



*Original article*

## QTL mapping for physiological traits affecting lead tolerance in the *Hordeum vulgare* L.

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### *Extended abstract*

#### **Introduction**

Human activities such as mining and industrial activities have increasingly affected soil contamination through the sedimentation of heavy metals. This issue is concerned as a global problem. All heavy metals are potentially poisonous, which vary based on their biologic available concentrations and the sensitivity of organisms which are exposed to the heavy metal. One of the main heavy metals is Lead. Lead is a potential pollutant that readily accumulates in soils and sediments. Pb is considered a major troublesome poison, crop productivity sharply declines in soils contaminated by Pb. The poisonous characteristics of lead on plants are attributed to chlorosis, declined growth, blackening of root system, upsets mineral nutrition and water balance, changes in hormone status, and the effects on the structure and permeability of membrane. In recent years, many plants have been destroyed due to the increased pollution of the earth by heavy metals, and which has led researchers are interested in soil improvement, the production and use of resistant. Most of the important agricultural traits, such as yield, grain quality and resistance, or tolerance of live and non-live stresses in nature, are complex qualitative traits. These traits are usually controlled by multiple quantitative traits (QTLs), which are strongly influenced by the genetic context of the creature plants, and the identification of mechanisms and genes associated with heavy metal tolerance. This research was carried to identification of QTL traits related to lead toxicity tolerance in barley.

#### **Materials and methods**

In this study, we used 94 double haploid from Dom and Rec parents that were planted in a completely randomized design with three replications in twenty-cm diameter pots. Then at 2- to 3-leaves stage, seedlings were treated by 0 (control) and 2000 ppm density lead nitrate. Physiological traits such as substomatal CO<sub>2</sub>, transpiration rate, photosynthesis rate, stomatal concentration and plant greenness after 24 under stress, were measured at the beginning heading stage. In order to investigate the frequency distribution of data and their variation under stress and normal conditions, Spss program version 23 was used. After observing the continuous and quantitative variation among progenies to studied traits, in

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order to locate the lead-tolerance genes, Using Mapchart 2.32 and MapQTLs Software, chromosomal regions associated physiological traits were identified on seven chromosome of barley.

## Results

The phenotypic Analysis of the studied traits showed a quantitative and continuous variation. the studied traits showed normal distribution in both non stress and stress conditions The genetic which is lead tolerance is controlled depending on the physiological indices of different parts in the genome of the barely. In this study No QTL was observed for the studied traits under normal conditions. among the traits sub-stomatal CO<sub>2</sub>, transpiration rate, plant greenness after 24 under stress, stomatal concentration, photosynthesis rate, QTL was observed Under stress conditions with lead, two QTL discovered for sub-stomatal CO<sub>2</sub> on chromosome 6, two QTL for transpiration rate on chromosome 3, four QTL for Stomatal Conduction on chromosomes 1 and 3, four QTL for photosynthesis rate on chromosomes 3 and 2, four QTL for plant greenness after 24 under stress on chromosomes 1, 4 and 5. Phenotypic variation justified by these QTLs varied 10.5% to 19.10%.

## Conclusion

The results of this study showed that the OWB mass mapping can be used as a proper mass mapping and as a model for studying the genetic of lead tolerance in plants. The results of this study also showed that various physiological traits of barely under lead stress conditions are manipulated by different genes. Precisely locating lead resistant QTL is the most important step in cloning and finding the function of the genes involved in lead resistance. In order to use QTL detection methods for understanding the attributes related to lead resistance, it is necessary to divide lead toxicity resistance into smaller traits. This task is achieved through considering the events occurring in lead poisoning and the determining the reason why some plants are tolerate.

**Keywords:** Chromosome, Lead Contamination, QTL Mapping, Resistance, Sensitivity

**Table 1. Analysis of variance (Mean squares) for morphological traits in two parents' of barley mapping population**

S.O.V	df	Plant greenness after 24 days under stress	Plant greenness after 12 days under stress	Photosynthesis rate
Lead	3	143.45**	94.22**	16.66**
Genotype	1	172.23**	10.00	0.36
Lead× Genotype	3	57.83**	84.74**	21.88**
Error	32	6.46	6.97	1.93
CV%		7.78	8.81	14.36

**Table 1. Continued**

S.O.V	Df	Stomatal Conduction	Transpiration rate	Sub-stomatal CO <sub>2</sub>	Leaf surface Temperature
Lead	3	0.002**	0.94**	33714.06**	35.32**
Genotype	1	0.001**	2.39**	71288.90**	42.02**
Lead× Genotype	3	0.003**	2.24**	36550.03**	54.09**
Error	32	0.000004	0.09	792.07	6.14**
CV%		3	14.92	10.26	8.25

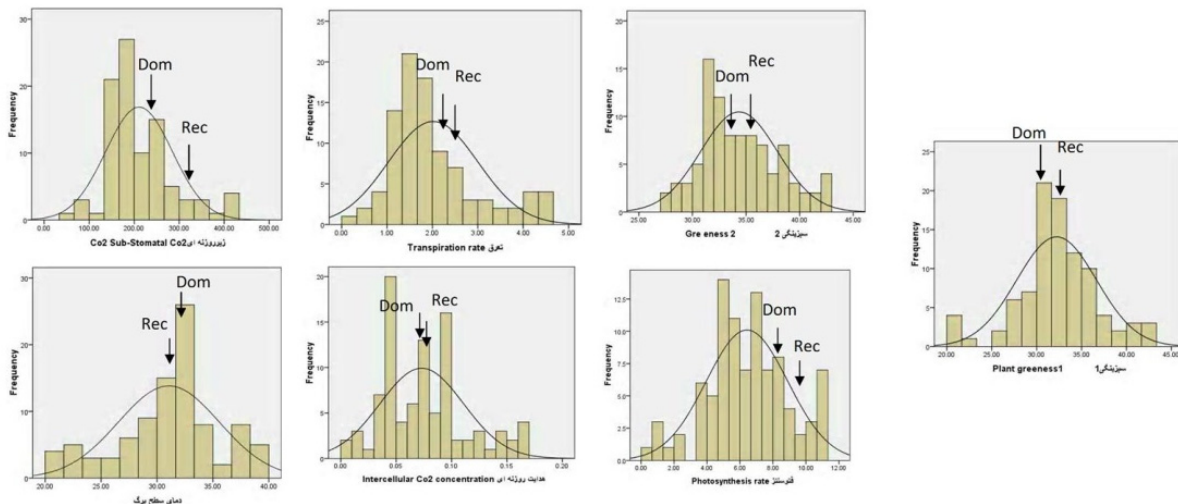
\*\* and \*, significant at 1% and 5% Probability levels, respectively

**Table 2. Mean comparison of Rec and Dom parents in different lead levels**

Parentes	Lead Doses (ppm)	Plant greenness after 24 days under stress	Plant greenness after 12 days under stress	Photosynthesis rate
REC	Control	35.6	31.6	9.18
	1000ppm	32.8	31	10.14
	1500ppm	34.2	30.34	9.38
	2000ppm	21.4	22.8	2.39
DOM	Control	34	31.4	8.96
	1000ppm	35.9	30.6	8.05
	1500ppm	34.8	30.14	8.51
	2000ppm	32.6	31.8	8.80
LSD		3.27	3.40	1.79

**Table 2. Continued**

Parentes	Lead Doses (ppm)	Stomatal Conduction	Transpiration rate	Sub-stomatal CO <sub>2</sub>	Leaf surface Temperature
REC	Control	0.076	2.26	342.80	31.60
	1000ppm	0.074	2.16	302	31.40
	1500ppm	0.078	2.10	315.60	30.34
	2000ppm	0.016	0.59	84	22.80
DOM	Control	0.074	2.24	292.40	31.40
	1000ppm	0.080	2.14	285	30.60
	1500ppm	0.066	2.50	281.80	30.14
	2000ppm	0.068	2.10	290.60	31.80
LSD		0.0025	0.38	36.31	3.19



**Fig. 1. Frequency distributions of studied traits in normal condition**

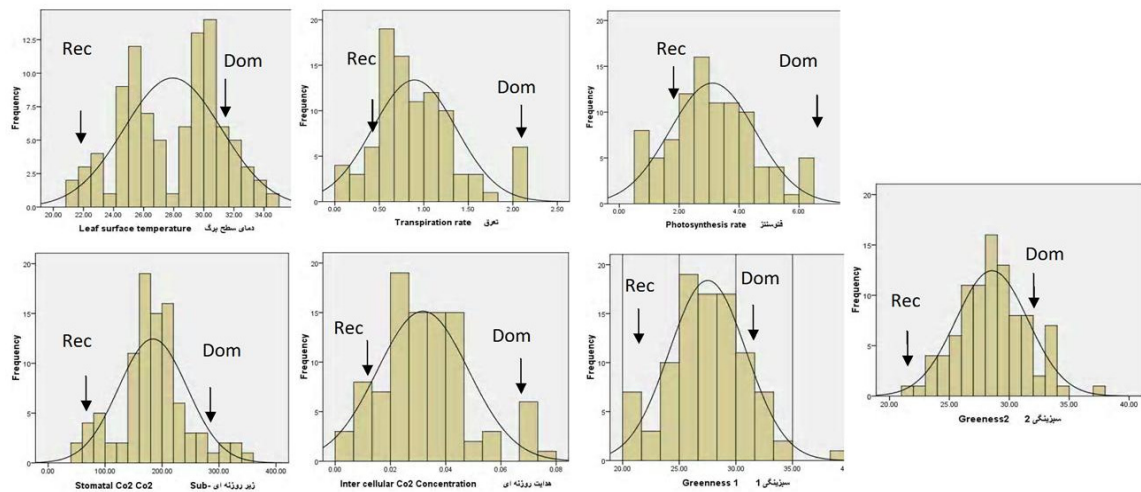


Fig. 2. Frequency distributions of studied traits in stress condition

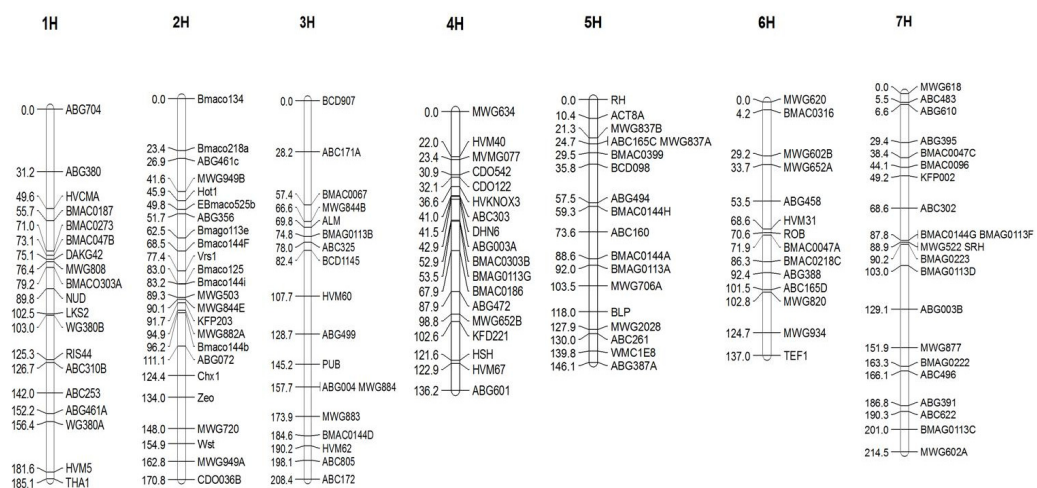


Fig. 3. The prepared linkage map in barley (Marcel et al, 2007)

Table 3. Statistics of traits studied under normal and stress condition

	Normal condition					
	Mean	Std.deviation	Kurtosis	Skewness	Min	Max
Temperature Leaf surface(°C)	31.06	4.523	0.065	-0.518	20.70	39.02
Sub-stomatal CO <sub>2</sub> (µmol/mol)	210.85	74.116	0.933	0.848	77	485
Transpiration rate(mmol m <sup>-2</sup> s <sup>-1</sup> )	2.02	0.987	0.238	0.732	0.29	4.48
Stomatal Conduction(mol m <sup>-2</sup> s <sup>-1</sup> )	0.07	0.00018	-0.035	0.054	0.01	0.17
Photosynthesis rate (µmol m <sup>-2</sup> s <sup>-1</sup> )	6.42	1.47	-0.156	-0.04	0.62	11.58
Greenness after 12 days stress(mol/cm <sup>2</sup> )	32.23	4.44	0.983	-0.313	22.5	44.05
Greenness after 24 days stress (mol/cm <sup>2</sup> )	34.35	3.58	-0.381	0.428	20.95	44.95

Table 3. Continued

	Stress condition					
	Mean	Std.deviation	Kurtosis	Skewness	Min	Max
Temperature Leaf surface(°C)	27.93	3.24	-0.895	-0.228	21.50	34.20
Sub-stomatal CO <sub>2</sub> (μmol/mol)	183.95	60.28	0.686	0.174	49.5	350
Transpiration rate(mmol m <sup>-2</sup> s <sup>-1</sup> )	0.89	0.467	0.084	0.056	0.15	2.06
Stomatal Conduction(mol m <sup>-2</sup> s <sup>-1</sup> )	0.03	0.00001	0.053	0.012	0	0.08
Photosynthesis rate (μmol m <sup>-2</sup> s <sup>-1</sup> )	3.11	0.12	-0.483	0.269	0.8	6.37
Greenness after 12 days stress(mol/cm <sup>2</sup> )	27.52	3.39	0.748	0.121	20.80	39.30
Greenness after 24 days stress (mol/cm <sup>2</sup> )	28.55	3.01	0.411	0.089	23.7	37.85

Table 4. Parameters of QTLs identified by employing MQM method for the studied traits in the OWB population under lead stress

Traits	Chromosome	Position (CM)	QTL	Marker interval	Expl%	Add	LOD	Permutation test
Transpiration rate	3H	40-100	<i>QH3Tr<sub>1</sub></i>	Alm-ABC325	12.5	-1.11	3.02	2.75
	3H	100-140	<i>QH3Tr<sub>2</sub></i>	Hvm60-Pub	13.1	0.97	2.93	
Stomatal conduction	1H	100-140	<i>QH1Sc</i>	Lks2-Ris44	11.4	3.6	3.14	2.85
	3H	40-100	<i>QH3Sc<sub>1</sub></i>	Alm-ABC325	13.1	-2.7	3.21	
	3H	10-100	<i>QH3Sc<sub>2</sub></i>	Bmac0067-Alm	12.4	2.6	3.14	
	3H	10-100	<i>QH3Sc<sub>3</sub></i>	MWG844B-Bmag0113b	12.8	-2.13	3.24	
Sub-stomatal CO <sub>2</sub>	6H	0-10	<i>QH6Ss<sub>1</sub></i>	Bmacoo47a-ABG388	17.3	4.3	3.72	2.02
	6H	50-100	<i>QH6Ss<sub>2</sub></i>	MWG620-MWG602B	15.2	8.9	2.84	
Plant greenness after 24 under stress	1H	50-150	<i>QH1Pg<sub>1</sub></i>	Nud- WG380B	11.3	-2.15	3.01	2.69
	1H	10-100	<i>QH1Pg<sub>2</sub></i>	HVCMA-Bmac0273	14.5	1.78	4.77	
	5H	50-100	<i>QH5Pg</i>	Bmac0144a-MWG706A	13.4	-2.31	4.21	
	4H	10-60	<i>QH4Pg</i>	CD0542-HVKNOX3	12.3	-3.17	3.53	
Photosynthesis rate	3H	40-100	<i>QH3Ph<sub>1</sub></i>	Alm-ABC325	10.5	-1.17	3.07	2.67
	3H	40-100	<i>QH3Ph<sub>2</sub></i>	MWG844B-Bmag113b	19.1	-0.91	3.85	
	2H	50-150	<i>QH2Ph</i>	ABG072-Zeo	13.8	1.03	2.93	
	3H	100-150	<i>QH3Ph<sub>3</sub></i>	Hvm60-Pub	11.6	0.95	2.99	

Table 5. Phenotypic correlation coefficients under normal conditions (values on upper triangular Matrix) and under stress conditions (values on stress triangular Matrix) in barley Genotype

Traits	1	2	3	4	5	6	7
Leaf surface Temperature	1	-0.41**	0.21*	-0.14	-0.14	-0.1	-0.21*
Sub-stomatal CO <sub>2</sub>	-0.56**	1	0.3**	0.29**	0.14	0.04	0.11
Transpiration rate	0.33**	0.26*	1	0.83**	0.79**	0.18	0.16
Stomatal conduction	-0.32**	0.6**	0.65**	1	0.80**	0.19	0.23*
Photosynthesis rate	-0.19	0.16	0.61**	0.72**	1	0.22*	0.25*
Greenness after 12 days stress	0.01	-0.11	-0.07	-0.01	0.13	1	0.59**
Greenness after 24 days stress	0.12	-0.11	0.02	0.01	0.09	0.8**	1

\*\* and \*, significant at 1% and 5% Probability levels, respectively

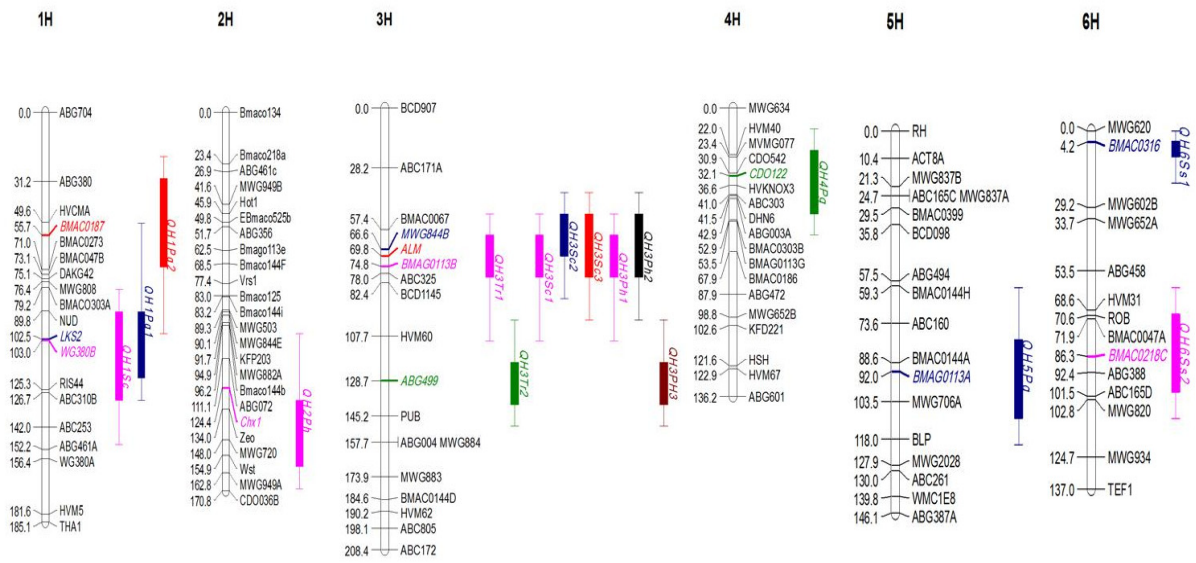


Fig. 4. Lead-related QTLs for studied traits and their position in the barley linkage map