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**Malting quality QTL validation and
Marker-Assisted Selection for malting
quality, winter growth habit and frost
tolerance in the winter x spring cross
'Nure x Tremois'**

Introduction

Plant breeding, in its conventional form, is based on phenotypic selection of superior genotypes within segregating progenies obtained from crosses. Phenotypic selection has led to the highly improved performance of crop species. However, breeding for quantitative traits, such as malting quality traits, has been difficult because the traits are affected by multiple genes (G), environments (E), and G x G and G x E interactions.

Selection for good malting quality often involves two approaches: prediction tests and micromalting. A number of methods have been proposed for predicting the quality potential of breeding lines without the need for micromalting and extract determinations (Davies 1992; Henry 1985; Morgan and Gothard, 1981). The advantage of prediction tests is to remove lines of obvious inferior quality early in the breeding process. However, the use of prediction methods has been limited to a few parameters (Burger *et al.*, 1985) and these methods have not eliminated the need for malting (Henry 1985). The standard method of assessing the quality potential of a progeny from a breeding program is by micromalting, followed by micromashing and analysis. However, the process of micromalting and micromashing is time-consuming and resource-intensive, despite recent advances in technology and procedures (Glennie-Holmes 1990). Furthermore, in the early stages of a barley breeding program only a small amount of grain is available for the assessment, so it is difficult to conduct micromalting. More efficient and feasible approaches for identifying genotypes with good malting quality are highly desirable.

Molecular marker-assisted selection (MAS) is an approach that has been developed to avoid the problems connected with conventional plant breeding, changing the selection criteria from selection of phenotypes towards selection of genes, either directly or indirectly. Molecular markers are clearly not environmentally regulated and are unaffected by the conditions in which the plants are grown and are detectable in all stages of plant growth. With the availability of an array of molecular markers and genetic maps, MAS has become possible both for traits governed by major genes as well as for quantitative trait loci (QTLs) (for a detailed review see Francia *et al.* 2005 and Collard *et al.* 2008).

Genetic Linkage maps have been developed in various barley crosses and used to detect and map QTLs for various traits, including grain and malt quality (for a recent review, see Fox *et al.* 2003). Information on the position of QTLs relative to marker loci provides the basis for MAS of quantitative traits. In barley, MAS is of particular interest for the development of genotypes with superior malting quality because thorough assessment of grain and malting quality traits is expensive and requires large grain samples; moreover, grain and malting quality traits are subject to considerable environmental variation and genotype x environment interaction. With MAS for QTLs that affect grain and malt quality, barley breeders could limit the breeding population to those progeny with the highest probability of having superior malting quality.

MAS may follow one of several strategies. One involves the genotyping of additional progenies from the mapping population (i.e., progenies that were not used for QTL detection). Application of this approach in barley has been used for QTLs

affecting malting quality (Han *et al.*, 1997; Romagosa *et al.*, 1999; Igartua *et al.*, 2000). Another MAS strategy involves backcrossing the mapping progenies to one of the parents. In barley, this has been used for QTLs affecting yield (Kandemir *et al.*, 2000a) and head shattering (Kandemir *et al.*, 2000b). A third MAS strategy involves intercrossing of selected progenies from the mapping population. For example, Zhu *et al.* (1999) crossed two doubled haploid lines from the Steptoe/Morex mapping population on the basis of their molecular marker genotypes in regions of the genome where QTLs have been detected for grain yield.

All of the strategies referred to above are restricted to materials derived directly from parents that had been used in QTL detection experiments. For MAS to be widely applicable in plant breeding programs, it would be desirable to transfer favorable QTL alleles into other materials. In barley, this has been used for QTLs affecting yield (Schmierer *et al.* 2004) and α -amylase activity (Ayoub *et al.* 2003).

Here, we report on a marker-based selection experiment of additional progenies from the 'Nure' x 'Tremois' mapping population involving winter-habit, frost tolerance and malt quality traits in the two-row barley cross. Francia *et al.* (2004) developed a molecular marker map for this cross, which was then used to detect and map QTL both for traits related to frost-tolerance and vernalization requirement (Francia *et al.*, 2004), and malting quality (Laidò *et al.*, submitted). The objectives of the present study were: 1) to validate the existence of QTLs in five regions of the barley genome that were reported to affect winter growth habit, frost tolerance and several malting quality

traits; and 2) to assess the effectiveness of marker-based selection to select winter type, malting, barley DH lines.

Materials and Methods

QTL position and effects

In respect to malting quality traits five QTL regions were considered in this experiment. The first region on chromosome 1H was the most important for its contribution to malting quality in the NxT cross, since it harboured QTLs for friability, wort viscosity, β -glucan content, hot water extract and acrospire growth. In particular in the large region of chromosome 1H included between the markers Bmac0399 and Bmag0382, 11 QTLs have been identified, mostly grouped in four clusters (Figure 1). For all these QTLs the parent 'Tremois' contributed favourable alleles, and the proportion of explained phenotypic variance ranged from 6.7% to 65.1% (Laidò *et al.*, submitted). On chromosomes 2H and 3H near Bmac0093 and HVM33 respectively, we have considered two QTLs for friability identified in NxT cross (Figure 1). The last two regions were on chromosome 5H in which two malting quality QTLs were detected with a positive allelic effect from the feeding parent 'Nure' (Figure 1). A first locus controlling HWE was identified between markers HvCBF8 and HvCBF4, with a positive effect of 'Nure' of 0.45. A second QTL carried by 'Nure', for wort viscosity, was mapped in the interval Hv635P2.4-E38M50-215 with a proportion of explained phenotypic variance of 24.3% (Laidò *et al.*, submitted).

The malting quality QTLs mapped on chromosome 5H are partially coincident with the two major low-temperature tolerance

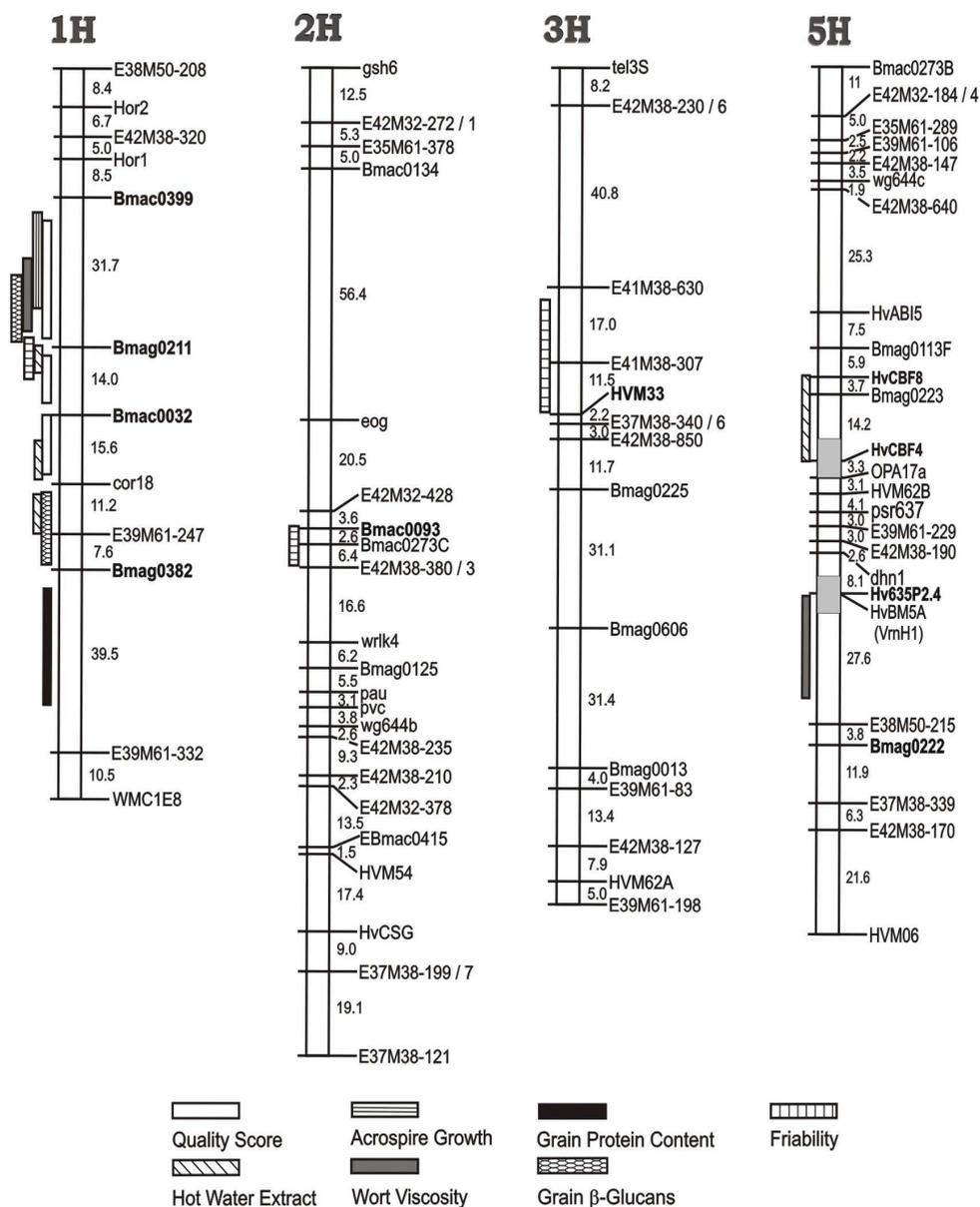


Figure 1: QTL map of chromosomes 1H, 2H, 3H and 5H of malting quality traits and grain yield in the NxT population (Laidò *et al.*, submitted). QTL bars drawn using the $\Delta 1$ -LOD support interval criterion are on the left of the chromosomes. Gray boxes inside chromosome 5H represent the frost tolerance QTLs *Fr-H2* (proximal) and *Fr-H1/VrnH1* (distal) as mapped by Francia *et al.* (2004) in the same population. Interval distances are in Kosambi cM and chromosomes are oriented with short arms at the top. Markers indicated in bold type are those used to represent or flank the QTL regions in this study.

QTLs, *Fr-H2* and *Fr-H1* (Figure 1), mapped in the 'Nure' (winter) x 'Tremois' (spring) population (Francia *et al.* 2004). The *Fr-H1* QTL of cold tolerance coincided with *VrnH1* (Francia *et al.*, 2004) and all the lines carried a 'Nure' allele at *Vrn-H1* locus (tagged by a candidate gene marker, *HvMB5A*), were classified as 'facultative' and 'winter', depending on allele at *Vrn-H2* candidate gene (*ZCCT-H*) on chromosome 4H.

In conclusion, in respect to the winter growth habit and frost tolerance three regions were considered in this experiment. These regions were as follow: near marker *HvCBF4* (*Fr-H2*), on the chromosome 5H; near marker *Hv635P2.4* (*Fr-H1/Vrn-H1*) on the chromosome 5H; and near marker *ZCCT-H* (*Vrn-H2*) on the chromosome 4H.

Plant material and markers used

Two hundred-ten doubled haploid (DH) lines were derived from the F1 generation of the 'Nure' x 'Tremois' cross. The winter parent 'Nure' is a frost tolerant feed cultivar, whereas the spring parent 'Tremois' is a frost susceptible malting variety. A random sample of 136 DH lines was used for marker map development (Francia *et al.*, 2004). We thus determined the alleles at the loci *Bmac0399*, *Bmag0211*, *Bmac0032*, *Bmag0382*, *Bmac0093*, *HVM33*, *HvCBF8*, *HvCBF4*, *Hv635P2.4*, *Bmag0222* and *ZCCT-H* for 78 DH lines that had not been used for mapping (Figure 1). Two *CBF* loci, *HvCBF4* and *HvCBF8*, were mapped using gene-specific primer as described by Francia *et al.* (2004). The molecular marker *ZCCT-H* was detected according to von Zitzewitz (2005). The eight SSR markers were screened for polymorphism using fluoresescently labeled primers and fragment analysis on a ABI PRISM 310

Genetic Analyzer (Applied Biosystems, Foster City, Calif.) according to Francia *et al.* (2004). Genomic DNA was extracted from young leaf tissue using a modified CTAB method (Saghai-Marooif *et al.*, 1984).

Selection strategy and field trial

The 78 DH lines, and 'Nure' and 'Tremois' were subjected to marker-assisted selection together with phenotypic one. Selection response was determined using a selection procedure based on a two-stage scheme. First, selection was based on molecular markers genotype, then conventional phenotypic selection was conducted on the selected genotypes.

The DH lines selected on the strength of molecular markers together with 'Nure' and 'Tremois' were sown in the last week of October ('autumn sowing') in 2005 at Fiorenzuola d'Arda, Northern Italy (44°55'N, 9°53'E, altitude 80 m a.s. l.), fertile clay-loamy soil pH 7.6, with 30-year average annual rainfall of 852 mm. A randomized block design was chosen, with three replications and a minimum plot size of 6.0 m² using 350 seeds/m².

Malting quality analysis

Malting quality traits have been measured for each DH lines selected on three field replications, using the kernel size fraction ≥ 2.0 mm. Grain protein content (GPC), friability, wort viscosity, hot water extract (HWE) and acrospire growth index were calculated according to Laidò *et al.* (submitted). A synthetic "quality score" (= *Score**, according to Gianinetti *et al.* 2005), obtained on the basis of HWE, wort viscosity and acrospire growth index, was calculated.

Results

MAS for malting quality traits

The molecular markers set flanking malting quality QTLs with major effect identified in the 'Nure' x 'Tremois' cross have been used to select among the 78 DH lines (named BNT), derived from the 'Nure' x 'Tremois' cross, lines with the allelic combination similar at the barley ideotype of winter high malting quality. This ideotype previously identified has been constituted from ten molecular markers, flanking the malting quality QTLs of the cluster on the chromosome 1H, the friability QTLs on the chromosome 2H and 3H, and the hot water extract and viscosity QTLs identified on the chromosome 5H.

In this study simultaneous MAS for five genomic regions has been conducted. The 78 DH lines BNT have been characterized at the ten molecular marker loci. On the basis of genotypic data eight DH BNT lines have been selected. The malting quality aptitude of the selected BNT lines has then been determined, in order to verify the presence and effect of malting quality QTLs (Laidò et al., submitted) considered in this experiment.

As expected, ANOVA pointed out that the two parents, Nure and Tremois, were significantly different for all the malting quality traits analysed. The malting parent 'Tremois' always outperformed 'Nure' for all the malting quality parameters (Table 1).

In this MAS programme to establish the correspondence between genotypic and phenotypic data the eight BNT lines have been selected in order to have three lines (BNT 149, BNT 156 and BNT 159) with an allelic combination partially (BNT 159) or completely (BNT 149 and BNT 156) different from the

ideotype of high malting quality and five lines (BNT 146, BNT 200, BNT155, BNT196 and BNT 221) with allelic structure almost equal to the ideotype.

Malting Quality																
Lines	Genotypic										Phenotypic					
	1H				2H	3H	5H				GPC (%)	Friability (%)	Wort Viscosity (cP)	HWE (%)	Acrospire Growth (0-1)	Score
	Bmac0399	Bmag0211	Bmac0032	Bmag0382	Bmac0093	HVM33	HvCBF8	HvCBF4	Hv635P2,4	Bmag0222						
BNT 149	B	A	A	A	B	A	B	B	B	B	10.72	56.32	1.70	67.65	0.49	-1.65
BNT 156	B	A	A	A	A	B	B	B	B	B	10.15	60.06	1.90	67.32	0.63	-2.09
BNT 159	B	B	B	A	B	B	B	B	B	B	10.12	71.50	1.69	71.14	0.52	-0.79
BNT 146	B	B	B	B	A	A	A	A	B	B	10.04	81.42	1.59	75.76	0.70	1.73
BNT 200	B	B	B	B	A	B	A	A	B	B	10.33	82.66	1.60	76.66	0.69	1.76
BNT 155	B	B	B	B	B	B	A	B	A	A	10.44	83.98	1.54	76.29	0.73	2.32
BNT 196	B	B	B	B	B	B	B	B	A	A	11.97	82.96	1.51	73.80	0.81	2.51
BNT 221	B	B	B	B	B	B	B	B	A	A	11.04	83.46	1.48	74.10	0.75	2.43
Nure	A	A	A	A	A	A	A	A	A	A	12.95	59.55	1.65	69.78	0.55	-0.20
Tremois	B	B	B	B	B	B	B	B	B	B	11.78	83.45	1.50	75.88	0.65	2.03
<i>DMS</i>											1.20	5.39	0.01	1.95	0.06	0.52

Table 1: Genetic and phenotypic malting quality traits data of the selected BNT lines and of the two parental

As expected the BNT 149 and BNT 156 lines have the lowest quality score and friability values, while the BNT 159 line, carrying in the region of chromosome 1H included between Bmac0039 and Bmac0032, and the ‘Tremois’ alleles at the friability QTLs on the chromosomes 2H and 3H, showed for friability, wort viscosity and hot water extract traits better values

than lines with an allelic combination completely different from ideotype.

The BNT 146, BNT 200, BNT155, BNT196 and BNT 221 lines carrying the 'Tremois' alleles in the region of chromosome 1H included between Bmac0039 and Bmac0382 showed high quality score values. In particular the BNT155, BNT196 and BNT 221 lines carrying the 'Tremois' alleles both in the region of chromosome 1H (Bmac0039-Bmac0382) and in the regions on chromosomes 2H and 3H near Bmac0093 and HVM33 respectively, and the 'Nure' alleles in the region of chromosome 5H included between Hv635P2.4 and Bmag0222. These 5 lines showed higher quality score values than the malting parent 'Tremois', although not statistically different from this last.

QTLs of frost tolerance and winter growth habit

As for frost tolerance among the eight BNT lines selected for malting quality, no one carried the 'Nure' alleles at both the two major low-temperature tolerance QTLs, *Fr-H2* and *Fr-H1*. In particular the BNT 146 and BNT 200 kept the 'Nure' alleles at the *Fr-H2* locus only, while the BNT155, BNT196 and BNT 221 lines carried the 'Nure' alleles at the *Fr-H1* locus (Table 2).

According to the model proposed by von Zitzewitz *et al.* (2005) the BNT 149, BNT 156, BNT 159, BNT 146, BNT 200 lines showed a spring growth habit because carrying the 'Tremois' allele at the locus *Vrn-H1*. While the BNT155, BNT196 and BNT 221 lines carried the 'Nure' allele at *Vrn-H1* locus and they were classified as 'facultative' or 'winter'. On the basis of the allele at *Vrn-H2* locus on chromosome 4H the BNT 155 line carrying the 'Tremois' allele had a 'facultative' growth habit while the BNT196

and BNT 221 lines keeping the 'Nure' alleles at both *Vrn-h* loci were classified as 'winter' (Table 2).

Lines	Frost Tolerance		Growth Habit		
	<i>Fr-H2</i> (HvCBF4)	<i>Fr-H1</i> (Hv635P2.4)	<i>Vrn-H1</i> (Hv635P2.4)	<i>Vrn-H2</i> (ZCCT-H)	Types
	<i>A</i>	<i>A</i>	<i>A</i>	<i>A</i>	<i>W</i>
BNT 149	B	B	B	-	S
BNT 156	B	B	B	-	S
BNT 159	B	B	B	-	S
BNT 146	A	B	B	-	S
BNT 200	A	B	B	-	S
BNT 155	B	A	A	B	F
BNT 196	B	A	A	A	W
BNT 221	B	A	A	A	W
Nure	A	A	A	A	W
Tremois	B	B	B	B	S

Table 1: Genetic growth habit and frost tolerance data of the selected BNT lines and of the two parental

Discussion

Our results validate the existence of major malting quality QTLs on chromosome 1H with effects on malt friability, wort viscosity, hot water extract and acrospire growth in the 'Nure' x 'Tremois' cross. The presence of linked clusters of major QTLs showing the same allelic contribution should have a positive effect for MAS of new malting varieties. In fact, in this work it was possible to observe that the interval among Bmac0399 and Bmac0382 is very important to increase the malting quality of the feeding parent cultivar 'Nure'. The BNT 159 line carrying the 'Tremois' alleles at the markers of the region of chromosome 1H included between Bmac0039 and Bmac0032 showed values statistically different from the BNT 149 and BNT 156 lines for all malting quality traits analysed.

Moreover, after comparing genotypic and phenotypic data it is interesting to note that the BNT 146, BNT 200, BNT155, BNT196 and BNT 221 lines, carrying the 'Tremois' marker alleles in the region of chromosome 1H included between Bmac0039 and Bmac0382, showed for all malting quality traits analysed values statistically different from the line BNT 159 carrying the 'Tremois' alleles only in the interval between Bmac0399 and Bmac0032. It is not possible to know whether the single QTLs of chromosome 1H are due to different linked genes or if they are the result of the pleiotropic effect of single key determinants. Given the close relationships among some malting quality traits (Burger and LaBerge, 1985), it seems logical that a single QTL might affect several traits pleiotropically. In this view, a fine mapping project of these

intervals and a positional cloning approach could answer these questions.

The friability QTLs on chromosomes 2H and 3H near Bmac0093 and HVM33 respectively, identified in 'Nure' x 'Tremois' cross had a proportion of explained phenotypic variance very low of 3.8% and 6.9% respectively (Laidò *et al.*, submitted). The same for the hot water extract QTL on chromosome 5H between markers HvCBF8 and HvCBF4, with a positive effect of 'Nure' of 0.45 and an explained phenotypic variance of 4.0%. For this reason the malt friability values among the five BNT lines with high malting aptitude were not significantly different. In the same way, the hot water extract values were very similar.

The wort viscosity QTL on the chromosome 5H, mapped in the interval between Hv635P2.4 and E38M50-215 and carrying the 'Nure' allele, it had a large effect on the explained phenotypic variance of 24.3% (Laidò *et al.*, submitted). The BNT 155, BNT 196 and BNT 221 lines carrying the 'Nure' alleles in the interval between Hv635P2.4 and Bmag0222, showed the quality score values statistically different from the BNT 146 and BNT 200 lines; moreover, they have very low values of wort viscosity.

As demonstrated, the selection of the loci with alternate large allelic effects, suitable combined, has permitted to select superior genotype combinations respect to the malting parent 'Tremois'. This is what we observed, at least for the synthetic score, for the lines BNT 155, BNT 196 and BNT 221. On the other hand, the role of the three minor allelic effect in the regions on the chromosome 2H, 3H and 5H (between HvCBF8 and HvCBF4) was not so evident. These results confirmed that MAS

for malting quality of major QTLs was more effective than MAS of minor QTLs.

The malting quality QTLs mapped on chromosome 5H were partially coincident with the frost tolerance loci *Fr-H2* and *Fr-H1/VrnH1* mapped in the NxT population (Francia *et al.*, 2004). This can be an useful result for the MAS of winter, frost tolerant and high malting quality lines. In fact as expected, all the selected lines with superior malting aptitude (BNT 155, BNT 196 and BNT 221) kept the 'Nure' allele at the *Fr-H1/VrnH1* frost tolerance locus. These lines have been analysed at the *Vrn-H2* locus on chromosome 4H and the BNT 196 and BNT 221 lines were classified as winter lines while the BNT 155 was classified as facultative.

In the present study, marker-assisted selection was conducted among progenies from the same cross in which the frost tolerance and malting quality QTLs have been mapped. This study confirm that the malting barley variety 'Tremois' carries desirable QTL for malting quality, but it also indicates that favorable malting quality QTL alleles may be found in germplasm not conventionally used in malting barley breeding programmes. Our results indicate that using the molecular marker set identified in this work the selection for winter, frost tolerance and high malting lines is possible, at least in material genetically derived from the 'Nure' x 'Tremois' cross. Marker-assisted selection may be an effective way to introduce winter growth habit, frost tolerance and malting quality alleles into elite germplasm. The introduction of winter growth habit, frost tolerance and malting quality alleles using advanced backcrossing with 'Nure' as donor parents is possible because the malting quality QTLs mapped on chromosome 5H are

partially coincident with the two major low-temperature tolerance QTLs, *Fr-H2* and *Fr-H1*, and *Fr-H1* locus is coincident with *VrnH1*. Following the *Fr-H2*, *Fr-H1/Vrn-H1*, *Vrn-H2* only loci to select winter, frost tolerance lines it is possible with the MAS. Here, we have show that marker-assisted selection is effective in selecting a superior sample of winter, low-temperature resistant and high quality genotypes from a segregating population. However, with selection based on marker genotypes alone, some phenotypically inferior genotypes may also be selected. The use of marker information in selection does not eliminate the need to gather reliable phenotypic data but it should permit breeders to allocate fewer resources to the evaluation of a reduced number of more advanced progenies that are more likely to carry favorable alleles at interest loci.

In conclusion, the molecular marker set prepared in this work resulted to be efficient to select, by means of MAS, superior barleys, at least in this cross combination cross. Further studies are necessary to understand which alleles, in the array of ten molecular markers, are present in different varieties to extend them use in most part of 'winter' x 'spring' combination cross.

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