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Efficiency of chromosomes 2H and 7H of barley in carrying the genes controlling callus induction and *in vitro* indicators of drought tolerance using immature embryo culture

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ABSTRACT

In order to locate the QTLs involved in the inheritance of callus induction and in vitro indices of drought tolerance in barley an experiment was carried out using a wheat-barley disomic addition lines. The results of analysis of variance revealed highly significant differences for callus primary fresh weight (CPFW), callus growth rate (CGR), callus relative fresh weight growth (CRFWG), callus relative growth rate (CRGR) and callus induction percentage (CIP) in the callus induction stage and CGR, RFWG, RGR, relative water content (RWC) and in vitro tolerance (INTOL) in the drought stress experiment indicating genetic variability and possible chromosomal localization of the genes involved in the genetic of callus induction and in vitro predictors of drought tolerance in barley. Mean comparison exhibited that most of the genes controlling callus induction and drought tolerance criteria are located on chromosomes 2H and 7H. The efficiency of added chromosome (EAC) indicated that chromosome 2H and 7H had the highest efficiency with positive effect for improvment of callus induction traits CPFW, CRFWG, CRGR and RFWG, RGR and INTOL in the drought stress stage, respectively.

Key words : Callus induction, drought stress, disomic addition lines, gene location, immature embryo.

INTRODUCTION

Tissue culture response (TCR) of immature embryos is under genetic control [28]. Immature embryos have been used frequently as an explant source in wheat tissue culture and for the initiation of wheat callus culture, but it is usually difficult to obtain immature embryos through out the year, and their suitable stage for culture is also strictly limited [8, 34, 35]. Immature embryo culture has several applications in crop improvement *per se*, including rapid generation advancement and to overcome the cross ability barrier in plants [12, 32]. For wheat, barley and maize, transgenic plants have been obtained using immature embryos as explant source [24, 53, 60].

Numerous studies have been conducted on the genetic control of plant tissue culture responses [56, 57]. The majority of QTL analyses of TCR traits were conducted with monocots such as rice, barley and maize, probably because of economical importance of these plant species [22]. In maize, chromosomes 1, 2, 3, 6 and 8 are associated with immature embryo TCR; moreover, either identical or tightly-linked QTL intervals on chromosomes 1 and 3 have been consistently identified in various studies [10, 32, 61].

Wheat immature embryo culture response has been extensively investigated using substitution lines, ditelosomic lines, nullisomic-tetrasomic, and monosomic lines in the late 1980s [28]. Wheat chromosomes 4B, 2D, 7B, 7D, 1D, 6BL, 2B and 2AL have been identified to have significant effects on immature embryo TCR [19, 20, 21, 46, 50]. Later, using chromosome recombinant lines, Ben Amer et al. [25, 26, 27] mapped QTLs on chromosomes 2B and 2D for plant regenerated from callus derived of immature embryos. Henry et al. [56, 57] reported a QTL on chromosome 2AS controlling green-point formation in calli from immature embryos. 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 [55]. Sexual hybridization of these species makes it possible to transfer agronomically useful genes from barley into wheat.

One of the main environmental abiotic stresses that is responsible for yield instability and limitations in cereal crops is drought stress, which affects practically every aspect of plant growth and metabolism. Improvement of productivity of crop plants under drought conditions becomes one of the important breeding program objectives [49]. Plant tissue culture techniques provide a promising and feasible approach to develop drought tolerant plants. Drought tolerance and productivity is one of the most difficult tasks for cereal breeders [49]. A number of useful wheat variants has been developed through tissue culture for drought tolerance [38, 33]. Although selection for genotypes with increased productivity in drought-prone environments has been an important aspect of many plant breeding programs, the biological basis for drought tolerance is still poorly understood [49]. Also, drough stress is highly heterogenous in time, space, degree of stress, growth stage and time of stress exposure [42] and is unpredictable.

Disomic addition lines in which a single pair of chromosomes from related species is added to the full chromosome complement carring the genes controlling drought tolerance indicators and form the starting point for cytogenetic transfer of genetic material into the genotypes under investigation [4, 18, 47]. These addition lines will be usefull in assigning genes controlling barley characters to particular barley chromosomes and also in determining the genetic similarity of individual barley chromosomes with wheat chromosomes. Furthermore , such addition lines could serve as the source material for transferring desirable characters from barley to wheat [43]. The drought stress could be induced in the plant cell cultures by adding different compounds to the nutrient medium such as, polyethylene glycol (PEG) which stimulates water stress by acting as osmotic agent which reduce the potential of the medium in where the cell are growing [3]. PEG of high molecular weight is a non-penetrating inert osmoticum lowering the water potential of nutrient solutions without being taken up or being phytotoxic [13].

Wheat-Barley disomic addition lines have been used to evaluate gene expression and physical mapping of barley [62] and the first wheat-barley disomic addition line was developed by Kruse [63] followed by Islam *et al* [43, 64].

The objectives of the present investigations were to: (i) locate the genes controlling callus induction characteristics in barley (ii) evaluate drought tolerance of wheat-barley disomic addition and (iii) screening *in vitro* indicators of drought tolerance.

MATERIALS AND METHODS

Plant genetic materials

The plant material 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 H1 to H7 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 [65] 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 genotypse 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 complete 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 Chen et al. [66] 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) [37], after 7, 14, 21 and 28 days of callus induction.

Calculation of CGR per replicatation was as: $CGR_1 = \frac{d_7}{7}$, $CGR_2 = \frac{d_{14} - d_7}{7}$, $CGR_3 = \frac{d_{21} - d_{14}}{7}$ 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 [30] as:
$CRGR = (\ln W_2 - \ln W_1)$/ 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 complete 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 were the traits CGR, RFWG, RGR, relative water content (RWC), callus growth index (CGI), reduction percentage (RP), relative tolerance and *in vitro* tolerance (INTOL) were calculated as follows:

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

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

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 et al. [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):
IT= RGR treatment / RGR control [30]

(v) Callus growth index (CGI) or increasing value of callus fresh weight was calculated [1] as:
$RFWG_{stress} = (W_1 - W_0)/W_0$ and $RFWG_{control} = (W_1 - W_0)/W_0$

$CGI = \frac{RFWG_{stress} - RFWG_{CONTROL}}{2}$

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 $RFWG_{stress}$ and $RFWG_{control}$, respectively.

(vi) Percetage of relative tolerance (Rt%) : Rt% = [a/b] × 100 [1]
where a = fresh weight under stress after 16 days and b = fresh weight after 16 days under control

(vii) Reduction percentage (R%): R%= (a-b) ×100 [1]
where a = fresh weight under stress after 16 days and b= fresh weight after 16 days under control.

Efficiency of added chromosomes (EAC): EAC was calculated for both experiments as:

$$ACE\% = \frac{Y_{DA} - Y_{CS}}{Y_{CS}} \times 100 \text{ [14, 17]}$$

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

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 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 [40, 54] and bread wheat [31, 48].

Table 1. Analysis of variance for callus induction characters using immature embryos

S.O.V	df	Mean Square					
		CPD_{mm}	$CPFW_{gr}$	CGR_{mm}	$CRFWG_{gr}$	$CRGR_{gr}$	$CIP_{\%}$
genotype	**8**	0.443^{ns}	0.0005**	0.041**	0.691**	0.00037**	0.024**
error	**36**	0.26	0.000008	0.001	0.002	0.000012	0.002

*** significant at 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 2H had the highest amount of CPD, CPFW, CGR, CRFWG, CRGR and CIP. Maximum amount of CPD was attributed to addition line 5H with no significant difference with 2H, accordingly most of the QTLs controlling callus induction characteristics are located on chromosome 2H, hence chromosomes 2H is suitable for improving wheat and barley tissue culture traits through interspecific and intergeneric hybridization.

Immature embryo was reported as the best tissue for callus induction and shoot regeneration [2, 45]. 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. [36] suggested that genotypes with high callus induction also caused an increase in the number of plants transferred to soil. Melahat et al. [5] showed significant correlation between callus induction frequency and regeneration capacity (r = 0.786*) in their immature embryo culture, indicated that these characteristics are genetically dependent on each other. Such genotypes which have high callus induction and regenerable callus frequencies are very desirable in tissue culture programs. In barley genes controlling tissue-culture traits have already been mapped on several chromosomes [52, 56].

In barley, QTLs associated with immature embryo TCR map to chromosomes including 2H 3H, 5H and 7H [41, 52]. In barley, chromosome 2H was shown to influence shoot regeneration. Komatsuda et al. [51, 52] identified the QTL *Shd1* (Shoot differentiation). This locus was mapped by RFLPs in the chromosomal region containing the *v* gene, which determines the 2-row/ 6-row ear type on 2HL, and it may be homoeoallelic to *Tcr-B3* of wheat. A further locus, *Qsr1* (Quantitative trait locus for shoot regeneration), was mapped recently by Mano et al. [58] in the centromere region of chromosome 2H, probably homoeoallelic to *Tcr-B1*, whereas another QTL controlling callus growth rate, *Qcg1* (Quantitative trait locus for callus growth), was located again on the long arm of chromosome 2H. Interestingly, genes modifying ear emergence time independently of environmental stimuli (vernalisation, photoperiod), 'earliness *per se*' genes (*eps*) were recently mapped in the centromere regions of chromosome 2B of wheat [7] and 2H of barley (Laurie et al. 1994), respectively. These genes act through the determination of the number and/or the rate of primordial initiation [6]. In similar way, the *eps* genes could influence the number and/or the rate of the differentiated cells in tissue culture as secondary pleiotropic.

The CIM analysis detected two loci on chromosome 2H and one locus on 5H controlling CGR [59]. 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 [23], and on chromosomes 2H and 3H in the Steptoe (ST) × Morex (MO) cross [58]. Immature embryos culture suggested that CGR is a polygenic trait and the effect of chromosome on this trait depend on the time of cycle life 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.

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

Genotype*	CPD		CPFW		CGR		CRFWG		CRGR		CIP	
1H	3.711	ab	0.027	c	0.199	b	0.866	g	0.029	d	98	a
2H	3.705	ab	0.049	a	0.203	b	1.793	a	0.048	a	96	a
3H	4.003	ab	0.029	bc	0.191	bcd	1.287	c	0.039	b	100	a
4H	3.267	b	0.019	de	0.174	d	1.122	d	0.035	bc	100	a
5H	4.295	a	0.032	b	0.198	bc	1.06	e	0.034	cd	100	a
6H	3.631	ab	0.025	c	0.179	cd	0.958	f	0.032	cd	92	a
7H	4.044	a	0.027	c	0.193	bcd	1.048	e	0.034	cd	100	a
Ch.s	3.612	ab	0.020	d	0.345	a	1.579	b	0.045	a	92	a
Betzes	3.763	ab	0.016	e	0.175	d	0.554	h	0.021	e	61.66	b

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

Efficiency of added choromosomes (EAC)

The efficiency of added chromosomes (Table 3) indicated that chromosomes 2H had the highest efficiency with positive effect for improvement of CPFW, CRFWG and CRGR. All disomic addition lines except 2H exhibited negative effect for improvment of CRFWG and CRGR, while all disomic addition lines except 6H revealed positive effect for improvment of CIP. Disomic

addition lines 4H indicated negative effect for improvment of all characters except CIP. The highest efficiency for improvement of CPD belonged to chromosome 5H.

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

genotype	CPD	CPFW	CGR	CRFWG	CRGR	CIP
1H	2.74	35	-42.31	-45.15	-35.55	6.52
2H	2.57	145	-41.15	13.55	6.66	4.34
3H	10.82	45	-44.63	-18.49	-13.33	8.69
4H	-9.55	-5	-49.56	-28.94	-22.22	8.69
5H	18.9	60	-42.60	-32.86	-24.44	8.69
6H	0.52	25	-48.11	-39.32	-28.88	0
7H	11.96	35	-44.05	-33.62	-24.44	8.69

Drought tolerance experiment

All of the tissue culture traits were influenced by the genotype in immature embryo culture at drought stress experiment. High significant differences were observed among the addition lines for CGR, RFWG, RGR, RWC 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 tolerace. Farshadfar et al. [14, 67] showed that the genes controlling salt and drought tolerance are also located on chromosome 4H and 5H. Molnar et al. [68] reported that the genes located on chromosome 4H of barley were able to increase water use efficiency of in wheat substitution lines.

Table 4. Analysis of variance for *in vitro* drought tolerance indicators using immature embryos

S.O.V	df	Mean Square				
		CGR_{mm}	$RFWG_{gr}$	RGR_{gr}	$RWC_{\%}$	INTOL
genotype	**8**	0.388**	0.132*	0.185**	6.29**	0.046**
error	**18**	0.029	0.043	0.0005	1.63	0.001

, significantly at 0.05 and 1% level of probability, respectively*

Table 5. Mean comparison of *in vitro* drought tolerance indicators using immature embryos

Genotype*	CGR		RFWG		RGR		RWC		INTOL	
1H	1.714	b	-0.733	c	-0.173	c	19.82	**b**	-8.412	b
2H	2.033	a	-0.561	bc	-0.059	b	19.17	**b**	-3.111	ab
3H	1.081	cd	-0.209	ab	-0.019	ab	28.60	**b**	-2.148	a
4H	1.260	cd	-0.389	abc	-0.033	ab	30.12	**b**	-1.588	a
5H	1.113	cd	-0.234	ab	-0.019	ab	29.44	**b**	-1.239	a
6H	0.964	d	-0.348	abc	-0.018	ab	40.81	**b**	-1.133	a
7H	1.367	c	0.0004	a	-0.0001	a	28.05	**b**	1.785	a
Ch.s	1.020	d	-0.348	abc	-0.771	d	33.31	**b**	-38.61	c
Betzes	1.089	cd	-0.297	ab	-0.023	ab	67.46	**a**	-1.762	a

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

The values of 2H, 1H, and 7H for CGR were significantly higher than recipient. Therefore these chromosomes are effective on CGR, respectively. Altough the value of 2H was significantly higher than 1H and 7H for CGR, but only chromosome 7H displayed positive RFWG and INTOL, hence the QTLs controlling *in vitro* indicators of drought tolerance are located on chromosome 7H. No significant difference was observed between disomic addition lines for

RWC, but as all disomic addition lines showed negative INTOL except 7H, therefore, the most desirable genes for improvement of drought tolerance through chromosome engineering are located on chromosome 7H.

Teulat et al. [9] reported that chromosomes 6H carry the genes responsible for RWC which is in agreement with the results of this experiment. Farshadfar et al [14] reported that addition line 6H had no significant difference with 4H but 4H is more outstanding.

Efficiency of added chromosomes (EAC)

The efficiency of added chromosome (Table 6) indicated that chromosome 2H had the highest efficiency with positive effect for improvement of CGR. Chromosome 7H exhibited maximum EAC with positive effect for improvement of RFWG, RGR and INTOL. Addition line 6H revealed the highest amount of RWC and CGI. Maximum RT% and R% was attributed to chromosome 3H. The results of EAC displayed that genes controlling *in vitro* indicators of drought tolerance are distributed on chromosomes 2H, 3H, 6H and 7H. Zhang [29] showed that QTLs of various physiological responses to drought in barley are different in various conditions (drought and well- watered).

Table 6. The EAC of *in vitro* indicators of drought tolerance using immature embryos

genotype	CGR	RFWG	RGR	RWC	INTOL	CGI	RT%	R%
1H	68.03	-110.63	77.56	-40.50	78.21	-854.54	-40.13	-11.82
2H	99.31	-61.20	92.34	-42.43	91.94	-559.09	17.7	35.43
3H	5.98	39.94	97.53	-14.13	94.43	-536.36	87.05	74.36
4H	23.52	-11.78	95.71	-9.58	95.88	-95.45	13.71	42.15
5H	9.11	32.75	97.53	-11.60	96.79	18.18	51.78	52.36
6H	-5.49	0	97.66	22.51	97.06	418.18	21.88	13.84
7H	34.01	100.11	99.99	-15.78	104.62	418.18	48.69	51.08

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 [69]. 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 70.56% 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 (Yan and Kang, 2003). INTOL and RGR are in group 1 (G1) with high correlation (acute angle) which introduced addition line 7H as drought tolerant. R% and Rt% were separated as group 2(G2) with high association and introduced chromosome 3H as the most drought tolerant but chromosome 3H showed negative RWC and CGI. RFWG, RWC and CGI discriminated chromosomes 7H, 6H and 7H as drought tolerant (group 3=G3), but as 6H displayed negative CGR and zero RFWG hence chromosome 7H is more desirable for improvement of drought tolerance. CGR was clustered as group 4 (G4) and introduced chromosome 2H as drought tolerant with negative EAC for RFWG, RWC and CGI. The vectors in the biplot revealed that (G1 and G3) and (G1 and G4) were independent (right angle), G4 and G3 showed negative correlation (obtuse angle). G1 and G2 exhibited positive correlation (acute angle). This procedure was also employed in chickpea (*Cicer*

arietinum L.) [71] for clustering stability statistics and in durum wheat (*Triticum turgidum* L.) [70] for screening selection criteria of different climate and water regime conditions

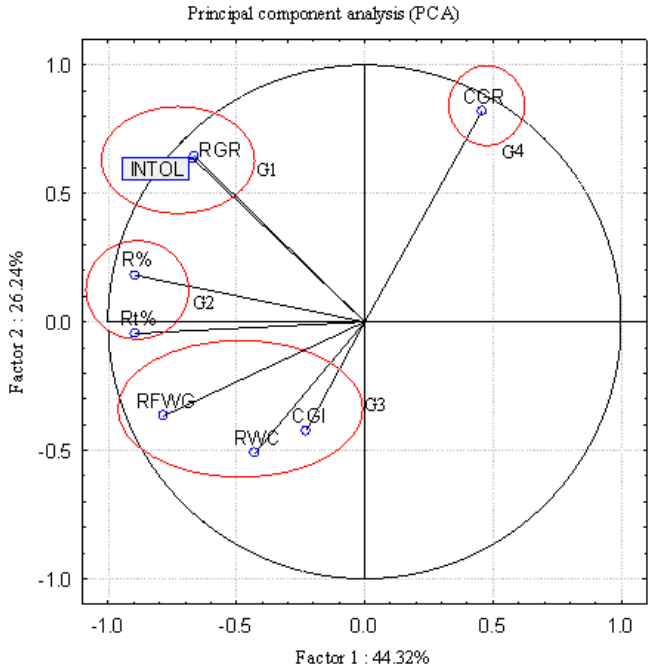

Fig. 1. Biplot analysis of *in vitro* indicators of drought tolerance in wheat-barley disomic addition lines using immature embryo culture

(ii) Ranking method

The estimates of *in vitro* indicators of drought tolerance (Table 6) indicated that the identification of drought-tolerant genotypes based on a single criterion was contradictory. For example, according to INTOL and RGR, the desirable drought-tolerant genotype was 7H, while according to R% and Rt% the desirable drought-tolerant genotype was 3H and with regard to CGI addition line 2H was the most drought tolerant.

To determine the most desirable drought tolerant genotype according to 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 to all indices, disomic addition line 7H showed the lowest 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 7H. The highest amount of EAC (Table 7) was also was attributed to this chromosome. The same procedures have been used for screening quantitative indicators of drought tolerance in wheat [70], in maize (*Zea mays* L.) [15], and in rye (*Secale cereale* L.) [16].

Table 7 (i). Ranks (R), ranks mean ($\overline{R}$) and standard deviation of ranks (SDR) of *in vitro* indicators of drought tolerance in disomic addition lines using immature embryo culture

Genotype	CGR	R	RFWG	R	RGR	R	RWC	R	INTOL	R	CGI	R	Rt%	R	R%	R	Sum	$\overline{R}$	SDR
1H	1.714	2	-0.733	8	-0.173	7	19.82	8	-8.412	8	-0.166	9	25.401	9	-3.594	9	60	7.5	2.329
2H	2.033	1	-0.561	7	-0.059	6	19.17	9	-3.111	7	-0.101	8	49.943	6	-2.075	6	50	6.25	2.375
3H	1.081	7	-0.209	2	-0.019	3	28.60	6	-2.148	6	-0.096	7	79.366	1	-0.824	1	33	4.125	2.642
4H	1.26	4	-0.389	6	-0.033	5	30.12	4	-1.588	4	0.001	5	48.25	7	-1.859	5	40	5	1.069
5H	1.113	5	-0.234	3	-0.019	3	29.44	5	-1.239	3	0.026	3	64.404	2	-1.531	3	27	3.375	1.06
6H	0.964	9	-0.348	5	-0.018	2	40.81	2	-1.133	2	0.774	1	51.714	5	-2.769	7	33	4.125	2.85
7H	1.367	3	0.0004	1	-0.0001	1	28.05	7	1.785	1	0.114	2	63.092	3	-1.572	4	22	2.75	2.052
CH.S	1.02	8	-0.348	5	-0.771	8	33.31	3	-38.61	9	0.022	4	42.43	8	-3.214	8	53	6.625	2.263
Betzes	1.089	6	-0.297	4	-0.023	4	67.46	1	-1.762	5	-0.031	6	56.959	4	-1.218	2	32	4	1.772

Table 7 continued

Genotype	CGR_{EAC}	R	$RFWG_{EAC}$	R	RGR_{EAC}	R	RWC_{EAC}	R	$INTOL_{EAC}$	R	CGI_{EAC}	R	$RT\%_{EAC}$	R	$R_{EAC}\%$	R	Sum	$\overline{R}$	SDR
1H	68.03	2	-110.63	7	77.56	6	-40.5	6	78.21	7	-854.54	7	-40.13	7	-11.82	7	49	6.125	1.726
2H	99.31	1	-61.2	6	92.34	5	-42.43	7	91.94	6	-559.09	6	17.7	5	35.43	5	41	5.125	1.807
3H	5.98	6	39.94	2	97.53	3	-14.13	4	94.43	5	-536.36	5	87.05	1	74.36	1	27	3.375	1.922
4H	23.52	4	-11.78	5	95.71	4	-9.58	2	95.88	4	-95.45	4	13.71	6	42.15	4	33	4.125	1.125
5H	9.11	5	32.75	3	97.53	3	-11.6	3	96.79	3	18.18	3	51.78	2	52.36	2	24	3	0.925
6H	-5.49	7	0	4	97.66	2	22.51	1	97.06	2	3418.18	1	21.88	4	13.84	6	27	3.375	2.263
7H	34.01	3	100.11	1	99.99	1	-15.78	5	104.62	1	418.18	2	48.69	3	51.08	3	19	2.375	1.407

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