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IDENTIFICATION OF LOCI INFLUENCING THE DROUGHT TOLERANCE OF BARLEY AND THE ANALYSIS OF THEIR SUITABILITY FOR MARKER-ASSISTED SELECTION

MAIN POINTS OF A PHD THESIS

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1. BACKGROUND AND AIMS

Drought and water deficiency are major yield-limiting factors throughout the world. Although Hungary, which is situated in the temperate zone and has a continental climate, does not count as an area of severe drought, rainfall quantities tend to fluctuate widely from one year to the other. The total annual rainfall was 476 mm in 2003 and 686 mm in the following year, causing substantial differences in winter wheat yield averages (Cseuz 2009). The rainfall varies not only in quantity, but also in annual distribution. There is often a lack of rainfall in summer, during the main vegetation period of cereals, and the drought is often associated with high temperature. In addition, the quantity of rainfall in spring has declined considerably over the last 100 years. It is thus clear that under Hungarian conditions the aim is to breed crops capable of tolerating drought in any stage in their life cycle, with the least possible yield reduction in response to drought stress (Lelley 1963). In Hungary drought tolerance is a decisive component in the yield stability of cereal species, but unlike the situation in desert countries, the primary aim is not to ensure the survival of the plants, but to maintain yield levels.

Cereal species have a broad genetic basis, and related wild species represent an important gene source for breeders. The landraces stored in gene banks, and varieties that are no longer cultivated, also form a useful pool of initial or crossing materials for the development of varieties with better tolerance to environmental factors. The selection of materials for a specific breeding purpose can be greatly simplified by the use of marker-assisted selection (MAS): instead of the time-consuming testing of genotypes, it is sufficient to check for the presence of a marker linked to the desired trait, from which it can be predicted whether or not the given genotype carries this trait. If MAS is to be applied, however, it is first necessary to identify suitable genetic markers. MAS has proved to be efficient in the case of phenotypic traits of agronomic importance, and for biotic resistance. In the case of complex traits such as drought tolerance, however, breeders have so far been unable to utilise MAS as few or any linked markers have been identified, and those available only have a slight phenotypic effect.

Drought tolerance is a quantitative trait, the development of which is influenced not only by a large number of genes but also by the environment (Ceccarelli 1987). Despite the great number of results achieved in basic and applied research, the genetic and physiological background of this trait is still insufficiently known. Finding the genes responsible for drought tolerance and proving their functions is thus one of the major challenges facing genetics today (Luo *et al*. 2002).

As well as being an important crop, barley (*Hordeum vulgare* L.) is also an ideal cereal for genetic modelling. It is a diploid species with a much smaller genome than that of wheat, and in general it has good drought tolerance due to its origin. The homoeology existing between certain

chromosomes of species belonging to the *Triticeae* tribe (Feuillet and Keller 2002) means that the results achieved with barley may lead to a better understanding of the complex process of drought tolerance in related species (e.g. wheat).

The primary aim of the present work was to identify loci that influence the drought tolerance of barley and to determine the role of these loci. As water deficiency or drought may occur in any phase of plant development, loci responsible for tolerance must be identified in several stages of development in order to distinguish between general quantitative trait loci (QTLs) and those specific to a single developmental phase. Markers linked to these loci could then be used for marker-assisted selection. To achieve these aims, the following tasks were outlined:

1. Selection of a mapping population suitable for the analysis of drought tolerance, based on preliminary experiments on the parental lines of barley mapping populations.
2. Testing of the lines in the selected population in several phases of development: in seedlings, young plants and plants grown to full maturity.
3. The parallel use and comparison of various systems for testing tolerance of osmotic stress and drought, with the emphasis on simple, rapid methods for the testing of large numbers of genotypes, but also paying attention to more complicated methods closer to natural conditions.
4. Identification of loci responsible for drought tolerance in various phases of plant development.
5. Separation of the QTLs identified into loci with a general effect or specific to a single phase of development.
6. Comparison of the expressed sequence tag (EST) markers linked to the QTLs with sequence databases in order to identify candidate genes.
7. Determination of whether the markers found to be linked to loci responsible for drought tolerance are suitable for MAS by testing them on a variety collection including genotypes of various origins.

2. MATERIALS AND METHODS

2.1. Plant materials

2.1.1. Selection of a population suitable for drought tolerance mapping

In preliminary experiments the seven parental lines of four barley mapping populations (‘DOM’ × ‘REC’, ‘Steptoe’ × ‘Morex’, ‘Brenda’ × ‘HS213’, ‘Brenda’ × ‘HS584’) were tested in the seedling and young plant stages to determine which parental pair differed to the greatest extent

for osmotic stress tolerance and was thus the most suitable for mapping studies. The spring barley genotypes 'Tadmor' and 'Er/Apm' were used as references, as numerous traits linked to drought tolerance were previously mapped on a crossing population originating from these lines.

2.1.2. The Oregon Wolfe Barley (OWB) population

Based on the results of the preliminary experiments the OWB population was chosen for the detailed analysis of drought tolerance. This is a doubled haploid (DH) population consisting of 94 DH lines, originally developed for the mapping of morphological traits. It is generally considered as a reference population for genetic mapping. The population was developed by the Canadian geneticist Bob Wolfe by consistently backcrossing the parental lines, genotypes carrying exclusively dominant ('DOM') or recessive ('REC') morphological traits (Wolfe and Franckowiak 1991).

2.1.3. Barley collection

In order to confirm the results achieved using this two-parental crossing population on material with greater genetic variability, 39 agronomically valuable cultivars and landraces were used from the association collection compiled by the International Center for Agricultural Research in the Dry Areas (ICARDA, Aleppo, Syria).

2.2. Methods

The parental lines of the mapping populations were tested in the seedling and young plant stages, while those of the OWB population were also tested in the adult stage. The testing of the full OWB population in all three developmental phases was carried out in a total of nine experiments (*Experiments 1–9*).

2.2.1. Seedling tests

The seeds were germinated on moist filter paper in transparent plastic boxes (23 × 29 cm). Each box contained ten seeds of each of seven genotypes. In the control treatments the seeds were moistened with tap water, and in the osmotic stress treatment with 15% (w/v) polyethylene glycol (PEG) 6000 (Merck, Darmstadt, Germany). Germination took place under controlled conditions (20°C, 12-h illumination) in a G-30 plant growth chamber (Conviron, Manitoba, Canada). The seven parental lines and the barley collection were tested in two independent replications and the OWB population (*Experiment 1*) in seven. Ten plants were tested in each replication and treatment of each genotype. The shoot and root lengths were recorded on the 8^{th} day.

2.2.2. Tests in the young plant stage

The germinated seeds were placed on nutrient solution (at approx. 1 week old) and were grown for 7 days in aquaculture in a plant growth chamber (PG-15, Conviron, Manitoba, Canada) with 14 h illumination and day/night temperatures of 18/13°C. The nutrient medium was Hoagland solution.

Osmotic stress was induced using nutrient solution containing 15 or 18% (w/v) PEG 6000 (Merck, Darmstadt, Germany). Treatment with 15% PEG was started a week after the beginning of aquaculture and after 7 days the PEG concentration was increased to 18% for a further 7 days. The shoot length was measured after each week of treatment, and the shoot dry weight at the end of the experiment, after the second week of treatment. In each case, plants grown on normal nutrient solution were used as a control. Irrespective of the experiment type, 8–15 plants were grown for each genotype. Both the seven parental lines and the OWB population were tested in two independent replications. In the experiment on the full OWB population (*Experiment 2*) parameters indicative of biomass production (shoot length, shoot dry weight) were determined, while in the case of the parental lines measurements were also made on relative water content (RWC), osmotic potential (OP) and osmotic adaptation (OA).

The relative water content of the plants was calculated using the equation RWC = (FW – DW) / (SW – DW) × 100 (Barrs and Watherley 1962), where FW = fresh weight, DW = dry weight and SW = saturated weight. Osmotic potential was recorded using an osmometer (Osmomat 030-D, Gonotec, Germany) on the basis of changes in freezing point, according to the method of Bajji *et al.* (2001).

In addition, an abbreviated version of the two-stage PEG test, involving only seven days of treatment with 15% (w/v) PEG, was carried out on the full OWB population (*Experiment 3*) and the barley collection. These tests were set up in a regulated greenhouse (IPK, Gatersleben, Germany), with additional illumination as required. This experiment was carried out in three independent replications for the OWB population and two for the barley collection. At the end of the experiment the shoot dry weight was recorded for both the control and treated plants.

2.2.3. Tests on the parental lines of the OWB population in the adult stage

Germinated seeds of the OWB population were planted two to a pot in pots containing 4 kg mixed soil and grown in a PGB-36 growth chamber (Conviron, Manitoba, Canada). For each genotype and treatment, six plants were sown in three replications. Until heading, the plants were grown with normal water supplies (80% of maximum water capacity), after which water was withheld from three pots per genotype until the soil dried to 40% of maximum water capacity. The treated plants were maintained at this water content value until maturity, while the control plants were given ideal water supplies. The relative water content of the flag-leaf was determined twelve days after the start of the stress treatment, while plant height, total yield, thousand-kernel weight and spike number per plant were recorded at full maturity.

2.2.4. Testing in a polythene tent (*Experiment 4*)

The experiment was set up in March 2005 in a polythene tent measuring 6 × 30 m at IPK (Gatersleben, Germany). The 94 lines of the OWB population were sown in three replications in a

random block design. All the plots were irrigated until heading. When 50% of the genotypes had started to head, irrigation was terminated on two plots. Unfortunately, the extent of water withholding proved to be too weak or too late, due to the water reserves of the soil. The water deficiency did not cause any significant change in the yield, only reducing the plant height. For this reason, only plant height data were processed from this experiment.

2.2.5. Greenhouse tests (*Experiment 5*)

The cereal seeds were planted one to a pot in March 2005 in pots containing 2 kg garden soil. Eight plants were grown for each genotype and the water supplies were ideal until heading. Ten days after the start of heading water was withheld from one of each pair of pots until the soil had dried to 40% of maximum water capacity. Water was then added on a weight basis. The control plants were grown with ideal water supplies until maturity. At harvest the height, total yield and thousand-kernel weight of the plants were determined.

2.2.6. Phytotron test (*Experiment 6*)

The lines of the OWB population were planted one to a pot in pots containing 2 kg of mixed soil and grown in the Martonvásár phytotron. Ten plants of each genotype were tested in a random block design with five replications. The temperature was 18/14°C for two weeks, 20/16°C for a further two weeks and finally 22/20°C. All the plants were given normal water supplies until heading. Drought stress was begun on the 5^{th} day after the spikes appeared. No water was supplied until the volumetric soil moisture content (VSMC) had dropped to 7.0 ± 0.5%. This value was then maintained until maturity. Seven days after the start of water withholding, samples were taken from the flag-leaf to determine RWC and osmotic parameters. The flowering date was recorded, while plant height, total yield and thousand-kernel weight were measured at full maturity.

2.2.7. Field testing system with controlled water supplies (*Experiment 7*)

This experiment was carried out under a movable rain shelter in the Martonvásár nursery. The polythene roof only covers the plots in the case of rain, to completely exclude natural rainfall. The water supplied to the area is regulated by an automatic drip irrigation system. Each of the six plots (3.4 × 5 m) under the shelter can be irrigated separately. Three of the six plots were given ideal water supplies, while the other three were subjected to drought stress.

The OWB population was sown on the experimental plots in March 2007. In each plot there were three seeds of each genotype, with four genotypes per row and a row distance of 15 cm, in a random block design with three replications. When 50% of the genotypes had begun to head, irrigation was withheld from three plots. At harvest, the moisture content of the treated plots at a depth of 10 cm was 6 VSMC %, compared with 28 VSMC % on the control plots. The plant density, plant height, total yield per plant and thousand-kernel weight were recorded at harvest.

2.2.8. Field experiment (*Experiment 8*)

In 2007, in addition to the field experiment with controlled water supplies, a further field experiment was set up in a random block design with three replications. With the exception of the water supplies, the block size, plant arrangement and experimental conditions were the same as those described for *Experiment 7*. At harvest the soil moisture content at a depth of 10 cm was 8 VSMC %.

2.2.9. Chemical desiccation in the field (*Experiment 9*)

Chemical desiccation tests on the OWB population were carried out in the IPK nursery (Gatersleben, Germany) in 2006. Each genotype was sown in four rows 1 m in length, with a row distance of 20 cm. Two rows of each genotype were sprayed with a 0.5% (w/v) aqueous solution of potassium chloride (KI) 14 days after heading. The yield of the main spikes of desiccated and control plants was determined after harvesting and threshing. The test on the barley collection was carried out in two independent replications. In this case the KI concentration was 1.5%.

2.3. Search for EST homology

The sequences of the EST markers located in the peak region of the QTLs found to influence several traits in at least two developmental phases were subjected to protein-based homology search. The EST marker sequences were downloaded from the GrainGenes database (http://wheat.pw.usda.gov/). The EST consensus sequences assembled for each locus (*unigenes*) were downloaded from the NCBI (http://www.ncbi.nlm.nih.gov) and HarvEST (http://www.harvest-web.org, barley, 1.72 #35) databases. The protein-based comparison was made using the BLASTX 2.2.19 program against the NCBI *non-redundant* database. The possible function of the sequences was deduced from that of the most similar rice orthologue.

2.4. Marker analysis

Total genomic DNA was isolated from the genotypes of the barley collection using the method described by Plaschke *et al.* (1995). The primer sequences of the microsatellite markers were downloaded from the GrainGenes database (http://wheat.pw.usda.gov/) and the polymerase chain reaction (PCR) was carried out according to Röder *et al.* (1993). The size of the PCR products was determined using a QIAxcel System (Qiagen GmbH, Hilden, Germany) fragment analyser.

2.5. Statistical analysis

The phenotypic data were subjected to analysis of variance and correlation analysis using the SPSS 16.0 statistical program. Tolerance indexes (TI) were calculated for each trait from the ratio

of parameters measured under treated and control conditions. The TI values for each phenotypic trait were mapped as parameters indicative of the degree of tolerance.

The QTLNetwork V2.0 program (Yang and Zhu 2005) was primarily used for QTL analysis, which applies the mixed-model based composite interval mapping (MCIM) method.

The consensus map compiled by Marcel *et al.* (2007) was used to compare the major drought tolerance-linked barley QTLs previously described or identified in the present work, with the help of the 'Cmap' function of GrainGenes.

3. RESULTS

3.1. Selection of the mapping population

3.1.1. Seedling tests on parental lines

The osmotic stress tolerance of the parental lines of five mapping populations was first tested in the seedling stage. For each genotype the PEG treatment resulted in a reduction in shoot length compared with the control. During osmotic stress the smallest shoot length was recorded for the genotypes 'DOM' and 'Tadmor'.

The tolerance index (TI) calculated from shoot length exhibited substantial differences between the genotypes, with the lowest value for 'Tadmor' (0.23) and the highest for 'REC' (0.82) (where TI = 1 represents absolute tolerance and TI = 0 absolute sensitivity). In this stage of development the root length exhibited close correlation with the shoot length (control shoot length and control root length: $r = 0.82$; treated shoot length and treated root length: $r = 0.77$; TI shoot length and TI root length: $r = 0.83$; significant at the $P \leq 0.01$ level).

When the parental lines of the mapping populations were compared, significant differences were found between the genotypes 'DOM' and 'REC' and 'Tadmor' and 'Er/Apm'.

3.1.2. Tests on parental lines in the young plant stage

In the experiment involving two-step osmotic treatment on the parental lines, detailed plant analysis was carried out after the 18% PEG treatment. During osmotic stress the increase in shoot length and biomass slowed considerably compared to the control. Among the nine genotypes, 'Steptoe' had the greatest shoot length and shoot dry weight after treatment, but the tolerance index was highest for 'DOM'.

An analysis of the relative water content of the plants indicated that in response to osmotic stress 'Morex' and 'Er/Apm' suffered the greatest reduction, while that of 'DOM' and 'REC' was more moderate. The best osmotic adaptation (OA) was observed for the genotype 'Er/Apm'.

When the parental lines of each mapping population were compared, the only significant difference on the basis of shoot length TI was found for 'DOM' and 'REC'. A similar tendency was found for the dry weight values.

Based on the results of seedling and young plant tests, and on the availability of genetic maps for the populations, the OWB population arising from a cross between 'DOM' and 'REC' was found to be the most suitable for further mapping analysis.

3.1.3. Tests on the parental lines of the OWB population in the adult stage

Before investigating the OWB population, information was required on the adult tolerance of the parental lines, so the tests made in the early stages of development were continued in the adult stage.

The 'DOM' and 'REC' phenotypes differed considerably: the first was rather short, with small spikes and good tillering, while the second was taller, with fewer tillers but larger spikes. Drought stress after flowering caused significant yield losses in both genotypes, but the yield of 'DOM' was somewhat greater. The thousand-kernel weight only declined significantly for 'REC'. A difference of 11 days was recorded between the flowering dates of the two genotypes.

3.2. Seedling tests on the OWB population

3.2.1. Phenotypic traits of the OWB population in the seedling stage

The lines making up the OWB population were tested in the seedling stage in seven independent replications (*Experiment 1*). The treatment significantly retarded shoot and root growth, but there were substantial differences between the lines, amounting to more than 10 cm in the case of both shoot and root length between the genotypes with the smallest and largest values.

3.2.2. Loci identified on the OWB population in the seedling stage

Six different traits were investigated in the seedling stage: control shoot length, treated shoot length, shoot length tolerance index, control root length, treated root length, root length tolerance index. In this stage of development, when all the traits were considered, a total of eight QTLs were identified at four different loci. In general one QTL was found for each trait, while two were found in two cases, located on chromosomes 2H, 5H and 7H.

3.3. Tests on the OWB population in the young plant stage

3.3.1. Phenotypic traits of the OWB population in the young plant stage

Lines from the OWB population were studied in two types of experiments in the young plant stage (*Experiment 2*: 7 days growth, 7 days 15% PEG treatment, 7 days 18% PEG treatment; *Experiment 3*: 7 days growth, 7 days 15% PEG treatment). Intensive growth was observed in this stage under control conditions. A comparison of the control data of the two independent

experiments (*Experiments 2 and 3*) showed that the dry weight of the plants increased 1.5 times on average during the third week. In response to the more severe osmotic stress (18% PEG) the population averages calculated from dry matter data were lower than those recorded a week earlier after the 15% treatment. As the data recorded for the two levels of osmotic stress originated from different experiments, the difference may have been due to the longer period of stress. It can be said in general, however, that the addition of 18% PEG to the nutrient solution caused such severe osmotic stress that the plants were only capable of growing to a slight extent, if at all.

3.3.2. Loci identified on the OWB population in the young plant stage

QTL analysis in the young plant stage was carried out on shoot length and shoot dry weight data, and on the tolerance indexes calculated for these traits. A total of 7 QTLs were detected, located at four loci on chromosomes 5H, 6H and 7H. With the exception of shoot dry weight, a single significant QTL was found for each trait.

3.4. Tests on the OWB population in the adult stage

3.4.1. Phenotypic traits of the OWB population in the adult stage

The drought tolerance of the lines belonging to the OWB population was examined in a total of six different tests in the adult stage. In contrast to the earlier stages of development, drought stress in the adult stage did not cause a significant reduction in all the traits. In the polythene tent experiment (*Experiment 4*) the stress applied proved to be too weak and did not cause significant changes in yield parameters, so only the shoot length data were processed from this experiment. By contrast, the water withholding in the greenhouse experiment (*Experiment 5*) had no significant effect on the shoot length, but caused a significant reduction in yield and thousand-kernel weight. This could probably be attributed to the excessive heat in the greenhouse in late spring and early summer, as there was also a decline in the shoot length of the control plants despite their ideal water supplies. In the phytotron experiment (*Experiment 6*), although the stress resulted in a significant decline in the yield average of the population, there was no change in the thousand-kernel weight, while in the rain shelter experiment (*Experiment 7*) the opposite was observed. This could probably be attributed to the fact that when plants are grown in pots there is generally only a small number of fertile shoots (often only 2 or 3), so the small kernel number per spike in the case of severe stress after flowering is the reason for reduced yields. If the kernel number is very low but the remaining kernels exhibit normal grain filling, the thousand-kernel weight does not reflect the negative effect of the stress. Under field conditions, on the other hand, the plants have better tillering, so a low level of water deficiency at a later stage may influence the thousand-kernel weight rather than the yield if there is nothing to restrict further tillering (e.g. high temperature). It was seen in the

phytotron experiment (*Experiment 6*) that water withholding significantly reduced the water content and osmotic potential of the plants, but to a very different extent for the individual genotypes.

3.4.2. Loci identified on the OWB population in the adult stage

A total of 20 QTLs were identified in the adult stage, each of which was able to influence one of the 16 traits investigated. Only one of these loci was found to have a significant effect on the given trait in one environment but not in the others. This was the QTL detected on chromosome 7H in the phytotron experiment, which influenced yield per plant and overlapped with QTLs identified in the young plant stage. The other 19 QTLs exerted a significant effect in all the adult stage experiments.

The QTLs with the greatest additive effect, influencing the thousand-kernel weight, yield per plant and shoot length, were located on chromosome 2H between 132.7 and 145.7 cM. All the traits related to the water balance were mapped to the 7H chromosome (50.3–55.9 cM) and coincided with the loci that influenced root growth in the seedling stage. In addition, loci responsible for yield and plant height were found on chromosomes 1H, 6H and 7H (the latter was located distally from the other locus identified on chromosome 7H).

3.5. Overlapping regions identified on the OWB population in several development phases

When all the QTLs identified in the present work were considered, five regions were found to influence several traits in at least two phases of development. These were located on the 2H (*QDr.ari-2H*), 5H (*QDr.ari-5H*), 6H (*QDr.ari-6H*) and 7H (*QDr1.ari-7H* and *QDr2.ari-7H*) chromosomes. The regions located on the 5H and 6H chromosomes were located close to the *GBS0318* and *GBR1052* markers. These two regions could only be detected under stress conditions and in the case of the tolerance indexes. The *QDr.ari-5H* region was found to influence the shoot and root length in the seedling stage and the shoot dry weight in the young plant stage, while *QDr.ari-6H* was linked with the shoot length tolerance index in the young plant and adult stages. The other three regions played a role under both control and stress conditions. The *QDr2.ari-7H* cluster was the only one of the five regions which had a significant effect on certain traits in all three developmental phases.

3.6. Homology between EST sequences and functional proteins

It would appear that the five QTL clusters identified in the present work could be attributed to the effect of individual genes, suggesting that the sequences of the EST markers coinciding with the most probable position (peak position) of the QTLs/genes could be used to draw conclusions on the

function of the regions. The candidate genes identified for the five regions included dehydration-responsive element-binding protein 2F (DREB2F), papain-like cysteine proteinase, aldehyde-dehydrogenase, trehalose-6-phosphate synthase and a serine/threonine protein phosphatase subunit.

3.7. Testing of the barley collection

3.7.1. Phenotypic data of the barley collection

The aim of analysing the barley collection was to test the regions found to be linked to drought tolerance in the OWB population in an independent genetic background. The 39 genotypes were examined under the same conditions as used for the OWB population, in seedling and young plant tests, and using chemical desiccation in the adult stage. The effect of the treatments was significant in all the experiments.

3.7.2. Testing of selected markers on the barley collection

Markers are only suitable for marker-assisted selection if they can be used on a large number of genotypes, simply and cheaply. These conditions are fully satisfied by microsatellite markers, so it is worth using this type of markers wherever possible. Among the EST sequences located in the peak regions of the five QTL clusters detected in the present work, the *GBM1359* and *GBM1498* markers found on chromosomes 2H (*Qdr.OWB-2H*) and 7H (*QDr2.OWB-7H*) are based on microsatellite polymorphism. In the other three cases, where there were no microsatellites in the peak region of the QTL cluster, the bordering 2.5 cM region was also studied. A GBM marker was found not far from the cluster on the 6H chromosome (*QDr.ari-6H*), but none were found for the regions identified on chromosomes 5H (*QDr.ari-5H*) and 7H (*QDr1.ari-7H*). The *GBM1359, GBM1404* and *GBM1498* microsatellite markers were thus used to analyse the strength of the correlation between the genetic background and phenotypic distribution of the barley collection.

Two allele types were identified for the *GBM1359* and *GBM1404* markers and three for *GBM1498* (two homozygous and one heterozygous). Genotypes heterozygous for the *GBM1498* marker were excluded from the statistical analysis. The distribution of allele types within the barley collection was not correlated with the origin of the genotypes or with whether they were landraces or cultivars.

The phenotypic values of the groups formed from the 39 barley genotypes on the basis of their alleles were compared in all three developmental phases and significant ($p \leq 0.05$) differences were found between the groups in six cases. For all three markers yield differences were found under stress conditions between genotypes carrying different alleles, but the greatest effect was observed for the *GBM1359* marker, with a difference of 36% between the two groups for the yield per spike. The shoot length values recorded with the *GBM1498* and *GBM1359* markers in the seedling stage also exhibited significant differences between the allele groups.

3.8. Novel scientific results

1. Identification of loci influencing the osmotic stress and drought tolerance of barley in several phases of development

After selecting the most suitable mapping population, the 94 lines of the OWB population were examined in three phases of development (seedling, young plant and adult plant stage) in various independent, replicated experiments modelling water deficiency. The phenotypic data were used for QTL analysis and a total of 38 QTLs influencing shoot and root length, shoot dry weight, yield parameters and physiological traits were identified. The joint consideration of the individual QTLs revealed five regions that influenced several traits related to drought tolerance in at least two phases of development. These regions were located on the 2H, 5H, 6H and 7H chromosomes. It was also proved that the effect of certain QTLs differed in different developmental phases, confirming that drought tolerance is a development phase-specific trait.

2. Determination of candidate genes for the QTLs using an EST-based map

The rice homologues exhibiting the greatest similarity to the EST markers located at the newly identified regions in barley were determined. As the rice genes are better annotated, they can help to draw conclusions on the possible functions of the genes/EST sequences known to be found in these QTL regions in barley. On the basis of the present results, the most likely candidate genes for the five regions were listed, and further studies are planned to determine their precise role.

3. Identification of markers for use in the marker-assisted selection of drought-tolerant barley genotypes

Drought tolerance analysis was carried out on a barley collection consisting of 39 genotypes in order to check the results obtained with the OWB population in an independent genetic background. The three selected microsatellite markers were genotyped on the variety collection in an attempt to find correlations between the allele type of the markers and the distribution of phenotypic values. With all three markers significant differences were found for the yield per spike between genotypes belonging to different allele types under stress conditions. The results suggest that markers *GBM1404, GBM1498* and *GBM1359* could be suitable for the marker-assisted selection of drought-tolerant genotypes, but this needs confirmation on a wider range of genetic material.

4. CONCLUSIONS AND RECOMMENDATIONS

In can be seen from the present experiments on drought tolerance that the results achieved in seedlings and young plants do not always conform with those obtained in tests on adult plants. This

suggests that osmotic and drought stress tolerance is a development phase-specific trait and that experiments should be carried out in various stages of development if loci influencing stress tolerance are to be identified.

The effect of regions associated with drought tolerance, identified on the two-parental mapping population, was confirmed on agronomically valuable lines with a different genetic background. These results could promote the application of marker-assisted selection even for traits as complex as drought tolerance. However, if the wide applicability of the three microsatellite markers selected on the basis of the present results is to be confirmed, further experiments will be required on a larger number of agronomically valuable barley lines.

The mean distance of the markers on the genetic map of the OWB population is 1.8 cM and the most probable location of the newly identified loci can be given with a precision of 0.0–5.9 cM. In addition to the candidate genes identified in the present work, this region contains numerous other genes which could also be responsible for the phenotypic effects detected. It is also possible that the effect of the QTLs identified, which influence several traits, is not due to the pleiotropic manifestation of single genes, but to that of several closely linked genes. The separation of these potential genes/loci, and the exact determination of the physical distance covered by these regions and of how many genes they contain, will only be possible after a physical map of barley has been compiled.

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