Effect of the *nud* Gene on Grain Yield in Barley

Delfina BARABASCHI[1], Enrico FRANCIA[2], Alessandro TONDELLI[1], Alberto GIANINETTI[1], Antonio M. STANCA[1] and Nicola PECCHIONI[2]

[1]CRA – Genomics Research Centre, Fiorenzuola d'Arda, Italy; [2]Department of Agricultural and Food Sciences, University of Modena and Reggio Emilia, Reggio Emilia, Italy

Abstract: Naked barleys are less yielding than the hulled ones while the reason for this difference has not been definitely clarified. To investigate the effect of the *nud* gene on yield, a barley doubled haploid (DH, Proctor × Nudinka) population was initially tested in three environments and a QTL study was run on the entire population as well as on two nud/NUD DH subpopulations. Among the agronomic traits studied, a QTL effect was found at *nud* locus on chromosome 7H only for yield and thousand grain weight (TGW), while a second QTL was found on 6H, although contributed by the naked parent. Other QTLs for TGW were identified on 2H, 3H and 5H. Most QTLs found in the entire population were confirmed by the study on the two groups. No interaction was observed between QTLs. To provide a more accurate evaluation of the effects of the *nud* gene upon grain yield, its components and other agronomic traits, sixteen naked advanced backcross (AB) BC5F2 lines in the hulled background of cultivar Arda were prepared and evaluated in a replicated yield trial for two years. The only differences found between AB lines and Arda in grain yield and TGW were due to hull weight (11.97% of kernel weight). No differences were observed in other traits such as grains/m^2, grains per spike, plant height, heading date and mildew resistance. In conclusion, we think to have clarified that the effect of the *nud* gene on yield is due to hulls, and we did not find any pleiotropic effect of *nud* on other traits. This suggests, together with the finding of a QTL contributed by the naked parent, that there is a great potential to improve naked barley up to the yield levels of hulled barley.

Keywords: advanced backcross lines; doubled haploids; naked barley; *nud* gene; QTL mapping; yield

Among crops of the *Poaceae* family, besides oat, also barley (*Hordeum vulgare* L.) shows a clear differentiation of grain type, which can be hulled or naked. While the grain is developing, the outer cases of the flowering structures (known as glumes: outer lemma and inner palea) normally fuse together and adhere tightly to the grain to form the hull that is typical of hulled (covered) barley. This barley grain type is mainly used for malting to produce beer and malt whisky, and as animal feed. Naked (hulless) grains do not require the abrasive 'pearling' needed to remove the indigestible hull of the covered grains, since they lack the 'cementing substance' between the pericarp and caryopsis (Gaines *et al.* 1985). Therefore naked types can be used in various food products with minimal processing and, more importantly, with the nutritious bran layer intact to get the full benefit of the whole grain (Liu 2007).

Thanks to the easy processing of its edible part, naked caryopsis is considered a key domestication character in barley, like in other cereals (Zohary & Hopf 2000; Salamini *et al.* 2002). Its higher digestible energy compared to hulled grain, the high content of dietary fibres (mainly β-glucans), key vitamins, minerals and proteins make wholegrain naked barley a healthy food (Bhatty 1986; Shewry 1993), also for the preparation of functional

foods that respect the EU health claims (Kinner *et al.* 2011).

Hulless genotypes are distributed worldwide: in East Asia they are a staple food, while in North America they are becoming increasingly popular as a new source of high-quality feed for pigs. Today the cultivated area devoted to hulless barley in Canada covers more than 40 000 ha (B. Rossnagel, personal communication). Nevertheless, the naked genotypes still have lower grain yields compared to the hulled varieties, and it has not yet been clearly demonstrated whether it is only a matter of lacking glumes or whether it also depends on other associated traits/factors. Thomason *et al.* (2009) studying the yield performances of hulless and hulled breeding lines found that a large part of the difference in grain yield in naked lines was due to the absence of the hull weight, although naked lines also had fewer heads per square meter and fewer grains per head.

The issue concerning the origin of the naked trait in the barley crop is still under debate. On the one hand, a single, monophyletic origin for naked barleys was proposed by Taketa *et al.* (2004); on the other hand, recent molecular evolutionary studies on the barley crop as a whole proposed two domestication sites for barley at least, one in the Fertile Crescent and the other in the Far East (Morrell & Clegg 2007; Saisho & Purugganan 2007). This could support the hypothesis of multiple origins at different locations also for naked types, as suggested by Barabaschi *et al.* (2007).

The naked trait is controlled by a single gene that was mapped at the *nud* locus on chromosome 7H (Fedak *et al.* 1972). The gene was recently cloned, using a positional cloning approach by Taketa *et al.* (2008): they revealed that an ethylene response factor (ERF) gene resides at *nud* and plays a putative role in controlling the formation of a lipid layer on the pericarp epidermis in covered barley. This layer corresponds to the cementing substance described by Gaines *et al.* (1985) as responsible for the adhesion of glumes to the pericarp, and which is absent in recessive naked types (Taketa *et al.* 2008).

Like the two-rowed/six-rowed (*Vrs1*/*vrs1*) gene, the *nud* mutation in barley was also suggested to have a considerable effect on several agronomical traits. For example, McGuire and Hockett (1981) detected genetic interactions of the *nud* and *Lks2*/*lk2* (long-awned/short-awned) loci with several malting quality traits. Choo *et al.* (2001) hypothesized a pleiotropic effect of the naked gene on plant height, whereas direct effects or linkage with other QTLs have been suggested for other quality-related traits. No associations with either smut or scald resistance, or with heading date, maturity and spike density were found by these authors. Three QTLs for pathogen resistance have been mapped in the *nud* region of barley: one for resistance to *Pyrenophora graminea* (Pecchioni *et al.* 1996), one for non-host resistance to leaf rust fungi (*Puccinia hordei-murini* and *Puccinia triticina*) (Niks *et al*. 2000), and one for non-host resistance to the rice pathogen *Pyricularia grisea* (Chen *et al.* 2003). These latter associations seem to depend in most cases on genetic linkage rather than on pleiotropy. For the first resistance trait, in fact, in a large naked/hulled germplasm collection it has been demonstrated that the naked phenotype was not associated with a higher susceptibility to leaf stripe (*Pyrenophora graminea*) compared to the hulled phenotype (Barabaschi *et al.* 2007). Therefore studies with appropriate genetic materials are necessary to figure out the effects of *nud* on grain yield and on other yield-related traits, as well as to develop efficient selection strategies for hulless barley breeding programs.

The first aim of this study was to investigate the effect of the *nud* gene on the agronomic performance of barley in a population of segregating doubled haploid (DH) lines, originating from a naked × hulled cross, in which a QTL study was performed. After having identified and quantified a QTL effect for yield at the *nud* locus, together with other QTLs on other chromosomes and for other agronomic traits, an additional QTL study was done in the two *nud*/NUD subpopulations, with the aim to eliminate the effect of the segregation of the *nud* gene. In a subsequent research, a population of naked advanced backcross (AB) lines, in which the *nud* gene had been introgressed into a hulled background, was studied. This population was prepared *ad hoc* in order to evaluate the effects of the *nud* gene upon grain yield and its components, including husk weighing, and not considered in an accurate way till now.

MATERIAL AND METHODS

Plant materials. Two different populations were used in replicated field trials, and characterized in terms of yield and other yield-related traits. The first population consisted of one hundred and one

(101) doubled-haploid (DH) lines, derived from the cross Proctor (hulled) × Nudinka (naked), on which a genetic map was built (Becker *et al.* 1995). Fifty-eight lines were naked, and forty-three were hulled. In addition, a set of 16 advanced backcross (AB) lines was developed by backcrossing the donor naked, two-rowed, barley cultivar Iabo to the recurrent hulled, two-rowed cultivar Arda, released and maintained at the CRA Genomic Research Centre (GPG) of Fiorenzuola d'Arda, Italy. The backcross program, which lasted over 10 years to complete, can be summarized as follows. Starting from BC1F1 each BC population was selfed in order to identify the recessive homozygous *nud*/*nud* plants in the progeny, and only these naked lines were backcrossed to Arda in the subsequent cycle. This scheme was applied until the BC5F2 generation was reached. BC5F3 progeny testing was then carried out on several families to separate the homozygous *nud* sister lines from the hulled ones.

Field trials and trait evaluation. A field evaluation of the 'PN' DH lines was carried out in 1995 in three replicated yield trials. Trials were performed at two contrasting locations: Fiorenzuola d'Arda (northern Italy, 44°55'N, 9°53'E, altitude 80 m a.s.l) with a fertile clay-loamy soil pH 7.6 and a 30-year average rainfall of 852 mm, and Foggia (southern Italy; 41°28'N, 15°32'E, altitude 75 m a.s.l.) with a clay-loamy soil pH 7.8, prone to mild drought with a 30-year average annual rainfall of 507 mm. In Fiorenzuola d'Arda, the DHs were sown in the last week of October (autumn sowing; FA) and in the first week of February (spring sowing; FS), while the trial in Foggia was sown in the last week of December (autumn sowing; FoA). In each field, a randomized complete block design with three replications was chosen, with a plot size of 3.4 m^2. Sowing was performed with 350 viable seeds/m^2 in Fiorenzuola d'Arda, and 300 viable seeds/m^2 in Foggia. Thousand grain weight (TGW), grain yield (GY), heading date (HD) and plant height (PH) were recorded for each plot at both locations, while resistance to powdery mildew (ML) was measured only in Fiorenzuola, since no disease was observed in the Foggia trial.

To evaluate the effect of the nud locus on agronomic performance when introgressed into a hulled elite background, the 16 nud-AB lines plus the two parents were grown in two replicated field trials during harvest years 2005 and 2006. Experiments were conducted at Fiorenzuola d'Arda (see above for details). A randomized complete block design was chosen, with three replications and a minimum plot size of 4.0 m^2 using a sowing density of 350 viable seeds/m^2. The two field trials were sown in the third week of November (autumn sowing) and harvested in the second week of July. Plants were grown according to the agronomic practices usually adopted for barley.

Fifteen agronomic traits were evaluated as follows. Grain yield was determined as total biomass of grains (t/ha), both on a plot basis (GY), after harvesting the seeds with a stationary cleaning device, and on a 1 m row basis (GY*), used for the calculations of the yield components (the grain yield in g obtained from the 1 m row was added to the machine-harvested one to get the plot-based yield record, GY). The 1 m row sample, hand-cut from each plot, was used for all the following measures. Biological yield (BY) at harvest maturity as total aboveground biomass is reported in tons per hectare (t/ha); harvest index (HI) was calculated as the ratio between GY and BY; thousand-grain weight (TGW) was estimated from the weight of three random samples of 100 grains per row. For the naked lines, the grain yield adjusted for glume weight (ADJ GY) was calculated from the data of four randomly selected ears per row which were hand-threshed and their grains and glumes were weighed separately. HI and TGW adjusted for glume weight (ADJ HI and ADJ TGW) were calculated as above. Stem density (i.e. the number of stems/m^2, STM), spike density (i.e. the number of spikes/m^2, SPKM), total grain number (number of grains/m^2, GM), and grain number per spike (GPS) were obtained as well. Plant height (PH) in cm was measured from the ground to the tip of the ear (excluding awns) at maturity, and averaged over ten stems. Heading date (HD) was recorded as the number of days from April 1st until emergence of at least 50% of the ears from the flag leaf sheath, on a plot basis. Severity of powdery mildew symptoms (ML) was rated visually according to a 1 (resistant) to 9 (susceptible) visual score.

Statistical and QTL analyses. Analyses of variance (ANOVA) as well as Dunnett's test for means comparison were carried out using the software Systat 12.0 (SPSS, Inc.1999, Chicago, USA).

Genome-wide QTL searches were conducted on the Proctor × Nudinka linkage map (Becker *et al.* 1995; http://www.wheat.pw.usda.gov, and Pecchioni, unpublished) using MapQTL® Version 5.0 (Van Ooijen 2004). QTL analyses were

carried out separately for each environment and for each trait, using genotype values averaged over the three blocks: GY, TGW, HD, PH and ML. After simple interval mapping (SIM), the forward selection procedure was followed, fixing significant regions with markers as cofactors in multiple QTL model (MQM) mapping. This procedure was repeated until a stable picture of the LOD profile was achieved. QTLs were declared according to the LOD threshold ranging from 2.5 to 3.3, as determined by the permutation test option provided in MapQTL (1000 permutations). A cross-validation of the QTLs found was performed by the backward elimination procedure after automatic cofactor selection and until a stable picture was achieved again.

For grain yield, an additional QTL analysis was performed in the two subpopulations, constituted by naked (58) and hulled (43) DH lines, in order to search for minor QT loci masked by the effect of the *nud* gene, and to look for interactions between the *nud* gene and possible additional interacting QTLs. In these cases a lower arbitrary LOD threshold of 2.0 was applied for each subpopulation mapping in order to prevent any QTLs being missed because of the relatively small experimental groups.

The interaction between the yield QTL at *nud* and other yield and TGW QTLs was tested by 2 × 2 factorial ANOVA using peak markers in order to search for epistatic (Q × Q) effects between the respective QT-responsible loci.

RESULTS

DHs (Proctor × Nudinka). One hundred and one DH lines of the Proctor × Nudinka population were evaluated in three environments in Italy in the same harvest year. Table 1 reports the mean values of the Nudinka and Proctor parents in each field trial, together with the means and ranges of the DH population. The effects of genotype, environment and their interaction were preliminarily evaluated for each agronomic trait using two-way ANOVA; both the two parents and the DH genotypes were included in this analysis (not shown). Since a significant genotype x environment interaction was observed for GY and other traits, data of the three environments were re-analysed

Table 1. Statistical parameters of yield and other agronomic traits measured in the Proctor × Nudinka DH population and parents

Trait	Locations	Nudinka	Proctor	Mean	Min. value	Max. value
GY (t/ha)	Fiorenzuola autumn (FA)	3.7	4.5	3.8	2.7	4.7
	Fiorenzuola spring (FS)	3.0	4.1	3.1	1.9	4.9
	Foggia autumn (FoA)	4.5	4.8	4.7	3.9	5.5
TGW (g)	Fiorenzuola autumn (FA)	40.7	34.0	38.5	28.7	46.7
	Fiorenzuola spring (FS)	39.3	38.0	39.1	33.3	50.7
	Foggia autumn (FoA)	25.0	27.8	26.0	20.4	32.0
PH (cm)	Fiorenzuola autumn (FA)	73.3	81.7	76.5	65.0	86.7
	Fiorenzuola spring (FS)	75.0	78.3	73.9	56.7	85.0
	Foggia autumn (FoA)	81.7	83.3	79.5	63.3	90.0
HD	Fiorenzuola autumn (FA)	57.3	50.7	54.1	40.3	61.3
	Fiorenzuola spring (FS)	65.3	64.0	65.4	60.3	75.0
	Foggia autumn (FoA)	22.3	20.3	22.1	16.0	28.3
ML (1–9)	Fiorenzuola autumn (FA)	0.7	0.3	2.1	0.0	6.3
	Fiorenzuola spring (FS)	2.0	2.3	1.4	0.0	4.3
	Foggia autumn (FoA)	nd	nd	nd	nd	nd

GY – grain yield; TGW – thousand grain weight; PH – plant height; HD – heading date (days from April 1st); ML – powdery mildew; nd – not determined

singularly to provide a ranking of the genotypes, as well as averages for QTL mapping of single environment data (Table 1). To evidence the role of the naked trait, a further analysis was done to compare the naked and hulled DH groups of the population (parents not included) within each environment by means of nested ANOVA. Naked/hulled was the main factor and genotype was the nested factor. The means of each agronomic trait for the two groups (58 naked vs. 43 hulled DH lines) are summarized in Table 2. The significance of the difference between the two group means are reported, based on the probability of the group effect calculated by nested ANOVA. Naked lines yielded significantly less than the hulled ones in all the three environments: from 8% less in FA and FoA to 20% less in FS. No differences were found for the other four agronomic traits in the trials conducted in Fiorenzuola, whereas in the FoA environment the group of hulled genotypes had a higher seed weight (TGW), was taller and flowered earlier than the group of naked DHs.

QTLs for yield and other agronomic traits in the naked × hulled DH population. As shown in Figure 1, a total of 12 QTLs, from 1 to 6 for each trait, were located on six chromosomes in the DH population. No QTLs were found on chromosomes 1H and 4H. As expected, QTL analysis revealed a major effect on GY of the genomic region on chromosome 7H harbouring the nud locus (peak marker). This QTL was consistent throughout the three environments analysed, with LOD values ranging from 6.49 to 13.14. The hulled parent Proctor contributed favourable alleles and the proportion of explained phenotypic variance ranged from 25.6% to 37.5%. Another QTL for GY (LOD = 8.49 and R^2 = 22.5%) was found on chromosome 6H although only in Foggia autumn, which was contributed by the naked parent Nudinka. At the same location, six regions distributed on four barley chromosomes harboured QTLs controlling TGW, explaining from 5.2% to 23.6% of the phenotypic variance (LOD of 3.28–11.81). In most cases, the hulled parent Proctor contributed favourable alleles. The QTL with the largest effect for TGW in terms of explained variance (23.6% R^2) was found on chromosome 7H, in correspondence with the nud gene.

Both GY and TGW were investigated thoroughly in order to remove the effect of the segregation of the 7H region at *nud* on the two traits, and to look for other 'hidden' QTLs. The DH lines were thus grouped into two subpopulations of 58 and 43 lines according to their grain phenotype, and a new QTL analysis for GY and TGW was carried out for each subpopulation. QTL searches were done following the same procedure as described for the entire population, although with a less stringent LOD threshold of 2.0. In Table 3 and Figure 1 all the QTLs detected in hulled and naked DH subgroups are compared with those present in the entire population. For GY, three additional QTLs on chromosomes 4H, 7H and 5H, specific to the FS and FoA environments, respectively, were mapped in the hulled DH subpopulation, while in the naked one, a QTL on 1H was added in the FoA environment. The only QTL of GY in the entire population that was different from *nud,* found at FoA on chromosome 6H (peak marker WG282), was detected in both the DH subpopulations. For TGW, for which six QTLs (including one at *nud*) were found at FoA, only one additional QTL was mapped on chromosome 3H (Q.Tgw-FoA.3H.2)

Table 2. Mean agronomic performance of 58 naked vs. 43 hulled DHs of the Proctor × Nudinka mapping population in three field trials in Italy; significant differences between the group pairs are designated with asterisks reported on the hulled DH columns

Trait	Fiorenzuola autumn		Fiorenzuola spring		Foggia autumn	
	naked DH	hulled DH	naked DH	hulled DH	naked DH	hulled DH
GY (t/ha)	3.7	4.1***	2.8	3.5***	4.5	4.9***
TGW (g)	38.4	38.6	38.6	39.7	25.1	27.2***
PH (cm)	75.5	76.6	72.9	75.0	78.5	80.7***
HD	70.1	69.4	65.5	65.3	21.5	22.9***
ML (1–9)	2.1	2.2	1.4	1.5	nd	nd

DH – doubled haploid; GY – grain yield; TGW – thousand grain weight; PH – plant height; HD – heading date (days from April 1st); ML – powdery mildew; nd – not determined; ***$P \leq 0.001$

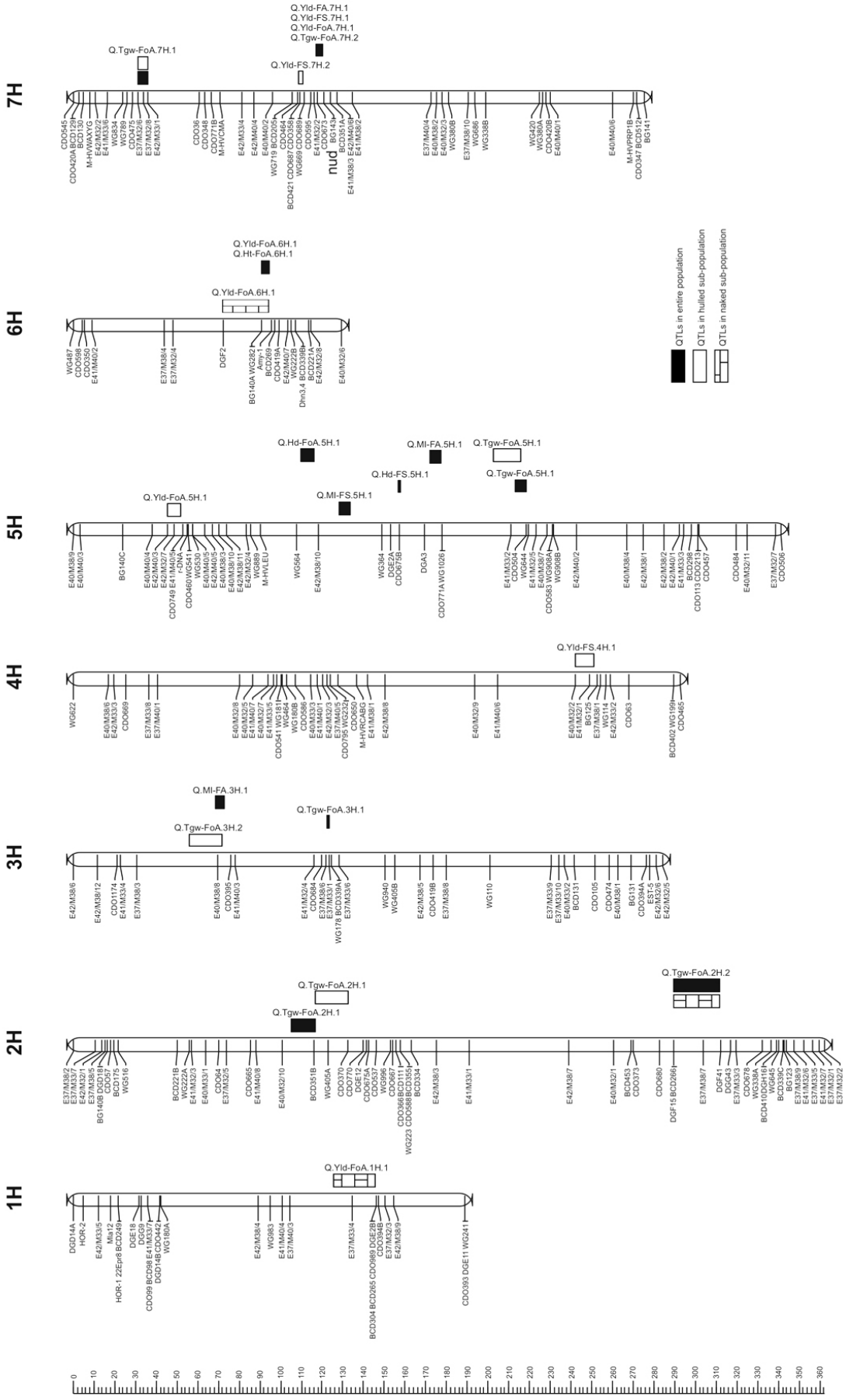

Figure 1. Chromosomal locations of mapped QTLs controlling yield and other agronomic traits in the DH population Proctor × Nudinka; the order of markers and the distances in centimorgans (cM, Kosambi) are based on the molecular map by Becker *et al.* (1995); the chromosome length scale is given on the left side and linkage groups are oriented with short arms at the top; loci are shown on the left side of chromosomes; bars on the right show QTL intervals above the LOD threshold

Table 3. QTLs for grain yield (GY) and thousand grain weight (TGW) detected in the entire Proctor × Nudinka population and in the two subpopulations of hulled and naked DHs; the names of QTLs not previously detected in the entire population are reported in the right-hand half of the QTL column

Trait	Genotypes used (*H^2)	QTL		Peak marker[1]	Peak position[2]	Chr-Bin[3]	LOD	Allelic effect[4]	R^2 %[5]
Grain yield Fiorenzuola autumn	all (*H^2 43%)	Q.Yld-FA.7H.1		*nud* (120.8)	120.8	7H-Bin 7	6.49	0.200	25.6
	hulled DHs (H^2 81%)	none							
	naked DHs (H^2 23%)	none							
Grain yield Fiorenzuola spring	all (H^2 82%)	Q.Yld-FS.7H.1		*nud* (120.8)	120.8	7H-Bin 7	9.45	0.329	35.0
	hulled DHs (H^2 79%)		Q.Yld-FS.4H.1	*E41/M32/1* (249.1)	249.1	4H-Bin 10	2.54	0.231	19.1
			Q.Yld-FS.7H.2	*WG669* (111.1)	111.1	7H-Bin 7	3.13	–0.374	24.2
	naked DHs (H^2 57%)	none							
Grain yield Foggia autumn	all (H^2 74%)	Q.Yld-FoA.6H.1		*WG282* (90.7)	90.7	6H-Bin 9	8.49	–0.169	22.5
		Q.Yld-FoA.7H.1		*nud* (120.8)	120.8	7H-Bin 7	13.14	0.204	37.5
	hulled DHs (H^2 30%)		Q.Yld-FoA.5H.1	*E42/M32/7* (48.7)	48.7	5H-Bin 4	2.70	0.110	19.1
		Q.Yld-FoA.6H.1		*WG282* (90.7)	90.7	6H-Bin 9	2.13	–0.108	15.1
	naked DHs (H^2 71%)		Q.Yld-FoA.1H.1	*E37/M33/4 (134.5)*	137.5	1H-Bin 12	2.21	–0.096	11.0
		Q.Yld-FoA.6H.1		*WG282* (90.7)	90.7	6H-Bin 9	6.45	–0.187	38.2
Thousand grain weight Foggia autumn	all (H^2 88%)	Q.Tgw-FoA.2H.1		*BCD351B* (115.9)	110.6	2H-(Bin 7)	3.30	0.563	6.0
		Q.Tgw-FoA.2H.2		*E37/M38/7* (303.9)	303.9	2H-Bin 13	8.31	0.896	14.8
		Q.Tgw-FoA.3H.1		*E37/M33/1* (123.4)	123.4	3H-Bin 7	3.28	–0.527	5.2
		Q.Tgw-FoA.5H.1		*CDO504* (218.4)	218.4	5H-Bin 11	5.04	0.658	8.4
		Q.Tgw-FoA.7H.1		*E37/M32/8* (33.6)	33.6	7H-Bin 3	5.98	–0.770	10.3
		Q.Tgw-FoA.7H.2		*nud* (120.8)	118.8	7H-Bin 7	11.81	1.142	23.6
	hulled DHs (H^2 87%)	Q.Tgw-FoA.2H.1		*WG405A* (122.8)	124.8	2H-(Bin 6)	4.44	1.007	21.0
			Q.Tgw-FoA.3H.2	*E40/M38/8* (69.7)	69.7	3H-Bin 3	2.21	–0.679	9.4
		Q.Tgw-FoA.5H.1		*E41/M33/2* (211.0)	211.0	5H-Bin 11	2.29	0.710	9.8
		Q.Tgw-FoA.7H.1		*E37/M32/8* (33.6)	33.6	7H-Bin 3	2.84	–0.788	12.5
	naked DHs (H^2 83%)	Q.Tgw-FoA.2H.2		*BCD266* (289.7)	289.7	2H-Bin 13	5.35	1.124	34.6

*H^2 – broad-sense hereditability; [1]peak marker with the highest LOD; the map position of the peak marker in cM is in brackets; [2]map position of the QTL peak in cM; [3]bin locus position as deduced from the Graingenes database (http://wheat.pw.usda.gov); [4]effects on the analyzed traits of the alleles from the parent Proctor; [5]R^2 (%) proportion of phenotypic variance explained by the QTLs; none – no QTL detected

Table 4. Agronomic performance of 16 advanced backcross (AB) lines subjected to the study, least-square mean of two years (2005 and 2006); trait codes are indicated in the text, $LSD_{0.05}$ and *P* values are reported in the last columns

	AB lines																$LSD_{0.05}$	*P* value
	8283	8284	8287	8288	8291	8292	8295	8296	8299	8300	8303	8304	8307	8308	8311	8312		
GY (t/ha)	4.0	4.7	4.0	5.1	4.5	4.6	4.7	4.2	4.2	4.3	4.3	4.4	4.5	4.7	4.6	5.0	0.87	0.323
GY* (t/ha)	3.7	4.4	3.7	4.8	4.2	4.3	4.4	3.9	3.9	4.0	4.0	4.1	3.9	4.4	4.3	4.2	0.80	0.302
ADJ GY (t/ha)	6.0	6.2	5.9	5.7	5.0	5.5	5.4	5.6	5.4	5.3	5.0	4.9	5.0	6.2	5.6	5.2	1.22	0.638
BY (t/ha)	12.2	13.0	12.8	12.1	11.8	12.0	11.4	12.5	11.9	11.4	11.1	11.7	10.1	13.5	12.2	11.6	2.53	0.716
HI	0.43	0.43	0.40	0.41	0.38	0.40	0.42	0.40	0.40	0.40	0.40	0.38	0.43	0.41	0.40	0.38	0.05	0.671
ADJ HI (t/ha)	0.49	0.51	0.46	0.48	0.43	0.46	0.48	0.46	0.46	0.47	0.46	0.44	0.51	0.47	0.46	0.45	0.05	0.455
TGW (g)	41.6	42.9	45.0	45.2	44.0	45.8	43.8	44.4	45.8	44.0	44.8	45.9	45.9	46.3	45.1	45.4	4.71	0.978
ADJ TGW (g)	47.5	48.8	51.4	51.8	49.6	51.6	49.3	50.2	52.2	50.9	51.1	52.5	53.0	51.8	51.3	52.2	5.27	0.948
STM	686	763	633	677	677	679	640	707	612	656	608	608	563	721	654	597	131	0.419
SPKM	676	747	617	664	665	662	629	696	599	648	602	597	553	705	644	591	131	0.456
GM	12 697	12 916	11 453	11 025	10 121	10 631	10 995	11 356	10 256	10 450	9 798	9 449	9 445	12 013	10 782	9 507	2 356	0.005
GPS	19	17	18	17	15	16	18	17	17	16	16	16	17	17	17	16	2.1	0.704
PH (cm)	69.2	67.5	70.0	73.3	70.0	70.8	71.7	67.5	68.3	70.0	70.8	69.2	66.7	70.0	69.2	69.2	9.34	1.000
HD	40.5	39.2	41.5	40.7	40.5	39.3	39.3	40.5	41.0	39.5	39.8	39.5	40.0	39.5	40.3	40.8	1.75	0.157
ML (1–9)	5.3	4.3	5.0	4.5	3.5	3.0	3.2	2.7	4.2	3.8	4.5	5.0	2.3	3.5	3.2	4.2	1.34	0.000

GY – grain yield on a plot basis; GY* – grain yield on a 1m row basis; ADJ GY – grain yield adjusted with glumes; BY – biomass yield; HI – harvest index; ADJ HI – harvest index adjusted with glumes; TGW – thousand grain weight; ADJ TGW – thousand grain weight adjusted with glumes; STM – number of stems/m^2; SPKM – number of spikes/m^2; GM – number of grains/m^2; GPS – number of grains per spike; PH – plant height; HD – heading date (days from April 1st); ML – powdery mildew

detected in the hulled subpopulation. The other QTLs mapped in the subpopulations either exactly or very probably coincided with the QT regions previously mapped in the entire population and were classified accordingly (Table 3 and Figure 1). None of the QTLs found for GY and TGW showed any significant Q × Q interaction with the yield QTL at *nud*, suggesting the absence of epistatic effects between the respective QT-responsible loci.

As shown in Figure 1, no QTLs for HD, PH or ML map at *nud* on 7H were found out; therefore no further searches on the two subpopulations were done. Two QTLs for HD were mapped on 5H from two different environments, and in separate bins. These QTLs explained 14.2% and 11.3% of the phenotypic variance, and the late alleles derived from the hulled parent Proctor in both cases. It is worth noting that one QTL for PH was found on chromosome 6H at FoA, in the same position as Q.Yld-FoA.6H.1. It explained 16.6% of the variance and the allele effect leading to taller plants derived from the naked parent Nudinka. Three QTLs were associated with resistance to *Blumeria graminis* (ML) at FS and FA, on chromosomes 3H and 5H, in regions not associated with yield QTLs.

Advanced backcross lines (Iabo × Arda). The 16 nud-AB lines were prepared after the DH study, and selected for having a naked caryopsis together with the plant and ear phenotype of the recurrent parent (Arda). They were characterized for agronomic traits at Fiorenzuola in 2005 and 2006. The effects of genotype, year and their interaction (G × Y) on these lines were evaluated by two-way ANOVA for each trait. Since no significant G × Y interaction was observed, all the values of the AB lines were averaged over the two years using least-square means and the $LSD_{0.05}$ was calculated for each trait (Table 4). The P value of the genotype effect calculated by the ANOVA for each trait is also reported in the supplemental table. Since the 16 nud-AB lines did not differ significantly in all the traits but two (GM and ML; Table 4), they were deemed sufficiently similar to be considered as a group. Because of the repeated backcrosses, the nud-AB lines are expected to have introgressed the nud locus into the genetic background of the recurrent parent; as for the DH lines, they should harbour much fewer stretches of other chromosomes from the hulless parent. Therefore they should represent a more appropriate genetic material than DH lines to study the effect of the nud locus, and provide results less biased by any linkage with other genes. For each trait, the least-square mean of the nud-AB lines was compared with the value of each parent in each year and over the two years averaged as well, using Dunnett's multiple comparison test (Table 5). Correspondingly, the significant mean differences reported in Table 3 refer to the contrast between either parent and the mean of the nud AB lines.

Across the harvest years 2005 and 2006, the naked ABs differed from Arda only in two traits: HI and TGW, which were both higher in the hulled parent (0.46, $P \leq 0.00$ and 149.7 g, $P \leq 0.01$). When the values of these two traits were adjusted by adding the hull weight (ADJ HI and ADJ TGW in Table 3), the differences between the naked ABs and Arda were no longer significant. None of the other traits differed significantly between the hulled Arda and its naked counterpart. Without the adjustment for hull weight (ADJ GY) the naked AB lines reached an average GY (4.5 t/ha) lower than that of Arda (5.1 t/ha), and the difference between the two yields corresponded well with the expected percentage of hull weight (10–13%, Bhatty *et al.* 1975). However, this difference was not statistically significant. On the other hand, the comparison between *nud*-AB lines and the donor parent Iabo revealed more differences, as would be expected because of the introgressed Arda's genetic background. The naked donor parent was worse in terms of HI and ADJ HI, it had fewer stems/m^2 (STM; $P \leq 0.001$), fewer spikes/m^2 (SPKM; $P \leq 0.005$) and higher number of grains per spike (GM; $P \leq 0.001$). The *nud*-AB lines were shorter and flowered earlier (HD) than Iabo (Table 3); moreover, they were less resistant to mildew (ML; $P \leq 0.001$). This pattern was essentially maintained in each single year (Table 5); however, differences in the levels of significance were found out. In particular in 2005, AB lines were also different from Iabo in plot yield, and in 2006 they were slightly different from Arda in the same trait, while they did not differ from Iabo in HI and ADJ HI.

DISCUSSION

In the first experiment we compared the group of naked *vs*. that of hulled DH lines, so that the random recombination of the *nud* locus with genes not associated with it could free the naked trait by the genetic background of the parents, with most unlinked traits randomly assorting in the two groups. The group of naked DH lines showed a lower yield potential than the hulled one in all

Table 5. Agronomic performance of *nud*-AB lines (least-square mean) compared to the recurrent parent Arda, hulled, and to the donor Iabo, naked, as observed in the two harvest years 2005 and 2006, and in both years; Dunnett's test was applied to compare the *nud*-AB lines with both the recurrent and the donor parents; significant differences in the two pairwise comparisons are designated with asterisks on the two parent columns

Trait	2005			2006			Two years average		
	Arda	*nud*-AB	Iabo	Arda	*nud*-AB	Iabo	Arda	*nud*-AB	Iabo
GY (t/ha)	5.0	4.8	3.1***	5.1*	4.2	4.6	5.1	4.5	3.9
GY* (t/ha)	5.5	4.7	3.8	5.2	5.0	5.2	5.3	4.8	4.5
ADJ GY (t/ha)	5.5	5.3	4.4	5.2	5.7	6.0	5.3	5.5	5.2
BY (t/ha)	12.6	12.3	11.7	10.5	11.6	13.3	11.6	12.0	12.5
HI	0.43**	0.38	0.32**	0.49***	0.43	0.39	0.46***	0.40	0.36*
ADJ HI (t/ha)	0.43	0.43	0.38*	0.50	0.50	0.46	0.46	0.47	0.42*
TGW (g)	45.6*	41.5	40.9	53.8***	48.0	45.1**	49.7**	44.7	43.0
ADJ TGW (g)	45.5	47.3	47.5	53.8	54.4	51.8	49.7	50.9	49.7
STM	727	710	493**	556	600	562	641	655	527**
SPKM	720	695	490*	532	592	562	626	643	526*
GM	11 977	11 292	9 340	9 685	10 197	11 488	10 830	10 744	10 413
GPS	17	16	19*	18	18	21**	17	17	20***
PH (cm)	73	76	85*	63.3	63.2	70***	68.3	69.6	77.5*
HD	39	39	51***	41	41	44***	40	40	48***
ML (1–9)	3.7	3.7	0.3***	4.3	4.0	0.0***	4.0	3.9	0.2***

GY – grain yield on a plot basis; GY* – grain yield on a 1m row basis; ADJ GY – grain yield adjusted for glumes; BY – biomass yield ; HI – harvest index; ADJ HI – harvest index adjusted for glumes ; TGW – thousand grain weight; ADJ TGW – thousand grain weight adjusted for glumes; STM – number of stems/m^2; SPKM – number of spikes/m^2; GM – number of grains/m^2; GPS – number of grains per spike; PH – plant height; HD – heading date (days from April 1st); ML – powdery mildew; *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

three environments, FS, FA and FoA (Table 2), suggesting either a direct effect of *nud* or linked with it. At least in FoA conditions, GY reduction could have been influenced by a lower TGW that was not adjusted for the absence of glumes in this experiment. In Foggia autumn, naked DHs differed from hulled ones also in terms of PH and HD. In this case, the association with a lower PH in the naked DH group is in agreement with the observations of Choo *et al.* (2001) for naked and hulled doubled haploid (DH) lines (like in our case derived from a single hulled × hulless cross), and of Thomason *et al.* (2009) in naked and hulled breeding lines. It could also suggest the existence of either pleiotropic or linked effects of the *nud* gene. However, the observation was not confirmed in the other two environments out of the three surveyed (Table 2).

The study continued with the QTL mapping of yield and other agronomic traits in the naked × hulled population. Our QTL analysis performed on the entire population identified a total of 12 chromosome regions that significantly affected one or more of the traits evaluated (Figure 1). Proctor and Nudinka contributed to QTLs differently, with Proctor alleles that increased the trait values in 9 QTLs vs. 5, respectively, which suggests the presence of transgressive genotypes in the population. Neither QTLs for HD nor those for PH were associated at *nud* on chromosome 7H. QTLs for HD were mapped on chromosome 5H, while for plant height on 6H (Figure 1). This observation further supported the hypothesis that the *nud* gene most likely has no direct effect on such traits, as already observed in two DH field trials, and later confirmed by the experiment with AB lines.

QTL studies are often affected by interactions with the environment (Veldboom & Lee 1996). The GY QTL on 7H at the *nud* locus was detected consistently over the three environments, and due to the direct effect of *nud* segregation. This GY QTL due to *nud* quantified a relatively large part of the variation most likely due to the *nud* gene (from 25.6% to 37.5%), larger than the grain yield differences between DH groups (Table 2) and larger than the expected difference due to glume weight (10–13%, Bhatty *et al.* 1975). However, such a QTL R^2 suggests, as a working hypothesis, rather an overestimation of the GY QTL explained variance by the QTL mapping software than the existence of a biological difference resulting from the glume loss. Interestingly, the only QTL of chromosome 6H that influenced GY and was different from *nud*, contributed by the lower yielding Nudinka, had the same map location as the QTL responsible for plant height (PH), contributed *in cis* by the shorter parent Nudinka (Figure 1 and Table 1). Given that we verified no interactions between the yield QTL at *nud* and this QTL, at present and with the available data no hypothesis can be reasonably proposed to explain the association of the two QT loci, if not simply a linkage.

A QTL with the highest effect on TGW (23.6% R^2) is also resident at the *nud* locus, although mapped in one environment only (FoA), while the two DH groups were also significantly different in TGW at FS (Table 2). The QTL for TGW found at FoA, together with the low and different TGW values found at the same Southern Italian location between the two DH groups, might suggest a differential behaviour of the naked vs. hulled DHs after a terminal drought stress. The largest TGW QTL on 7H could have been due to either the *nud* gene itself or a linked locus for the trait; however, the trait is also affected by other loci on chromosomes 2H, 3H and 5H, as shown in Table 2, which taken together explain a significant part of the variation.

Table 3 shows additional QTLs mapped after eliminating the segregation of *nud* on 7H for the two traits (GY and TGW) that were shown to be ruled by a QTL mapped at that locus. This study led to the confirmation of the QTLs found in the entire population which were different from *nud*. In addition, it led to the discovery of a few new loci, four for GY and at least one for TGW, whose effects were likely hidden in the entire population by the segregation of the *nud* region, although accounting for a significant part of the variation. The fact that no interaction was found between all the QTLs detected for the two traits suggested that epistatic effects between QT-responsible loci, including *nud*, did not significantly occur. Interestingly, and as already mentioned for the 6H QTL in the entire population, yield was not driven only by the QTLs contributed by the hulled parent (Table 3). The QTL study as a whole identified the QTL effect on yield due to the *nud* gene. However, it also mapped the 6H QTL contributed by the naked parent, and confirmed by QTL mapping in the two DH groups, together with other QTLs for TGW on 2H, 3H and 5H, as well as for other traits. This highlights the potential for naked barley breeding inside naked germplasm as well, and it also confirms the presence of transgressive DHs for yield in the Proctor × Nudinka population.

To better clarify the reasons for the difference in agronomic performance between hulled and naked barleys, we developed a set of 16 advanced backcross lines carrying the recessive allele (naked phenotype) at the *nud* gene to reduce the effects of genetic background as well as of gene interaction (Tsujimoto 2001). To create this kind of population two different cultivars were employed, Arda and Iabo, better adapted to Italian conditions than Proctor and Nudinka. The average agronomic performance of *nud*-AB lines compared to the two parents is summarized in Table 5. By fixing the large majority of the genome with the contribution of Arda after five backcrosses, *nud*-AB lines were expected to show an ADJ GY equal to that of the hulled recurrent parent if the *nud* gene *per se* had no direct effects on yield apart from the lack of glumes, and if the *nud* gene had no pleiotropic effects. This is indeed what we observed: with or even without the adjustment for glume weight (apart in 2006), no significant difference in GY was noticed between the AB lines and the hulled parent Arda. Nor significant differences were observed for most of the other traits (Table 5). The introgression of the *nud* locus in the Arda background only resulted in significant changes in HI and TGW. Without adjustment, as happens in normal barley cropping, we presume that the lower TGW of hulless lines was driving the lower HI with respect to Arda. In fact, after adjustment for glumes, the two differences were significant no longer (Table 5).

More differences were found between the *nud*-AB lines and the donor naked cultivar Iabo. The lower HI of the donor parent compared to the AB lines is still different even after adjustment for glume weight. This could be due to the significantly taller plant of the relatively old cultivar Iabo compared to the AB lines. In fact, the AB lines have a smaller plant height, which is equal to the recurrent parent Arda. On the other hand, the significantly lower number of stems per square metre of Iabo most likely prevented us from observing a significant difference in BY between the *nud*-AB lines and the naked cultivar (Table 5). As for the yield components, Iabo compensated for the lower number of plants m^{-2} with a higher number of seeds per spike, thus showing the grain yield equal to the AB lines.

Notably, our measurements of the weights of naked caryopses and of their glumes, obtained by hand-threshing four ears of 1 m row of each AB line, showed that the difference between the grain yield adjusted for glume weight (ADJ GY) and the yield of naked grains (GY*) represented the 11.97% of the ADJ GY, as an average of the 16 AB lines, which fits well with the expected percentage of hull weight (10–13%, Bhatty *et al.* 1975).

After the two experiments, the use of such advanced backcross materials strongly suggests that the *nud* gene *per se,* when properly introgressed into a hulled background, only leads to irrelevant differences in yield, together with a reduction in grain size, as observed by Choo *et al.* (2001) and Thomason *et al*. (2009). This in turn leads to a HI reduction, exclusively due to the absence of the glume weight.

In contrast to Choo *et al.* (2001), although in different environments and materials, our results did not confirm that the *nud* locus had pleiotropic effects either on PH or on STM, on GPS, on HD, or on susceptibility to mildew (ML). This could mean that the associations observed for example by Thomason *et al.* (2009) and Choo *et al.* (2001) with fewer heads/plants per m^2, and by Thomason *et al.* (2009) with fewer grains per spike were rather due to different, non-isogenic, hulless genotypes used, and/or to environmental factors, or to grain damage conditions that reduced the emergence of hulless barley, than to the direct or even pleiotropic effects of the *nud* gene.

Naked barley breeding has been underexploited for many years with respect to the hulled cultivar development, and the naked germplasm available for crosses is often inferior compared to the hulled one. In conclusion our work, with the use of AB lines after a DH population study, demonstrated that there is a potential to improve naked barley until it reaches hulled barley.

Acknowledgements. The authors would like to thank M. Baronchelli and N. Faccini for their excellent technical assistance. We also wish to thank Mss. I. Vaccari, I. Tagliaferri and M. Polledri for their valuable field assistance. The study was partially supported by grants from the National Projects AGRO-NANOTECH of the Mi.P.A.F. (Ministero delle Politiche Agricole e Forestali), and FISR QUA.SI.CER. – QUAlità e SIcurezza dei CEReali of the MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca).

References

Barabaschi D., Campani L., Francia E., Toubia-Rahme H., Valé G.P., Gianinetti A., Delogu G., Stanca A.M., Pecchioni N. (2007): Haplotype struc-

ture around the *nud* locus in barley and its association with resistance to leaf stripe (*Pyrenophora graminea*). Plant Breeding, **126**: 24–29.

Becker J., Vos P., Kuiper M., Salamini F., Heun M. (1995): Combined mapping of AFLP and RFLP markers in barley. Molecular and General Genetics, **249**: 65–73.

Bhatty R.S. (1986): The potential of hulless barley – a review. Cereal Chemistry, **63**: 97–103.

Bhatty R.S., Berdahl J.D., Christison, G.I. (1975): Chemical composition and digestible energy of barley. Canadian Journal of Animal Science, **55**: 759–764.

Chen H., Wang S., Xing Y., Xu C., Hayes P.M., Zhang Q. (2003): Comparative analyses of genomic locations and race specificities of loci for quantitative resistance to *Pyricularia grisea* in rice and barley. Proceeding of the National Academy of Sciences of United States of America, **100**: 2544–2549.

Choo T.M., Ho K.M., Martin R.A. (2001): Genetic analysis of a hulless × covered cross of barley using doubled-haploid lines. Crop Science, **41**: 1021–1026.

Fedak G., Tsuchiya T., Helgason S.B. (1972): Use of monotelotrisomics for linkage mapping in barley. Canadian Journal of Genetics and Cytology, **14**: 949–957.

Gaines R.L., Bechtel D.B., Pomeranz Y. (1985): A microscopic study on the development of a layer in barley that causes hull caryopsis adherence. Cereal Chemistry, **62**: 35–40.

Kinner M., Nitschko S., Sommeregger J., Petrasch A., Linsberger-Martin G., Grausgruber H., Berghofer E., Siebenhandl-Ehn S. (2011): Naked barley – Optimized recipe for pure barley bread with sufficient beta-glucan according to the EFSA health claims. Journal of Cereal Science, **53**: 225–230.

Liu R.H. (2007): Whole grain phytochemicals and health. Journal of Cereal Science, **46**: 207–219.

Morrell P.L., Clegg M.T. (2007): Genetic evidence for a second domestication of barley (*Hordeum vulgare*) east of the Fertile Crescent. Proceeding of the National Academy of Sciences of United States of America, **104**: 3289–3294.

McGuire C.F., Hockett E.A. (1981): Effect of awn length and naked on malting quality of Betzes barley. Crop Science, **21**: 18–21.

Niks R.E., Fernandez E., van Haperen B., Bekele Aleye B., Martinez F. (2000): Specificity of QTLs for partial and non-host resistance of barley to leaf rust fungi. Acta Phytopathologica et Entomologica Hungarica, **35**: 13–21.

Pecchioni N., Faccioli P., Toubia-Rahme H., Valè G., Terzi V. (1996): Quantitative resistance to barley leaf stripe (*Pyrenophora graminea*) is dominated by one major locus. Theoretical and Applied Genetics, **93**: 97–101.

Saisho D., Purugganan M.D. (2007): Molecular phylogeography of domesticated barley traces expansion of agriculture in the Old World. Genetics, **177**: 1765–1776.

Salamini F., Özkan H., Brandolini A., Schäfer-Pregl R., Martin W. (2002): Genetics and geography of wild cereal domestication in the Near East. Nature Reviews Genetics, **3**: 429–441.

Shewry P.R. (1993): Barley seeds proteins. In: MacGregor A.W., Bhatty R.S. (eds.): Barley: Chemistry and Technology. AACC, St. Paul, 1–5.

Taketa S., Kikuchi S., Awayama T., Yamamoto S., Ichii M., Kawasaki S. (2004): Monophyletic origin of naked barley inferred from molecular analyses of a marker closely linked to the naked caryopsis gene (*nud*). Theoretical and Applied Genetics, **108**: 1236–1242.

Taketa S., Amano S., Tsujino Y., Sato T., Saisho D., Kakeda K., Nomura M., Suzuki T., Matsumoto T., Sato K., Kanamori H., Kawasaki S., Takeda K. (2008): Barley grain with adhering hulls is controlled by an ERF family transcription factor gene regulating a lipid biosynthesis pathway. Proceeding of the National Academy of Sciences of United States of America, **105**: 4062–4067.

Thomason W.E., Brooks W.S., Griffey C.A., Vaughn M.E. (2009): Hulless barley seeding rate effects on grain yield and yield components. Crop Science, **49**: 342–346.

Tsujimoto H. (2001): Production of near-isogenic lines and marked monosomic lines in common wheat (*Triticum aestivum*) cv. Chinese Spring. The Journal of Heredity, **92**: 254–259.

Van Ooijen J.W. (2004): MapQTL5, software for the mapping of quantitative trait loci in experimental populations. Plant Research International, Wageningen.

Veldboom L.R., Lee M. (1996): Genetic mapping of quantitative loci in maize in stress and non stress environments: I. Grain yield and yield components. Crop Science, **36**: 1310–1319.

Zohary D., Hopf M. (2000): Domestication of Plants in the Old World. 3rd Ed. Oxford University Press, New York.

Received for publication September 15, 2011
Accepted after corrections February 9, 2012

Corresponding author:

Dr. Delfina Barabaschi, PhD., CRA – Genomics Research Centre, Via San Protaso 302, 29017 Fiorenzuola d'Arda, Italy
e-mail: delfina.barabaschi@entecra.it