Mol Breeding (2017) 37: 28
DOI 10.1007/s11032-017-0641-9

Mapping gibberellin-sensitive dwarfing locus *Rht18* in durum wheat and development of SSR and SNP markers for selection in breeding

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Received: 25 May 2016 / Accepted: 16 February 2017 / Published online: 1 March 2017

Abstract Gibberellin-sensitive dwarfing gene *Rht18* was mapped in two durum wheat recombinant inbred lines (RIL) populations developed from crosses, Bijaga Yellow/Icaro and HI 8498/Icaro. *Rht18* was mapped within genetic interval of 1.8 cM on chromosome 6A. Simple sequence repeat (SSR) markers *S470865SSR4*, *barc37* and *TdGA2ox-A9* specific marker showed co-segregation with *Rht18* in Bijaga Yellow/Icaro population consisting 256 RILs. Effect of *Rht18* on plant height was validated in HI 8498/Icaro RIL population which segregated for *Rht18* and *Rht-B1b*. *Rht-B1b* from HI 8498 showed pleiotropic effect on plant height and coleoptile length, on the other hand, *Rht18* did not show effect on coleoptile length. The SSR and SNP markers linked to *Rht18* were also validated by assessing their allelic frequency in 89 diverse durum and bread wheat accessions. It was observed that 204 bp allele of *S470865SSR4* could differentiate Icaro from rest of the wheat accessions except HI 8498, suggesting its utility for selection of *Rht18* in wheat improvement programs. *Rht18* associated alleles of *TdGA2ox-A9*, *IAW4371* and *IAW7940* were absent in most of the tall Indian local durum wheat and bread wheat, hence could be used to transfer *Rht18* to bread wheat and local durum wheat. SSR marker *barc3* showed high recombination frequency with *Rht18*, though it showed allele unique to Icaro. Since semidwarf wheat with GA-sensitive dwarfing genes are useful in dry environments owing to their longer coleoptile, better emergence and seedling vigor, *Rht18* may provide a useful alternative to widely used GA-insensitive dwarfing genes under dry environments.

Keywords Durum wheat · Gibberellin-2-oxidase · Plant height · *Rht18* · Semidwarf wheat

Introduction

Wheat consumption worldwide is estimated to surpass 817 million tonnes by 2030. To meet the estimated demand, production would need to increase by 23-45% from the current production level. This target has to be achieved despite limited resources, change in climatic conditions and decreasing acreage under wheat cultivation in dry environments. Present increase in wheat production has been achieved through introduction of semidwarfing genes and selection for grain yield. Semidwarf plants do not lodge under high fertility conditions, exhibit greater harvest index and thereby contribute to increase in grain yield. Therefore, introduction of dwarfing genes in cereal crops was crucial to increase grain yield during the green revolution (Hedden 2003). However, gibberellin-insensitivity conferred by semidwarfing genes *Rht-B1b* and *Rht-D1b* not only affects culm elongation but also many other gibberellic

Electronic supplementary material The online version of this article (doi:10.1007/s11032-017-0641-9) contains supplementary material, which is available to authorized users.

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acid (GA)-dependent developmental processes such as α-amylase production in germinating seeds (Bhagwat and Bhatia 1994), root elongation (Bai et al. 2013), coleoptile length (Botwright et al. 2001) and leaf expansion (Appelford and Lenton 1991). These pleiotropic effects of GA-insensitive semidwarfing genes can lead to reduction in yield.

In low-precipitation dry land wheat-growing regions, deep seed placement is a better option to obtain sufficient moisture to initiate germination. Earlier studies have shown that dwarfing genes *Rht-B1b* or *Rht-D1b* exhibit strong negative correlation with coleoptile length and seedling vigor (Rebetzke et al. 2001; Richards 1992). The *Rht-B1b* and *Rht-D1b* wheat cultivars with a short coleoptile have difficulty in emerging from deep sowing, particularly when sown at a depth more than 4 to 5 cm, which can result in poor seedling establishment. In contrast, wheat cultivars with GA-sensitive dwarfing gene and long coleoptiles emerge with higher frequency especially when sown deep or where stubble has been retained (Allan et al. 1962; Rebetzke et al. 2005). Field studies have demonstrated that GA-sensitive semidwarf wheats emerge significantly better at 11 cm sowing depth than GA-insensitive semidwarf wheats. In wheat genotypes with *rht* (tall) and *Rht8*, longer coleoptile showed positive association with greater number of emerged seedlings, greater seedling area and seedling biomass, and negative association with shallower crown depth (Rebetzke et al. 2007). Comparisons between tall and semidwarf wheat varieties showed that reduced plant height due to GA-sensitive dwarfing genes is associated with increased grain yield (Rebetzke and Richards 2000), thus have potential for improving wheat establishment through greater coleoptile length and seedling vigor. GA-sensitive dwarfing gene *Rht13* was found to be associated with reduced peduncle length with significantly greater biomass, yield, harvest index, grain number and spike number (Rebetzke et al. 2011). Another GA-sensitive dwarfing gene *Rht12* substantially reduced plant height without altering seedling vigor and significantly increased spikelet fertility in common wheat under favorable sowing environment (Chen et al. 2013). In evaluation of breeding potential of GA-sensitive *Rht18* from tetraploid wheat in the background of common wheat, *Rht18* moderately reduced plant height and increased harvest index without affecting seedling vigor, root growth and coleoptile length (Yang et al. 2015). Considering these advantages, GA-sensitive dwarfing genes are needed for diversification of reduced height genes in wheat breeding programs to facilitate access to soil moisture at deeper sowing in dry environments.

GA-responsive height reducing genes such as *Rht4* (2BL), *Rht5* (3BS), *Rht8* (2DS), *Rht9* (5AL), *Rht12* (5AL) and *Rht13* (7BS) have been mapped and DNA markers linked to these genes reported in bread wheat (Ellis et al. 2005). However, there are very few reports on genetic studies of GA-sensitive dwarfing genes in durum wheat. Out of the 22 dwarfing genes reported in wheat 16 are sensitive to GA, of which five genes (*Rht14*, *Rht15*, *Rht16*, *Rht18* and *Rht19*) are available in durum wheat (Konzak 1987). These induced *Rht* mutants have better emergence potential, since their coleoptile length and gibberellic acid (GA) sensitivity is unaltered. In a genetic mapping study using three F_2 populations, *Rht14*, *Rht16*, and *Rht18* appeared to be allelic and linked to *barc3* on chromosome 6A (Haque et al. 2011). The genes were mapped at 11.7 to 28.0 cM distance from *barc3*. Using a marker at such a long distance would result in to very high chances of false positive selection in breeding programs. The present study was undertaken with the following objectives: (1) mapping of GA-sensitive dwarfing gene *Rht18* in durum wheat and development of closely linked simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) for efficient selection and deployment of *Rht18* in durum wheat breeding. (2) Validation of newly identified markers in diverse wheat genetic backgrounds to assess their usefulness in wheat improvement programs.

Materials and methods

Plant material

Icaro (Ic; PI503555) is a GA-sensitive dwarf mutant obtained from Cv. Anhinga, carrying semidominant dwarfing gene *Rht18*, with long coleoptile and high breeding value (Konzak 1987; Maluszynski and Szarejko 2003). It was crossed with two Indian durum wheat cultivars Bijaga Yellow (BY) and HI 8498 (HI) and two sets of recombinant inbred line (RIL) populations (BY/Ic-256 RILs and HI/Ic-134 RILs) were developed by single-seed descent method. Bijaga Yellow is a tall durum wheat cultivar released in 1965 for cultivation under rainfed conditions. HI 8498 is a cultivar carrying GA-insensitive *Rht-B1b*, with semidwarf

plant type and short coleoptile. It was released in 1999 for cultivation under high fertility conditions. BY/Ic population was used for mapping *Rht18* while HI/Ic population was mainly used to validate the effect of *Rht18* on plant height in the background of *Rht-B1b*. A set of 89 wheat accessions composed of durum cultivars, local durums and bread wheat cultivars was also included in the study to test allelic variation of markers linked to *Rht18*.

Field trials and phenotype analysis

The RIL populations were planted at experimental farm of Agharkar Research Institute, Pune (18^0 31′ N, 73^0 55′ E) during regular crop seasons of 2013–14 (date of sowing November 17, 2013) and 2014–15 (date of sowing November 07, 2014). Fields were irrigated after 42 days from sowing when most of the RILs were at booting stage. Plant height was determined as the measurement of the main tiller from soil surface to the top of the ear (excluding awns) at maturity. Coleoptile length was measured for ten seedlings of each genotype as per method described by Li et al. (2011). In brief, ten similarly sized seeds from each RIL were placed side by side in a straight line, 0.5 cm apart with the germ end downward on the germination paper. The germination paper was folded, rolled and placed vertically in a container with nutrient solution (Bai et al. 2013). Coleoptile length was measured at emergence of first leaf through coleoptile (Zadoks scale 10) and expressed as a mean of 10 measurements. Similar experiment was conducted to test the response of Bijaga Yellow, HI 8498 and Icaro to exogenous gibberellic acid (GA_3, 100 μM) in nutrient solution. Control seedlings were treated with nutrient solution only. Response to gibberellic acid was measured in terms of elongation of coleoptile at Zadoks scale 10. The differences in coleoptile length due to GA_3 treatment and dwarfing genes were tested for significance by Student's *t*- test.

Development of SNP and SSR markers

Since Icaro has been reported as sensitive to exogenous GA_3 (Konzak 1987) and also showed response to exogenous GA_3 at Zadoks scale 10 in the present study (Supplementary Fig. S1; Table S1); it was hypothesized that Icaro may be carrying altered GA biosynthesis or GA inactivation pathway. Gibberellin 2-oxidase-A9 (*TaGA2ox-A9*) was reported on chromosome 6A with putative function of inactivation of active GA (Pearce et al. 2015), therefore, genomic DNA sequence of this gene was used to design PCR primers to amplify *GA2ox-A9* in Bijaga Yellow, Icaro and HI 8498. A DNA fragment of 5.1 kb comprising complete ORF as well as 5′ and 3′ regulatory regions was amplified using primer pair TdGA2ox-A9F/TdGA2ox-A9R and HiFi HotStart polymerase, Kapa Biosystems. Amplicon was ligated in pSC-A-amp/kan vector and transformed into SoloPack competent cells using Strataclone PCR Cloning Kit, Agilent Technologies, USA. Four clones for each insert were sequenced on ABI PRISM® 3100-*Avant* Genetic Analyzer. *GA2ox9* homoeologue from A-genome (*TdGA2ox-A9*) was verified by comparing it with three scaffolds containing sequences of A, B and D homoeologues of *GA2ox9* kindly shared by Dr. Andrew Phillips, Rothamsted Research, Harpenden, UK. DNA Sequence polymorphism observed at −182 bp (C/GT) in 5′ regulatory region of *TdGA2ox-A9* was targeted to develop co-dominant marker differentiating between alleles of *TdGA2ox-A9* in Bijaga Yellow and Icaro. Primers were designed by following strategy reported earlier for development of SNP-based co-dominant markers for disease resistance genes in rice (Ramkumar et al. 2015). The primers were used to map *TdGA2ox-A9* in segregating RILs. Nucleotide sequences of *TdGA2ox-A9* derived from Bijaga Yellow, Icaro and HI 8498 were submitted to GenBank (Accession numbers KX163067, KX163068, and KX163069). The scaffold 470865-6AL containing *GA2ox-A9* was further searched for presence of SSR using SSR identification tool (SSRIT) available at http://archive.gramene.org/db/markers/ssrtool. Total four putative SSRs were identified and tested for polymorphism. On the basis of their map position, six SNP markers were selected from high density consensus SNP map of tetraploid wheat (Maccaferri et al. 2015) and IWGSC wheat genome sequence repository (available at https://wheat-urgi.versailles.inra.fr/Seq-Repository/Genes-annotations) to test their putative linkage with *Rht18*. PCR markers specific to these SNPs were designed by keeping SNP at 3′ end of either forward or reverse primer (Supplementary Table S2) as described by Ellis et al. (2002).

Mapping of *Rht18*

Genomic DNA was extracted from tender leaves of RILs by a modified Cetyltrimethylammonium bromide

(CTAB) method (Rogers and Bendich 1985). Since *Rht18* was assigned to chromosome 6A in earlier report (Haque et al. 2011), a total of 62 SSR markers from chromosome 6A were tested for polymorphism between parental genotypes. Polymorphic markers were used initially to genotype a subset of 139 RILs and framework map was generated for chromosome 6A using MapMaker version 3.0 (Lander et al. 1987). Considering near centromere position of *Rht18* locus, eight markers flanking *Rht18* were used to genotype BY/Ic population of 256 RILs to obtain further resolution in the map. HI/Ic population was also segregating for *Rht-B1*, therefore, a locus specific marker for *Rht-B1* (Ellis et al. 2002) from chromosome 4B was used for genotyping and single-marker regression analysis was carried out to estimate effect of *Rht-B1* on plant height and coleoptile length. Map of chromosome 6A was drawn using the program MapChart 2.1 (Voorrips 2002). Markers linked to *Rht18* locus were tested on 89 diverse durum and bread wheat accessions to study their allelic variation in the germplasm. Markers flanking to *Rht18* were tested on 89 diverse wheat genotypes to determine frequency of their alleles in germplasm.

Statistical analysis

The differences in phenotype due to GA_3 treatment and dwarfing genes were tested for significance by Student's *t*- test. Genotypic (σ^2_g) and phenotypic variances (σ^2_p) were estimated as $\sigma^2_g = (MS_v - MS_e)/r$ and $\sigma^2_p = \sigma^2_g + \sigma^2_e$, respectively, and were used further to calculate heritability ($h^2 = \sigma^2_g/\sigma^2_p$), where r is number of replications, MS_v and MS_e are mean sum of square for genotype and residual error, respectively. Relationships between the traits were examined by Pearson correlation coefficient.

Results

Phenotype evaluation

Significant differences were observed between plant height of Bijaga Yellow (102.8 to 105.3 cm), Icaro (57.9 to 64.3 cm) and HI 8498 (74.2 to 74.8 cm) over two wheat-growing seasons. Detail data in parental genotypes and two RIL populations over two seasons is presented in Table 1. Very high broad-sense heritability was observed for plant height (0.95 to 0.97). In both the RIL populations, plant height measured across 2 years showed significant correlation ($P < 0.01$; Supplementary Table S3). Coleoptile length showed significant correlation with plant height in HI 8498/Icaro population. Though correlation was observed between coleoptile length and plant height (2013–14) in Bijaga Yellow/Icaro, the magnitude of correlation coefficient was low. Analysis of variance showed that genotype, environment and their interaction (G × E) had significant effect on plant height ($P < 0.01$) in both the populations (Supplementary Table S4), however, high broad-sense heritability values suggested that genotype had major contribution to variation in plant height. Bi-modal distribution for plant height was observed in BY/Ic population, suggesting that plant height is governed by a single major locus in the population (Fig. 1a). RILs with plant height less than 85 cm were classified as dwarf lines and RILs with plant height greater than 85 cm were considered tall. BY/Ic population consisted of 97 dwarf lines and 159 tall lines. Average plant height of dwarf lines was 64 cm while that of tall lines was 98 cm over two seasons. Plant height was skewed towards semi-dwarf phenotype in HI/Ic population because of presence of two dwarfing genes in the population (Fig. 1b). All the internodes in Bijaga Yellow were significantly longer than that of HI 8498 and Icaro. Peduncle and second internode showed significant differences for length among Bijaga Yellow, Icaro and HI 8498 (Supplementary Fig. S2). However, lengths of third and fourth internodes of Icaro and HI 8498 were not significantly different. Contrasting values for coleoptile length were observed in Bijaga Yellow (10.2 ± 0.8 cm), HI 8498 (6.7 ± 1.0 cm) and Icaro (9.1 ± 0.6 cm). Coleoptile length in BY/Ic and HI/Ic RILs ranged from 7.1 to 14.2 cm and 5.5 to 14.8 cm with mean 10.3 and 8.8 cm, respectively (Fig. 1c; Table 1). Application of exogenous GA_3 (100 μM) showed 46.2% increase in coleoptile length in Icaro, suggesting its sensitivity towards GA_3. Bijaga Yellow also showed moderate sensitivity towards GA_3 application (Supplementary Fig. S1; Table S1).

Development of SNP and SSR markers linked *Rht18*

Three SNP markers out of six could differentiate between BY and Ic, while only two (*IWA4371* and *IWA7940*) could give reproducible amplification in RIL population. Nucleotide sequence of 4.2 kb was derived for putative *TdGA2ox-A9* from Bijaga Yellow,

Table 1 Plant height and coleoptile length observed in Bijaga Yellow/Icaro and HI 8498/Icaro RIL populations

Population	Trait	Parents: mean ± SD (cm)		RILs (cm)		h^2
		P_1	P_2	Range	Mean	
Bijaga Yellow[a]/Icaro[b]	Plant height (2013–14)	102.8 ± 5.7	58.5 ± 4.4	44–118	84.6	0.95
	Plant height (2014–15)	105.3 ± 4.9	64.3 ± 2.6	43–116	86.8	0.97
	Coleoptile length	10.2 ± 0.8	9.1 ± 0.6	7.1–14.2	10.3	0.91
HI 8498[a]/Icaro[b]	Plant height (2013–14)	74.2 ± 1.6	57.9 ± 4.2	51–123	84.1	0.96
	Plant height (2014–15)	74.8 ± 3.5	61.2 ± 6.2	50–107	76.7	0.97
	Coleoptile length	6.7 ± 1.0	9.1 ± 0.6	5.5–14.8	8.8	0.91

[a] P_1

[b] P_2

Icaro and HI 8498 (GenBank accessions KX163067, KX163068 and KX163069). Derived sequence comprised of three exons of 489, 324 and 231 bp separated by two introns of 814 and 677 bp. Upstream 5′ (1388 bp) and downstream 3′ (314 bp) regions were also partially sequenced. Sequence alignment of Bijaga Yellow and Icaro showed sequence variation at −182 bp (C/GT) in 5′ regulatory region and single nucleotide polymorphism (SNP) in exon 1 (+438 bp) as well as exon 3 (+2435 bp). SNP in exon 3 region resulted in substitution of amino acid proline (Bijaga Yellow) by serine (Icaro). A co-dominant PCR marker targeting sequence variation at −182 bp in 5′ regulatory region could differentiate between alleles of *TdGA2ox-A9* present in Bijaga Yellow and Icaro (Fig. 2). The marker was subsequently used for genotyping of RILs and mapping *TdGA2ox-A9*. A PCR primer pair targeting SNP observed between HI 8498 and Icaro at +2196 bp was also developed but it failed to give consistent amplification due to A/T rich flanking region. Four putative SSRs were identified from the scaffold 470865-6AL containing *TdGA2ox-A9*. However, only one SSR *S470865SSR4* with trinucleotide repeat $(GTA)_n$ showed polymorphism between Bijaga Yellow (186 bp) and Icaro (204 bp) that was used further for linkage mapping.

Mapping of *Rht18*

Since *Rht18* is located on chromosome 6A, a total of 62 SSR markers on 6A were tested for polymorphism among parents. Out of these, 18 polymorphic SSRs were used to develop genetic linkage map of chromosome 6A initially in a set of 139 RILs of BY/Ic population. No recombinants were observed between *Rht18*, *gwm82* and *barc37* (Fig. 3). *S470865SSR4* and *TdGA2ox-A9* specific marker developed in present study also showed co-segregation with *Rht18*. Both the SNP markers (*IAW4371* and *IAW7940*) showed only one recombinant with *Rht18* in 139 RILs. The genetic linkage distance between *Rht18* and *barc3* was 9.4 cM with 14 recombinants. To resolve this cluster further, additional 117 RILs form the same population were also used for mapping. Genotype data for 8 markers flanking *Rht18* was produced for a total of 256 RILs, which showed no recombinants between *Rht18*, *TdGA2ox-A9*, *S470865SSR4* and *barc37*. This cluster was flanked by *barc118* and *IWA4371* within an interval of 1.8 cM. In HI/Ic population *barc118* showed five recombinants with *Rht18* among RILs carrying wild allele of *Rht-B1*. Unexpectedly, *TdGA2ox-A9*, *S470865SSR4* and *barc37* showed monomorphic alleles in Icaro and HI 8498, therefore, could not be mapped in HI/Ic population. Regression analysis showed that *Rht18* did not have significant effect on coleoptile length BY/Ic and HI/Ic populations, while *Rht-B1* showed significant pleiotropic effect on coleoptile length and plant height in HI/Ic population. Additive effect showed that *Rht18* reduced plant height by 16.95 cm in BY/Ic RILs. Allelic variation of markers *S470865SSR4*, *barc118*, *TdGA2ox-A9*, *IAW4371*, *IAW7940* and *barc3* linked to *Rht18* locus were tested on 89 diverse durum and bread wheat accessions (Supplementary Table S5). It was observed that 204 bp allele of *S470865SSR4*

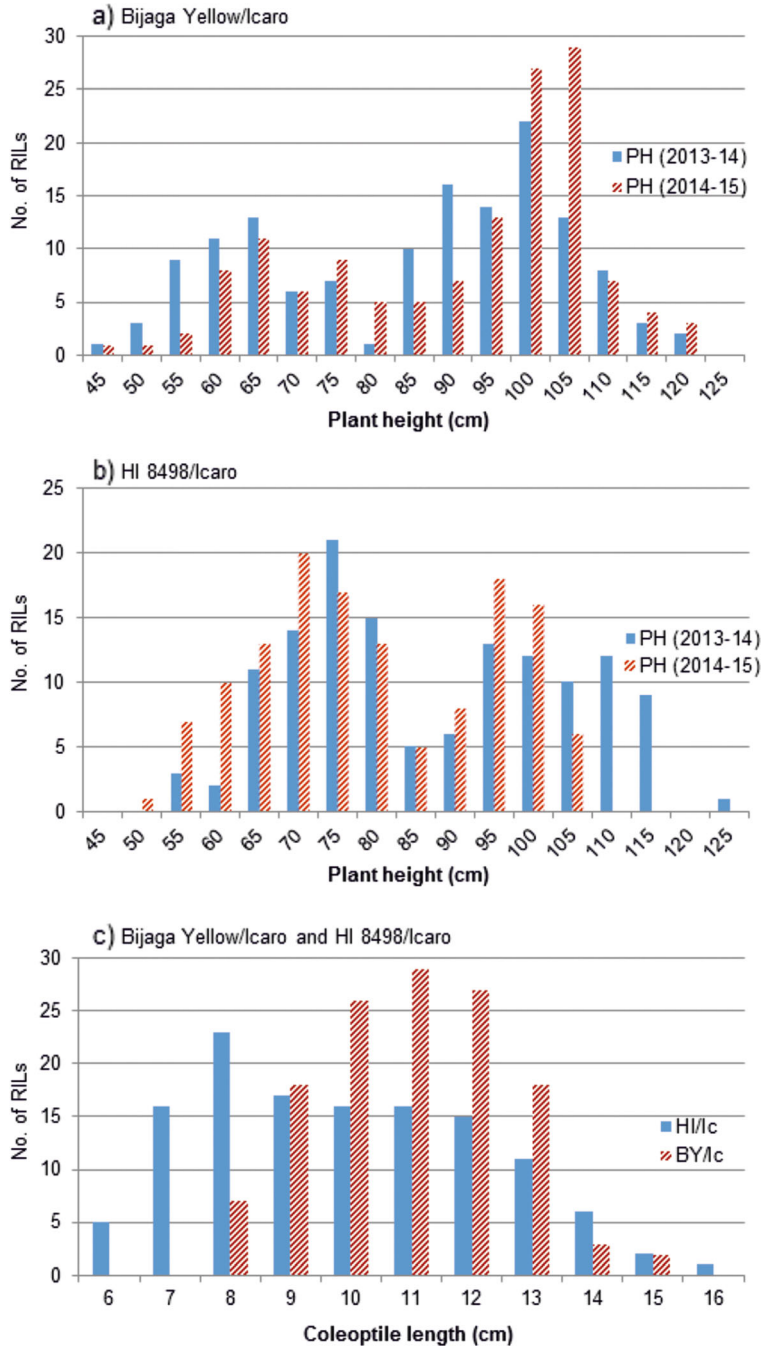

Fig. 1 Frequency distribution of plant height (**a**, **b**) and coleoptile length (**c**) in Bijaga Yellow/Icaro and HI 8498/Icaro RIL populations

could differentiate Icaro from rest of the wheat accessions except HI 8498. Similarly, *barc3* showed 217 bp allele which was also unique to Icaro. *Rht18* associated alleles of *TdGA2ox-A9*, *IAW4371* and *IAW7940* were absent in most of the tall Indian local durum and bread wheat.

Discussion

Semidwarf wheats with GA-sensitive dwarfing genes are known to be useful in dry environments owing to their longer coleoptile, better emergence and seedling vigor, thus, may provide a useful alternative to widely

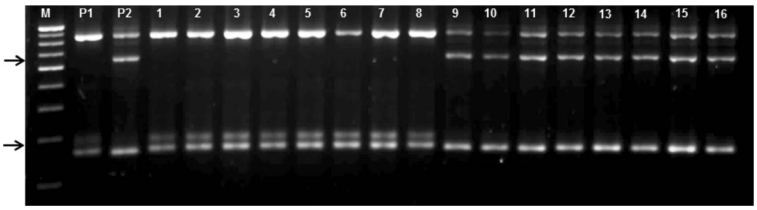

Fig. 2 *TdGA2ox-A9* specific marker showing 282 bp fragment specific to allele from Bijaga Yellow and 633 bp fragment specific to allele from Icaro (indicated by *arrows*). M-100 bp marker, P_1-Bijaga Yellow (tall), P_2-Icaro (*Rht18*), 1 to 8-tall RILs, and 9 to 16 dwarf RILs with *Rht18*

used GA-insensitive dwarfing genes under dry environments. Therefore, it is important to develop markers for GA-sensitive dwarfing genes, which would subsequently facilitate development of wheat genotypes better adapted to semiarid conditions by marker-assisted breeding. This study was undertaken to characterize GA-sensitive dwarfing gene *Rht18* at molecular level to enable its exploitation in wheat breeding program. In BY/Ic and HI/Ic RIL populations, *Rht18* was mapped within interval of 1.8 cM flanked by *barc118 and IWA4371*, moreover, *TdGA2ox-A9*, *S470865SSR4* and *barc37* showed no recombinants with *Rht18* in a population of 256 RILs (Fig. 3). Results showed that SSR and SNP markers reported in the present study are more closely linked to *Rht18* than *barc3* reported earlier (Haque et al. 2011). Therefore, *S470865SSR4*, *barc37*, *TdGA2ox-A9* and *IWA4371* assure more precise selection of *Rht18* as compared to earlier reported marker. The identified markers were tested on 89 diverse durum and bread wheat accessions to survey their allele frequency in different genetic backgrounds. It was observed that *S470865SSR4* could discriminate between Icaro and all the other accessions except HI 8498, therefore, can be used to transfer *Rht18* in diverse wheat genetic backgrounds (Supplementary Table S5). Icaro specific allele of SNP marker *IWA4371* and *TdGA2ox-A9* was absent in most of the tall Indian local durum as well as bread wheat carrying *Rht-B1b* allele, however, their frequency was high in durum cultivars. The results suggested that *S470865SSR4* can be useful to select donor allele in diverse genetic backgrounds, while *IWA4371* and *TdGA2ox-A9* specific markers will have limited use in the background of local durums and bread wheat. Although *barc3* showed an allele unique to Icaro, comparatively large linkage distance was observed between *barc3* and *Rht18* in present study as well as earlier report (Haque et al. 2011), limits its utilization in marker-assisted selection for *Rht18* due to higher chances of false positives.

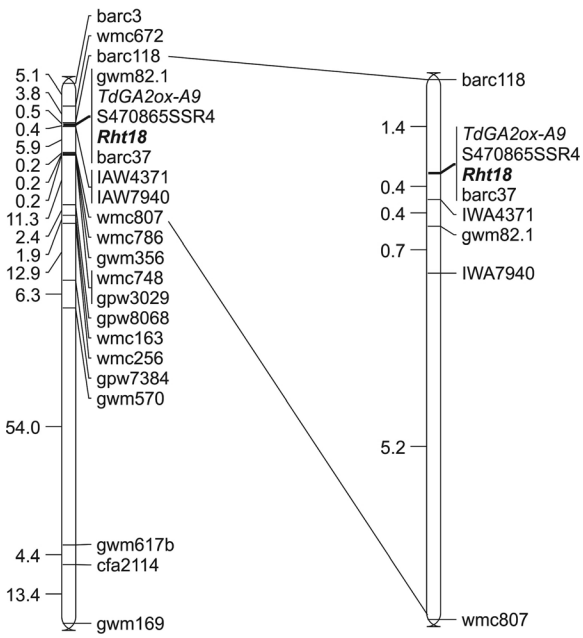

Fig. 3 Genetic linkage map of chromosome 6A showing SSR and SNP markers linked to *Rht18* in Bijaga Yellow/Icaro population. Genetic map on left side was initially derived using a subset of 139 RILs. Map on right side was derived using full set of 256 RILs to obtain further resolution

Additive effect showed that *Rht18* reduces plant height by 16.95 cm in BY/Ic population, which is in agreement with earlier results where GA-sensitive dwarfing genes were reported to reduce plant height in common wheat (Chen et al. 2013; Rebetzke and Richards 2000; Rebetzke et al. 2012). *Rht-B1b* from HI 8498 showed pleiotropic effect on plant height and coleoptile length, on the other hand, *Rht18* did not show any significant effect on coleoptile length (Table 2). Therefore, *Rht18* may help in better seedling establishment due to longer coleoptile under limited moisture conditions, thereby improving crop stand and higher grain yield than wheat carrying *Rht-B1b*. The additive effect on plant height reduction is shared by *Rht18* and

Rht-B1b in HI/Ic population, however, no significant difference for plant height was observed between dwarf RILs carrying either *Rht18* or *Rht-B1b* (Supplementary Fig. S3; Table S6). This suggests that *Rht18* has dwarfing effect comparable to that of *Rht-B1b*. Moderate height reducing effect of *Rht18* on plant stature, without any negative effect on coleoptile length and root growth in the background of Chinese winter wheat has also been reported recently (Yang et al. 2015).

Chromosomal region of *Rht18* in Icaro coincides to the QTL for plant height, coleoptile length, and leaf width identified earlier in bread wheat cross between Chuan-Mai18 and Vigour18 (Spielmeyer et al. 2007). A consistent QTL for plant height was identified near centromere on chromosome 6A in meta-QTL study conducted using three bread wheat doubled haploid populations (Griffiths et al. 2012). In durum wheat *Rht14* and *Rht16* were also reported to be allelic to *Rht18* and mapped near *barc3* on chromosome 6A (Haque et al. 2011). All these reports in bread and durum wheat suggest the presence of a major height reducing gene on chromosome 6A.

TdGA2ox-A9 specific marker showed perfect association with variation in plant height due to *Rht18* in BY/Ic population, however, the marker showed high frequency of donor allele in durum cultivars and could not be considered as diagnostic marker. Nevertheless, the marker could be used to map *TdGA2ox-A9* on chromosome 6A and its map position coincides with *Rht18* locus as demonstrated in present study. Overexpression of *OsGA2ox9* and *PvGA2ox9b* was shown to be associated with reduction in plant height along with increase in tiller number and root length in rice and switch grass (Lo et al. 2008; Wuddineh et al. 2015). Similar results were also reported in wheat, wherein overexpression of bean *GA2ox* yielded gibberellin-deficient dwarf and semi-dwarf phenotype in wheat transformants (Hedden and Phillips 2000). However, linkage between *Rht18* and *TdGA2ox-A9* identified in present study is indirect evidence, therefore, needs to be studied further on a comparatively larger mapping population for fine mapping or by comparing Icaro with its wild-type parent Anhinga for expression of the gene and its effect on levels of active gibberellins in developing stem.

Table 2 Effect of *Rht18* and *Rht-B1b* on plant height and coleoptile length in Bijaga Yellow/Icaro and HI 8498/Icaro RIL populations

Gene	Trait	R^2%	P value	Additive effect
Bijaga Yellow/Icaro				
Rht18	Plant height	85.5	0.00	16.95
Rht18	Coleoptile length	0	ns	–
HI 8498/Icaro				
Rht18	Plant height	28.4	0.00	9.00
Rht18	Coleoptile length	0	ns	–
Rht-B1	Plant height	32.2	0.00	9.65
Rht-B1	Coleoptile length	36.6	0.00	1.45

Conclusion

Effect of *Rht18* on plant height was shown in two durum wheat recombinant inbred lines populations. *Rht-B1b* from HI 8498 showed pleiotropic effect on plant height and coleoptile length, on the other hand, *Rht18* did not show effect on coleoptile length. *Rht18* was mapped on chromosome 6A in a population of 256 RILs within interval of 1.8 cM. The gene showed co-segregation with newly developed co-dominant SSR marker *S470865SSR4* with very rare Icaro-type allele of 204 bp, hence could be very useful for selection of *Rht18* in diverse breeding material including bread and durum cultivars carrying *Rht-B1b* as well as tall local durum. Closely linked SNP markers were also identified that are amenable to high-throughput systems in breeding programs. These SNP markers are more useful for selection of *Rht18* in the background of bread wheat and local durum.

Acknowledgements Authors wish to thank Dr. M. D. Bhagwat for initiating development of mapping populations and useful suggestions. Authors are grateful to Dr. Andrew Phillips, Rothamsted Research, Harpenden, UK, for providing scaffolds containing sequences of three homoeologues of *TaGA2ox9*. Authors wish to thank three anonymous reviewers for constructive suggestions on the manuscript. Financial support by the Science and Engineering Research Board, Department of Science and Technology, New Delhi under Start-Up Research grants for Young Scientists-SB/FT/LS-243/2012 to RP is gratefully acknowledged. The Junior Research Fellowship by Agharkar Research Institute to PV under project GEN15 is also gratefully acknowledged.

Compliance with ethical standards

Conflict of interest The research was partially funded by the Science and Engineering Research Board, Department of Science and Technology, New Delhi (SB/FT/LS-243/2012) and the Agharkar Research Institute. The authors declare that they have no conflict of interest.

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