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Chromosome location of ISSR markers and genes controlling seed germination under drought stress in wheat-barley disomic addition lines

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Abstract: Alien disomic addition lines are valuable genetic resources for breeding programs under wide hybridization. The present study was carried out to identify the chromosome location of ISSR markers and genes controlling seed germination under drought stress in wheat-barley disomic addition lines. Germination experiment was performed at 0, -4 bar and -8 bar levels in a completely randomized design with three replications. The Germination Index, Germination Percentage, Coefficient of Germination Velocity, Mean Germination Time, Mean Germination Daily, and Plantlet Growth Speed were measured. Based on karyotype analysis, the presence of addition chromosomes was confirmed. The IS_{10} (0.494) and IS_{15} (0.395) primers showed the greatest polymorphism among the addition lines. The primers amplified most parts of the chromosomes 2H, 3H, and 7H, indicating these ISSR primers are located on these chromosomes. It is also indicated that most of the genes responsible for the seed germination under drought stress are located on chromosomes 4H and 5H.

Key words: Addition lines; ISSR marker; Germination index; Cytogenetic indices.

Introduction

Wheat (*Triticum aestivum* L.) as one the most important food resources for the human that is at risk of biotic and abiotic stresses. Drought tolerance is a key trait in increasing and stabilizing wheat productivity in dry areas worldwide (1, 2). Increasing wheat gene pools to enrich the resistance to such stresses is one of the most important objectives of researchers and wheat breeders. Wheat wide hybridization makes it possible to transfer agronomical useful genes from wild species into cultivated plants. Several useful alien genes have been transferred from wild species into wheat. Barley can be applied as a donor of the genetic material into cultivated wheat through wide crosses, in terms of tertiary gene pools in wheat improvement programs (3, 4). Such addition lines have been applied to determine chromosome location of different target genes (5, 6). Attempts to hybridize the two species began in the early 20th century, but the first demonstrably successful cross was reported by Kruse (7). Encouraged by his success, attempts were made in several countries, aimed at producing new hybrids and progenies. The Chinese Spring-Hope substitution line series was used to determine the chromosomal location of genes in Chinese Spring that permit crossability with Betzes barley (8-10).

Wheat–barley disomic addition lines (2H, 3H, 4H, 6HS, 7H, 1HS (isochromosome) were produced and identified using molecular cytogenetic methods from hybrids between winter wheat line Mv9kr1 and the German two-rowed winter barley Igri (11, 12). To increase the allelic variation in wheat/barley introgressions, new wheat/barley disomic addition lines were developed containing the 2H, 3H, 4H, 6H and 7H chromosomes of the six-rowed Ukrainian winter barley cultivar Manas (6). Identification of the genes responsible for drought tolerance in barley (*Hordeum vulgare* L.) will facilitate understanding of the molecular mechanisms of drought tolerance, and also facilitate the genetic improvement of barley through marker-assisted selection or gene transformation (2). To identify the disomic alien addition lines carrying different chromosomes, the following indices including the morphological traits, the chromosomal karyotype, behavior of meiotic chromosome pairing in F_1 plants obtained from the cross between the two alien addition lines, biochemical and molecular markers have been used (5, 13).

Wheat-barley addition lines have been studied to characterize the expression of alien genes in the wheat genome and to draw up a physical map of the barley genome (4). The results of this research indicated that the abnormality of the locus (activity and non-activity) was a normal phenomenon in wheat-barley addition lines. Inter-Simple Sequence Repeat, Polymerase Chain Reaction (ISSR-PCR) technique was used to detect some molecular markers associated with drought tolerance. Five ISSR primers were used and revealed 78% polymorphism. The primers produced 12 bands, which could be used as molecular markers in barley breeding programs (4). Drought tolerance genes and/or quantitative trait locus (QTLs) could be cloned and transform to increase crop tolerance (15, 16).

Different molecular markers such as various PCR-based molecular markers, localization of QTLs, mar-

ker-assisted selection are powerful tools for gene mapping, identification of specimens, analysis of population structure, and genetic diversity (17, 18). To date, many studies have reported on the application of molecular markers in genetic mapping of barley, QTLs analysis (19), association mapping (20), microsatellite markers (21), , genetic structure, and relationships between morphological and molecular markers of North African barley germplasm (22), biochemical markers (23) Simple Sequence Repeat (SSR) (25). RAPD markers (44),

The objective of the present study is to identify the chromosomal localization of ISSR markers and genes controlling seed germination indices of drought tolerance in barley using wheat-barley disomic addition lines.

Materials and Methods

Plant materials

In this study, seven disomic addition lines, each containing one pair of barley (Betzes cultivar) chromosomes transmitted into the wheat genetic background (Chinese Spring), were used (Table 1).

Cytogenetic experiments

To identify and confirm the alien barley chromosome karyotype, the seeds were placed on a moistened filter paper in a Petri dish for 48 h for germination. After cold treatment, collected root tips (7 to 10 mm long) were pretreatment with 0.1% α -bromonaphthalene for 3 h at RT. After pretreatment of roots the fixation, hydrolyzing and staining of the chromosomes were done with Levitsky solution, 1N sodium hydroxide and hematoxylin, respectively. The root tips were squashed gently, then five metaphase cells were photographed from each addition line and the statistical parameters were analyzed by MicroMeasure 3.3 software. The following cytogenetic characters were measured: long arm (L), short arm (S), the total length of chromosome (CL), arm ratio (AR), centromeric index (CI) and the type of chromosomes (K.F). As a standard measure for the chromosome length, a relative value is used. Its absolute length is expressed as the percentage of the total sum of the length of all chromosomes in each metaphase plate (24).

Molecular experiments

The DNA extraction was carried out using modified CTAB (27) for genotypes. The quantity and quality of the extracted DNA were determined using 0.8% (w/v) agarose gel and spectrophotometric method. Polymerase Chain Reaction (PCR) was performed in a volume of 20 µL including (50 ng) DNA, 2 mM MgCl2, 0.05 MM of each dNTP, 0.2 µM primer, one unit of Taq DNA Polymerase enzyme and a reaction buffer to 1x.

Table 1. List of wheat- barley disomic addition lines and parents.

No.	Chromosome addition lines
1	1H
2	2H
3	3H
4	4H
5	5H
6	6H
7	7H
8	Chinese Spring (Ch.S.), Recipient
9	Barley (Betzes cultivar), (H.V), Donor

The thermal cycles consisted of a first stage of the denaturation at 95 °C for 5 minutes and 40 thermal cycles. In each cycle, the time and temperature of the denaturation were 30 seconds and 95 °C, respectively. The initiation time of primer attachment was 45 seconds and the temperature varied for each primer (Table 2). Also, the time and temperature of chain extension were 60 seconds and 72 °C, respectively. The final development was performed in 7 minutes at 72 °C temperature. In this experiment, a 2% (w/v) agarose gel with 1% TBE buffer was used for loading polymerase chain reaction products (PCR) and then the gel was stained in ethidium bromide solution (one microgram per microliter). The Gel Document (Quantum ST4) was used to represent the bands. Finally, using the Darwin 5 software, the data were analyzed.

The Polymorphic Information Content (PIC) was calculated (26) as follows: where Pi is the frequency for the *ith* allele among a total of n alleles.

$$\mathrm{PIC} = 1 - \sum_{i=1}^{n} p_i^2$$

Seed germination experiment

To determine the chromosomal location of genes controlling seed germination traits under osmotic stress conditions, wheat-barley addition lines were treated with two osmotic stress levels and (-4 bar and -8 bar and control) in PEG8000, using a factorial experiment in a completely randomized design with three replications. The following traits were measured: Germination index (GI), Coefficient of Germination Velocity (CGV), Germination percentage (GP), Mean Germination Time (MGT), Mean Germination Daily (MGD) and Plantlet Growth Speed (PGS).

Germination Index (GI) = (10×n1) + (9×n2) + · · · + (1×n10) where n1, n2 . . . n10 = number of germinated seeds on the first, second and subsequent days until the 10th day; 10, 9 . . . and 1 are weights given to the number of germinated seeds on the first, second and subsequent days, respectively.

In the GI, maximum weight is given to the seeds germinated on the first day and the minimum to those germinated later on. The lowest weight would be for seeds germinated on the 10th day. Therefore, the GI emphasizes both the percentage of germination and its speed. A higher GI value denotes a higher percentage and rate of germination (28, 29).

Mean Germination Time (MGT) (day) = MGT=Pf ·x/Pf where f=Seeds germinated on day x.

The lower the MGT, the faster the population of seeds has germinated (30).

Germination Rate Index (GRI) (%day) = GRI=G1/1 + G2/2 +· · ·+ Gx/x where G1=Germination percentage × 100 at the first day after sowing, G2=Germination percentage × 100 at the second day after sowing

The GRI reflects the percentage of germination on each day of the germination period. Higher GRI values indicate higher and faster germination after modifica-

tion.

Coefficient of Velocity of Germination (CVG) = (CVG)(%day) = N1 + N2 + · · · + Nx/100 × N1T1 + · · · + NxTx where N=Number of seeds germinated each day, T=Number of days from seeding corresponding to N.

The CVG indicates the rapidity of germination. It increases when the number of germinated seeds increases and the time required for germination decreases).

Time Spread of Germination (TSG) (day) = TSG = the time in days between the first and last germination events occurring in a seed lot.

The higher the TSG value, the greater the difference in germination speed between the 'fast' and 'slow' germinating members of a seed lot (31).

Statistical procedure

Statistical parameters such as analysis of variance, mean comparison, cluster analysis have been done carried out by SPSS24 and MicroMeasure software 3.3.

Results

Cytogenetic achievement

The study of chromosomes metaphase of wheat-barley addition lines showed the presence of additional chromosomes for all lines. The Tjio and Hagber (32) method was used to identify all seven pairs of barley chromosomes (1H, 2H, 3H, 4H, 5H, 6H, 7H) transmitted to the genetic background of wheat, then these chromosomes were applied to make synthetic karyotype named as H.Vs karyotype (Fig.1).

However, to confirm the identification of the addition lines (1H, 2H, 3H, 4H, 5H, 6H and 7H), recipient parent (Ch.S.), donor parent (barley), and the H.Vs chromosome karyotype were classified based on Ward method. The karyotype characteristics including total chromosome length, long arm length, short arm length, centromeric index, and long to short arm ratio were used to cluster analysis (Fig. 2). The donor parent and karyotype derived from the transmitted chromosomes (H.Vs) were located in the same group. Therefore, it could be stated that the additional chromosomes were correctly identified.

Molecular data

To identify the chromosome location of ISSR markers in barley chromosomes, the 12 ISSR primers were used and 9 out of 12 revealed sharp bands. The location of the ISSR markers bands on the wheat-barley addition lines are shown in Table 2 and Figure 3.

The ISSR primers were able to identify 58 marker locations, of which 8 were similar bands and the others were polymorph. The IS_5 and IS_{13} primers showed the greatest band numbers (9 bands) and the IS_{15} primer showed the lowest band numbers (4 bands). The banding pattern of nine lines using the IS_5 primer is shown in Figure 3. Each genotype showed an average of 32.33 bands for nine primers, and the 7H line with 36 bands had the highest number of bands and the 6H line with the 25 bands had the least band numbers. The results are presented in Table 3. The lowest percentage of polymorphism belonged to IS_{13} and IS_{16} primers with 66.67% and 75%, respectively, and the highest polymorphism belonged to IS_{10}, IS_{11}, IS_{14} and IS_{15} primers with 100%; the mean percentage of polymorphism was 88.86%. The average number of bands produced by each primer for the nine lines was 6.44 and the average number of polymorph bands was 5.56. The highest PIC pertained to IS_{10} (0.494) and IS_{15} (0.395) primers. The 1H addition line had a high distance with both wheat and barley parents based on the cytogenetic and molecular markers.

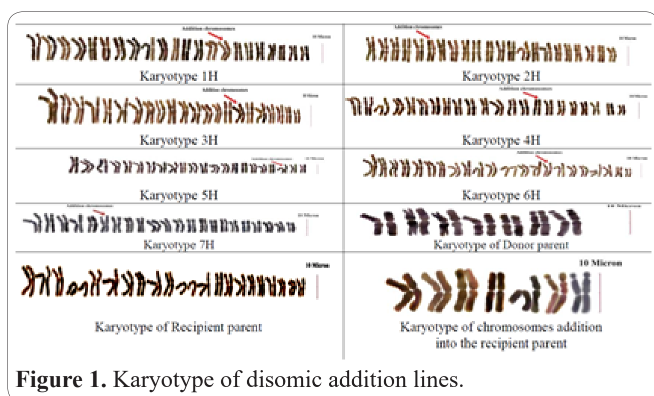

Figure 1. Karyotype of disomic addition lines.

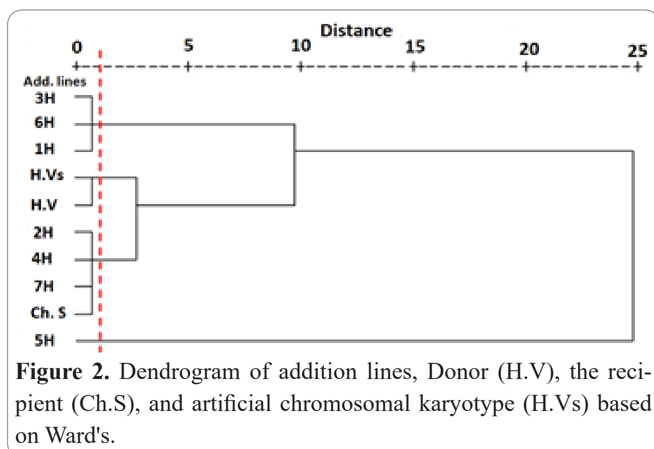

Figure 2. Dendrogram of addition lines, Donor (H.V), the recipient (Ch.S), and artificial chromosomal karyotype (H.Vs) based on Ward's.

Table 2. The location of the ISSR markers on the barley chromosomes.

Barley chromosome no.	ISSR primers
1H	IS_5, IS_6, IS_{10}, IS_{12}, IS_{13}, IS_{14}, IS_{16}
2H	IS_9, IS_{11}, IS_{12}, IS_{13}, IS_{15}, IS_{16}
3H	IS_9, IS_{10}, IS_{11}, IS_{12}, IS_{13}, IS_{15}
4H	IS_{10}, IS_{11}, IS_{12}, IS_{13}, IS_{16}
5H	IS_9, IS_{10}, IS_{12}, IS_{13}, IS_{14}, IS_{15}
6H	IS_9, IS_{10}, IS_{11}, IS_{12}, IS_{13}, IS_{14}, IS_{16}
7H	IS_5, IS_{10}, IS_{11}, IS_{12}, IS_{14}, IS16

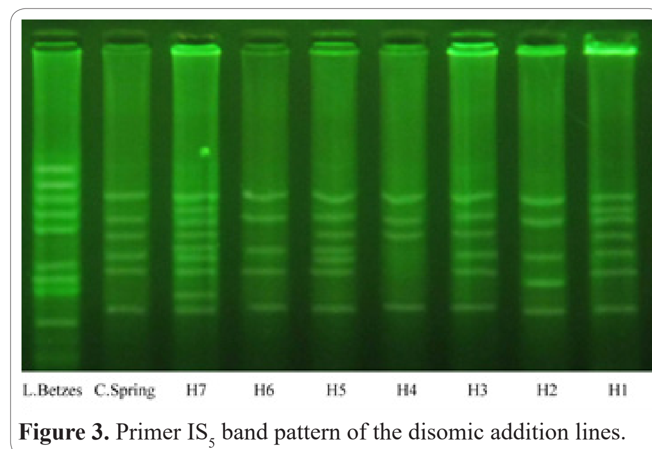

Figure 3. Primer IS_5 band pattern of the disomic addition lines.

Table 3. Polymorphic percentages, the total number of bands, and the polymorphic information content of the primers

Primer Code	Primer sequence	Number of Proliferation place	Number of Polymorphic places	Polymorphism %	PIC
IS5	5'- AGAGAGAGAGAGAGAGC-3'	9	8	88.89	0.302
IS9	5'- CTCTCTCTCTCTCTCTG-3'	5	4	80	0.326
IS10	5'- GAGAGAGAGAGAGAGA Rc-3'	5	5	100	0.494
IS11	5'-ACACACACACACACACC-3'	5	5	100	0.346
IS12	5'-TGTGTGTGTGTGTGTGG-3'	8	7	87.5	0.247
IS13	5'- AGAGAGAGAGAGAGAGYT-3'	9	6	66.67	0.296
IS14	5'- GACAGACAGACAGACA-3'	5	5	100	0.316
IS15	5'- GGATGGATGGATGGAT-3'	4	4	100	0.395
IS16	5'-DBDACACACACACACACA-3'	8	6	75	0.241
Mean		6.44	5.56	88.67	0.329

Table 4. Similarity matrix (Dice similarity coefficient) for the addition lines bases on ISSR marker.

Addition lines	1H	2H	3H	4H	5H	6H	7H	Ch.S.
2H	0.603							
3H	0.638	0.828						
4H	0.500	0.724	0.690					
5H	0.621	0.638	0.638	0.845				
6H	0.509	0.774	0.660	0.811	0.830			
7H	0.586	0.810	0.810	0.707	0.690	0.679		
Ch.S.	0.552	0.707	0.672	0.810	0.793	0.792	0.724	
H.V.	0.509	0.472	0.509	0.434	0.453	0.509	0.604	0.453

Genetic similarity and cluster analysis based on the ISSR

The genetic similarity of the addition lines with dice similarity coefficients varied from 0.434 to 0.845 (Table 4). The mean similarity among all genotypes was 0.65, therefore, there is a fairly variation among entries based on the ISSR marker. The average similarity between just addition lines was 0.695, so the similarity between addition lines was higher than the total average similarity. The mean similarity of the addition lines with the recipient parent was 0.72. This high similarity coefficient indicated that the barley chromosomes did not have a significant effect on the genetic background of the recipient parent based on the ISSR primers. Donor and recipient parents had a high genetic distance (0.453). The 4H, 5H and 6H addition lines had the highest similarity (0.810, 0.793 and 0.792, respectively) with the recipient parent and had the maximum distance (0.443, 0.445 and 0.509) with the donor parent. And the 7H line had the highest similarity (0.604) with the donor parent. The similarity of this addition line with the donor parent showed that the primers amplified most parts of the barley chromosomes 7H. The 1H addition line had the lowest similarity (0.552) with the wheat parent and revealed intermediate similarity (0.509) with the donor parent.

The addition lines were classified into four groups by the UPGMA method based on the Dice similarity coefficient (Fig.4). However, the 4H, 5H, 6H addition lines and Chinese Spring. Located in the group1, the next cluster included 2H, 3H, and 7H. The third group included the 1H line and the fourth group consisted barley (H.V.) parent.

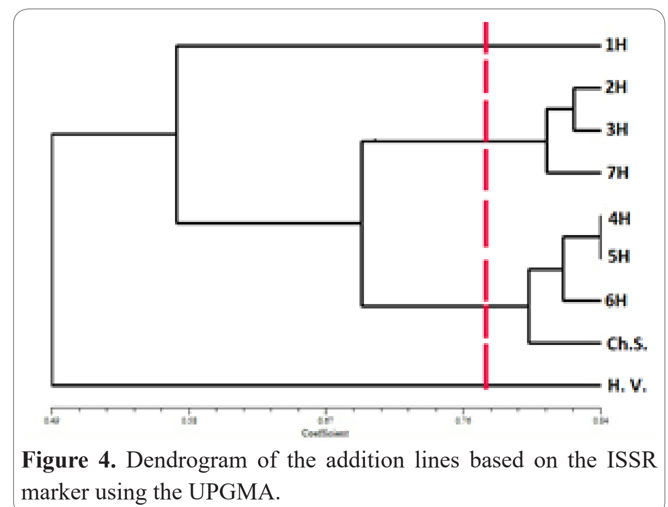

Figure 4. Dendrogram of the addition lines based on the ISSR marker using the UPGMA.

Chromosomal location of genes controlling germination indices of drought tolerance

Analysis of variance showed significant differences (P<0.01) for all traits at different stress levels (Table 5). Interaction between line × stress levels was significant (P<0.01) for all traits. The significance of the interaction was indicative of the different reactions of the chromosomes at a different level of stress.

The comparison of the wheat-barley addition lines in the -4 bar osmotic stress experiment is shown in Table 6. The chromosome 7H (5.2) had the highest value of the GS trait. However, there was no significant difference between 7H and 4H, 5H, and CS wheat. The highest amount of MTG belonged to the barley (5.92) and chromosome 3H (5.56). The greatest MDG index (13.79) pertained to the CS wheat but had no significant difference with chromosomes 4H, (12.43), 7H (11.96) and 5H (10.95).

Table 5. The analysis of variance of germination traits under in vitro conditions for entries at three levels of drought stress

Sources of variation	DF	Germination Speed (GS)	Mean Time Germination (MTG)	Mean Daily Germination (MDG)	Speed Plantlet Growth (SPG)	Germination Vigor (GV)	Germination Index (GI)	Germination Percentage (GP)
Addition line	8	1.562**	0.100**	0.100**	0.003**	0.784**	0.080**	20.043**
Stress level	2	1.562**	1.843**	28.650**	0.097**	24.997**	6.007**	125.966**
Line × stress	16	0.349**	0.134**	0.779**	0.001*	0/346**	0.058**	4.493**
Error	54	0.039	0.020	0.175	0.0001	0.029	0.018	0.585
CV		11.08	6.41	15.31	1.87	11.51	5.42	11.47

** = Significant at 0.01 probability level.

Table 6. The comparison between addition lines of germination traits with Duncan method under osmotic stress

Addition Lines	GS		MTG		MDG		SPG		GV		GI		GP	
	-4 bar	-8 bar	-4 bar	-8 bar	-4 bar	-8 bar	-4 bar	-8 bar	-4 bar	-8 bar	-4 bar	-8 bar	-4 bar	-8 bar
1H	3.49b	0.89b	4.39bc	4.88c	9.46b	3.70ab	0.23ab	0.13bc	0.62bc	0.18ab	6.61ab	2.46cd	65.00b	31.67a
2H	0.79d	0.40d	4.67bc	7.56a	2.33d	1.74c	0.23ab	0.13ab	0.15d	0.08bc	6.33ab	3.44abc	15.00d	15.00bc
3H	0.62d	0.38d	5.56ab	4.42c	2.10d	1.67c	0.19bc	0.11c	0.19d	0.06c	5.44bc	2.92bcd	15.00d	11.67c
4H	4.53ab	0.85bc	4.20c	7.15a	12.43ab	4.07a	0.24a	0.14ab	1.58a	0.18ab	6.80a	3.85ab	81.67ab	30.00ab
5H	4.51ab	1.28a	4.19c	6.51ab	10.95ab	4.26a	0.24a	0.15a	1.34a	0.21a	6.81a	4.49a	80.00ab	40.00a
6H	1.88c	0.42d	4.12c	4.42c	5.83c	1.79c	0.24a	0.13ab	0.31cd	0.14abc	6.82a	2.92bcd	35.00c	13.33c
7H	5.2a	0.35d	4.18c	5.18bc	11.96ab	1.54c	0.24a	0.12bc	0.91b	0.07bc	6.82a	2.16d	90.00a	13.33c
Ch.S.	4.41ab	0.31d	4.30bc	4.44c	13.79a	1.34c	0.23ab	0.13ab	0.88b	0.05c	6.70ab	2.89bcd	83.33a	10.00c
H.V	0.61d	0.64c	5.92a	3.83c	2.08d	2.26bc	0.17c	0.12bc	0.14d	0.10abc	5.08c	3.50abc	16.67d	16.67bc

Common letters(S) in each column means no significant difference between genotypes for that specific character in the row.

The chromosomes 4H (0.24), 5H (0.24), 6H (0.24), and 7H (0.24) had the most SPG value showing the highest effect on SPG. The chromosomes 4H (1.58) and 5H (1.34) had the highest contribution to controlling the GV trait. The addition lines 4H (6.80) 5H (6.81) 6H (6.88), and 7H (6.82) had the most impact on the GI trait. The 7H (90) and Chinese Spring (83.33) had the greatest role in genetic control of the GP index.

The comparison of the wheat-barley addition lines in the -8 bar osmotic stress experiment was shown in Table 6. The chromosome 5H (1.28) had the greatest value for the VG trait. The highest amount of MTG belonged to 2H (7.56) and 4H (7.15). The highest MDG index (4.26) was significantly related to the 5H. The chromosome 5H (0.15) had the highest value for the SPG trait. However, there was no significant difference between chromosomes 5H and 4H, 6H, and 2H, indicating a similar effect on the SPG trait. The 5H (0.21) had the highest GV value, although there was no significant difference between chromosomes 1H and 4H and 6H, showing the same role to control the SPG trait. The addition line 5H (4.49) had the greatest amount of GI and there was no significant difference between 5H and 4H (3.84) and 2H (3.44), having the most effect on the GI trait. The 5H (40) and 1H (31.67) had the greatest role in genetic control of the GP index.

The dendrogram based on the germination characteristics for two stress conditions by Ward method is presented in Figure 5. All entries were classified into three groups. The first group included the barley parent with 2H and 3H lines, which had the weakest performance based on the measured characteristics under osmotic stress conditions. The second group consisted of 4H and 5H lines, holding the high values in most germination traits under osmotic stress conditions. The third group consisted of the recipient parent with 1H, 6H, and 7H lines, which had a moderate impact on most germination traits.

Discussion

The barley chromosomes in the addition lines was confirmed by karyotype matching. The study of metaphase cells of wheat-barley addition lines showed the presence of additional chromosomes for all lines. The Tjio and Hagber method (32) was used to identify all seven pairs of barley chromosomes (1H, 2H, 3H, 4H, 5H, 6H, 7H) transmitted to the genetic background of wheat. In this study, the main factor to discriminate the karyotypes H.V and H.Vs was the short arm length and the mean of the total length of the chromosome traits. Other karyotype traits were identical and all chromosomes were metacentric.

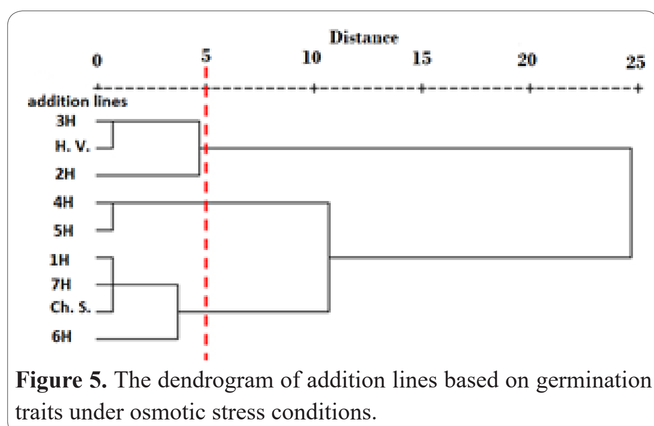

Figure 5. The dendrogram of addition lines based on germination traits under osmotic stress conditions.

Nearly half of the Hordeum species being polyploids (tetraand hexaploids), including allo- and autopolyploids, the genus *Hordeum* is a good model to study speciation through polyploidization (33).

Development of addition lines will help researchers speed up the process of different genes transfer and chromosome mapping of genes. In addition, gene transfer through addition lines greatly reduces the likelihood of negative and harmful gene transfer to the recipient plants (3, 33).

To identify the alien chromosome in wide crosses, different indices including Agro-morphological, cytogenetic, biochemical, and molecular markers have been reported. Molecular markers and molecular cytogenetic markers are being upgraded to continue (3). The desirable genetic diversity was observed among the addition lines based on the ISSR marker. The IS_{10} and IS_{15} primers showed a better polymorphism. In this study, these primers have been amplified by the chromosomes 2H, 3H and 7H. The 1H line had a high distance with the donor and recipient parents according to the cytogenetic and molecular marker methods. The PIC varies from zero to 0.5, and the high amount of PIC indicates the Moderate polymorphism for that locus in the studied genotypes. The IS_{10} and IS_{15} primers were fast in determining the genetic distance between addition lines, and the primers IS_{12}, $IS_{13,}$ and IS_{16} with the lowest PIC found to be slow. The primers used in this study amplified more regions of the barley chromosomes 2H, 3H, and 7H. Based on the studied primers, the 2H, 3H, and 7H lines were more similar to the donor parent.

Identification of the genes responsible for drought tolerance in barley (*Hordeum vulgare* L.) will facilitate understanding of the molecular mechanisms of drought tolerance, and also assists the genetic improvement of barley through marker-assisted selection or gene transformation. Determining the most tolerant variety by using the ISSR markers can be used as a screening basis for drought tolerance in breeding programs (2). Molecular characteristics of barley (*H. vulgare* L.) genome for drought-tolerant cultivars selection were studied using SSR and ISSR markers. Based on these markers, drought tolerance and drought-sensitive genotypes were identified. The presence of late embryogenesis abundant (LEA) genes was correlated with the drought tolerance in the studied cultivars; hence these genes confer drought tolerance trait (34). Wheat-Barley disomic addition lines have been used to evaluate gene expression and physiological mapping of barley. Of the 4014 transcripts detected in barley 365, 271, 265, 323, 194, and 369 were detected in wheat–barley disomic chromosome addition lines 2(2H), 3(3H), 4(4H), 7(5H), 6(6H), and 1(7H), respectively, (35). The CYP710A8 (cytochrome P450 subfamily) genes were mapped on chromosome 3 in barley (3H) and wheat (3A, 3B, and 3D), and the expression of CYP710A8 genes increased in the 3H addition line, indicating that it is responsible for stigmasterol accumulation. (36).

In this study, the significance of the interaction between addition lines × stress levels was indicative of the different reactions of the chromosomes at a dif-

ferent level of stress. The interaction between genotype and environment is the reaction of different genotypes in the environments, and such an interaction causes a reduction in the relationship between phenotypic expression and genotypic values. Genotype by environment interactions (GEI) can also alter community structures and dynamics (37). For instance, the stability of ectomycorrhizal fungal communities can be altered as a consequence of the interactions of plant genotypes with drought conditions. Assessing and modeling GEI is a key objective in evolutionary science (38, 39).

The overall consideration of the indices using Stress Tolerance index (STI), Germination stress index (GSI), and physiological Multiple Selection Index (MSI) indicated that most of the genes responsible for the inheritance of drought tolerance predictors are located on chromosomes 4H and 5H, hence they can be transferred for the breeding of drought tolerance in barley through chromosome engineering and for mapping QTLs by the molecular breeding procedures. Association between field (STI) and laboratory (GSI) indicators of drought tolerance showed that GSI can be considered as an early selection criterion for drought tolerance (40). Disomic addition lines in which a single pair of chromosomes from related species is added to the full chromosome complement of the recipient, can be used to identify chromosomes carrying the genes controlling drought tolerance indicators and form the starting point for cytogenetic transfer of genetic material into the genotyped under investigation (41, 1). The overall results showed that salinity is a polygenic character, chromosomal locations 4H, 5H and 6H in barley known as gene location for controlling salt tolerance (42). Morgan (43) reported that the most of the genes involved in the inheritance of osmoregulation (RWC) are located on chromosome 4H of barley.

The barley chromosomes in the addition lines was recognized by karyotype matching. The main factor to discriminate the karyotypes H.V and H.Vs was the short arm length and the mean of the total length of the chromosome traits. The desirable genetic diversity was observed among the addition lines based on the ISSR marker. The IS_{10} and IS_{15} primers showed a better polymorphism. In this study, these primers have been amplified by the chromosomes 2H, 3H and 7H. The 1H line had a high distance with the donor and recipient parents according to the cytogenetic and molecular marker methods. The chromosomes 4H and 5H were the most superior in all traits under the osmotic stress conditions. Osmotic stress studies were highly matched with cytogenetic traits. The 3H, 4H, and 6H addition lines were the most similar to barley parent and the difference between wheat and barley was due to the effect of chromosomes 3H, 4H, and 6H. Molecular studies based on ISSR primers were less coordinate with germination tests under drought stress conditions. It would be suggested that QTLs amplified by these primers were not related to drought tolerance in barley.

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