2

QTLs for malting quality in the 'Nure' x 'Tremois' mapping population

QTL alleles from a winter feed type can improve malting quality in barley

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Abstract: In recent years, mapping key genes and/or QTLs (Quantitative Trait Loci) responsible for single malting quality traits in 'spring x spring' barley populations has been the main genetic approach to improve the efficiency of selection for malting quality. A genetic map derived from a 'winter x spring' two-rowed barley population Nure x Tremois (NxT), where malting quality traits were segregating, has been recently developed. Our objective was to map QTLs for malting quality from two years of trials in two contrasting locations (Northern and Southern Italy) in the NxT 'winter x spring' cross. The results allowed us to identify QTLs on six chromosomes, with a main cluster on chromosome 1H. For wort viscosity and malt extract, the favourable alleles at the two QTLs on chromosome 5H were carried by the winter feeding parent 'Nure'. Six QTLs for grain yield were also added to the map. Doubled-haploid lines with malting quality higher than the spring cultivar 'Tremois' were individuated in the NxT population. These higher quality lines all showed either a facultative or a winter growth habit, and a level of frost tolerance comparable to that of the winter, tolerant parent 'Nure'. This showed that, at least in the present cross, positive contributions to malting quality can be found in winter feed barleys.

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Introduction

Barley (*Hordeum vulgare* L.) grains are used to manufacture a variety of human foods and are also valuable as livestock feed. The most economically important use is for malting and brewing (Edney 1996); for this purpose, maltsters require grains of known varieties with consistent quality, and specify strict quality criteria for accepting new varieties.

Selection of varieties with the complex range of traits necessary to produce high quality malt is a difficult task, and a great number of parameters have been proposed in order to tag malting quality (Briggs 1998; Fox et al. 2003). Testing all malting quality parameters for each genotype is not only expensive, but also requires the availability of sufficient grains, because only one or few tests can be performed on single plants seeds at an early stage of the barley breeding process. These factors limit the effectiveness of phenotypic selection to improve malting quality, and increase the interest of breeders for simple and cheap analyses to identify superior genotypes.

In this view, mapping key genes and/or quantitative trait loci (QTL) associated with malting quality traits has recently been the main genetic approach to improve the efficiency of selection. At least 156 distinct malting quality QTLs for 19 traits have been reported in nine barley mapping populations, as summarized by Zale et al. (2000) and Fox et al. (2003). QTLs have been mapped for hot water extract (HWE), friability, wort viscosity, β-glucan and grain protein content (GPC), diastatic power, α- and β-amylase activity, and β-glucanase activity, all commonly used traits to test malting quality of breeding lines (MacGregor et al. 1996; Briggs 1998; Fox et al. 2003). By means of marker-assisted selection

(MAS) of the QT regions, both the time of release and the cost for the evaluation of the malting aptitude of the selected lines should be reduced (Fox et al. 2003). Nevertheless, in spite of the large number of mapped QTLs, few examples exist in literature in which QTL analysis and MAS have been applied to the genetic improvement of malting barley; in these studies only about half of the introgressed QTLs significantly improved the malting quality traits (Han et al. 1997; Igartua et al. 2000).

From a breeding point of view, it does not exist a single barley ideotype universally accepted, describing a malting variety. It is well known that two-rowed barleys are preferred for malting quality all over the world except in the USA and in Mexico, where six-rowed barleys are used mainly for this purpose (Riggs and Kirby 1978). The best malting barley cultivars have the spring growth habit and breeding for malting quality is generally practiced inside the spring germplasm, that has been shown by RFLPs to have a narrower gene pool (Backes et al, 2003), and rather distinct (Faccioli et al, 1995) from that of the winter types.

In barley, as in other members of the *Triticeae*, winter growth habit is due to the requirement of an external signal to the plant to shift from vegetative to reproductive growth; this signal can be completion of a vernalization requirement and daylength of sufficient duration (Dubcovsky et al. 2006). The genetic basis of vernalization response in cultivated barley can be described by using a two loci (*Vrn-H1* and *Vrn-H2*) epistatic model, whose candidate genes (Hv*BM5A* and *ZCCT-H*) have been recently characterized (von Zitzewitz et al. 2005). Besides vernalization requirement, resistance to low temperature is necessary for overwintering genotypes grown in areas with subzero winter temperatures. In fact, winter varieties that can survive winter

would warrant, in the autumn sowing, a higher yield in respect to spring type cultivars, thanks to their longer growing cycle (Cattivelli et al., 1994); nevertheless, in Central and Northern Europe the spring-sowing of malting barley is the most common practice (Pržulj et al. 1998).

Due to milder winters, in the recent years, winter barley acreage has increased in geographic areas of Northern and Central Europe, and a certain progress has been made to release winter malting varieties. There are currently some winter malting varieties recommended for 2007 by the UK Home-Grown Cereals Authority (HGCA; http://www.hgca.com/), known as 'Flagon', 'Pearl' and 'Cassata' (all two-rowed). In spite of this, no winter malting varieties have been recommended up to now by the American Malting Barley Association (AMBA) in America (http://AMBAinc.org/). Since the first report of Schildbach (1990), that indicated winter barleys as generally of lower malting quality, it is still debated if presently available winter malting lines are really of equal quality than spring ones (Spunar et al., 2000).

Moreover, few studies have been done on the level of frost tolerance of malting barley genotypes. It has been recently reported the development of a new barley map derived from a two-rowed cross 'winter' x 'spring' (Francia et al. 2004). The winter parent 'Nure' is a frost tolerant feed cultivar, whereas the spring parent 'Tremois' is a frost susceptible malting variety. The genetic map built on the 'Nure' x 'Tremois' (NxT) doubled-haploid (DH) is currently the only one where malting quality traits were expected to segregate together with winterhardiness and vernalization requirement, and this represented a valuable tool to investigate in a unique genetic system with multiple agronomical traits, such as malting quality and tolerance to abiotic stresses.

By using the DH population NxT, the objective was to study the genetic basis of the quantitative variation observed for malting quality, together with grain yield, in a winter x spring barley cross.

Materials and Methods

Plant materials and field trials

The NxT segregating population composed of 136 doubled-haploid (DH) lines (Francia et al., 2004) has been used in this work.

The NxT population has been sown in four field trials over two different years (2002 and 2003) and two contrasting locations: Fiorenzuola d'Arda (3 trials), 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, and Foggia (1 trial), Southern Italy (41°28'N, 15°32'E, altitude 75 m a.s.l.), cla y-loamy soil pH 7.8, prone to mild drought (30-year average annual rainfall of 507 mm).

The field trials in Fiorenzuola d'Arda were sown in the last week of October ('autumn sowing') in 2002 and 2003, and in the first week of February ('spring sowing') in 2003. The field trial performed in Foggia was sown in the last week of December 2003. At each location a randomized block design was chosen, with three replications and a minimum plot size of 6.0 m^2 using 350 seeds/m^2 in the field trials at Fiorenzuola d'Arda, and 300 seeds/m^2 in the field trial at Foggia.

At the location of Foggia, a pre-sowing inputs included 92 Kg ha^{-1} of P_2O_5 and 20 Kg ha^{-1} of N; the remaining 50% of N (20 Kg ha^{-1}) was applied in one date as top dressing. At Fiorenzuola, no pre-

sowing input was given, and 100% N (40 Kg ha^{-1}), divided in equal amounts, was applied in two dates as top dressing.

Malting quality evaluation

Kernel size fraction ≥ 2.0mm was obtained by using 100g samples of each line with an Octagon 200 test sieve shaker (Endecotts Ltd., England), and all the subsequent analyses were done after removal of screenings (kernels < 2.0mm).

Malting quality traits have been measured for each genotype on two field replications. Grain Protein Content (GPC) has been evaluated as % of dry weight, using the NIR (Near Infra-Red) spectrometer InfraAlyzer 500 (Bran+Luebbe, Germany) after derivation of a setting curve (reflectance/protein tenor) suitable for the material. Grain β–glucan were determined with an enzymic method (mixed-linkage β-glucan kit, Megazyme, Ireland) according to McCleary and Codd (1991).

Malting was performed on 60g samples of barley seed, with an Automatic Micromalting System (Phoenix Biosystems, Australia). The following malting cycle (168-h) was used: 7.5-h steep at 15℃, 8-h air rest at 19℃, 9-h steep at 15℃, 6-h air re st at 19℃, 0.5-h steep at 15℃, 88.5-h germination at 15℃, 48-h kil ning at 30-80℃. Friability was determinated with a Friabilime ter (Pfeuffer GmbH, Germany; European Brewery Convention 1998), using 25g for each sample on about 50g of malt obtained from modification. Acrospire growth index was calculated, on the dry malt, according to Gianinetti et al. (2005). Hot water extract (HWE) rate has been calculated following a small-scale infusion mashing (Institute of Brewing, 1997) modified as indicated by Gothard et al. (1980). Wort viscosity has been measured with a rotational viscometer (Brookfield, USA; Institute of Brewing, 1997).

A synthetic “quality score” (= *Score** of Gianinetti et al. 2005), obtained on the basis of HWE, wort viscosity and acrospire growth index, was calculated.

Statistical and QTL analyses

Analysis of variance (ANOVA) was carried out using the software Systat 9.0 (SPSS, Inc.1999, Chicago, IL, USA). For every parameter of malting quality and yield Least Significant Difference (5% LSD) was calculated and used to discriminate amongst genotypes; broad sense heritability ($H^2 = \sigma_g/\sigma_p$) was calculated for all the traits.

QTL analyses were performed for all malting quality traits (GPC, grain β-glucan, friability, acrospire growth, HWE, wort viscosity, quality score) and were carried out with mean values over environments, on the basis of the absence of GxE interactions. Whereas for yield there was found significant GxE interaction, the QTL analyses were conducted separately on data derived from each single field trial. Genome-wide QTL searches were conducted on the ‘Nure’ x ‘Tremois’ linkage map reported by Francia et al. (2004) and von Zitzewitz et al. (2005) by using two packages, the software PLABQTL (Utz and Melchinger 1996), and the software . With PLABQTL, a LOD (Log-Likelihood) threshold of 3.0 and a 2.0 cM scan interval were applied. After a preliminary analysis using Simple Interval Mapping (SIM), the markers with the highest LOD value were used as cofactors for Composite Interval Mapping (CIM). This procedure was repeated until a stable picture of the LOD profile was achieved. Finally, a cross-validation of the QTLs found was performed by means of a number of replicates in resampling of 1000. With the package MapQTL® Version 5.0 (Van Ooijen 2004), the QTL analysis was

carried out with the multiple QTL model (MQM) mapping, and a LOD (Log-Likelihood) threshold of 2.5, calculated by means of the permutation test option provided in MapQTL, was considered as evidence for the existence of a QTL. In both analyses, the additive effect and percentage of variation explained by an individual QTL were also estimated. Once considered that the two softwares found identical QTLs, that these were all cross-validated, and that additive effects and percentage of variation explained were slightly lower from the computations of MapQTL, the ouptut of this last software was chosen to be presented as result.

Results

Phenotypic data

Analysis of variance (ANOVA) pointed out that for all the malting quality traits the effect of genotypes, location (the four field trials), sowing dates (at Fiorenzuola, autumn and spring sowing 2003) and years (at Fiorenzuola, autumn sowing 2002 and 2003) were all significant. The interactions genotype x location, genotype x sowing date and genotype x year were not significant for almost all the quality traits. Since no GxE interactions were found, this suggested to perform QTL analyses of malting quality traits on the averages of the four trials.

As summarized in Table 1, ANOVA pointed out that the two parents were significantly different for all the malting quality traits, with the exception of GPC. As expected, ‘Tremois’ always outperformed ‘Nure’ for the malting quality parameters, as averages of four different trials Friability, wort viscosity, HWE and quality score showed a H^2(%) greater than 50% (with a maximum value of 57.7% for wort viscosity), whereas acrospire growth and

GPC recorded low (13.8%) and very low (6.1%) genetic effects, respectively.

With respect to grain yield, significant effects of genotypes, locations, sowing dates (at Fiorenzuola, autumn and spring sowing 2003) and years (at Fiorenzuola, autumn sowing 2002 and 2003) were found. Moreover, the interactions 'genotype x location' and 'genotype x sowing date' were significant. For this reason it was decided to perform QTL analysis of grain yield on the data derived from each single field trials. The feeding parent 'Nure' always yielded more grain than 'Tremois', although only for Yield in autumn sowing 2002 and 2003 at Fiorenzuola the two parents were significantly different (Table 1).

Trait	Max Value	Mean	Min Value	$LSD_{0.05}$	H^2 (%)	Nure	Tremois
Malting Quality							
GPC (%)	13.78	12.35	11.23	1.18	6.1	12.88	11.95
Grain β-Glucans (%)	6.31	4.28	2.71	0.30	54.5	5.0	3.9
Friability (%)	88.27	75.48	55.76	5.39	54.3	58.55	83.57
Acrospire Growth (0-1)	0.72	0.61	0.48	0.06	13.8	0.57	0.64
Wort Viscosity (cP)	1.60	1.52	1.46	0.01	57.7	1.60	1.49
HWE (%)	78.12	73.62	68.15	1.95	52.5	69.81	75.98
Quality Score	2.71	1.26	-0.39	0.52	53.6	-0.20	2.03
Yield							
N-A2 (Fiorenzuola)	7.94	5.62	3.19	1.23	60.8	7.01	5.43
N-A3 (Fiorenzuola)	7.26	5.14	2.90	1.38	48.0	7.26	4.35
N-S3 (Fiorenzuola)	5.06	3.78	2.15	0.82	65.5	4.98	4.27
S-A3 (Foggia)	5.27	4.52	3.28	0.81	34.5	5.27	4.98

Table 1: Statistical parameters for malting quality traits and grain yield measured in the NxT population and parents.

The range of variation of the DH values was in many cases higher than the range of variation of the parental values. In other terms, for all quality traits except GPC and friability, a significant transgressive segregation was observed in the DH population, at least in comparison with the values of one parent, suggesting bi-

parental allelic contributions to malting quality traits. As for grain yield, the DH lines never overtook the higher yielding parent 'Nure', while in all the four trials lines were found yielding less than 'Tremois'.

Among the 136 NT lines, eleven showed a malting quality score greater than ‘Tremois’, and Table 2 was constructed accordingly, descending from the highest quality scoring NT line to ‘Tremois’. In Table 2, a summary of individual values for all the malting quality traits, frost tolerance, growth habit and grain yield, together with alleles at three marker genes is shown. Four lines - NT140, NT135, NT141 and NT80 - significantly outperformed the quality score of the malting parent ‘Tremois’ ($P \leq 0.005$, LSD test), and, accordingly, they have been highlighted in bold (Table 2). Line NT140 had the highest quality score value of the DH population (2.71), together with the highest HWE (78.12%); moreover, this line showed for all traits higher or equal values than ‘Tremois’. All DH lines carried a 'Nure' 'A' allele at Fr-H1/VrnH1 and Fr-H2 loci on 5H chromosome, while they were randomly assorted as alleles 'A' and 'B' at VrnH2 on chromosome 4H. For such genetic compositions (Von Zitzewitz et al, 2005), in accordance to their field records of heading date delay, NT135, NT141 and NT80 were classified as facultative lines (F), carrying a 'winter' allele at *VrnH1* locus, and a 'spring' allele at *VrnH2*. Line NT140, putatively a winter type from marker scores, nevertheless reported a heading date delay higher, but not statistically different from that of parent ‘Tremois’ (Table 2). Remaining ones were winter and facultative types; interestingly, none of the 11 DHs of equal or higher malting quality than ‘Tremois’ were spring genotypes, carrying 'B' alleles at both *Vrn* loci (Table 2). With regard to frost tolerance, NT140 and NT80 had the highest winter field survival

Table 2: Average values of malting quality traits, frost tolerance, growth habit, and grain yield performances of 11 NxT lines plus parents. Only the doubled haploids with the synthetic quality score higher than **2.0** are listed, ordered for decreasing synthetic quality score. In bold the DH lines belonging to the highest ranking group for quality score. Alleles at three molecular marker loci: Hv*CBF4* (peak marker of *Fr-H2*), Hv*BM5A* (peak marker of *Fr-H1* and of *VrnH1*) and *ZCCT-H* (peak marker of *VrnH2*) are shown as well. Yield locations are coded as in Table 1 and Fig. 1.

	Quality Score	*GPC* (%)	*Grain β-Glucans* (%)	*Friability* (%)	*Acrospire Growth* (0-1)	*Wort Viscosity* (cP)	*HWE* (%)	*Winter Survival* (% of Nure)[a]	*Fr-H2*[b]	*Fr-H1 /VrnH*[b]	*VrnH2*[b]	*Heading Date Delay*	*Growth Habit*[c]	*Yield* N-A2 (t/ha)	*Yield N-A3* (t/ha)	*Yield S-A3* (t/ha)	*Yield N-S3* (t/ha)
NT 140	2.71	11.49	3.8	86.51	0.70	1.49	78.12	103.45	A	A	A	39.8	(W)	6.51	4.31	4.30	5.14
NT 135	2.65	12.97	3.7	82.85	0.70	1.49	77.50	89.66	A	A	B	34.5	F	7.07	6.69	4.65	4.70
NT 141	2.60	13.05	2.7	82.57	0.72	1.50	77.00	100.00	A	A	B	37.0	F	7.69	6.43	4.61	4.97
NT 80	2.55	12.27	4.1	80.89	0.70	1.47	76.41	103.45	A	A	B	38.5	F	7.00	5.46	3.33	4.49
NT 20	2.33	12.99	4.6	84.32	0.66	1.48	76.95	100.00	A	A	B	33.8	F	6.37	6.04	3.95	4.77
NT 113	2.30	12.69	4.0	85.07	0.63	1.48	77.91	103.45	A	A	B	37.2	F	6.85	6.39	3.57	4.15
NT 40	2.26	12.66	4.4	83.30	0.65	1.49	77.32	96.55	A	A	A	45.5	W	5.74	4.98	4.25	4.48
NT 74	2.21	13.19	3.4	79.75	0.69	1.47	74.66	100.00	A	A	A	-	W	5.73	4.50	2.63	4.01
NT 131	2.14	12.46	5.5	79.39	0.68	1.48	74.97	100.00	A	A	A	63.3	W	6.19	5.85	3.36	4.81
NT 137	2.08	12.40	5.1	78.85	0.66	1.52	76.94	103.47	A	A	A	62.3	W	6.07	5.36	2.76	4.60
NT 114	2.06	11.58	4.0	88.27	0.67	1.47	74.81	93.10	A	A	B	36.0	F	5.97	5.50	4.62	3.48
TREMOIS	*2.03*	*11.95*	*3.9*	*83.57*	*0.64*	*1.49*	*75.98*	*58.62*	*B*	*B*	*B*	*30.0*	*S*	*5.43*	*4.35*	*4.27*	*4.98*
NURE	*-0.20*	*12.88*	*5.0*	*58.55*	*0.57*	*1.60*	*69.81*	*100.00*	*A*	*A*	*A*	*51.3*	*W*	*7.01*	*7.26*	*4.98*	*5.27*
Mean	1.26	12.35	4.28	75.48	0.61	1.52	73.62	82.33				37.3		5.62	5.14	3.78	4.52
$LSD_{0.05}$	0.52	1.18	0.30	5.39	0.06	0.01	1.95	34.41				13.9		1.23	1.38	0.82	0.81

(103.45% of 'Nure'), whereas NT141 had the same value of the resistant parent, and NT135 reached about 90% of the tolerant parent.

Surprisingly, it came out from the present cross that all the 11 malting barleys reported in Table 2, with the malting quality score equal or greater than the spring cultivar 'Tremois', were highly tolerant to frost, with much higher winter survival values than the parent contributing most malting quality QTLs. These field winter survival observations (taken by the data of Francia et al. 2004) were in accordance with the 'A' scoring of the peak marker alleles at both the frost tolerance QTLs, *FrH2* and *FrH1* (Table 2).

None of the 11 DH lines statistically yielded more than the parent 'Nure'; it can also be observed that, as expected from growth habit, these facultative and winter type lines performed in average better after autumn sowing, than after spring sowing, especially in the Northern Italian location (Table 2).

QTL analyses

Quantitative trait loci for malting quality parameters and for grain yield were detected with both CIM and MQM mapping analyses in the NxT population. These last results are summarized in Table 3 and Figure 1. Gray boxes inside chromosome 5H of Fig. 1 represent the two loci of frost tolerance, and of frost tolerance and vernalization requirement *Fr-H2* (proximal) and *Fr-H1/VrnH1*(distal), mapped by Francia et al. (2004). In total 25 QTLs, 19 for malting quality traits and six for grain yield, have been located on the map.

Chromosome 1H was the most important for its contribution to malting quality in the NxT cross, since it harboured QTLs for all the traits considered. In particular in the large region of

Trait	QTL	Interval [a]	Chr	LOD	Additive effect [b]	R^2(%) [c]
GPC	1	Bmag0382 - E39M61-332	1H	5.73	-0.29	29.1
	2	wrlk2 - OPA17b	6H	3.94	0.23	18.7
Grain β-Glucans	1	Bmac0399 - Bmag0211	1H	6.63	0.35	27.9
	2	cor18 - Bmag0382	1H	4.48	0.25	16.9
Friability	1	Bmag0211 - Bmac0032	1H	28.77	-5.58	65.1
	2	Bmac0093 - E42M38-380/3	2H	3.07	-1.45	3.8
	3	E41M38-630 - HVM33	3H	5.08	-1.82	6.9
Acrospire Growth	1	Bmac0399 - Bmag0211	1H	6.60	-0.03	31.1
	2	dhn6 - E39M61-181	4H	8.13	-0.02	22.9
Wort Viscosity	1	Bmac0399 - Bmag0211	1H	10.75	0.02	39.5
	2	Hv635P2.4 - E38M50-215	5H	4.55	-0.01	24.3
HWE	1	Bmag0211 - Bmac0032	1H	12.84	-1.23	21.1
	2	Bmac0032 - cor18	1H	6.73	-0.94	11.3
	3	cor18 - E39M61-247	1H	5.33	-0.74	6.7
	4	Hv*CBF8* - Hv*CBF4*	5H	2.97	0.45	4.0
Quality Score	1	Bmac0399 - Bmag0211	1H	7.28	-0.33	13.8
	2	Bmag0211 - Bmac0032	1H	18.35	-0.45	34.1
	3	Bmac0032 - cor18	1H	5.48	-0.27	8.5
	4	E41M38-198 - aba3	4H	6.93	-0.21	8.7
Grain Yield	1	E42M32-378 - EBmac0415	2H	5.08	0.33	13.7
N-A2 (Fiorenzuola)	2	OPA17a - psr637	5H	9.56	0.46	27.0
Grain Yield	1	EBmac0415 - HvCSG	2H	13.80	0.51	36.9
N-A3 (Fiorenzuola)	2	Bmag0223 - OPA17a	5H	11.53	0.45	28.6
Grain Yield	1	dhn6 - E41M38-198	4H	13.24	0.39	34.5
N-S3 (Fiorenzuola)						
Grain Yield	1	Bmac0093 - E42M38-380/3	2H	5.87	0.19	19.3
S-A3 (Foggia)						

Table 3 Summary of QTLs detected in the NxT population by using the multiple QTL model (MQM) mapping and a LOD threshold of 2.5.

chromosome 1H included between the markers Bmac0399 and Bmag0382, 11 QTLs out of 19 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% (Table 3). The first cluster between markers Bmac0399 and Bmag0211 contained the most effective QTL for acrospire growth (31.1% R^2), for wort viscosity (39.5% R^2), for grain β-glucan (27.9% R^2), and the second QTL in terms of explained variance (13.8% R^2) for the quality score (Table 3). The second cluster was found in the neighbouring Bmag0211-Bmac0032 interval. It included the three most important QTLs of friability (with the absolute highest LOD value, 28.77, and R^2 65.1%), quality score (LOD 18.35, R^2 34.1%), and HWE (LOD 12.84, R^2 21.1%) (Table 3). The third cluster was mapped in the interval Bmac0032-cor18 included only two QTLs of HWE (LOD 6.73, R^2 11.3%) and quality score (LOD 5.48, R^2 8.5%). At last the fourth cluster was found in the interval cor18-Bmag0382 in which two QTLs of HWE (LOD 5.33, R^2 6.7%) and grain β-glucan (LOD 4.48, R^2 16.9%) were found.

Other QTLs were found on other chromosomes, for GPC (1H and 6H), friability (2H and 3H), viscosity and quality score (4H) (Fig. 1 and Table 3).

No malting quality QTLs have been mapped on chromosome 7H.

Interestingly, two malting quality QTLs detected on chromosome 5H showed a positive allelic effect from the feeding parent 'Nure' (Fig. 1 and Table 3). A first locus controlling HWE was identified between markers Hv*CBF8* and Hv*CBF4*, with a positive effect of 'Nure' of 0.45 (LOD 2.97, R^2 4.0%) partially overlapped with the *Fr-H2* frost tolerance QTL. A second QTL carried by 'Nure', for

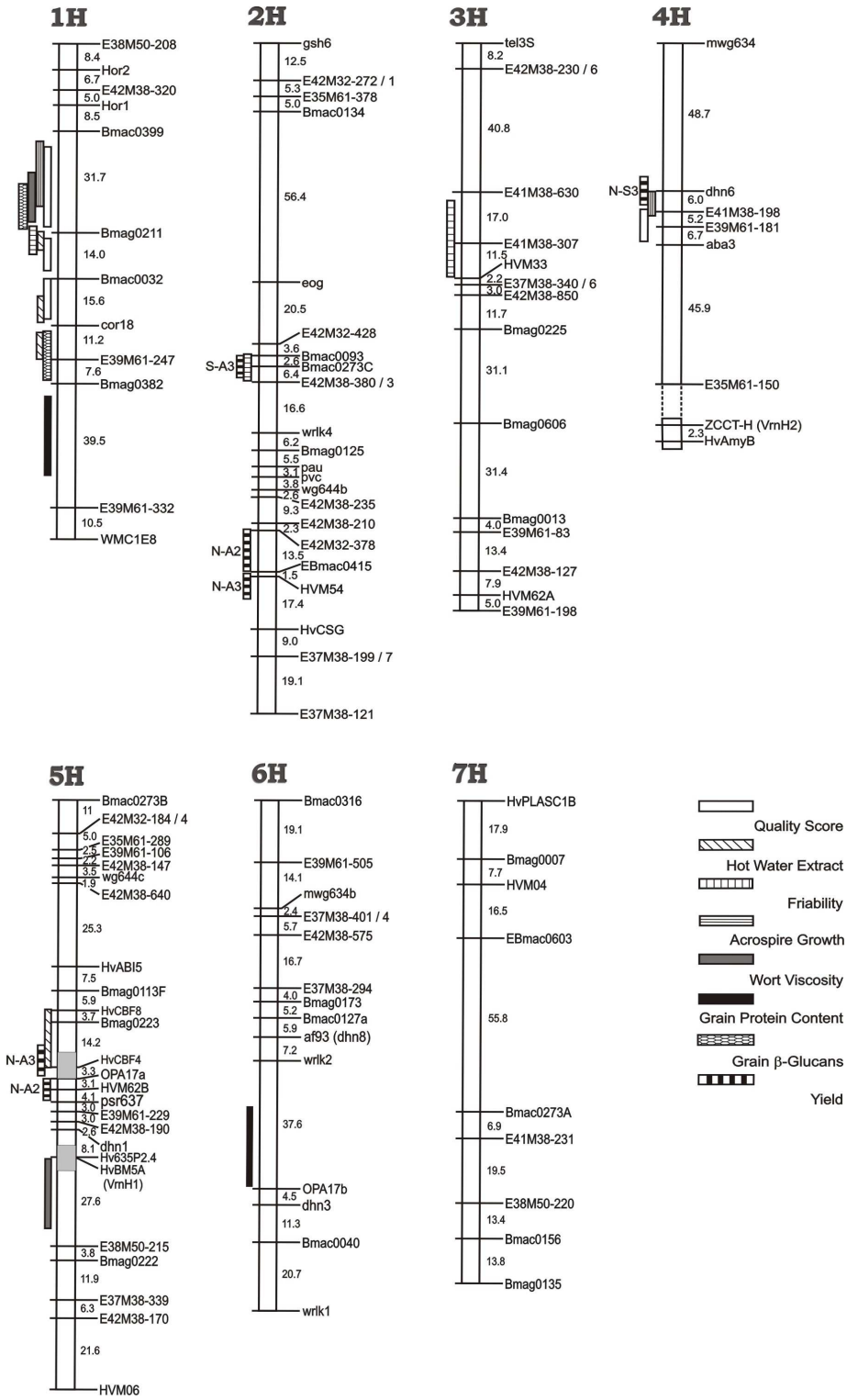
1H
E38M50-208
8.4
Hor2
6.7
E42M38-320
5.0
Hor1
8.5
Bmac0399
31.7
Bmag0211
14.0
Bmac0032
15.6
cor18
11.2
E39M61-247
7.6
Bmag0382
39.5
E39M61-332
10.5
WMC1E8
2H
gsh6
12.5
E42M32-272 / 1
5.3
E35M61-378
5.0
Bmac0134
56.4
eog
20.5
E42M32-428
3.6
S-A3
Bmac0093
2.6
Bmac0273C
6.4
E42M38-380 / 3
16.6
wrlk4
6.2
Bmag0125
5.5
pau
3.1
pvc
3.8
wg644b
2.6
E42M38-235
9.3
N-A2
E42M38-210
2.3
E42M32-378
13.5
EBmac0415
1.5
N-A3
HVM54
17.4
HvCSG
9.0
E37M38-199 / 7
19.1
E37M38-121
3H
tel3S
8.2
E42M38-230 / 6
40.8
E41M38-630
17.0
E41M38-307
11.5
HVM33
2.2
E37M38-340 / 6
3.0
E42M38-850
11.7
Bmag0225
31.1
Bmag0606
31.4
Bmag0013
4.0
E39M61-83
13.4
E42M38-127
7.9
HVM62A
5.0
E39M61-198
4H
mwg634
48.7
N-S3
dhn6
6.0
E41M38-198
5.2
E39M61-181
6.7
aba3
45.9
E35M61-150
ZCCT-H (VrnH2)
2.3
HvAmyB
5H
Bmac0273B
11
E42M32-184 / 4
5.0
E35M61-289
2.5
E39M61-106
2.7
E42M38-147
3.5
wg644c
1.9
E42M38-640
25.3
HvABI5
7.5
Bmag0113F
5.9
HvCBF8
3.7
Bmag0223
14.2
N-A3
HvCBF4
3.3
OPA17a
3.1
N-A2
HVM62B
4.1
psr637
3.0
E39M61-229
3.0
E42M38-190
2.6
dhn1
8.1
Hv635P2.4
HvBM5A
(VmH1)
27.6
E38M50-215
3.8
Bmag0222
11.9
E37M38-339
6.3
E42M38-170
21.6
HVM06
6H
Bmac0316
19.1
E39M61-505
14.1
mwg634b
2.4
E37M38-401 / 4
5.7
E42M38-575
16.7
E37M38-294
4.0
Bmag0173
5.2
Bmac0127a
5.9
af93 (dhn8)
7.2
wrlk2
37.6
OPA17b
4.5
dhn3
11.3
Bmac0040
20.7
wrlk1
7H
HvPLASC1B
17.9
Bmag0007
7.7
HVM04
16.5
EBmac0603
55.8
Bmac0273A
6.9
E41M38-231
19.5
E38M50-220
13.4
Bmac0156
13.8
Bmag0135
Quality Score
Hot Water Extract
Friability
Acrospire Growth
Wort Viscosity
Grain Protein Content
Grain β-Glucans
Yield

Figure 1 (Last page): QTL map of malting quality traits and grain yield in the NxT population. QTL bars drawn using the ∆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 given in Kosambi cM and chromosomes are oriented with short arms at the top. To differentiate for each field trial the grain yield QTLs, codes of the four locations are given at left of the QTL bars. N-A2 and N-A3 = Northern Italy (Fiorenzuola) Autumn sowing 2002 and 2003; N-S3 = Northern Italy (Fiorenzuola) Spring sowing 2003; S-A3 = Southern Italy (Foggia) Autumn sowing 2003.

wort viscosity, was mapped in the interval Hv635P2.4-E38M50-215 (R^2 24.3%).

As for grain yield, six QTLs have been identified, with a positive effect of the winter parent 'Nure' (Table 3). For the Yield in autumn sowing 2002 and 2003 at Fiorenzuola were been identified four QTLs in close linkage, two QTLs on the chromosome 2H, the first one was mapped for Yield in autumn sowing 2002 at Fiorenzuola in the interval E42M32-378-EBmac0415 (LOD 5.08 and R^2 13.7%), whereas the second one for Yield autumn sowing 2003 at Fiorenzuola in the interval EBmac0145-HvCSG (LOD 13.80, R^2 36.9%). The other two QTLs were identified on chromosome 5H, the first one was for Yield autumn sowing 2003 at Fiorenzuola (LOD 11.53 and R^2 of 28.6) overlapping with the *Fr-H2* frost tolerance QTL (Fig. 1), whereas the second QTL for Yield in autumn sowing 2002 at Fiorenzuola in close linkage with *Fr-H2* frost tolerance QTL was mapped in the interval OPA17a-psr637 (LOD 9.56 and R^2 of 27.0). For the Yield in spring sowing 2003 at Fiorenzuola only one QTL was mapped on chromosome 4H in the interval dhn6 - E41M38-198 (LOD 13.24 and R^2 34.5%). At last, in the interval Bmac0093-E42M38-380/3 on the chromosome 2H for Yield in autumn sowing 2003 at Foggia one only QTL (LOD 5.87 and $R^2$19.3%) was mapped.

Discussion

As expected in a feeding x malting barley cross (NxT), considerable differences among the DH lines for all the malting quality traits were found (Table 1). The observed broad sense heritabilities for grain β-glucan, friability, wort viscosity, HWE and quality score from the four field trials resulted quite high for these malting quality traits, particularly for wort viscosity. On the other hand, very low H^2(%) were observed for GPC and acrospire growth, 6.1 and 13.8 respectively. This higher influence of the environment on these two traits in the segregating population, with respect to the genetic effects, was also demonstrated by the very small difference between the parents. However, grain beta-glucan and wort viscosity parental values are quite similar and the segregation range for grain beta-glucan is quite wide, whereas wort viscosity is not. This pointed out that the differences in progeny are not always reflected in the differences of the parents, as in the parental lines can bring different genes operating for the trait.

The quality score parameter used in this work has been recently reported by Gianinetti et al. (2005); it can be used to rank malting genotypes. In our study four loci have been identified in the NxT population for the quality score and, as expected, all are partially or entirely coincident with the QTLs for the three parameters that are used to calculate this synthetic score (acrospire growth, wort viscosity and HWE).

Taking into account GPC, grain β-glucan, friability, acrospire growth, wort viscosity and HWE, a total of 15 malting quality QTLs have been identified (Fig. 1). QTL analyses revealed major loci in clusters on chromosome 1H (Fig. 1 and Table 3). On this

chromosome nine malting quality QTLs have been mapped and, among these, eight loci showed the allelic contribution of the parent 'Tremois' (Table 3). The two QTL clusters with highest LOD, localized in the intervals Bmac0399-Bmag0211 (grain β-glucan, wort viscosity and acrospire growth) and Bmag0211-Bmac0032 (friability and HWE) are colinear with two genomic regions described in the QTL consensus mapping work of Fox et al. (2003). The authors hypothesized that the grouping of QTLs for differently measured traits could be likely explained as a physiological interdependence among these traits; this could have also been the case in our work, at least for the coincident QTL of friability and HWE (see also Briggs 1998). The other two HWE loci mapped on 1H in intervals Bmac0032-cor18 and cor18-E39M61_247 (Fig. 1) could be part of the second and/or the third cluster, respectively, reported by Fox et al. (2003) on this chromosome, whereas the grain β-glucan locus identified in interval cor18-Bmag0382 could correspond to the malt β-glucan QTL mapped by Han et al. (1995). If the single QTLs of chromosome 1H are due to different linked genes or are the result of the pleiotropic effect of single key determinants remains to be determined. In this view a fine mapping project of these intervals and a positional cloning approach could answer these questions.

On the other hand, the presence of linked clusters of major QTLs showing the same allelic contribution should have a positive effect for MAS of new malting varieties, since, after increasing the genome coverage, it should lead to a better efficiency in assisted improvement of malting quality elite lines.

On chromosome 5H two malting quality loci (HWE and wort viscosity) have been mapped. The HWE QTL is colinear with a GPC QTL mapped in the 'Steptoe' x 'Morex' population by Hayes

et al. (1993) and it could be part of a cluster reported on this chromosome by Fox et al. (2003). The wort viscosity QTL corresponds with a α-amylase activity QTL mapped by Hayes et al. (1993) and it could correspond to a more distal cluster reported by Fox et al. (2003). It is notherworty that in the same region of this wort viscosity locus two putative candidate genes (*CesA2* and *A3*) involved in cellulose synthesis (Burton et al. 2004) have been identified.

Both QTLs mapped on chromosome 5H (Fig. 1) are partially coincident with the frost tolerance loci *Fr-H2* and *Fr-H1/Vrn-H1*, mapped in the NxT population by Francia et al. (2004). Moreover, the positive effect on malting quality of these two QTLs is driven by the frost tolerant winter parent 'Nure' (Table 3). This might be an useful result for MAS of winter, frost tolerant, malting barley lines.

No QTLs have been mapped on chromosome 7H in the NxT population, in spite of the large QTL effects on malting quality found in other maps (e.g. Han et al., 1995; Fox et al., 2003). This can be due to a low density of markers in this specific chromosome, and/or to lack of segregation for the malting quality traits between the two parents.

For all malting quality traits considered in this work we observed the presence of positive transgressive segregants (Table 1), wich was suggesting the existence of alleles carried out by both parents, afterwards found by QTL mapping (Table 3). Malting quality QTLs contributed by both parents is not a case limited to our study, but is a well-known fact (e.g. Thomas et al. 1995; Oziel et al., 1996; Collins et al., 2000).

The loci with alternate allelic effects, suitably combined, could have created superior genotype combinations respect to the

parent ‘Tremois’. This was what we observed, at least for the synthetic score, for the DHs NT140, NT135, NT141 and NT80, and, for other single quality traits, for all the DHs presented in Table 2. It was noteworty, and not expected, that all the selected superior lines had kept the ‘Nure’ alleles at both the *Fr-H2* and the *Fr-H1/Vrn-H1* frost tolerance loci (Table 2). This is confirmed by the winter survival phenotypic values that were very similar to those of the winter parent ‘Nure’, except NT135. On the other hand, no malting quality superior NT line outperformed ‘Nure’ in terms of grain yield (Table 2).

According to the model proposed by von Zitzewitz et al. (2005), all the lines of Table 2 carried a ‘Nure’ allele at *Vrn-H1* locus (tagged by a candidate gene marker, Hv*MB5A*), and were thus classified as ‘facultative’ and ‘winter’, depending on allele at *Vrn-H2* candidate gene (*ZCCT-H)*. Phenotypic measures, taken from data of Francia et al. (2004), confirmed the prediction, except for the line NT140, that had a phenotypic value of heading date delay similar to that of ‘facultative’ lines. Markers alleles of candidate genes have been confirmed; we are currently repeating the test of vernalization requirement. No spring (B-B) DH lines, out of 136, were found among the top malting quality ones. This could be due to a relatively high importance for malting of the above-reported quality QTL on 5H in linkage in *cis* with the vernalization requirement locus *Vrn-H1* (Fig. 1); further support is given by the monomorphy of the lines at *Vrn-H1* (Table 2).

In conclusion, the NxT, winter x spring, population represented a useful genetic material to unravel the genetic bases of malting quality in barley, different from all the spring crosses previously evaluated. It also demonstrated the feasibility of the molecular breeding for superior winter-habit malting barleys at least in

combination with ‘Nure’. In particular, some markers and QTL clusters have been suggested to be tagged for MAS of winter malting barley.

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