164.285
5H	80.21	21.84	26.31	22.71	-56.61	-80.603	76.312	0
6H	23.05	92.85	73.68	10.94	60.84	-3.017	114.492	85.714
7H	65.36	-86.55	-105.26	10.54	-644.06	-230.172	48.305	-78.571

Screening *in vitro* indicators and drought tolerant genotypes

(i) Biplot analysis method

To better understand the relationships, similarities and dissimilarities among the *in vitro* 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 [17]. The relationships among different indices are graphically displayed in a biplot of PCA₁ and PCA₂ (Fig. 1). The PCA₁ and PCA₂ axes which justify 77.14% 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 vitro* indices [36]. INTOL, RGR, Rt%, CGI and RFWG are in group 2 (G2) with high correlation (acute angle) which introduce addition line 4H as drought tolerant. R%, RWC and CGR were separated as group 1 (G1), 3 (G3) and 4 (G4), respectively discriminated chromosome 4H, 1H and 5H as drought tolerant, but as 5H displayed negative INTOL and CGI, hence it is discarded as drought tolerant and 1H was considered as low tolerant. The vectors in the biplot revealed that G1 and G3 were independent (right angle), while G1 and G4 showed negative correlation (obtuse angle). G1 and G2 almost exhibited positive correlation (acute angle). This procedure was also employed in chickpea (*Cicer arietinum* L.) [10] for clustering stability statistics and in durum wheat (*Triticum turgidum* L.) [18] for screening selection criteria of different climate and water regime conditions

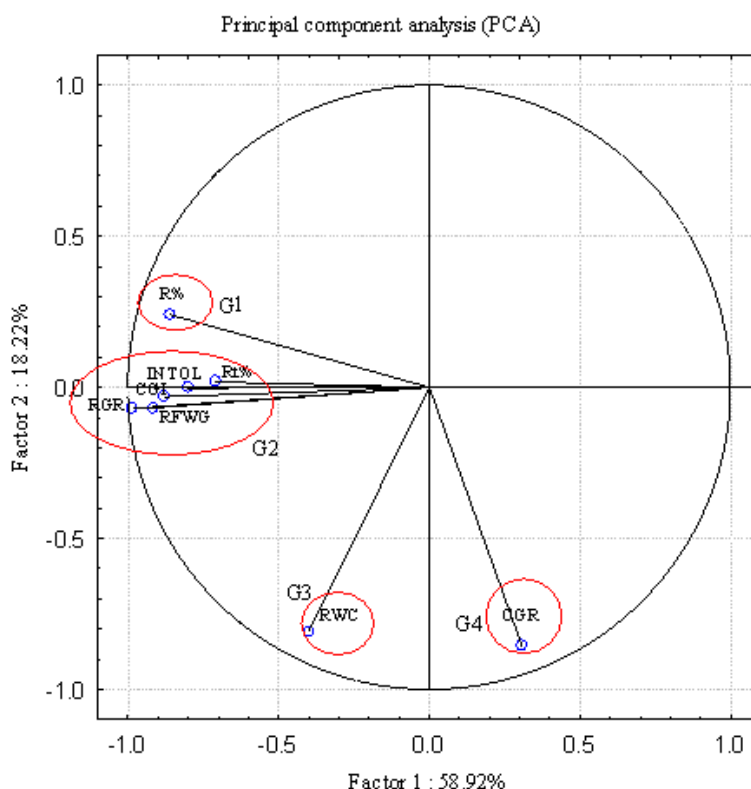


Fig. 1. Biplot analysis of *in vitro* indicators of drought tolerance in wheat-barley disomic addition lines using mature embryo culture

Table 6. Ranks (R), ranks mean ($\bar{R}$) and standard deviation of ranks (SDR) of *in vitro* indicators of drought tolerance in disomic addition lines using mature embryo culture

Genotype	CGR	R	RFWG	R	RGR	R	RWC	R	INTOL	R	CGI	R	Rt%	R	R%	R	Sum	$\bar{R}$	SDR
1H	1.191	5	0.059	3	0.001	4	79.999	1	0.031	3	0.409	2	87.843	6	-0.6	6	30	3.75	1.83
2H	0.856	9	0.333	2	0.01	2	70.532	5	0.0009	4	0.267	3	110.814	3	0.2	3	31	3.875	2.29
3H	1.088	7	-0.169	6	-0.012	6	68.914	8	-1.559	8	-0.071	8	124.965	1	0.8	2	46	5.75	2.76
4H	1.204	4	1.735	1	0.053	1	75.331	3	0.943	1	1.546	1	116.051	2	0.9	1	14	1.75	1.16
5H	1.712	2	-0.186	7	-0.014	7	77.133	2	-0.924	7	0.045	7	78.787	7	-1.4	7	46	5.75	2.31
6H	1.169	6	-0.017	5	-0.005	5	69.738	6	-0.231	5	0.225	5	95.848	4	-0.2	5	41	5.125	0.64
7H	1.571	3	-0.444	9	-0.039	9	69.486	7	-4.390	9	-0.302	9	66.272	8	-2.5	8	62	7.75	2.05
CH.S	0.950	8	-0.238	8	-0.019	8	62.856	9	-0.590	6	0.232	4	44.686	9	-1.4	7	59	7.375	1.68
Betzes	1.767	1	0.058	4	0.003	3	73.058	4	0.572	2	0.081	6	95.813	5	-0.1	4	29	3.625	1.59

Table 7. Ranks (R), ranks mean ($\bar{R}$) and standard deviation of ranks (SDR) of *in EAC* in disomic addition lines using mature embryo culture

Genoty	CGR _E	R	RFWG	R	RGR _{EAC}	R	RWC	R	INTOL	R	CGI _{EAC}	R	RT% _{EA}	R	R _{EAC} %	R	Sum	$\bar{R}$	SDR
pc	ac		EAC				EAC		EAC				C						
1H	25.36	4	124.78	3	105.26	3	27.27	1	105.25	2	76.29	2	96.57	5	57.14	5	25	3.125	1.45
2H	-9.89	7	239.91	2	152.63	2	12.21	4	100.15	3	15.08	3	147.98	3	114.28	3	27	3.375	1.59
3H	14.52	6	28.99	5	36.84	5	9.63	7	-164.23	6	-130.6	6	179.65	1	157.14	2	38	4.75	2.12
4H	26.73	3	828.99	1	378.94	1	19.84	3	259.83	1	566.37	1	159.7	2	164.28	1	13	1.625	0.91
5H	80.21	1	21.84	6	26.31	6	22.71	2	-56.61	5	-80.6	5	76.31	6	0	6	37	4.625	1.99
6H	23.05	5	92.85	4	73.68	4	10.94	5	60.84	4	-3.01	4	114.49	4	85.71	4	34	4.25	0.46
7H	65.36	2	-86.55	7	-105.26	7	10.54	6	-644.06	7	-230.17	7	48.3	7	-78.57	7	50	6.25	1.75

(ii) Ranking method

The estimates of *in vitro* 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 RWC, the desirable drought-tolerant genotype was 1H, while according to CGR the desirable drought-tolerant genotype was 5H and with regard to the indices RFWG, RGR, INTOL, CGI and R% genotype 4H was the most drought tolerant.

To determine the most desirable drought tolerant genotype based on the all indices mean rank and standard deviation of ranks of all *in vitro* drought tolerance criteria were calculated and based on these two criteria the most desirable drought tolerant genotypes were identified.

In consideration of all indices (Table 6), disomic addition line 4H showed the best mean rank and low standard deviation of ranks in stress condition, hence it was concluded that most of the QTLs involved in the inheritance of *in vitro* drought tolerance criteria are located on chromosome 4H. The highest amount of EAC (Table 7) was also attributed to this chromosome. The same procedures have been used for screening quantitative indicators of drought tolerance in wheat [18], in maize (*Zea mays* L.) [3] and in rye (*Secale cereale* L.) [5].

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