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**ARTICLE**

# Segregation- and association based mapping of loci influencing osmotic tolerance in barley

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**ABSTRACT** Identifying quantitative trait loci (QTLs) influencing abiotic stress tolerance could speed up the breeding process via marker assisted selection (MAS), however the accuracy of QTL analysis is a limiting factor to use successfully the identified marker alleles. We used a new approach to map more accurately the loci affecting osmotic tolerance in barley, which permit the introduction of MAS in the breeding process of new and more tolerant varieties against abiotic stresses.
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**KEY WORDS**

barley, osmotic tolerance
association mapping
QTL-analysis

Abiotic stress tolerance character – including osmotic- and drought tolerance - in plants are known as quantitatively inherited „polygenic" traits. These traits are generally affected by small number of quantitative trait loci (QTLs) and environmental factors as well. The final goal of such studies is to use the identified advantageous marker alleles for marker assisted selection of genotype with higher phenotypic value. However, their usability is limited in the case of "small effect loci", because of the high support interval of the identified QTLs. The support interval is generally a large region covering 10 to 30 cM (Kearsey and Farquhar 1998), therefore for accurate mapping the fine mapping of loci of interest are needed. This step is time consuming and expensive, and could avoid by using more independent replicates under more environmental conditions as it was proved by Price (2006). The aim of our study reported here was to map QTLs affecting osmotic tolerance in barley with high accuracy using high number of independent replications.

## Material and Methods

Plant material: 94 Double haploid (DH) lines and the parents (DOM, REC) of the Oregon-Wolfe Barley (OWB) population were examined at two different developmental stages. The population is mapped with 643 markers (the marker data were kindly provided by Nils Stein, IPK-Gatersleben, Germany). The full length of the genetic map covers an 1164 cM region with an average 1.8 cM distance between markers. For investigating marker and phenotype association 40 barley genotypes are selected, including varieties and wild relatives.

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Screening system: Germination stage: tests were carried out on filter paper in plastic boxes moistened with 15 m/V% polyethylene glycol (PEG 6000, Sigma) and with distilled water as control. The seeds were germinated and measured after 8 days. The shoot and root lengths were determined and their ratio under stress and non-stress conditions was used as a Tolerance Index for osmotic tolerance. The experiment was replicated independently 9 times. Seedling stage: Germinated seedlings were grown in half-strength Hoagland solution in the first week, and in complete Hoagland solution from the second week. The osmotic stress was induced by adding 15 m/V % PEG to the solution from the second week, and was replicated independently 3 times. Three additionally replicates were also performed applying 18 m/V% PEG solutions to provoke osmotic stress. The Tolerance Index was calculated from the shoot dry weight and shoot length data at the end of the third week.

Statistical analysis: The significant differences between mean values were determined by one-way analysis of variance (ANOVA). The distribution of the phenotypic data was tested by Statistica 6.0 (STATSOFT Inc, 2001). For QTL analysis, the phenotypic data measured under control and osmotic stress treated environment were used. The position of the QTLs was determined in each replicates by interval mapping method using the software MapQTL 5.0 (Van Ooijen 2003). The positions of the QTLs calculated in the independent replicates were compared, and the same QTLs determined at least 2 replicates were collected in both investigated developmental stage. The accurate positions of the QTLs were calculated from their positions in the separate experiments, taking the arithmetic means of the individual cM values.

**Table 1.** Summary of the result of QTL analysis.

| Chr /cM | DNM | Affected traits | Average LOD value | Effect came from |
|---|---|---|---|---|
| 1H, 61.05 cM | ≤ 0.7 cM | SLC, SLT, SDW, SDWT, RLC, RLT | 2.6 | REC parental line |
| 2H, 88.26 cM | ≤ 0.1 cM | SDWC, SDWT, SLT | 2.9 | DOM parental line |
| 3H, 163.00 cM | ≤ 1.4 cM | SLT, SLTI | 2.5 | REC parental line |
| 5H, 69.40 cM | ≤ 0.6 cM | SLT, SLTI, RLTI, SDWT | 2.7 | DOM parental line |
| 7H, 76.75 | ≤ 0.2 cM | SLC, SLT, SDWT | 2.4 | REC parental line |
| 7H, 82.60 cM | ≤ 0.8 cM | SLC, SLT, SLTI, RLC, SDWC, SDWT | 3.2 | REC/DOM parental line |

Chr: chromosome; cM: centimorgan; DNM: distance from the nearest marker; SLC: shoot length control, SLT: shoot length under osmotic stress; SLTI: Tolerance Index calculated from shoot length data; RLC: root length control, RLT: root length under osmotic stress; RLTI: Tolerance Index calculated from root length data; SDWC: shoot dry weight control; SDWT: shoot dry weight under osmotic stress

## Results and Discussion

For the traits (shoot lengths under control and stress treated environment, root length under control and stress treated environment, shoot length Tolerance Index and root length Tolerance Index) investigated in the 9 independent replicates at germination stage we determined 105 QTLs. Most of the QTLs affect more than one traits (i.e. shoot and root length), therefore only 47 independent QTLs were identified. From that number only a subset of QTLs were recognized in more replicates (16 QTLs), which can show the great influence of environmental factors. From 16 QTLs only 3 were drought specific that means they were found only in osmotic stress treated environment. These QTLs were located on the chromosome 3H, 5H and 7H.

In seedling stage we investigated the shoot lengths (under control and osmotic stress treated conditions) and shoot dry weights (control and treated) and Tolerance Indexes calculated from them. For those traits we determined in 6 replicates 108 QTLs, from which only 42 were independent. From these 42 QTLs only 7 were recognized in more than one replicates and only one QTL was specific for osmotic stress (locating on chromosome 3H).

When we compared the results obtained in the 2 developmental stages we found only 6 QTLs which were determined in each developmental phase; this result indicates the strong developmental stage specificity of the identified loci. From this 6 QTLs 3 QTLs were determined more than one replicates in each phases and 3 were determined more than one in 1 phase and only once in other developmental phases. Only 2 QTLs were classified as osmotic stress specific QTLs (were located on chromosome 3H and 5H, Table 1).

The position of QTLs were determined as described by Price (2006), therefore we obtained support intervals with a range less than 1 cM (in average, see Table 1). As a next step, we have already screened the osmotic tolerance of 7 barley lines (Brenda, Steptoe, Morex, Tadmor, Er/Apm and 2 wild genotypes) in germination and seedling stage. In our current experiments we are screening the osmotic tolerance of the remaining 33 genotypes, and after genotyping by the identified markers we analyze the phenotype-marker allele associations. The result of these studies could answer the question: Whether this new approach makes it possible to use identified marker alleles for marker assisted selection of osmotic stress tolerant line?

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