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Pleiotropic effects of the *sdw1* locus in barley populations representing different rounds of recombination

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ABSTRACT

Background: In the present study populations, representing different rounds of recombination were used for the analysis of phenotypic effects associated with the *sdw1/denso* locus. Other studies have mostly focused only on one type of population. Many different QTLs mapped at the same position as the *sdw1/denso* locus may indicate a pleiotropy of this gene or a tight linkage between genes conditioning quantitative traits. To date, results of studies have not unequivocally proven either of these two phenomena.
Results: Both breeding and molecular mapping experiments were undertaken to examine 200 single seed descent (SSD) and 60 doubled haploid (DH) lines obtained from the Maresi (with a semi-dwarfing gene) and Pomo cross combination. They were evaluated for the type of juvenile growth habit and certain agronomic traits were measured after harvesting. The estimates of mean values, standard errors and significance of effects were analyzed. In terms of the analyzed characteristics, the greatest variability was obtained for genotypes with the prostrate growth habit. Microsatellite markers (SSR) were also used to identify co-segregation with the *sdw1/denso* locus and Bmag0013, Bmag0877, Bmag0306b markers were linked the closest. A partial linkage map of chromosome 3H with the *sdw1/denso* semi-dwarfing gene was constructed and QTLs were identified.
Conclusions: Our experiments confirmed the impact of the semi-dwarfing gene on plant height, heading and flowering date both in SSD and DH populations, which may indicate pleiotropy. Moreover, a partial linkage between *sdw1/denso* locus and grain weight per spike and 1000-grain weight was found in the SSD population.

1. Introduction

In recent years, a lot of research has been carried out in the field of mapping crop genomes, including barley, *e.g.* The North American Barley Genome Mapping Project finally leading to the creation of a physical, genetic and functional sequence assembly of the barley genome based on barley cv. Morex [1]. Exploring a genome sequence and its regulatory mechanisms is crucial to the development of effective breeding strategies and unlocking the full potential of natural genetic variation for crop improvement. Barley has been considered a model genome for genetic research into cereals. There are more than 460,000 public ESTs, collections of aneuploids and translocation lines, several thousand SNP markers for high throughput genotyping, genetic maps featuring nearly 5000 genes, several bacterial artificial chromosome (BAC) libraries, and a couple TILLING populations and efficient transformation procedures [http://www.barleygenome.org], [2,3,4,5,6,7,8,9].

Modern, high-yielding barley varieties are mostly related to the sources of semi-dwarfness associated with the *sdw1/denso* locus. Initially, *sdw1* and *denso* were considered different genes because they were derived from different sources: the *sdw1* gene from cv. Jotun and *denso* from cv. Diamant. However, they have been proved to be allelic [10,11], by mapping to the same region of the long arm of chromosome 3H [12,13,14]. The type of juvenile growth habit is the morphological marker of the *sdw1/denso* gene. Plants possessing the semi-dwarfing gene are characterized by prostrate growth, whereas plants with its dominant allele are characterized by erect growth.

Genetic maps with molecular markers and also phenotypic observations allowed spots in the genome to be found which are responsible for the development of economically important quantitative traits. The influence of the *sdw1/denso* locus on various agronomic and physiological characteristics has been observed in numerous studies. Some QTLs for heading date, grain yield, thousand-grain weight and plant height have been localized in the *sdw1/denso* region [12,15]. In addition to reduced plant height, semi-dwarf plants are observed to have an increased time to heading, late maturity, decreased thousand grain weight and plant yield, and a high level of beta-glucan [16]. For the *sdw1/denso* locus, research has

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focused on modifications of major crop related traits, while its anatomical effects or the identification of proteins involved in barley semi-dwarfness have been analyzed only to a small extent. Such studies offer an approximate understanding of the effects of this gene [17,18]. Many QTL for quantitative traits localized at the *sdw1/denso* region may be the result of tight linkage between genes controlling these traits or pleiotropy effects of the *sdw1/denso* gene [19]. In the presence of complete linkage (no recombination) or pleiotropy, relationships between quantitative traits are significant and similar in different populations. If linkage between genes conditioning quantitative traits is not complete, correlations in DH derived from F_1 hybrids are expected to be higher than in single seed descent (SSD) populations [20,21,22].

The aim of the present studies was to compare two barley populations representing different rounds of recombination: derived by a DH system and the SSD technique. In the DH system, lines were developed from F_1 hybrids and in that population only one round of recombination was fixed. In the case of the single seed descent technique it was applied up to F_6 — this means that six rounds of recombination in that population were utilized. SSR markers reported in the literature as being specific to barley chromosome 3H [2,6] were exploited to investigate the genetic polymorphism of barley lines derived from the same cross by two different techniques and to identify markers co-segregating with the *sdw1/denso* locus. Juvenile growth habit was observed and several important agronomic traits were measured. Statistical analysis allowed the identification of QTL associated with *sdw1/denso* in barley.

2. Materials and methods

2.1. Plant material

Plant material consisted of two homozygous populations of spring barley (*Hordeum vulgare* L.), DH and SSD, derived from the cross Maresi/Pomo. The cv. Maresi is a two-rowed, hulled, German brewing cultivar, whereas the cv. Pomo is a six-rowed, hulled, Finland fodder cultivar. In addition, Maresi possesses the semi-dwarfing gene (*sdw1/denso*) from the cv. Diamant, an X-ray mutant of the cv. Valticky, being in the pedigree of cv. Maresi. The DH population was derived from F_1 hybrids by crossing with *Hordeum bulbosum* [23] and the SSD population was obtained by the single seed descent technique, which was applied up to the F_6 generation. As many as 200 SSD $F_{6/9}$ (the SSD technique was applied from F_2 up to F_6, whereas in the experiment the F_9 generation was examined) and 60 DH lines along with their parental genotypes were examined in the field experiment carried out in a completely randomized design with three replications. In each plot, seeds were sown in six rows, 4 m long, 20 cm apart, with each row containing 200 seeds. The studied lines were evaluated for the type of juvenile growth habit. Plant height (PH, cm) was measured at full maturity. Heading date (HD) was calculated as the number of days between the 1st of January and the day when approximately 2 cm of awns were visible in 50% of stems. Flowering date (FD) was calculated as the number of d between the 1st of January and the day when 50% plants had visible spikes. After harvest, the length of spike (LS, cm), number of grains per spike (NGS), grain weight per spike (WGS, g), 1000-grain weight (TWG, g) and grain yield per plot (GY, g) were measured.

3. Methods

15-d-old leaf tissue was collected for molecular analysis. Genomic DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega). The group of 40 microsatellite markers specific for barley chromosome 3H was screened for DNA polymorphism of parental genotypes. Markers displaying polymorphism were selected for genotyping DH and SSD lines and mapped with the *sdw1/denso* locus on chromosome 3H using JoinMap 4.1 software.

PCR conditions were as follows: 1 μL of 50 ng DNA template and 5 μL of microsatellite PCR Kit (Qiagen) containing primers added in appropriate volumes. PCR reactions were performed on a PCT-225 MJ Research thermocycler and included an initial 5 min of denaturation at 95°C, followed by 30 cycles of 94°C for 1 min, primer dependent annealing temperature for 1 min, 72°C for 2 min and final extension at 72°C for 10 min with a hold at 4°C. PCR products were separated with an Applied Biosystems 3130 Genetic Analyzer using capillary electrophoresis and fluorescence detection.

Data were submitted for analysis of variance (R system, lme4 library) to obtain the estimates of mean values, their standard errors and significance of differences between row type of spike and growth type classes. The significance level was set to $\alpha = 0.05$, unless stated otherwise. Pearson correlation coefficients were computed to analyze relationships between traits. QTL mapping was done in Windows QTL Cartographer [24] version 2.5, using the interval mapping [25] method. Significant thresholds for declaring the presence of QTLs were estimated [26] from 1000 permutations of data at the significance level $\alpha = 0.01$ and step size equal to 1 cm.

4. Results

Both DH and SSD lines obtained from hybrids between two-rowed and six-rowed cultivars showed large variation in their spike morphology. In the DH population, there were 27 two-rowed and 33 six-rowed lines. In the SSD population, 94 two-rowed and 106 six-rowed lines were observed. A set of 14 SSR markers showed polymorphism between Maresi and Pomo cultivars.

Based on SSR polymorphism in the *sdw1/denso* locus with correlation to growth habit in juvenile stage, barley lines can be classified into two groups: 1) lines carrying recessive alleles at the *sdw1/denso* locus with prostrate growth habit and 2) lines possessing dominant alleles with erect growth habit. Polymorphic markers were applied to construct the map of a segment of the long arm of barley chromosome 3H. Three markers were recorded to be the closest to the *sdw1/denso* locus. Bmag0013 was linked directly into the locus (0 cm), while Bmag0877 was mapped at a distance of 3 cm in both populations and Bmag0306b was mapped at a distance of 4.5 cm and 7.6 cm in SSD and DH lines, respectively. In the case of the DH population, three traits exhibited LOD score profiles exceeding the thresholds (Table 1). For SSD, there were five such traits (Table 2).

In the analyzed SSD population, 112 lines were distinguished with erect growth habit and 88 lines were characterized by prostrate growth habit at juvenile stage. Among the DH lines, 32 and 29 genotypes were found with erect and prostrate growth habit, respectively. The value of the chi-square test is 2.88 for SSD and 0.17 for DH and this is not significant at $P = 0.05$, which means that the segregation of alleles in the *sdw1/denso* locus did not differ significantly from the expected ratio of 1:1.

The average values of analyzed quantitative traits depending on the type of row or growth habit were similar in both DH and SSD lines (Table 3). Semi-dwarf genotypes were characterized by considerably lower plant height and later flowering. Stems in semi-dwarf SSD lines were shorter than those in erect lines by on average 11 cm

Table 1
The QTL mapping for barley DH Maresi/Pomo population.

Trait	Threshold	LOD_{max}	Location	Additive effect
LS	3.1	0.97	Bmag0877 + 0.7 cm	0.33
NGS	2.5	0.99	Bmag0877 + 1.7 cm	-1.55
WGS	2.5	1.69	Bmag0853 + 1.2 cm	0.14
TGW	2.4	0.99	Bmag0853 + 1.2 cm	0.86
GY	2.5	0.86	Bmag0225 - 1.5 cM	3.34
PH	2.1	**15.15**	Bmag0013 + 0.3 cm	-9.63
HD	2.3	**5.65**	Bmag0013 + 0.3 cm	1.22
FD	2.6	**3.77**	Bmag0877 - 1.3 cM	0.79

Table 2
The QTL mapping for barley SSD Maresi/Pomo population.

Trait	Threshold	LOD_{max}	Location	Additive effect
LS	2.2	0.81	HVM33 - 0.6 cm	0.51
NGS	2.5	1.77	Bmag606 + 0.4 cm	2.72
WGS	2.1	**2.67**	Bmag306b - 0.5 cm	0.98
TGW	2.1	**4.64**	Bmag606 + 0.4 cm	-3.18
GY	1.9	1.64	Bmag0013 - 0.9 cm	8.59
PH	2.1	**38.15**	Bmag0013 + 0.1 cm	-8.35
HD	2.4	**10.62**	Bmag0877 + 1.2 cm	0.93
FD	2.4	**8.61**	Bmag0877 + 1.2 cm	0.65

(two-rowed) and 15 cm (six-rowed) and semi-dwarf DH lines by almost 11 cm (two-rowed) and 14 cm (six-rowed). For most of the studied traits in the SSD population significant differences were observed between two- and six-rowed groups of lines and between groups with prostrate and erect growth habits (Table 4). In DH lines, significant differences were noted for traits depending on the type of row, with the exception of the grain yield per plot, which did not differ significantly for two-row and six-row genotypes. Between the erect and prostrate DH lines significant differences were recorded for the number of grains per spike, grain weight per spike, plant height, and heading and flowering date. In the case of the type of spike row relative to the juvenile growth habit (AxB in Table 4) differences were significant only for 1000-grain weight and grain weight per spike for DH and SSD lines, respectively.

For both populations, the correlation of traits was analyzed. Based on the analyses of data distribution and significance tests (for $^{*}\alpha = 0.05$ and $^{**}\alpha = 0.01$) of Pearson correlation coefficients (*cc*), several relationships were found between observed traits. No significant differences in trait correlations between DH and SSD populations were observed, so the following relationships are presented for data from both groups, together. PH turned out to be negatively correlated with HD (*cc* = -0.595**) and FD (-0.554**), irrespective of the type of growth and type of row. For all groups of genotypes, there was a strong positive correlation (>0.75**) between HD and FD.

For bimodal traits, where the two groups were determined by type of row *r* (2 — two-rowed, 6 — six-rowed), the following positive correlations were found: NGS–WGS ($r = 2{:}0.764^{**}$, $r = 6{:}0.774^{**}$), LS–GY ($r = 2{:}0.271^{**}$, $r = 6{:}0.335^{**}$), and also WGS–TGW ($r = 6{:}0.829^{**}$), LS–WGS ($r = 6{:}0.2^{*}$), NGS–TGW ($r = 6{:}0.294^{**}$), PH–NGS ($r = 6{:}0.251^{**}$), PH–WGS ($r = 6{:}0.486^{**}$), and PH–TGW ($r = 6{:}0.505^{**}$). In these last cases, data distribution for $r = 2$ does not allow for reasoning from the correlation coefficient (it is distorted by some outliers/outlying genotypes); however, some positive correlations can be observed (Fig. 1). The significant positive correlation between two traits in both populations indicates partial linkage. No traits were found where the relationships differed according to the type of growth (Fig. 2).

Molecular mapping showed that the *sdw1/denso* gene co-segregated with a major QTL controlling not only plant height, heading and flowering date for both DH and SSD populations (Fig. 3), but also grain weight per spike and thousand grain weight for SSD lines (Fig. 4). The SSR marker Bmag0013 was selected as the most strongly associated with the QTL for these traits. The statistical significance of the relationship between most SSR markers (among them also Bmag0013) and grain yield per plot was observed for SSD lines, but LOD, which defines the certainty the QTL location at a specific location on a chromosome, was below the critical value. The results of QTL analysis indicate a less precise QTL analysis for DH than SSD populations, which is associated most likely with its small number of lines. This also had an impact on the distance mapping which is slightly different in the DH and SSD lines, but the order of SSR markers is the same.

5. Discussion

The present research confirmed the influence of the *sdw1/denso* locus on plant height, heading and flowering date in both populations, and additionally on grain weight per spike and 1000-grain weight in SSD alone. Some QTLs for heading date, thousand grain weight, grain yield, spikelet number per spike, grain number per spike, ear length, resistance to leaf rust have been localized in the *sdw1/denso* region [27,28,29,30]. One of the main QTL for plant height was detected on barley chromosome 3H and linked to Bmag0013 for both analyzed homozygous populations. The effect of this gene is about a 10 cm reduction of plant height in comparison to plants with a normal growth habit. In the present study, the marker Bmag0013 was placed in the same position as the *sdw1/denso* gene. This indicates a co-localization of the plant height QTL with the *sdw1/denso* locus. Jia et al. [31] identified a single nucleotide mutation in intron 2 of the *sdw1/denso* gene and mapped a single nucleotide polymorphism (SNP) marker to chromosome 3H. Quantitative trait locus analyses have revealed that plant height is co-segregated with this SNP [31]. These studies correspond with our observations, where semi-dwarf SSD and DH lines were shorter than erect lines.

In our study, the *sdw1/denso* locus has been associated with higher grain yield and simultaneously with an increased number of tillers. It is widely known that the introduction of semi-dwarf genes into cultivated plants results in an increase in yield potential. On the other hand, grain yield is a complex trait and several different regions of the plant genome appear to be responsible for this trait [32,33,34]. Thomas et al. [16] mapped a QTL controlling plot yield on chromosomes 3H, associated with the *sdw1/denso* locus, and 6H in a cross between Blenheim and breeding line E224/3, similar to the

Table 3
Means and standard errors of traits analyzed DH and SSD populations (for parental cultivars standard error is given in parentheses).

			LS	NGS	WGS	TGW	GY	PH	HD	FD
Maresi			7.98 (0.04)	20.98 (0.43)	0.86 (0.05)	41.22 (1.43)	305 (8.66)	76.67 (3.18)	12.67 (0.33)	18.33 (0.67)
Pomo			5.40 (0.08)	64.93 (1.02)	2.46 (0.02)	37.98 (0.74)	260 (5.77)	82.33 (1.45)	14.00 (0.58)	17.67 (1.20)
DH lines	2-rowed	p	7.39	23.35	1.17	50.03	249.64	69.40	14.21	19.57
		e	7.24	29.05	1.28	46.69	244.44	86.81	11.39	17.33
	6-rowed	p	6.48	55.50	1.82	32.71	252.87	66.22	14.81	19.67
		e	6.42	59.18	2.07	34.82	252.69	87.21	13.08	18.49
Mean standard error			0.19	1.15	0.05	0.83	7.96	1.20	0.28	0.26
SSD lines	2-rowed	p	7.63	23.11	1.12	48.36	252.07	69.24	14.23	19.20
		e	7.35	24.17	1.22	51.04	230.00	85.34	12.71	18.02
	6-rowed	p	6.71	54.59	1.71	31.14	241.45	65.61	14.88	19.49
		e	6.59	58.14	1.96	33.59	226.47	81.24	13.03	18.38
Mean standard error			0.09	0.51	0.02	0.35	4.13	0.97	0.17	0.20

p: prostrate growth habit; e: erect growth habit.

Table 4
Significance levels for comparison of groups of lines differentiated in spike row (A) and juvenile growth habit (B) in barley DH and SSD populations.

	Source of variation	LS	NGS	WGS	TGW	GY	PH	HD	FD
DH lines	A	<0.001	<0.001	<0.001	<0.001	0.426	0.045	<0.001	0.016
	B	0.600	<0.001	<0.001	0.626	0.722	<0.001	<0.001	<0.001
	A × B	0.809	0.382	0.121	<0.001	0.721	0.138	0.045	0.046
SSD lines	A	<0.001	<0.001	<0.001	<0.001	0.469	<0.001	<0.001	<0.001
	B	0.036	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	A × B	0.411	0.011	<0.001	0.757	0.395	0.735	0.314	0.834

situation in our studies. The *sdw1*/*denso* dwarfing gene (present in Blenheim) was associated with an increase in yield. This is in contrast to the Blenheim and Kym cross combination in which an association has been found between the *sdw1*/*denso* gene and lower single plant and thousand-grain weights. Powell et al. [35] showed that associations between the *sdw1*/*denso* gene and undesirable traits can be due, at least in part, to linkage disequilibrium and could be reduced by additional rounds of recombination. This is particularly important

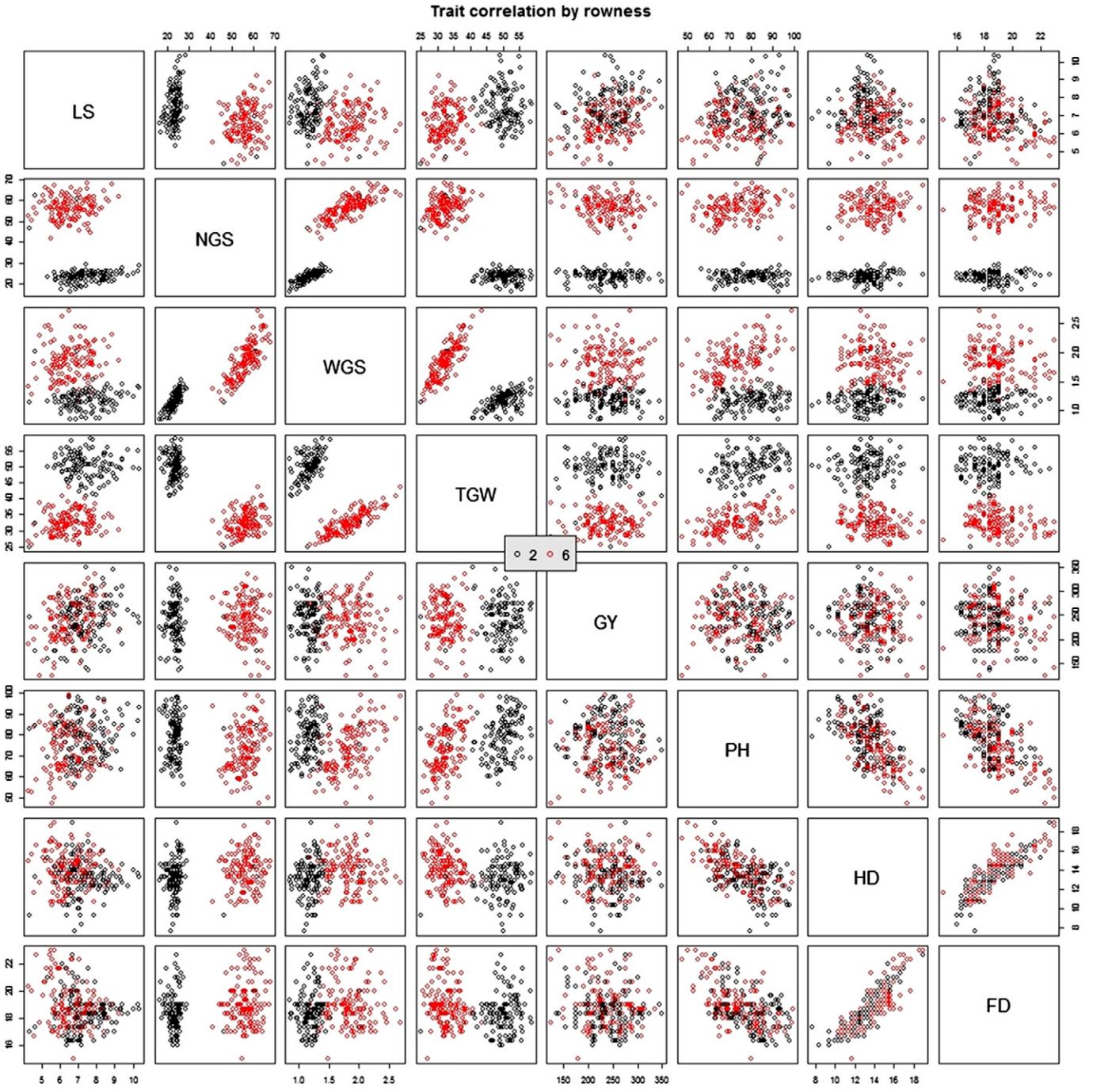

Fig. 1. Correlation plots for all analyzed traits in two-rowed (black) and six-rowed (red) DH and SSD barley lines.

Fig. 2. Correlation plots for all analyzed traits in DH and SSD barley lines with prostrate (red) and erect (black) growth habit.

where doubled haploids are concerned, since mapping populations have only gone through one round of recombination. It may be that the *sdw1/denso* gene is linked to a yield QTL in the population generated from this cross.

In the present study, populations representing different rounds of recombination were used in the analysis of phenotypic effects associated with the *sdw1/denso* locus. Other studies have mostly focused only on one type of population. Many different QTLs mapped at the same position as the *sdw1/denso* locus may indicate a pleiotropy of this gene or a tight linkage between genes conditioning the mentioned traits [36]. Snape and Simpson [37] used doubled haploid lines produced by the *H. bulbosum* method to investigate the pleiotropic effects of the *sdw1/denso* semi-dwarfing gene. This gene, which was present in the parental variety Maris Mink, caused a reduction in plant height, delayed ear emergence, reduced grain size and decreased yield in progenies. However, the doubled haploids were derived from F_1 plants and the single round of recombination may have been insufficient to break any linkage groups. Single seed descent lines and F_1-derived doubled haploid populations should be examined to see if any character associations with the dwarfing gene can be broken by providing further opportunities for recombination. Barua et al. [12] observed genetic factors influencing heading dates to be linked to the *sdw1/denso* locus on barley chromosome 3H and to three RAPD and one RFLP marker located on chromosome 6H. Results from their study showed that the *sdw1/denso* locus either has a pleiotropic effect on heading date or is very tightly linked to this trait.

In our study, the effects of *sdw1/denso* locus on plant height, heading and flowering date were recorded in both populations, which may

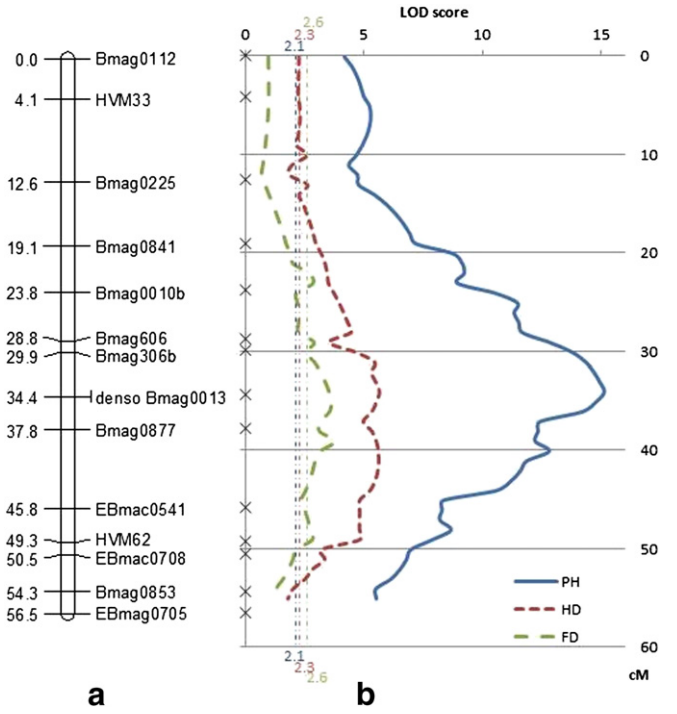

Fig. 3. Partial linkage map of chromosome 3H with the *sdw1/denso* semi-dwarfing gene (a) and QTL controlling plant height (PH), heading date (HD) and flowering date (FD) (b) in DH population based on the simple sequence repeat polymorphism.

indicate pleiotropy. This seems to be obvious in the light of the results presented by Jia and coworkers [31], who identified the gene *Hv20ox2* encoding oxidase GA20, which is involved in gibberellin biosynthesis, in the *sdw1/denso* locus. As is known, gibberellin influences plant growth and earliness [38]. On the other hand, QTLs for grain weight per spike and 1000-grain weight were found only in SSD populations, which indicate a partial linkage between the *sdw1/denso* locus and loci determining grain weight per spike and 1000-grain weight. Our results did not fully explain the problem of the genetic bases of several existing QTLs concerning agronomical traits in the *sdw1/denso* locus. Undoubtedly, the results of barley genome sequencing will enable an understanding of the relative contribution of pleiotropy and linkage in the genetic control of semi-dwarfism and other traits correlated with reduced height. Therefore, a more consistent harmonized approach between molecular and agronomic research is needed. In addition, it is also crucial to know the *sdw1* gene sequence to distinguish the *sdw1* alleles, which may exist in modern barley cultivars and breeding lines. For that purpose, association studies should be performed on broad plant materials of different genetic backgrounds.

Author contribution

Proposed the theoretical frame: AK, KM; Conceived and designed the experiments: AK, KM; Software development: AK, KM, HĆ; Contributed reagents/materials/analysis tools: AK, KM, HĆ; Wrote the paper: AK, KM; Performed the experiments: AK, KM; Analyzed the data: AK, KM, HĆ.

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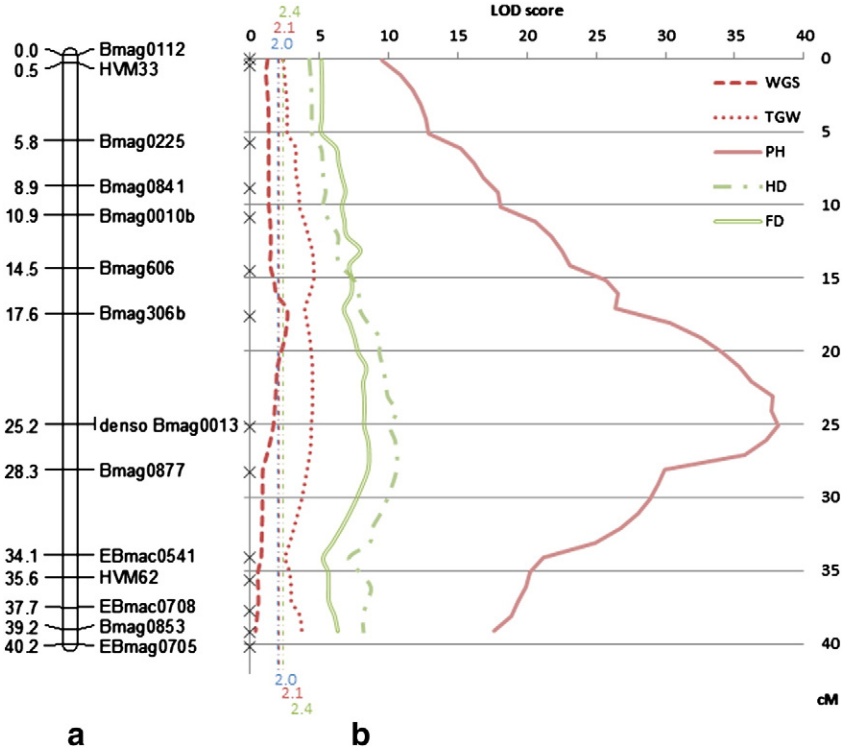

Fig. 4. Partial linkage map of chromosome 3H with the *sdw1/denso* semi-dwarfing gene (a) and QTL controlling grain weight per spike (WGS), 1000-grain weight (TGW), plant height (PH), heading date (HD) and flowering date (FD) (b) in SSD population based on the simple sequence repeat polymorphism.

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