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Full Length Research Paper

Determination of chromosomes that control physiological traits associated with salt tolerance in barley at the seedling stage

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Salt stress is one of the most important abiotic stresses, and plays an important role in reducing the yield of crops worldwide. It is now recognized that tolerance to salinity is genetically and physiologically complex and also inherited quantitatively. Barley is a short-season, early maturing, diploid and self pollinating crop, thus it is an ideal model plant for genetic and physiological studies of salinity tolerance. In order to map the genes/QTLs for salinity tolerance in barley, 72 doubled-haploid lines derived from a cross between 'Steptoe' and 'Morex' were used in an experiment using a randomized complete factorial design with three replications. The phenotypic traits under study included: chlorophyll contents, chlorophyll fluorescence (Fo, Fv, Fv/Fm), proline and carbohydrate rates, relative water content (RWC) and dry and wet weight of plant. Analysis of variance results showed that there were significant differences among the lines and different levels of salinity for all the traits. The strongest correlation was observed between dry and wet weight of plant (r = 0.95). QTL analysis was performed using the genetic linkage map derived from 327 RFLP molecular markers and QTL cartographer software with the composite interval mapping method. Phenotypic variations that were explained by these QTLs, ranged from 10.64 to 24.20. The highest and lowest phenotypic variances were related to chlorophyll content (*Q3cls*) and Fv/Fm (*Q1fv/fms*), respectively. LOD values ranged from 2.77 to 6.33. The highest LOD scores were attained for Fv/Fm on chromosome 2H. Physiological traits associated with salt tolerance in this population were mapped to chromosomes 1H, 2H, 5H and 7H.**

Key words: Barley, QTL, salinity, stress, tolerance.

INTRODUCTION

Salinity is an important limitation factor to crop production in many agricultural areas and is receiving much attention from plant breeders. Salt tolerance is a complex trait that involves multiple physiological and biochemical mechanisms and numerous genes. The identification of genes whose expression enables plants to tolerate salt stress is essential for breeding programs, but relatively little is known about the number, the location and inheritance of genes that are responsible for higher salt tolerance. QTL analysis using functional molecular marker maps provides detailed information about both location and function of genes involved in the polygenical inherited trait of salt tolerance (Weidner et al., 2006). There has been much interest in studying quantitative traits of agronomic importance, disease resistance (Young, 1996), drought tolerance (Teulat et al., 2001; Diab et al., 2004), and many other traits for biotic and abiotic stress tolerance in barley. Barley provides an excellent system for genome mapping and genetic studies, due to (1) its diploid nature, (2) low chromosome number (2n=14), (3) relatively large chromosomes (6 to 8 µm), (4) high degree of self fertility and (5) ease of hybridization (Benneth and Smith, 1976).

Screening a large number of genotypes for salt

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tolerance is not easy. To avoid the necessity of growing plants for long periods of time to measure biomass or yield, practical selection techniques can be based on physiological traits (Munns et al., 2002). Screening for a trait associated with a specific mechanism is preferable for screening salt tolerance itself, as measuring the effect of salt on biomass or yield of a large number of lines is not feasible. Hence, measuring of physiological traits associated with salt tolerance can be a good criterion to determine their tolerance. The most important physiological traits that can be used for screening of salt tolerance, are osmotic regulation, proline content, water soluble carbohydrate, relative water content (RWC), ABA content, water use efficiency and carbon isotope discrimination (Li et al., 2007). The complexity of barley responses to salt has been widely studied during germination, in seedlings and plants. Mano and Takeda (1997) found QTLs for salt tolerance at germination on chromosomes 1H, 4H, 5H and 6H and at different locations, for seedling tolerance of salt on chromosome 1H, 2H, 5H and 6H.

The main objective of this study was to detect QTLs associated with salt tolerance in a DH population derived from ‘Steptoe’ and ‘Morex’ via physiological components of salt tolerance.

MATERIALS AND METHODS

The materials for this study comprised of a doubled haploid population derived from a cross between two lines of spring barley, namely ‘Steptoe’(CI15229) and ‘Morex’ (CI15773) (Hayes et al., 1993; Kleinhofs et al., 1993) together with both parents. These were utilized to determine salt tolerance. In brief, the population is derived from an F1 hybrid of this cross by a modified ‘Hordeum bulbosum’ technique, as described by Chen and Hayes (1989). The DHLs were developed by the Oregon State University Barley Breeding Program and kindly provided by Hayes (Department of Crop and Soil Science, Oregon State University, Corvallis, OR 973314501, USA). This experiment was carried out in hydroponic system at the Faculty of Agriculture, University of Zabol, Zabol, Iran. It was arranged as a completely randomized factorial, with 3 replications and 4 levels of salinity (0, 5, 10, 15 dS/m). Seeds of barley (*Hordeum vulgare*) were germinated at 25°C in dark conditions. Seedlings were hydroponically grown with Hoagland nutrient solution (Hoagland and Arnon, 1950) in a growth chamber. For differential display, 2-week old plants were treated with Hoagland solution containing NaCl. The treatments lasted for 30 days after which the following parameters were determined: Chlorophyll content, chlorophyll fluorescence (Fo, Fv, Fv/Fm), proline and carbohydrate rate, relative water content (RWC) and dry and wet weight of plant. A SPAD-502 Chlorophyll-Photometer was used to measure chlorophyll content. We measured chlorophyll content in fresh leaves, in the first part of the leaf, medium part of leaf and last part of leaf as an average of three leaves. Chlorophyll fluorescence was measured using the Chlorophyll flourometer (Handy PEA) and we recorded parameters associated with chlorophyll fluorescence: Fo, Fv and Fv/Fm. Proline and carbohydrate contents were measured according to Bates et al. (1973) and Irrigoyen et al. (1992), respectively. RWC was calculated as follows:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Where FW = leaf fresh weight, DW = leaf dry weight, and TW = leaf turgid weight.

Statistic evaluation for experiment data was arranged in 2 parts:

1. The evaluation of phenotype data was conducted by means of variance and correlation analysis.
2. QTL analysis was carried out using the genetic linkage map derived from 327 RFLP molecular markers and QTL cartographer software with the composite interval mapping method (Figure 1).

Analysis of variance (ANOVA) was performed using PROC ANOVA procedure in SAS (SAS Inst. Inc., Cary, Nc.). Heritability of entry was estimated as broad sense heritability by h2 = σ2g / σ2g + σ2e (Therrien, 2003; Falconer, 1981). Simple correlation analysis was carried out for all characteristics of salt tolerance using entry means and PROC CORR procedure in SAS (SAS Inst. Inc., Cary, Nc.).

The molecular marker linkage map (Kleinhofs et al., 1993; Hayes et al., 1993) (current at http://barleygenomics.wsu.edu/) developed by the North American Barley Genome Mapping Project from doubled haploid line population derived from ‘Steptoe/Morex’ F1s, was used for mapping of salt 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 performed using WinQTL cartographer 2.5 (Wang et al., 2007). A series of 1000 permutations was run to determine the experiment-wise significance level at P = 0.05 of logarithm of the odds ratio (LOD) for the traits (Churchill and Doerage, 1994).

The genome was scanned at 2 cM intervals and the window size was set at 10 cM. Cofactors were chosen using the forward-backward method of stepwise regression. The percentage of phenotypic variance explained by a specific QTL value (R2) was taken as the peak QTL position as determined by WinQTL cartographer 2.5. The LOD peaks were considered to indicate the most likely position of QTL effects. 95% confidence intervals were calculated by 1000-bootstrap re-sampling (Lebreton et al., 1998), as proposed in the WinQTL cartographer 2.5 package.

RESULTS AND DISCUSSION

All the traits measured in response to different levels of salinity differed strongly among the lines of ‘Steptoe/ Morex’ DH population. The analysis of variances of the 72 doubled haploid lines and their parents (‘Steptoe’ and ‘Morex’) for nine characters is presented in Table 1. These results pointed out clearly that there was a high degree of phenotypic variation in the population and that it was suitable for QTL analysis. Hayes and Iyambo, (1994) and Gibson et al. (1994) reported similar variation within the ‘Steptoe/Morex’ DHLs population for grain quality traits. The difference between the means of doubled haploids (XDHS) and their midparent was not significant for all traits. Results shown in Table 2 indicate that the 72 DHLs in this study are representative of the total possible DHLs from the cross ‘Steptoe/Morex’ and that the studied traits are mostly controlled by additive gene effects. The best DH when compared with the best parent showed significantly higher values for two traits, which are dry and wet weight of plant. Bregitzer and Campell (2001) in a study to determine the QTLs associated with plant regeneration in this population also reported transgressive segregation. This phenomenon of “transgressive variation” could be interpreted as

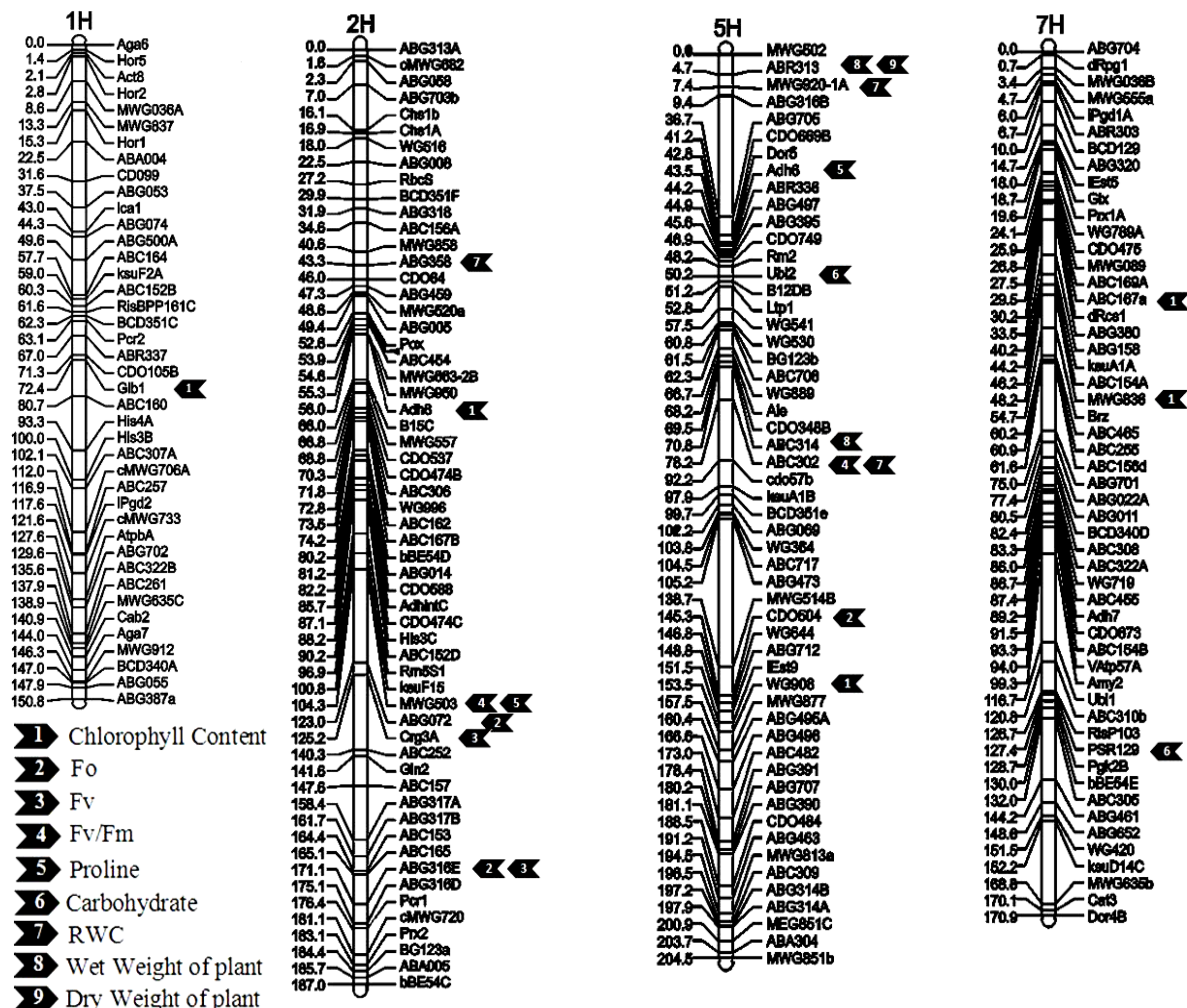

Figure 1. QTLs map for salt tolerance attributes, based on data from 72 doubled haploids from a cross between Steptoe and Morex, identified by composite interval mapping. Distances are in cM (calculated using the Kosambi mapping function).

favourable alleles combining from both parental lines. The proportion of phenotypic variation explained by each QTL and all QTLs together depends on heritability of the trait as well as on the portion of revealed QTLs. Narrow sense heritabilities (h^2) presented in Table 2 showed high values ranging from 22 to 75% for all traits. Heritability provides an estimate of how much variability is due to genetic factors. Correlations among all traits are given in Table 3. The maximum correlation was observed between dry and wet weight of plant (r = 0.95**). Highest LOD score (6.3) was obtained for the fv/fm parameter (*Q1fv/fms*) on chromosome 2H.

Physiological traits associated with salt tolerance were mapped to chromosomes 7H, 4H, 1H and 6H by Ellis et al. (1997). Mano and Takeda (1997) found QTLs for seedling tolerance of salt on chromosome 1H, 2H, 5H and 6H. In this study, we found QTLs for the Steptoe/Morex population on chromosome 1H, 2H, 5H and 7H (Table 4). Some of these QTLs were located in the same region on the chromosome. For example, The QTLs for dry and wet weight of plant on chromosome 5H and the QTLs, *Q1fv/fms* and *Q2fv/fms* on chromosomes 5H and 2H were co-localized with *Q1prs* and *Q3rwcs*, respectively. These co-locations could be either due to the linkage between two genes or the pleiotropy effect of one gene. In the later case, the correlation between traits will never be broken. Pleiotropy controls the common sub-fraction of the traits, and therefore, would result in the concurrent increase or decrease of the correlated traits when we select for only one trait. The results also revealed many QTLs for individual trait located on different chromosomes like chlorophyll content on four chromosomes 1H, 2H, 5H and 7H. It indicated that these traits were controlled by multiple genes distributed widely on the barley genome. The power of QTL detection can be increased by decreasing the variation caused by the

Table 1. Results of analysis of variance of 9 salt tolerance traits in 72 Steptoe/Morex doubled haploid lines (DHLs) barley population and their two parents.

S.O.V	D.F	Mean squares								
		Chlorophyll contents	Fo	Fv	Fv/fm	Proline	Carbohydrate	RWC	Wet weight of plant	Dry weight of plant
Salinity	3	10.37**	4267.64**	194194.16**	0.003**	205.01**	10.82**	3270.92**	4.935**	0.020**
Line	73	1.51**	1707.69**	14555.46*	0.001**	2.29**	0.45**	143.01**	0.159**	0.001**
S×L	219	0.80**	649.77^{ns}	11316.77^{ns}	0.001**	1.42^{ns}	0.26**	69.192^{ns}	0.041**	0.0003**
E	592	0.485	644.72	10621.33	0.001	1.30	0.18	92.85	0.007	0.0001
C.V		19.90	8.23	7.34	3.43	0.13	6.11	14.75	16.44	28.68
R^2		52.39	42.29	39.60	36.23	58.59	52.53	39.18	89.52	71.93

* and **, significant at 0.05 and 0.01 probability level, respectively; ns, non significant.

Table 2. Simple statistics, genetic gain and heritability of 9 salt tolerance traits in a population of 72 Steptoe/Morex DHLs barley and their two parents.

Item	Chlorophyll contents	Fo	Fv	Fv/Fm	Proline	Carbohydrate	RWC	Wet weight of plant	Dry weight of plant
Steptoe (P_1)	3.647	318.917	1413.000	0.775	871.410	7.060	59.583	0.440	0.034
Morex (P_2)	4.023	291.500	1388.000	0.790	871.11	7.470	67.667	0.688	0.052
P_1-P_2	-0.376^{ns}	27.417^{ns}	25.000^{ns}	-0.015^{ns}	0.297^{ns}	-0.414^{ns}	-8.083^{ns}	-0.248**	-0.018^{ns}
$X_P = (P_1+P_2)/2$	3.835	305.208	1400.500	0.782	871.262	7.262	63.625	0.564	0.043
Min_{DHs}	2.728	282.000	1294.83	0.750	870.040	6.630	57.833	0.260	0.23
Max_{DHs}	4.470	351.583	1480.92	0.755	872.080	7.560	72.750	0.844	0.080
Rang	1.742	69.583	186.084	0.045	2.040	0.930	14.917	0.584	0.057
$X_{DHs}{}^a$	3.492	308.642	1404.040	0.778	870.924	7.044	65.353	0.507	0.041
SD_{DHs}	0.353	11.889	35.001	0.009	0.428	0.190	3.425	0.115	0.010
CV_{DHs}	10.102	36.852	2.493	1.216	0.049	2.700	5.241	22.62	23.77
X_{DHs}-X_P	-0.343^{ns}	3.434^{ns}	3.540^{ns}	-0.004^{ns}	-0.338^{ns}	-0.218^{ns}	1.728^{ns}	-0.057^{ns}	-0.002^{ns}
$GG^b = B_{DH}{}^c$-$B_P{}^d$	0.447^{ns}	32.666^{ns}	67.920^{ns}	0.005^{ns}	0.670^{ns}	0.090^{ns}	5.083^{ns}	0.156*	0.028**
h^2	0.470	0.619	0.222	0.373	0.380	0.428	0.516	0.742	0.750

* and **, significant at 0.05 and 0.01 probability level; ns, non significant. [a]Mean of doubled haploids; [b]genetic gain; [c]best doubled haploid; [d]best parent.

Table 3. Simple correlations for salt tolerance traits in the 72 Steptoe×Morex doubled haploid (DH) line barley population and their two parents.

	Chlorophyll contents	Fo	Fv	Fv/Fm	Proline	Carbohydrate	RWC	Wet weight of plant	Dry weight of plant
Chlorophyll contents	**1**								
Fo	0.111ns	1							
Fv	0.102ns	0.235*	1						
Fv/Fm	0.114ns	-621**	-0.044ns	1					

Table 3. continue.

Proline	0.089ns	0.030ns	-0.168ns	0.146ns	1				
Carbohydrate	0.025ns	0.040ns	-0.034ns	0.003ns	0.235*	1			
RWC	-0.035ns	-0.063ns	-0.069ns	0.094ns	-0.033ns	0.159ns	1		
Wet weight of plant	0.344**	-0.075ns	0.051ns	0.156ns	-0.005ns	0.422**	-0.009ns	1	
Dry weight of plant	0.313**	-0.077ns	0.055ns	0.158ns	-0.050ns	0.421**	-0.079ns	0.950**	1
	Chlorophyll contents	Fo	Fv	Fv/Fm	Proline	Carbohydrate	RWC	Wet weight of plant	Dry weight of plant
Chlorophyll contents	1								
Fo	0.111[ns]	1							

* and ** present the significant difference of 0.05 and 0.01 level of probability, respectively.

Table 4. Quantitative trait loci (QTL) for 9 salt tolerance traits identified by composite interval mapping for 72 Steptoe/Morex doubled haploid (DH) barley lines population.

Trait	QTL name	Chr. name	Nearest marker	QTL pos.[a]	QTL interval	LOD score[b]	Allelic effect	R^{2c}	Total R^{2d}
Chlorophyll Content	*Q1cls*	1H	Glb1	72.4	67.0-86.5	4.57	0.16	18.67	68.24
	Q2cls	2H	Adh8	56	53.9-63.3	3.01	-0.12	11.67	
	Q3cls	5H	VAtp57A	153.5	149.7-157.8	2.77	0.12	10.64	
	Q4cls	7H	ABC167a	29.5	24.1-30.1	3.98	0.19	15.87	
	Q5cls	7H	MWG836	48.2	42.2-56.8	2.95	-0.16	11.39	
Fo	*Q1fos*	2H	ABG072	123	113.3-133.1	3.74	4.46	12.10	38.81
	Q2fos	2H	ABG316E	171.1	166.5-174.1	4.22	-5.43	14.27	
	Q3fos	5H	CDO504	145.3	140.7-146.8	3.70	-4.22	12.34	
Fv	*Q1fvs*	2H	Crg3A	125.2	122.8-135.7	2.91	68.79	14.81	29.50
	Q2fvs	2H	ABG316E	171.1	165.1-18/1.1	2.88	-58.09	14.69	
Fv/Fm	*Q1fv/fms*	2H	MWG503	120.3	112.8-124.9	6.33	-0.005	24.20	39.63
	Q2fv/fms	5H	ABC302	80.2	72.1-90.2	3.62	0.009	15.43	
Proline	*Q1prs*	2H	MWG503	122.3	107.7-125.0	3.31	-0.167	14.35	28.21
	Q2prs	5H	Adh6	43.5	32.4-44.2	3.27	-0.169	13.86	
Carbohydrate	*Q1cas*	5H	Ubi2	50.2	48.2-52.2	3.27	0.16	15.44	29.03
	Q2cas	7H	PSR129	127.4	126.7-128.7	2.66	0.10	13.59	
RWC	*Q1rwcs*	2H	ABG358	43.3	40.8-46.0	3.42	1.35	14.07	41.82
	Q2rwcs	5H	MWG920-1A	7.4	5.5-13.8	3.60	1.38	14.92	
	Q3rwcs	5H	ABC302	86.2	70.8-91.0	2.91	-1.27	12.83	
Wet weight of plant	*Q1wwps3*	5H	ABR313	7.4	0.0-7.1	3.75	0.035	17.02	29.38
	Q2wwps3	5H	ABC314	70.8	68.2-76.8	2.81	-0.030	12.36	
Dry weight of plant	*Qdwps3*	5H	ABR313	4.7	0.0-6.9	4.51	0.003	20.48	20.48

[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.

environment as well as by the background genome. Environmental variation can be decreased by repeated phenotypes measurements. Our detected regions need to be more precisely mapped because a QTL may be expressed in one environment but not in another, or expressed strongly in one environment but weakly in another, and/or expressed very differently and with opposite effects in different environments. One of the major goals of QTL mapping is to select markers linked to genes contributing to variation in the trait of interest. QTL consistency across different environments and background is an important component for marker-assisted selection (MAS). Genetic dissection of the quantitative traits controlling the adaptive response of crops to abiotic stress is a prerequisite for application of MAS in breeding programs.

REFERENCES

Bates S, Waldem RP, Teare ED (1973) Rapide determination of free praline for water stress studies. Plant Soil, 39: 205-207.

Benneth MD, Smith JB (1976) Nuclear DNA Amounts in Angiosperms. Philos. Trans. R. Soc. London, Biol. Sci. 274: 227-274.

Bregitzer P, Campbell RD (2001) Genetic markers associated with green and albino plant regeneration from embryogenic barley callus. Crop Sci. 41: 173-179.

Chen F, Hayes PM (1989) A comparison of *Hordeum bulbosum*-mediated haploid production efficiency in barley using *in vitro* floret and tiller culture. Theor. Appl. Genet. 77: 701-704.

Churchill GA, Doerage RW (1994) Empirical threshold values for quantitative trait mapping. Genetics, 138: 963-971.

Diab AA, Béatrice T-M, Dominique T, Neslihan Z, Ozturk DB, Mark ES (2004) Identification of Drought-Inducible Genes and Differentially Expressed Sequence Tags in Barley. Theor. Appl. Genet. 109: 1417-1425.

Ellis RP, Forster BP, Waugh R, Bonar N, Handley LL, Robinson D, Gordon D, Powell W (1997) Mapping physiological traits in barley. New Phytol., 137: 149-157.

Falconer DS (1981) Introduction to Quantitative Genetics. Longman, New york.

Gibson LA, Bowman JGP, Oberthur LE, Blake TK (1994). Determination of genetic marker associated with ruminant digestion of barley. Proc. West. Section Am. Soc. Anim. Sci. 45: 317-320.

Hayes PM, Iyambo OE (1994) Summary of QTL effects in the Steptoe×Morex population. Barley Genet. Newsl. 23: 98-143.

Hayes PM, Liu BH, Knapp SJ, Chen F, Jones B, Blake T, Franckowiak J, Rasmmusson D, Sorrells M, Ullrich SE, Wesenberg D, Kleinhofs A (1993) Quantitative trait locus effects and environmental interaction in a Sample of North American barley germplasm. Theor. Appl. Genet. 87: 392-401.

Hoagland DR, Arnon DI (1950). The water culture method for growing plants without soil. California Agric. Exp. Station Circular, p. 347.

Irigoyen J, Emerich J, Sanches-Diaz M (1992). Water stress induced changes in concentrations of praline and total soluble sugars hn nodulated alfalfa plants.

Kleinhofs A, Kilian A, Saghai maroof MA, Biyashev RM, Hayes PM, Qchen F, Laption N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skaden R, Steffeson BJ (1993). A molecular, isozyme and morphological map of the barley (*Hordeum vulgare* L.) genome. Theor. Appl. Genet. 86: 705-712.

Lebreton CM, Visscher PM, Haley CS, Semikhodskii A, Quarrie SA (1998) A nonparametric bootstrap method for testing close linkage vs. Pleiotropy on coincident quantitative trait loci. Genet. 150: 931-943.

Li G, Zhang G, Lance R (2007). Recent Advances in Breeding Barley for Drought and Saline Stress Tolerance. Advances Molecular Breeding Toward Drought Salt Tolerant Crops, pp. 603-626.

Mano Y, Takeda K (1997) Mapping quantitative trait loci for salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare L*). Euphytica. 94: 263-272.

Munns R, Husain S, Rivelli AR, James RA, Condon AG, Lindsay MP, Lagudah ES, Schachtman DP, Hare RA (2002) Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. Plant Soil, 247: 93-105.

Teulat B, Borries C, This D (2001) New QTLs identified for plant waterstatus, water-soluble carbohydrate and osmotic adjustment in a barley population grown in a growth-chamber under two water regimes. Theor. Appl. Genet. 103: 161-170.

Therrien MC (2003) Heritability estimates for forage quality in barley. Barley Genet. Newslett. 3:16-17.

Wang S, Basten CJ, Zeng ZB (2007) Windows QTL cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. Available at http://statgen.ncsu.edu/qtlcart/wQTLcart.htm .

Weidner A, Varshney RK, Buck-Sorlin GH, Stein N, Graner A, Borner A (2006). QTLs for salt tolerance: Comparison of barley mapping populations. Pflanzenzüchtung und Genomanalyse, pp. 123-130.