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QTLs Analysis Controlling Physiological Traits of Barley under Arsenic Stress

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ABSTRACT: Arsenic (As) is a metalloid substance that can exist in inorganic or organic form and results in heavy-metal-induced plant toxicity via reactive oxygen species production. A population of 72 F1 double haploids derived from the Morex × Steptoe cross, and the two parental lines ,was used to identify the quantitative trait loci (QTLs) associated with arsenic tolerance, and determine the contribution of each QTL on trait diversity in the barley. This study were carried outunder normal and arsenic stress conditions, in two randomized complete block designs with three replications under hydroponic system at Agricultural Biotechnology Research Institute, Zabol University, Zabol, Iran, in 2013. physiological traits including Chlorophyll content, proline content, water soluble carbohydrate (WSC), relative water content (RWC), cell membrane stability (CMS) and chlorophyll fluorescence (Fo, Fm) were measured. QTL analysis was performed using the genetic linkage map derived from molecular marker of RFLP and QTL cartographer software (version 2.5) with composite interval mapping (CIM) method. In general we found 23 QTLs for the traits (15 and 8 QTL under control and arsenic stress condition, respectively). Phenotypic variations that were explained by these QTLs changed from 11.10 to 27.31. The highest and the lowest phenotypic variations were related to proline content and water soluble carbohydrate (WSC) QTLs (Qpro5H1.nand Wsc6H2.n) in both conditions, respectively. LOD values ranged from 2.66 to 5.14.The lowest and the highest LOD scores were attained for the QTLs of chlorophyll florescence of arsenic stress condition (QFm7H1.s) and proline content in control condition (Qpro5H2.n).QTLs QFo5H.n and QFo5H.s is ranged from 148.8cM of chromosome 5H, controlling Fo and QTLs Qch7H.n and Qch7H.s in Location from 14.01cM of chromosome 7H, controlling Chlorophyll contents were quite stable. Therefore, if this result would repeat in different environment, years and genotypes, it can be used in marker assisted selection.

Key words: Arsenic stress, Barley, Physiological traits, QTL mapping

INTRODUCTION

Among the heavy metals (HM), arsenic is one of the major environmental health concerns for human. For plants, arsenic is a nonessential element and is toxic to them. Arsenic is readily taken up by crop plants, enters food chain and causes food Safety problem. It is mobilized in the environment through a combination of volcanic emissions, combustion of fossil fuels and the use of arsenical pesticides and herbicides. Arsenic toxicity is associated with interference in the ATP synthesis, protein degradation, induction of apoptosis and inhibition of enzymatic activity in plants (Sinha *et al*., 2013). Trivalent arsenical species have high affinity for sulfhydryl groups and can bind to reduced cysteines in peptides and proteins, deactivate some enzymes and that results in root dysfunction (Kuramata *et al*., 2013).Leaf chlorophyll content is a good indicator of photosynthesis activity, mutations, stress condition and nutritional status of plants (Naumann *et al*., 2008). Iron (Fe) plays a vital role in many enzymatic reactions of chlorophyll synthesis. Iron absorption and transport is more affected by arsenic toxicity in comparison to other micronutrients, which induces Fe deficiency chlorosis in plants (Munns and Tester, 2008). Chlorophyll content decreased in sorghum and barley shoots due to a Fe shortage in response to arsenic toxicity (Shaibur *et al*., 2008; Shaibur and Kawai, 2011). Mechanisms of arsenic tolerance and detoxification in plants is including root exudates, and transporting arsenic-glutathione conjugates in to vacuoles(Sally *et al*., 2010). Mohammadi *et al*., (2008) reported 8 QTLs, which were involved in controlling the chlorophyll content on chromosome 2, 3, 4 and 7 in a double haploid population of barley. Photosystem II activity, evaluate through chlorophyll fluorescence. Photosystem II is involved in the electron transport chain which is very sensitive to environmental stresses (Guo *et al*, 2008; Gu *et al*, 2012). A significant correlation was reported between the fluorescence index and carbon fixation under stress conditions, in rice (Guo *et al*., 2008), alfalfa (Smethurst and Shabala, 2003), soybean (Yin *et al*., 2010) and wheat (Zhang *et al*., 2010).

Today, proline accumulation in different organs of plants is one of the assessment indexes for selection of varieties under stress condition, (Leinhose and Bergman1995). Aminoacids (AAs) play significant roles in metal binding, antioxidant defense, signaling and and accumulation of these elements in plants during heavy metal stress. Under mild arsenic stress, the amino acids content, specifically proline, increases and these factors play an important role in reducing the accumulation of arsenic in plant (Kumar *et al*., 2014). Almost all morphological and physiological traits that affect the yield are quantitative traits. The genes that contribute to these complex traits are usually several in number (polygenes) and the classic genetic is not able to investigate the gene behavior in controlling them (Wang, 2003).Most of these traits are difficult to use in breeding due to high genotype-by-environment interaction. Arsenic-induced changes in physiological and morphological traits of plants are useful to investigate how plants show tolerance against arsenic toxicity (Shaibur and Kawai, 2011). Selection at genetic level is now possible with the identification of quantitative trait loci (QTL) associated with the stress, but genotype-by-environment interactions are still a problem, since QTL analysis depends on phenotypic characteristics, which in most cases is influenced by the environment. The efficiency of QTL is highly dependent on appropriate phenotype, as well as the traits that selected for screening and their heritability (Wo´jcik- Jagla *et al.* 2013). Due to the rapid growth in the field of genetic information for QTL and transmission stages of information to the plants with different methods, the main problem in transfer of QTLs by using marker-assisted backcrossing, is complete various information and how to interpret them (Khalily *et al*, 2014).Determine the number and the types of genes controlling the quantitative traits such as yield, and its related quantitative traits is an important step in plant molecular breeding (Cooper *et al*., 2009). Barley (*Hordeum vulgare* L.) is the world's fourth most important cereal crop after wheat, rice and maize. It is grown in a range of extreme environments and has adapted to diverse environmental conditions (Anonymous, 2013). Barley has been widely used in different QTL analyses with only seven pairs of chromosomes, (Ahmed *et al*. 2013; Close *et al*. 2009). Several QTLs linked to physiological traits of double haploids lines derived from a cross between Morex and Steptoe, identified under saline conditions (Aminfar *et al*., 2011; Siahsar and Narouei , 2010) and drought stress (Fakheri and Mehravaran, 2014).

Hydroponic is a method of growing plants using mineral nutrient solutions, in water, without soil. A popular variation of hydroponic systems is the nutrient film technique or NFT (Allan Cooper, 1965), whereby a very shallow stream of water containing all the dissolved nutrients required for plant growth is recirculated past the bare roots of plants. Main advantage of NFT hydroponics is the capability of producing very high yields and flow hydroponics include the relatively low start-up cost and ease of use (Domingues *et al*., 2012). Marker assisted selection could make it possible to study the traits of plantlets, and analyzing several traits on a single DNA sample, then using markers is a faster technique compared to investigating the plant phenotype. Marker assisted selection may solve problems associated with genotype × environment interactions and improve the selection efficiency to find arsenic tolerant plants. The objective of the present study was identifying QTLs involved in genetic variation of physiological traits, assessing the genetic effect of each QTL on quantitative traits and determining the molecular markers linked to QTLs in order to apply marker assisted selection in future.

MATERIALS AND METHODS

A population of 72 F1-derived doubled haploid lines (DH) from the cross "Steptoe/Murex" and their two parents were utilized to determine the quantitative trait loci (QTLs) associated with arsenic tolerance. The DHs were developed through a modified *'Hordeum bulbosum*' technique, as described by Chen and Hayes (1989), by the Oregon State University Barley Breeding Program and were kindly provided by Hayes (Department of Crop and Soil Science, Oregon State University, Corvallis, USA). This experiment was carried in two randomized complete block designs with three replications at the Faculty of Agriculture, University of Zabol, Zabol, Iran in 2013. For this purpose, a sheet of Whatman filter paper was laid in the petri dishes (15 cm diameter petri dish) and disinfected seeds were placed on wattman paper in Petri-dish,. Germinated seeds were moved to a hydroponic system and nourished with Hoagland nutrient solution. Macro nutrients were added to the solution. Micro-nutrients were provided from a prepared stock solution and iron from a chelated-iron solution (Table 1). A 67 µmol sodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) was applied as stress treatment. Plants exposed to arsenic stress for a week and then physiological traits were measured. Chlorophyll content and chlorophyll fluorescence were measured using a chlorophyll meter (Hansatech-Model-cl-ol) and a chlorophyll fluorometer (Handy PEA) respectively (Habash *et al*., 1985). Relative water content (RWC) was calculated by the following equation (Pessarakli, 1999) RWC = (LFW-LDW) / (LTW-LDW) × 100 where LFW, LTW and LDW are leaf fresh weight (g), leaf turgid weight (g) and leaf dry weight (g), respectively. Cell membrane stability (CMS) estimated by placing 10 leaf disks in the vials containing 20 ml of distilled water for 24 hours and measuring the electrical conductivity of the solution. Combined analysis of variance was performed using proc GLM procedure in SAS with ver. 9.2 (SAS Inst. Inc., Cary, Nc.).

The RFLP map and the S × M bin map downloaded from the. NABGP website (http://barleygenomics.wsu.edu/ arnis/linkage maps/maps-svg.html) and used for mapping of arsenic tolerance traits. This map comprises 327 markers with an average density of 3.75 Cm (Kleinhofs *et al*., 1993; Hayes *et al*., 1993). QTL analysis was conducted separately for each trait in each condition. Analyses were performed using Win QTL cartographer 2.5 (Wang *et al*., 2007). Composite interval mapping (CIM) was used to detect QTLs and their effects. The lowest LOD score and the lowest walking speed chosen for QTL analyses was 2.5 and 0.1 cM, respectively. Composite interval mapping (CIM) was employed to detect QTLs and estimate the magnitude of their effects (Jansen and Stam, 1994; Zeng, 1994) using model 6 of the Zmapqtl program module.

The percentage of phenotypic variance explained by a specific QTL value (R2) was taken as the peak QTL position as determined by Win QTL cartographer 2.5. The LOD peaks were considered to indicate the most likely position of QTL effects.

Table 1: Composition of Hoagland nutrient solution (Taiz and Zeiger, 1998).

Macro elements	The Molecular weight (g/mol)	Concentration of basic solution (g/l)	The volume of basic solution(ml)
KNO_3	110.10	110.10	6
$Ca(NO_3)_2$-$4H_2O$	236.16	236.16	4
$NH_4H_2PO_4$	115.08	115.08	2
$MgSO_4$-$7H_{2O}$	246.48	246.49	1
Microelements	-	-	-
KCl	74.55	1.864	2
H_3BO_3	61.83	0.773	2
$MnSO_4$-H_2O	169.01	0.169	2
$ZnSO_4$-$7H_2O$	287.54	0.282	2
$CuSO_4$-$5H_2O$	249.68	0.062	2
H_2MoO_4(85%MoO_3)	161.97	0.040	2
NaFeDTPA(10%Fe)	558.50	30	0.3-1

*, ** and ^{ns} Significant (α= 5%), highly significant (α= 1%) and non significant, respectively

RESULTS AND DISCUSSIONS

Combined analysis of variance of 72 doubled haploid lines and their parents ('Steptoe' and 'Morex') showed that effect of environment and Genotype were highly significant (P≤0.01) for all of the studied traits. Also Effect of Genotype × Environment was highly significant for all traits except for RWC (Table 2). A high proportion of the genetic diversity was observed for the physiological traits of double haploid lines, which is useful in screening arsenic tolerante genotypes. The same results were reported by Fakheri and Mehravaran (2014) and Khalili *et al*. (2014).

A. QTL analysis under control and arsenic stress conditions

The results of QTL analysis for 72 double haploid lines under control and arsenic stress condition are presented in table 3 and 4. In general we found 23 QTLs for all studied traits (15 and 8 QTL under control and arsenic stress condition, respectively). Phenotypic variations that were explained by these QTLs changed from 11.10 to 27.31. The highest and the lowest phenotypic variations were related to proline content and water soluble carbohydrate (WSC) QTLs (Qpro5H1.nand Wsc6H2.n) in both conditions, respectively.

Table 2: Analysis for different trait of 72 barley doubled haploid lines and their two parents (Steptoe × Morex) in normal and arsenic stress conditions.

		Mean squares						
S.O.V	**DF**	**Chlorophyll contents**	**Prolin**	**WSC**	**RWC**	**CMS**	**Fm**	**FO**
Environment	1	8234.95**	44413.69**	97.83**	19837.83**	36205.58**	1.4**	452.5**
Block (environment)	2	0.933^{ns}	0.0026^{ns}	0.007**	90.65**	0.415^{ns}	0.002**	0.034^{ns**}
Genotype	73	38.85**	402.57**	0.28**	478.83**	183.74**	0.035**	4.56**
Genotype × Environment	73	20.91**	181.34**	0.061**	519.64^{ns}	88.55**	0.008**	1.68**
Error	146	0.512	20.91	0.001	28.32	1.36	0.001	0.027
C.V (%)		6.84	6.85	7.71	6.02	5.16	4.20	6.58
R^2 (%)		98.8	99.9	99.6	91.8	99.2	94.2	99.1

Table 3: Detected QTLs for different traits in 72 barley doubled haploid lines and their two parents (Steptoe × Morex) in normal conditions.

Plant traits	QTL	Chromosome	Nearest marker	QTL position[a]	QTL interval (95%)	LOD score[b]	Allelic effect	R^{2c}	Total R^{2d}
Chlorophyll contents	$Qch2H_1.n$	2H	Bbe54D	80.21	79.8 -82.20	2.79	-1.38	12.38	41.28
	$Qch2H_2.n$	2H	ABC152D	90.21	88.20 -94.70	4.06	-1.64	17.33	
	Qch7H.n	7H	BCD129	14.01	12.30 -80.15	2.85	-2.50	11.57	
Proline content	$Qpro5H_1.n$	5H	ABG473	114.21	101.70 -132.40	4.27	2.40	27.31	52.96
	$Qpro5H_2.n$	5H	ABG390	183.11	182.20 -190.50	2.66	1.84	13.59	
	$Qpro5H_3.n$	5H	ABG314E	197.21	196.50 -202.90	2.85	1.69	12.06	
WSC	QWsc3H.n	3H	MWG584	29.31	20.10 -32.10	4.53	-0.085	18.42	96.05
	$QWsc6H_1.n$	6H	ABG458	47.81	47.20 -51.60	3.16	0.084	12.28	
	$QWsc6H_2.n$	6H	ABC169E	52.81	51.80 -55.80	2.68	0.083	11.10	
	$QWsc7H_1.n$	7H	WG789A	24.11	16.40 -26.70	5.03	0.10	20.79	
	$QWsc7H_2.n$	7H	dRcsl	32.21	29.50 -34.90	4.54	0.10	20.16	
	$QWsc7H_3.n$	7H	ABC156d	65.61	64.30/ -74.70	2.97	-0.082	13.30	
CMS	Qcms7H.n	7H	MWG555a	5.71	0.00 -16.70	3.66	1.55	17.65	17.65
Fo	QFo5H.n	5H	ABG712	148.8	142 -155.40	2.79	0.025	12.26	25.15
	QFo6H.n	6H	ABC170E	74.61	70.40 -75.70	2.83	-0.025	12.59	

[a]QTL position expressed in cM, from origin of the linkage group (end of short arm); [b]peak value of the LOD; [c]proportion of phenotypic variance explained by the QTL.
[d]total phenotypic variance explained by the model.

LOD values ranged from 2.66 to 5.14.The lowest and the highest LOD scores were attained for the QTLs of chlorophyll florescence of arsenic stress condition (QFm7H1.s) and proline content in control condition (Qpro5H2.n). Leaf chlorophyll content is one of the most important physiological traits related to photosynthesis activity in plants under stress. Since the most important morphological and physiological traits affecting yield are quantitative and gene blocks are involved in the incidence them, classic genetic is not able to investigate the behavior of genes controlling quantitative traits as separate genes. Three QTLs were identified for chlorophyll content in normal condition that had significant additive effects and were detected and mapped to chromosomes 2H, 2H and 7H, that each of them explained the 12.23, 17.33, 11.57 % and a total of 41.13% of the total phenotypic variation, respectively.

Table 4: Detected QTLs for different traits in 72 barley doubled haploid lines and their two parents (Steptoe × Morex) in arsenic stress condition.

Plant traits	QTL	Chromosome	Nearest marker	QTL position [a]	QTL interval (95%)	LOD score [b]	Allelic effect	R^{2c}	Total R^{2d}
Chlorophyll contents	Qch7H.s	7H	BCD129	14.01	12.00-15.50	3.11	0.054	18.7	28
Proline content	QPro2H.s	2H	WG516	21.60	16.10- 25.80	2.74	5.45	16.50	21
WSC	QWsc3H.s	3H	ABG316A	3.11	0.00-11.10	3.41	-0.116	17.05	35.54
CMS	Qcms2H.s	2H	ABG703b	7.01	5.40-17.90	3.01	-4.47	14.10	33.74
Fm	QFm5H.s	5H	MWG813a	194.51	192.10- 195.50	3.33	-0.24	15.16	36.28
	$QFm7H_1$.s	7H	ABR303	6.71	6.40 – 14.00	5.14	-0.36	24.82	36.29
	$QFm7H_2$.s	7H	dRCSL	30.21	29.90 -31.90	3.19	0.27	14.44	36.28
Fo	QFo5H.s	5H	ABG712	148.81	142.70 – 155.20	2.97	0.37	14.14	32.56

[a]QTL position expressed in cM, from origin of the linkage group (end of short arm); [b]peak value of the LOD; [c]proportion of phenotypic variance explained by the QTL.
[D]total phenotypic variance explained by the model.

These QTLs were located near Bbe54D, ABC152D and BCD129 markers at n 80.21, 90.21 and 14.01 cM. A QTL were detected for chlorophyll content in stress conditions. This QTL located on chromosomes 7H, near BCD12 markers at 14.01 cM, which explained about 11.57 % of phenotypic variation. Wang *et al*. (2003) detected 6 QTLs associated with chlorophyll content that mapped on chromosome 1 and 4. Mohammadi *et al*., (2008) reported that 8 QTLs were involved in controlling chlorophyll content on chromosome 2, 3, 4 and 7 in a double haploid population of barley.

Five QTLs were linked to chlorophyll content of double haploid barley (derived from the cross between Morex and Steptoe), was identified at saline conditions in a hydroponic system, which explained 68.24 percent of phenotypic variation (Aminfar *et al*., 2011).Wang et al. (2003) stated that the QTL controlling chlorophyll content located in different genomic regions at different stages of growth.

Three QTLs were detected for leaf proline content on chromosome 5H at 80.21, 90.21 and 14.01 cM. Major QTL, Qpro5H1.n, with LOD score of 4.18 justified largest share of the total variance. The role of two other QTL, Qpro5H2.n and Qpro5H3.n, were less that, in general 25.65 % of variation was explained. In arsenic stress condition, this QTL located on chromosome 2H at 21.60 cM. Qpro2H.s had WG516 marker with a phenotypic variance of 16.50 and an additive effect of 5.45. Amino acids during of heavy metals stress periods including arsenic, play significant role in metal binding, antioxidant defense and nutrient accumulation in plants. In mild arsenic stress, the amino acids content, especially proline, increases and results in lower as accumulation in plant tissues (Kumar *et al*., 2014). In the previous studies (Christopher *et al*., 2008) it was shown that proline alleviate the effect of NaCl and drought stress on carbon dioxide fixation and Rubisco activity in crops. Aminfar *et al*. (2011), detected two QTLs for proline content which accounted for 28.21% variance in double haploid lines derived from a cross between Morex and Steptoe, under control and saline conditions. In another study (Fakheri and Mehravaran, 2014), four QTLs were detected on chromosome 1H, 2H, 2H and 5H which associated with leaf proline content in well-watered and drought conditions. Six QTLs (Qwsc3H.n, Qwsc6H1.n, Qwsc6H2.n, Qwsc7H1.n, Qwsc7H2.n and Qwsc7H3.n) were detected on chromosome 3H, 6H, 6H, 7H, 7H and 7H which associated with total soluble sugars (TSS). These six QTLs explained a high percentage of phenotypic variance (96.06) major effect QTL of Qwsc7H1.n with a peak close to WG789A marker had a LOD score of 5.03 and accounted for the highest variations of TSS. Under arsenic stress condition, one QTL associated with TSS, was located on chromosome 3H at 3.11 cM. This QTL linked to ABG316A marker and explained 17.05 % of f phenotypic variance. Water-soluble carbohydrates (WSC) Soluble carbohydrates are an important source of carbon for grain filling. Photosynthesis restriction during environmental stress is a reason of soluble carbohydrate loss in crops (Yang *et al*., 2007). Siahsar and Narouei (2010), reported two and three QTLs associated with WSC under different conditions of salinity and mean of two conditions, respectively. Fakheri and Mehravaran, (2014) found three QTLs involved in WSC on chromosome 2H and 5H. Under stress conditions, degradation of membranes occurs and more content will leak out of the cell. Lipid peroxidation is considered to be one of the reasons for membrane deterioration in stress conditions (Popova *et al*., 2009). Only one QTL for cell membrane stability (CMS) was located on chromosome 7H at 5.71 cM. The QTL linked to MWG555a marker and controlled about 17.65% of phenotypic variation in control condition and had an additive effect of 1.55. Under arsenic stress condition, one QTL (Qcms2H.s) was mapped on chromosome 2H near ABG703b marker and accounted about 14.10% of phenotypic variations of CMS with a negative additive effect.

Chlorophyll fluorescence is a measure of photosynthetic performance and is widely used as a tool for the evaluation of environmental stress (Behra *et al*., 2002; Grafts *et al*., 2004). Photosystem II (PS II) involved in electron transport chain and is highly sensitive to environmental stresses. Photosynthesis rate, the capacity of NADPH and ATP pools and quantum yield of PS II, decreases by environmental plants (Maxwell and Johanson, 2002). Under saturating light intensities, when the reaction centers of chlorophyll are in an active, open state and the fluorescence yield is minimal (Fo) (Maxwell and Johnson, 2002). Under control condition, two QTLs (QFo6H.n and QFo5H.n) for Fo detected and mapped on chromosome 5H and 6H at 74.61 and 148.81 cM which accounted about 24.85% of phenotypic variations with a negative additive effect. Only one QTL (QFo5H.s) for Fo detected on chromosome 5H at 148.81 cM near ABG712 marker and controlled about 14.14% of variances with an additive effect of 0.037, Under arsenic stress condition. Detected QTLs explained about 24.85 and 32.65% of total variation of Fo in control and arsenic stress conditions, respectively. Siahsar and Narouei (2010) detected three QTLs associated with Fo on chromosome 5H in saline stress. Exposing to a high intensity, short flash of light, catalyzes the reduction of all primary quinone acceptor of chloroplast. This transiently closes all PSII reaction centers, which prevents energy of PSII being passed to downstream electron carriers. During the flash, the fluorescence reaches the level reached in the absence of any photochemical quenching, known as maximum fluorescence (Fm) (Maxwell and Johnson, 2002). Under arsenic stress condition, three QTLs associated with Fm on chromosome 5H, 7H and 7H at 194.51, 6.71 and 30.21 cM that accounted about 54.42% of variations for this trait. The LOD score for these QTLs were 3.33, 5.14 and 3.19, respectively. The additive effects of these QTLs were -0.24, -0.36 and 0.27 respectively. Siahsar and Narouei (2010) detected six QTLs associated with Fm on chromosome 5H under saline conditions. In another study four QTLs were detected for Fm variations on chromosome 2H and 5H, at well-watered and drought conditions (Fakheri and Mehravaran, 2014).

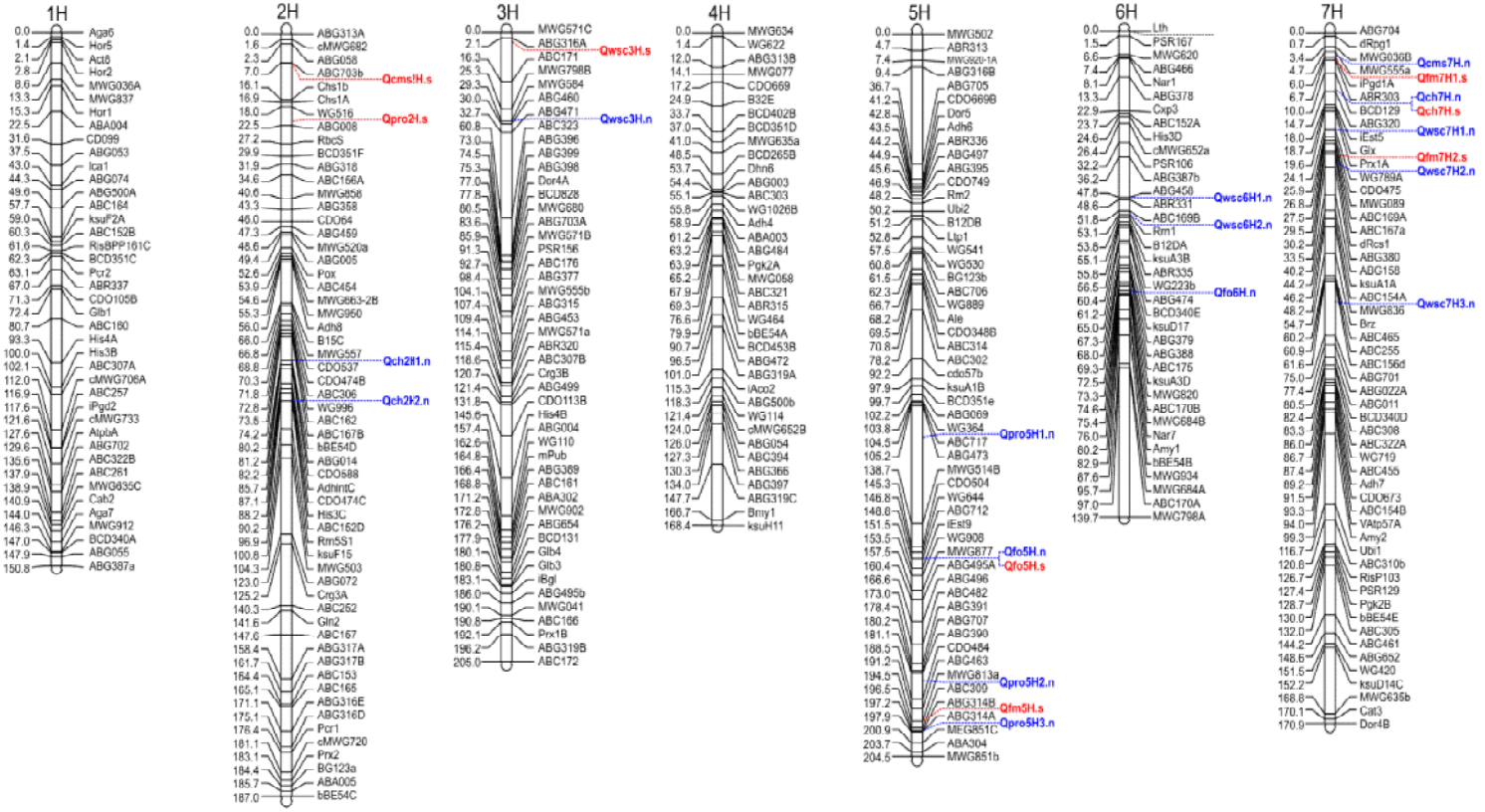

Fig. 1. QTLs of heavy metals absorption traits in 72 barley doubled haploid lines in non-stress and arsenic stress conditions.

Fluorescence index was used as a tool to detect QTLs under drought stress in wheat (Czyczylo-Mysza *et al*. 2011), rice (Gu *et al*., 2012) and barley (Guo *et al.,* 2008; Wo´jcik-Jagla *et al.,* 2013) and also under Saline conditions in barley (Aminfar *et al*., 2011). Czyczylo-Mysza *et al*. (2013) have been used QTLs for leaf chlorophyll fluorescence parameters to determine the efficiency of photosynthesis in wheat and its association with biomass productivity and yield. Although QTL studies provide useful information about the genetic factors that control trait inheritance, there are limitations in applying this method like low heritability of some traits, and performing accurate experiments and analyzing models for identification of QTLs within breeding programs (Shahinnia *et al*., 2014). Using segregating populations, saturated molecular map and proper experimental designs have solved this problem to some extent. Selecting the major and stable QTLs is a proper method to screening paternal lines in breeding programs to enhance productivity of agronomic traits. In the QTL analysis, the value of additive effect has a great effect on genetic variations of genotypes (Zhu *et al*., 1999). Discoverin and transfering of valuable QTL alleles from unadapted donor lines into established elite inbred lines and pyramiding QTL alleles in a genotype stated as suitable methods in performing marker assisted selections (Dadley, 1993 and Zhu *et al*., 1999). QTL analysis is difficult in polyploid crops like wheat. Due to its simple inbreeding diploid genetics barley is an excellent experimental model for other cereals such as wheat, which have more complex polyploid genomes and genetics. It is possible to identify homologous regions for QTLs in cereals using the same types of markers to construct the genetic map (Bezant *et al*., 1997). The aim of plant breeders is producing genotypes with a high and stable yield production under different environmental conditions (Yadav *et al*. 2003). This is the objective of barley breeders as it is grown in a range of extreme environments and have adapted to diverse environmental conditions. QTLs QFo5H.n and QFo5H.s is ranged from 148.8 cM of chromosome 5H, controlling Fo and QTLs Qch7H.n and Qch7H.s in Location from 14.01 cM of chromosome 7H, controlling Chlorophyll contents were quite stable. Producing a stable yield is possible via finding the QTLs with the least Q×E interaction and producing genotypes adapted to different environmental conditions via pyramiding the QTLs. In considering the above factors, the first important factor for plant breeding is the stability of detected QTLs. Stable QTLs show relative gene expression stability and is able to overcome the Q×E interaction. In the present study, almost all detected QTLs were unstable; in other word different QTLs were associated with a particular trait under control and arsenic stress conditions.

It is important for multiple-environments analysis QTLs to detect environment-dependent QTLs (Siahsar and Naroui, 2010). In addition, different error values in different experiments, may also lead to QTL instability. QTL analysis using a variety of genotypes and populations under a range of locations and over several years is needed to introducing stable QTLs.

CONCLUSION

The present study was the first investigation on QTL analysis of physiological traits of double haploid population of barley under the arsenic stress condition in Iran. In total, 23 QTLs were detected for studied traits. QTLs QFo5H.n and QFo5H.s is ranged from 148.8 cM of chromosome 5H, controlling Fo and QTLs Qch7H.n and Qch7H.s in Location from 14.01 cM of chromosome 7H, controlling Chlorophyll contents were quite stable. Therefore, if this result would repeat in different environment, years and genotypes, it can be used in marker assisted selection.

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