Biological Forum – An International Journal **7**(1): 1277-1289(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Mapping Genomic Regions Controlling Heavy Metals in Barley under Nickel Stress

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(Received 28 March, 2015, Accepted 28 April, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: In order to map genomic regions controlling traits related to uptake of heavy elements in the Steptoe/Morex doubled haploid lines of barley with their parents under nickel stress and non stress conditions, an experiment was conducted, at the Institute of Agricultural biotechnology (Biocenter), University of Zabol, in two randomized complete block design with two replications, in 2014. Heavy metals absorption traits, including Root's Ni, Shoot's Ni, Root's Ni/Shoot's Ni, Root's Zn/Shoot's Zn, Root's Cu/Shoot's Cu, Root's Mn/Shoot's Mn and Root's Fe/Shoot's Fe ratios were measured. QTL analysis was carried out using genetic linkage map derived from 327 molecular marker of RFLP and QTL cartographer 2.5 software with composite interval mapping method. Analysis of variance showed significant difference between the lines for all the studied traits in nickel stress and non stress conditions. The maximum correlation in non stress and stress conditions were observed between Root's Ni/Shoot's Ni ratio, respectively. We found 20 QTLs (12 and 8 QTLs for non stress and nickel stress conditions, respectively.) for the studied traits. Phenotypic variances that were explained by these QTLs changed from 10.84 to 22.70, respectively, for Root's Ni/Shoot's Ni ratio (QNi-r/s4H.2n) and Root's Ni (QNir2H.2n) in non stress condition. LOD scores were ranged from 2.50 to 4.98, respectively, for the QTLs of Root's Ni / Shoot's Ni ratio (QNi-r/s4H.1s) and Root's Cu/Shoot's Cu ratio (QCu-r/s7H.1n) in nickel stress and non stress conditions. Morex had higher role in transport of effective allele in heavy metal absorption traits. In general, mapped QTLs did not possess required stability in two studied conditions.

Key words: Barley, Heavy metals, Mapping, Nickel stress, QTL

INTRODUCTION

The heavy metals as a result of human being activities such as mining, smelting and galvanizing, energy and fuel production, extreme agriculture, sludge wastes and military operations have a significant role in environmental pollution (Nedelkoska and Doran, 2000). Since the beginning of the industrial revolution, the biosphere heavy metals levels are increasingly growing and toxicity of the heavy metals and environmental pollutions resulting from them cause damage to the agricultural lands (Gisbert *et al.,* 2003). These metals create risks for primary and secondary consumers and ultimately human beings. Nickel is one of the chemical elements that exist in various forms in the environment (rivers, lakes, oceans, soil, air and drinking water, as well as the bodies of plants and animals). Soil and sediments, are the major source of Nickel (Smialowicz *et al.,* 1984). Nickel as a heavy metal plays an important role in plants. This element does not have toxic effect on the plant at low concentrations, but at high concentrations is toxic for them (Baycu *et al.,* 2006). Nickel causes chlorosis in leaf and finally continues as necrosis and then leaf becomes completely dark (Abdel Latif *et al.,* 1988). Monni *et al.* (2001) showed that total amount of the chlorophyll has decreased in leaves of *Phaseolus vulgaris* wax bean that had grown in the mineral form of Nickel in treatment. The reduction of photosynthesis has also been reported in treatment with organic form of Nickel in the cabbage leaves (Molas, 2002). The studies conducted on various forms of Nickel in barley by Molas and Brown (2004) showed that although the degree of toxicity of chemical forms of Nickel was different, the morphological and anatomical pathology types were similar. The role of other heavy elements in the plant is not negligible. Zn deficiency in grains, especially barley, maize and rice is common. The effects of Zn deficiency, growth arrest are gradually achieved and therefore vegetative organs especially leaf as a photosynthetic apparatus get in trouble. As a result of this, the construction of the photosynthesis materials is also disrupted and the formation of reproductive organs damaged and therefore the number of grains per spike and grain weight decreases in the barley.

Therefore, grain yield also decreases by the effect of damage to the components of yield. In Graham and MacDonald study (2000), the increased Zn showed the effect of this element in the improvement of yield and seed weight in wheat. The amount of Cu in plants is usually equal to 2-20 ppm in dry matter. Cu uptake by plants primarily depends on the ionic concentration amount of the soil solution and the ionic competition of other cations in uptaking this element is inconsequential (Chaudhry and Loneragan, 2000). Approximately, 70% of Cu can be found in chloroplasts (Alturk and Helal, 2004). Cu deficiency in wheat causes spikes shrinkage and the seed at the end of the spikes is not even formed (Graham and Mcdonald, 2000). Mn contributes in composition of photosynthesis and respiration enzymes and prevents nitrate accumulation in plant tissues. The growth reduction, yellows, loss of plant height, pollen grains sterility and reduced number of tiller per plant are of Mn deficiency complications (Ziaeian and Malakoti, 1988). Fe by establishing appropriate vegetative growth through increasing the leaf number and area, participation in photosynthesis, increased height and dry matter provides the formation and development of yield components and consequently grain yield. Although Fe does not participate in chlorophyll structure, its deficiency causes a decrease in chlorophyll amount, finally the green color of the leaves tend to be yellow that this phenomenon is called chlorosis (Pinto *et al.,* 2005). The conventional plant breeding methods have very useful achievements for improving agronomic traits in barley. But, there is need for communities' purity and access to advanced breeding generations in order to select superior lines. The development of molecular markers technology has made it possible to provide high-density genome linkage maps for lots of crops including barley. The selection in early generations of breeding programs has been made possible and its efficiency meliorated with the rapid growth of dense linkage maps based on molecular markers and finding a place for quantitative trait loci (QTL) and use it by marker assisted selection (MAS) (Han *et al.,* 1997, Ayoub *et al.,* 2003). In this method, the co-segragation of the quantitative traits and molecular markers is examined and finally, the number of genes (effective factors), their performance type, and the effect of each one is estimated and QTL location is detected on the genome. The QTL knowledge, the number and effects of QTLs can help breeders to understand the genetic control of plant traits and selection assistance in order to improve and breed the plants (Broman and Speed, 1999). In a study using Atomic Absorption Spectrometry, mapping of QTL associated with the absorption of Cu, Fe, Mn and Zn was done in wheat shoot under Cu stress that was observed as one of the biggest QTLs in Cu tolerance on the chromosome 5DL (Balint *et al.,* 2007). Therefore, the purpose of this study is QTLs mapping, estimate of the impact of each of them, determination of molecular markers associated with them and offers them for use in marker-assisted selection in early generations of breeding programs.

MATERIALS AND METHODS

In order to map QTLs of some of the traits related to uptake of heavy elements of barley under nickel stress and non-stress conditions, seventy-two doubled haploid lines with their parents were examined in hydroponics environment. The study population, from F1 hybrids resulting from the Steptoe (CI15229) /Morex (CI15773) conflux by modified Hordeum bulbosum method, analyzed by (Chen and Hayes, 1989), has been prepared by (Hayes, 1992) through barley breeding program of Oregon State University in America. The population along with their parents were planted at the Institute of Agricultural Technology (Biocenter) University of Zabol in 2014. The experiment was conducted in two randomized complete block projects with two replications. First, 72 doubled haploid lines of barley and their parents were carefully disinfected. For this purpose, the seeds were initially washed with water and dishwashing liquid, then were placed in alcohol (96%) for twenty seconds. After washing with distilled water, they were placed in a solution of sodium hypochlorite (diluted in distilled water with the ratio of one to nine) for 50 seconds. Then, they were washed several times with sterile distilled water (Fakheri and Khalegh Babaki, 2014). After this stage, they were soaked in sterile distilled water for 24 hours. Then, the disinfected seeds were cultured in the sterile petri dishes of 15 cm containing Whatman paper. After 10 days, the seedlings that had grown enough in petridishes, were transferred to a hydroponic system containing Hogland nutrient solution. For the more influence, the solution PH was adjusted on the 5 (Peralta Videa *et al.,* 2002). In order to obtain a final solution, the major nutrients (macro elements), separate from the basic solution were added as 1.2 and micronutrients (micro elements), except Fe, had a basic solution. After every 3 days, the nutrient solution given to the plants within each pan was discarded and fresh nutrient solution was replaced. The temperature was set at about 26°C. After a week and the seedlings compatibility with hydroponic conditions, different concentrations of Nickel ($NiCl_2$): 1, 2.5, 5 mM were added respectively by replacing the culture medium every 2 days to the culture medium.

In this study, Root's Ni, Shoot's Ni, Root's Ni/Shoot's Ni, Root's Zn/Shoot's Zn , Root's Cu/Shoot's Cu, Root's Mn/Shoot's Mn and Root's Fe/Shoot's Fe ratios (ppm) were separately measured in roots and shoots on 10 samples of 72 doubled haploid lines of barley and their parents in the 10-leaf stage, in both stress and non-stress conditions, in two replications by Flame Atomic Absorption Spectrometer, model Konik Won M300 (the product of Barcelona, Spain) equipped with a conventional pneumatic nebulizer. A hollow cathode lamp, model Konik-Tech were used for measuring Cu, Zn, Mn, Nickel and Fe. The most sensitive wavelengths were used for the Cu in 324.8 nm, Fe in 248.3 nm, Zn in 213.9 nm, Mn in 279.5 nm and Nickel in 232.0 nm. In order to prepare the samples for measurement by an atomic absorption apparatus, initially, the samples obtained from root and shoot were separately placed inside the oven at a temperature of 74°C for 48 hours until completely dry. Then, each sample was crushed in a porcelain mortar until just be completely shattered. One gram from every shattered sample was poured into the porcelain bush and then every bush was transferred into the furnace. The furnace temperature was adjusted to 650°C for 5 hours. After cooling the furnace, 5 ml of 2N hydrochloric acid was added to plant and was slightly heated on the heater until the acid digestion stage to be completed. The sample was smoothen by two layers filter paper and was brought to volume by 2 time's deionized distilled water (Tabande *et al.,* 2013, Jones *et al.,* 1991). The analysis of variance was performed for nickel stress and non-stress conditions. The simple statistical statistics (descriptive) was estimated. The differences between parents (P1- P2) with LSD resulting from the analysis of parent's variance and the differences between parent's average and average of the doubled haploid lines ($\overline{x}_{DH} - \overline{x}_P$) were compared with LSD resulting from the analysis of doubled haploid lines variance along with parents. The transgressive segregations in a positive and negative direction were calculated by means of $G_P = B_{DH}-B_P$ and $G_N = W_{DH}-W_P$ respectively, in which G_P and G_N are transgressive segregations in a positive and negative direction, and B_{DH} and W_{DH} are the best and worst doubled haploid and B_P and W_P are the best and the worst parent. The transgressive segregations were compared with LSD resulting from the analysis of doubled haploid lines variance along with parents. The private heritability of traits were calculated using the formula (Johnson *et al.,* 1955). The simple phenotypic correlation between traits were calculated for the non-stressed and Nickel stress conditions. The statistical analysis was performed with SAS software, version 9.2. The molecular marker linkage map of barley was retrieved from http://barleygenomics.wsu.edu and was used for mapping traits mentioned (Kleinhofs and Graner, 2001, Fakheri and Mehravaran, 2013, Kleinhofs *et al.,* 1993). This map is fairly saturated, consisting of 327 RFLP markers with a length of 1226.3 and an average interval of 3.75 cM that has been prepared by North American barley genome mapping project (NABGMP) (Kleinhofs and Graner, 2001, Kleinhofs *et al.,* 1993). QTL analysis was separately performed for non-stress and nickel stress conditions. The composite interval mapping (CIM) method was used to determine the QTLs and estimate of their effects (Fakheri and Khalegh Babaki, 2014, Fakheri and Mehravaran, 2013, Mahdinejad *et al.,* 2014). To identify QTLs, at least LOD of 2.5 and scanning distance of 2 cM (software default) were considered. The cofactors were determined by forward-backward regression method. In addition to the determination of QTL and the effect of each QTL, phenotypic variance that was justified by each QTL and by the total QTLs in a multiple regression model was calculated in the peak position of the QTL. The LOD peaks revealed QTL position. QTL effects in the peak position of the QTL and approximately 95% of the QTLs was obtained. To determine whether the two adjacent peaks are representative of single QTL or whether each one is related to a separate QTL, the LOD values decline between the two peaks was used (If there was a fall between two adjacent peaks as LOD≥2 or LRS≥9.21, it should be considered two separate QTL) (Kim and Rieseberg, 1999, Fakheri and Mehravaran, 2013, Fakheri and Khalegh Babaki, 2014, Mahdinejad *et al.,* 2014). Percentage of phenotypic variance justified by each QTL, were determined. The QTL analysis was conducted using the WinQTL Cartographer 2.5 software.

RESULTS AND DISCUSSION

In nickel stress and non-stress conditions a significant difference ($p \leq 0.01$) was observed between the lines in term of all the traits studied (Table 1). This issue implies that there was a considerable diversity in the population studied. Since this population is doubled haploid lines, therefore the variation in the population is mostly due to the increases effects.

Siahsar and Naroui (2010), Aminfar *et al.* (2011) have reported a similar diversity in this population for physiological traits under salt stress in hydroponic environment. The simple statistics (descriptive) of traits studied (Table 2) showed that Steptoe to Marx in non-stress conditions showed greater quantities for the traits of the Root's Ni/Shoot's Ni, Root's Zn/Shoot's Zn , Root's Cu/Shoot's Cu, Root's Mn/Shoot's Mn and Root's Fe/Shoot's Fe ratios and for other traits showed lower quantities.

Table 1: Analysis of variance of 72 barley doubled haploid lines and their parents (Steptoe and Morex) for 7 traits related to the absorbtion of heavy metals in non-stress condition.

S. O. V.	DF	Mean Square						
		Root's Ni (ppm)	Shoot's Ni (ppm)	Root's Ni / Shoot's Ni (ppm)	Root's Zn / Shoot's Zn (ppm)	Root's Cu / Shoot's Cu (ppm)	Root's Mn / Shoot's Mn (ppm)	Root's Fe / Shoot's Fe (ppm)
Block	1	0.10^{ns}	0.0000^{ns}	0.03^{ns}	0.04^{ns}	0.009^{ns}	0.005^{ns}	0.006^{ns}
Line	73	1043.89^{**}	980.49^{**}	0.96^{**}	0.95^{**}	1.51^{**}	2.19^{**}	0.16^{**}
Error	73	7.94	13.94	0.02	0.02	0.007	0.03	0.01
C.V (%)		9.99	13.59	13.09	13.27	7.71	15.09	10.10
R^2 (%)		99.24	98.59	97.50	97.78	99.52	98.44	94.03

Table 1Continued: Analysis of variance of 72 barley doubled haploid lines and their parents (Steptoe and Morex) for 7 traits related to the absorbtion of heavy metals in Nickel stress condition.

S. O. V.	DF	Mean Square						
		Root's Ni (ppm)	Shoot's Ni (ppm)	Root's Ni / Shoot's Ni (ppm)	Root's Zn / Shoot's Zn (ppm)	Root's Cu / Shoot's Cu (ppm)	Root's Mn / Shoot's Mn (ppm)	Root's Fe / Shoot's Fe (ppm)
Block	1	0.72^{ns}	41.40^{ns}	0.04^{ns}	0.06^{ns}	0.003^{ns}	0.003^{ns}	0.009^{ns}
Line	73	26691.98^{**}	37098.94^{**}	1.17^{**}	1.15^{**}	2.15^{**}	0.83^{**}	0.95^{**}
Error	73	40.35	75.57	0.02	0.02	0.0097	0.01	0.008
C.V (%)		6.03	7.70	13.13	13.00	8.28	11.70	7.04
R^2 (%)		99.84	99.79	97.91	98.14	99.55	98.20	99.15

* and ** significant at 5 and 1% probability levels, respectively and ns, not significant

The difference between the parent for traits of Root's Ni, Shoot's Ni at one percent probability level ($p \leq 0.01$) and for traits of Root's Mn/Shoot's Mn ratio at 5 percent probability level ($p \leq 0.05$) were significant and for other traits ($p > 0.05$) were non-significant. In Nickel stress conditions, for traits of Root's Ni/Shoot's Ni ratio, Root's Zn/Shoot's Zn ratio Steptoe showed higher quantities than Marx and for other traits, Marx showed higher quantities than Steptoe (Table 2). The differences between parents for traits of Root's Zn/Shoot's Zn ratio at one percent probability level ($p \leq 0.01$) and for traits of Root's Ni/Shoot's Ni and Root's Fe/Shoot's Fe ratios at 5 percent probability level were significant ($p \leq 0.05$) and for other traits were non-significant ($p > 0.05$). Since the population resulting from the Steptoe and Marx confluence of barley is not made for the routine use to modify and isolate the top lines and the aim of formation of the population is the mapping of seed quality QTLs; therefore, the lack of significant difference between parents for some physiological traits under nickel stress is not unexpected. But, since the lines differences were significant for all studied traits; therefore, a considerable variation exists in the investigated population for studied traits and QTL analysis will lead to identification of the traits controller QTLs. Fakheri and Mehravaran (2014) in the investigation of physiological traits of the population in drought stress conditions reported that the differences between parental lines for all studied traits, except for grain yield were non-significant. Fakheri and Mehravaran (2014) in the investigation of agronomic traits of this population in drought stress conditions reported that the differences between parental lines for all studied traits were non-significant. These authors stated that since the population resulting from the Steptoe and Marx confluence is not made for the routine use to modify and isolate the top lines, this issue is justifiable. The differences between the doubled haploids average and parent's average in non-stress conditions, for the traits of Root's Ni/Shoot's Ni and Root's Cu/Shoot's Cu ratios at one percent probability level ($p \leq 0.01$) and for the traits of Root's Zn /Shoot's Zn ratio at 5 percent probability level were significant ($p \leq 0.05$) and for other traits were non-insignificant ($p > 0.05$). The difference between the doubled haploids average and parent's average in Nickel stress conditions for all traits at one percent probability level was significant ($p \leq 0.01$) except the traits of Root's Zn /Shoot's Zn and Root's Mn /Shoot's Mn ratios that was non-significant ($p > 0.05$). In two conditions, parent's average had been in the progenies variation range and the best and worst genotypes were obtained from each parent. This issue indicated the existence of transgressive segregation in both positive and negative directions. In non-stress conditions, the worst doubled haploid lines compared to the worst parent, showed lower quantities and these quantities for all traits at one percent probability level ($p \leq 0.01$) and for traits of Root's Ni/Shoot's Ni and Root's Cu/Shoot's Cu ratios at 5 percent probability level were significant ($p \leq 0.05$). In stress conditions, the worst doubled haploid lines compared to the worst parent, except the Root's Cu /Shoot's Cu ratio showed lower quantities and these quantities for all traits at one percent probability level ($p \leq 0.01$) were significant and for the trait of Root's Cu/Shoot's Cu ratio at 5 percent probability level ($p \leq 0.05$) and for Shoot's Ni was non-significant ($p > 0.05$). In non-stress conditions, the best doubled haploid lines compared to the best parent, showed higher quantities except Root's Ni /Shoot's Ni ratio, and these quantities for all traits at one percent probability level ($p \leq 0.01$) were significant except Shoot's Ni that was non-significant ($p > 0.05$). In stress conditions, the best doubled haploid lines compared to the best parent, showed higher quantities and these quantities for all traits at one percent probability level ($p \leq 0.01$) were significant. So, for most of the traits there was transgressive segregation in both positive and negative directions. This phenomenon indicates that a lot of positive and negative enhancer and reducer alleles have been scattered between the two parental lines for these traits. In other words, there is diversity for the traits studied, among progenies resulting from the Steptoe and Marx confluence. So, QTL analysis will lead to the identification of one or some QTLs. Siahsar and Naroui (2010), Aminfar et al. (2011) have reported transgressive segregation in this population for physiological traits of barley under salt stress. Fakheri and Mehravaran (2014) for physiological traits in this population and, Fakheri and Mehravaran (2013) for agronomic traits under drought stress have reported transgressive segregation. The phenotypic variation coefficients of all studied traits in both environments were greater than the genetic variation coefficients. But, in many cases, their differences were small (Table 2). This issue reflects the low effects of environmental factors in their estimation. The private heritability of traits in non-stress conditions was in the range of 88.08-99.04% and in nickel stress conditions was in the range of 95.82-99.70. The heritability of under study traits were high, therefore they can be used to select genotypes with high yield. Regarding available changes to some of the traits, it was determined that the selection would be effective for their improvement. However, the efficiency of selection depends on the expected genetic improvement. Those traits which have high heritability and genetic progress, may be controlled by additive effects of genes. Additionally, the high estimates of heritability and genetic progress may be due to the low environmental variance of traits (Panse, 1957).

Table 2: Descriptive statistics, phenotypic and genotypic coefficients of variation, heritability and genetic advance for the absorption heavy elements traits in 72 barley doubled haploid lines and their parents (Steptoe and Morex) for non-stress condition.

Simple statistics	Root's Ni (ppm)	Shoot's Ni (ppm)	Root's Ni / Shoot's Ni (ppm)	Root's Zn / Shoot's Zn (ppm)	Root's Cu / Shoot's Cu (ppm)	Root's Mn / Shoot's Mn (ppm)	Root's Fe / Shoot's Fe (ppm)
Steptoe (P1)	18.29	17.48	1.07	0.61	1.44	1.05	1.15
Morex (P2)	178.14	179.86	0.99	0.97	0.32	0.98	0.96
P1- P2	-159.85**	-162.38**	0.08^{ns}	-0.36^{ns}	1.12^{ns}	0.07*	0.19^{ns}
$\bar{x}_P$= (P1 + P2) / 2	98.21	98.67	1.03	0.79	0.88	1.01	1.06
W_{DHs}	8.52	7.09	0.31	0.29	0.14	0.19	0.42
B_{DHs}	69.34	73.14	3.54	4.54	4.37	6.68	1.91
Range	60.82	66.04	3.22	4.25	4.23	6.48	1.49
$\bar{x}_{DHs}$	26.26	25.49	1.21	1.12	1.11	1.24	1.01
SD_{DHs}	14.65	13.9	0.70	0.69	0.87	1.06	0.29
$C.V_{DHs}$	55.81	51.39	58.40	62.49	78.98	85.77	28.76
$\bar{x}_{DHs} - \bar{x}_P$	-71.95**	-73.18**	0.18^{ns}	0.32*	0.23**	0.23^{ns}	-0.05^{ns}
$G_N=W_{DH}-W_p$	-9.77**	-10.39**	-0.68**	-0.32*	-0.18*	-0.79**	-0.54**
$G_p=B_{DH}-B_P$	-108.8**	-1.6.72^{ns}	2.47**	3.57**	2.93**	5.63**	0.76**
GCV(%)	80.69	80.04	57.14	61.63	78.67	84.39	27.47
PCV(%)	81.31	81.19	58.62	63.05	79.05	85.73	29.27
$GC_{5\%}$	46.64	44.75	1.38	1.36	1.78	2.1	0.52
h2 (%)	98.49	97.19	95.0	94.96	99.04	96.9	88.08

Ns: Not significant, * and **: significant at 5 and 1% probability levels, respectively and: GG_N, downward genetic gain; GG_P, upward genetic gain; B_{DHs}, DH with maximum trait value;W_{DHs}, DH with minimum trait value; Range, Extent of the variation; Bp, parent with higher trait value; Wp, parent with lower trait value; PCV, phenotypic coefficient of variation; genotypic coefficient of variation; $GC_{5\%,}$ genetic gain for 5% selection index; h2, narrow sense heritability

Table 2 Continued: Descriptive statistics, phenotypic and genotypic coefficients of variation, heritability and genetic advance for the absorption heavy elements traits in 72 barley doubled haploid lines and their parents (Steptoe and Morex) for Nickel stress condition.

Simple statistics	Root's Ni (ppm)	Shoot's Ni (ppm)	Root's Ni / Shoot's Ni (ppm)	Root's Zn / Shoot's Zn (ppm)	Root's Cu / Shoot's Cu (ppm)	Root's Mn / Shoot's Mn (ppm)	Root's Fe / Shoot's Fe (ppm)
Steptoe (P1)	49.00	14.68	3.34	1.39	0.04	0.77	0.76
Morex (P2)	326.93	260.26	1.26	1.15	0.47	1.01	0.59
P1- P2	-277.93^{*}	-245.58^{*}	2.08^{ns}	0.24^{ns}	-0.43^{**}	-0.24^{ns}	0.17^{*}
$\bar{x}_P$= (P1 + P2) / 2	187.97	137.47	2.30	1.27	0.26	0.89	0.68
W_{DHs}	7.75	9.37	0.20	0.22	0.06	0.10	0.34
B_{DHs}	387.85	479.10	3.83	4.28	6.38	3.56	3.84
Range	380.09	469.72	3.62	4.06	6.32	3.46	3.50
$\bar{x}_{DHs}$	102.99	112.08	1.18	1.13	1.22	1.06	1.30
SD_{DHs}	113.93	136.49	0.73	0.77	1.04	0.65	0.69
$C.V_{DHs}$	110.62	121.78	62.52	68.08	85.52	61.75	53.38
$\bar{x}_{DHs}- \bar{x}_P$	-84.97^{**}	-25.39^{**}	-1.13^{**}	-0.14^{ns}	0.96^{**}	0.17^{ns}	0.62^{**}
$G_N=W_{DH}-W_p$	-41.25^{**}	-5.31^{ns}	-1.06^{**}	-0.93^{**}	0.02^{*}	-0.67^{**}	-0.25^{**}
$G_p=B_{DH}-B_P$	60.92^{**}	218.84^{**}	0.49^{**}	2.89^{**}	5.91^{**}	2.55^{**}	3.08^{**}
GCV(%)	109.64	120.66	62.95	66.34	87.05	60.68	53.70
PCV(%)	109.81	120.90	64.31	67.60	87.44	61.80	54.16
$GC_{5\%}$	238.02	280.39	1.52	1.52	2.12	1.28	1.4
h2 (%)	99.70	99.59	95.82	96.3	99.1	96.42	98.3

Ns: Not significant, * and **: significant at 5 and 1% probability levels, respectively and: GG_N, downward genetic gain; GG_P, upward genetic gain; B_{DHs}, DH with maximum trait value;W_{DHs}, DH with minimum trait value; Range, Extent of the variation; Bp, parent with higher trait value; Wp, parent with lower trait value; PCV, phenotypic coefficient of variation; genotypic coefficient of variation; $GC_{5\%,}$ genetic gain for 5% selection index; h2, narrow sense heritability

Those traits which do not have simultaneously high heritability and genetic progress, presumably are under control of non-additive gene effects (dominance and epistasis) (Liang and Walter, 1968). In five percent selection severity, for non-stress conditions, expected genetic progress amount that has been expressed as a percentage of the mean varied from 0.52 for the trait of Root's Fe/Shoot's Fe ratio, up to 46.64 for Root's Ni and in Nickel stress condition from 1.28 for the trait of Root's Mn/Shoot's Mn ratio up to 280.39 for Shoot's Ni. In both conditions, the traits of Root's Ni and Shoot's Ni had a very good heritability and a high genetic progress. Thus, in the heritability of these traits there is presumably additive genetic control.

The high heritability and little genetic progress of some traits, showed that presumably noadditive gene effects have a significant share in their inheritance. In non-stress and Nickel stress conditions, there was maximum correlation between the traits of Root's Ni, Shoot's Ni (0.77 and 0.94) ($p \leq 0.01$) (Table 3). The high correlation between heavy elements may be due to isotope of the controller QTLs or cohesion between them. Additionally, the variety in a trait may describe the variety of other traits (peliotropy) the (Siahsar *et al.*, 2008). QTLs and their position, markers, 95% confidence approximation, LOD score, their additive effect and coefficient of determination in non-stress and Nickel stress conditions are shown in Table 4. In total, 20 QTLs was obtained (12 and 8 QTLs for non-stress and nickel stress conditions, respectively) for the studied traits. Phenotypic variances that were explained by these QTLs for non-stress conditions varied from 10.84 to 22.70, respectively, for Root's Ni/Shoot's Ni ratio (QNi-r/s4H.2n) and Root's Ni (QNir2H.2n) and for Nickel stress conditions from 11.41 to 18.86, respectively, for Root's Cu/Shoot's Cu ratio (QCu-r / s1H.s) and Root's Ni (QNir5H.s). LOD score in non-stress conditions was obtained in the range of 2.61 to 4.98, respectively, for QTLs of Root's Ni/Shoot's Ni ratio (QNi-r / s4H.2n) and Root's Cu/Shoot's Cu ratio (QCu-r / s7H.1n) and in Nickel stress conditions was obtained in the range of 2.50 to 3.68, respectively, for QTLs of Root's Ni/Shoot's Ni ratio (QNi-r / s4H.1s) and Root's Ni (QNir5H.s). In this experiment, QTLs QNir5H.s and QNis5H.s in Nickel stress conditions, adjacent to marker IEst9 on chromosome 5H, were in the same position that represents the correlation between these traits. This correlation may be due to linkage or peliotropic effect between the traits. Allelic effects (additive) of these QTLs in Table 4 explain the positive and negative correlation between these traits. The isotope QTLs with the same allelic effects (additive) have a positive correlation and the isotope QTLs with different allelic effects (additive) have negative correlation. Isotope of several QTLs cause traits high correlation. Poor and very poor correlations between traits is also justified by the lack of a common QTL (Fakheri and Mehravaran, 2014). Also, overlap different traits QTLs in a chromosomal region can confirm the existence of the pleiotropic phenomenon or severe linkage between the QTLs in a chromosomal region. The importance of recognition of pleiotropy and linkage is in that if the correlations between the traits be due to the the pleiotropy, breaking of this correlation is not possible through the selection. This phenomenon may also be due to existence of the cluster genes in that region of the chromosome. The cluster genes of different traits may cause QTLs overlapping.

Nevertheless, to understand whether the nature of controller areas more than one trait is due to pleiotropy, gene linkage, or cluster genes, a high-density map is required for mapping. The co-position QTLs with the same allelic effects (increase), indicate transition of them from a common parent (Siahsar and Narouei, 2010). This isotope QTL with parent P1 (Steptoe) was transferred to the progeny. By additive effect of QTLs in nonstress conditions in total 7 QTLs through parent P1 and 5 QTLs through parent P2, and in nickel stress conditions, 7 QTLs through parent P1 and 1 QTL through parent P2 were transferred to the progeny. So, in total of two conditions, (P1: 14 and P2: 6) parent P1 (Steptoe) has had a greater role in uptake of heavy elements. It is difficult to compare the results of this experiment with the other researcher's experiment results, because the linkage maps have been made by different communities and comparing them with each other is very difficult (Ding *et al.*, 2011). In this study, the number of mapped QTLs in nickel non-stress conditions was more than nickel stress (14 vs 6). The different mapped QTLs have been scattered in every seven chromosomes of the barley, But 40 percent of those were located on chromosome 5H (3 and 5 from 20 QTLs respectively in Nickel stress and non-stress conditions) and 25% of them were located on genome 1H (4 and 1 QTLs, respectively, in non-stress and Nickel stress conditions). So, it seems that these two chromosomes have a crucial role in controlling the traits of uptaking heavy elements in relation to nickel stress. 5QTLs on the genome 1H (four and one QTLs, respectively, in non-stress and nickel stress conditions), 2 QTLs on the genome 2H (2 QTLs in non-stress conditions), 1 QTL on the genome 3H (1 QTL in Nickel stress conditions), 2 QTLs on the genome 4H (one and one QTLs, respectively, in non-stress and Nickel stress conditions), 8 QTLs on the genome 5H (3 and 5 QTLs, respectively, in non-stress and Nickel stress conditions), 2 QTLs on the genome 7H (2 QTLs in Nickel stress conditions) had been located. In a study, QTL mapping for the absorption of Cu, Fe, Mn and Zn in wheat seeding shoot took place under Cu stress that was observed as one of the biggest QTLs in Cu tolerance on the chromosome 5DL and effective QTLs in term of Mn were on chromosome 3BL and 3AL and the centromic region in the chromosome 3B played an important role in the regulation of Fe (Balint *et al.*, 2007). In another study, in order to map the QTL in rice for tolerate the toxicity of Zn and its relation were performed to Fe, Al and Mn controller QTLs that three QTLs were observed for resistance to ZN^{2+} toxicity on chromosomes 1, 3 and 10, respectively (Dong *et al.*, 2006). In a study for effective QTL mapping in barley under Mn stress, three sources of Mn productivity were determined in Mel1 (Pallotta *et al.*, 2000).

Table 3: Simple correlation in 72 barley doubled haploid lines and their parents (Steptoe and Morex) for non-stress (down) and nickel stress (up) conditions.

Characteristics	Root's Ni (ppm)	Shoot's Ni (ppm)	Root's Ni / Shoot's Ni (ppm)	Root's Zn / Shoot's Zn (ppm)	Root's Cu / Shoot's Cu (ppm)	Root's Mn / Shoot's Mn (ppm)	Root's Fe / Shoot's Fe (ppm)
Root's Ni (ppm)	1	0.94^{**}	-0.10^{ns}	-0.06^{ns}	-0.21^{ns}	0.17^{ns}	-0.18^{ns}
Shoot's Ni (ppm)	0.77^{**}	1	-0.29^{*}	-0.09^{ns}	-0.14^{ns}	0.17^{ns}	-0.12^{ns}
Root's Ni / Shoot's Ni (ppm)	0.31^{**}	-0.30^{**}	1	0.12^{ns}	-0.18^{ns}	0.04^{ns}	-0.19^{ns}
Root's Zn / Shoot's Zn (ppm)	0.06^{ns}	0.11^{ns}	-0.08^{ns}	1	0.12^{ns}	-0.11^{ns}	0.16^{ns}
Root's Cu / Shoot's Cu (ppm)	-0.07^{ns}	-0.16^{ns}	0.11^{ns}	-0.19^{ns}	1	-0.19^{ns}	0.16^{ns}
Root's Mn / Shoot's Mn (ppm)	-0.02^{ns}	0.0006^{ns}	-0.05^{ns}	0.03^{ns}	0.02^{ns}	1	-0.13^{ns}
Root's Fe / Shoot's Fe (ppm)	0.14^{ns}	0.004^{ns}	0.18^{ns}	0.14^{ns}	0.11^{ns}	-0.05^{ns}	1

* and ** significant at 5 and 1% probability levels, respectively and ns, not significant

The main goals of plants breeders is modification of the genotypes with high yield and superior quality in order to have necessary stability in different environments (Yadav *et al.*, 2003). This issue is also true especially for barley breeders, because this multifunctional product is planted all over the world with different climatic conditions. For breeding purposes, the first issue is stability in emerging mapped QTLs that may be the candidates for marker assisted selection. Stability of QTLs in different environments and fields of genetics is the most important part of marker assisted selection. To assess the stability of QTLs effects, mapping community should be studied in environmental conditions and different genetic fields. One of the reasons that cause a QTL to be located in different regions of the genome is the occurrence of inside and outside chromosomal events including chromosomal inversion and translocation. It should be noted that this issue becomes more important when the two target population to have a greater distance from each other. Another reason could be the effect of the environment. When breeding populations are tested in the various environments, they usually show the effect of genotype × environment (Mahdinejad *et al.*, 2014, Fakheri and Mehravaran, 2014, Fakheri and Khalegh Babaki, 2014, Hayes *et al.*, 1993). In this mode, at least some of the genes divulge QTLs that show E × QTL interaction. E × QTL interaction appears as a change in the number of QTLs in different environments or change in the size of their effect in different environments (Hayes *et al.*, 1993).

Table 4: QTLs of heavy metals absorption traits in 72 barley doubled haploid lines in non-stress condition.

Characteristics	QTL	Chromosome	Nearest marker	QTL position	QTL interval (95%)	LOD score	Additive effect	R^2 (%)	Total R^2(%)
Root's Ni	QNir2H.1n	2H	ABG008	22.50	19.30-27.20	3.80	9.60	20.01	33.87
	QNir2H.2n	2H	BCD351F	30.90	29.0-35.60	4.55	9.37	22.70	36.56
Shoot's Ni	QNis1H.n	1H	ABG074	47.30	43.40-49.30	3.33	9.24	18.00	39.77
Root's Ni / Shoot's Ni	QNi-r/s1H.1n	1H	His3B	100.00	97.30-102.10	2.91	0.29	14.71	28.40
	QNi-r/s4H.2n	4H	BCD453B	90.70	79.90-96.70	2.61	-0.23	10.84	40.51
Root's Zn / Shoot's Zn	QZn-r/s5H.1n	5H	ABC706	66.30	63.80-70.40	4.32	-0.38	19.28	41.16
	QZn-r/s5H.2n	5H	ABC302	79.20	78.70-86.10	2.95	0.30	13.61	42.65
	QZn-r/s5H.3n	5H	WG644	146.80	145.40-153.80	3.43	-0.18	13.65	44.47
Root's Cu / Shoot's Cu	QCu-r/s7H.1n	7H	Ubi1	119.70	112.50-125.80	4.98	0.40	18.99	51.82
	QCu-r/s7H.2n	7H	bBE54E	131.00	130.00-136.20	4.08	0.38	15.88	48.72
Root's Fe / Shoot's Fe	QFer/s1H.1n	1H	ABC257	116.90	115.30-121.60	3.29	0.11	14.96	36.22
	QFer/s1H.2n	1H	cMWG733	125.60	121.60-127.00	2.63	0.10	12.90	34.16

Table 4 Continued: QTLs of heavy metals absorption traits in 72 barley doubled haploid lines in nickel stress condition.

Characteristics	QTL	Chromosome	Nearest marker	QTL position	QTL interval (95%)	LOD score	Additive effect	R^2 (%)	Total R^2(%)
Root's Ni	QNir5H.s	5H	iEst9	151.50	149.30-154.70	3.68	-52.27	18.86	28.96
Shoot's Ni	QNis5H.s	5H	iEst9	151.50	147.80-156.50	2.73	-54.00	14.12	26.18
Root's Ni / Shoot's Ni	QNi-r/s4H.1s	4H	WG464	76.60	76.30-78.50	2.50	0.40	14.53	16.40
	QNi-r/s5H.2s	5H	ABG314B	197.20	196.60-199.90	3.43	-0.42	14.44	41.32
Root's Zn / Shoot's Zn	QZn-r/s3H.1s	3H	ABG319B	196.20	189.00-203.20	2.98	0.29	14.06	33.32
	QZn-r/s5H.2s	5H	MWG920-1A	7.40	0.00-24.40	2.60	0.27	12.10	33.32
Root's Cu / Shoot's Cu	QCu-r/s1H.s	1H	CDO99	31.60	22.50-38.70	2.59	-0.37	11.41	37.01
Root's Fe / Shoot's Fe	QFe-r/s5H.s	5H	ABG463	191.20	190.50-193.70	3.61	0.46	17.45	32.91

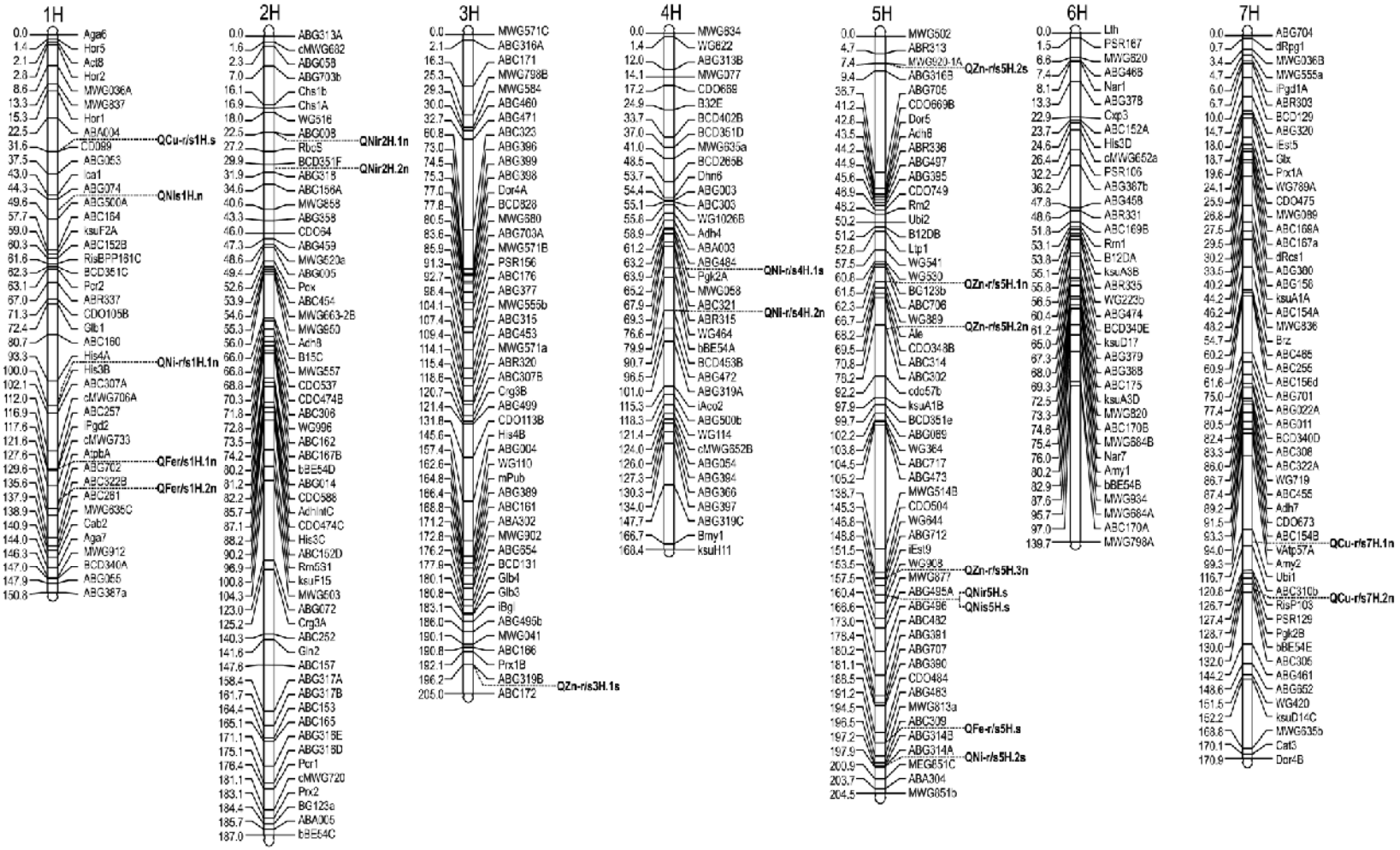

Fig. 1. QTLs of heavy metals absorption traits in 72 barley doubled haploid lines in non-stress and nickel stress conditions.

In two under study conditions, mapped QTLs did not have the required resistance. In other words, for a trait in two under study conditions, various QTLs were obtained. Or, they have slightly different locations or their allelic effects were different. At QTL analysis, it is possible that the location of a QTL to be determined in a specific place, whiles its actual place to be located several cM from it (Kearsey and Farquhar, 1998). At QTL analysis, repetition of an experiment in several environments can have a great importance, because some QTLs in a private environment and without repeating were not identified. Although the plant community, software, mapping function and the number and type of markers used are also factors that may not be alike in the results achieved (Siahsar and Narouei, 2010, Kearsey and Farquhar, 1998). The environmental factors, including Nickel stress, impress the quantitative magnitudes of the traits. In other words, the amount of variety may be varied in different degrees of Nickel stress and leads to instability of QTLs. Additionally, the different error quantities in different experiments may also lead to instability of QTLs. Therefore. In order to use QTLs in improving agricultural statistics, there is need to study on years, places, different genetic areas and various populations.

CONCLUSION

This research is the first report of QTL analysis related to traits of heavy elements uptake in the Steptoe/Morex doubled haploid lines in hydroponic conditions under nickel stress. In this study, 20 QTLs for traits related to uptake of heavy elements were examined and identified in which the mapped QTLs did not possess required stability in two studied conditions.

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