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ORIGINAL PAPER

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Characterization of a dwarf gene in *Brassica rapa*, including the identification of a candidate gene

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Abstract Dwarf genes have been valuable for improving harvestable yield of several crop plants and may be useful in oilseed *Brassica*. We evaluated a dwarf gene, *dwf2*, from *Brassica rapa* in order to determine its phenotypic effects and genetic characteristics. The *dwf2* mutant was insensitive to exogenous GA_3 for both plant height and flowering time, suggesting that it is not a mutation in the gibberellin biosynthesis pathway. The dwarf phenotype was controlled by a semidominant allele at a single locus. Near-isogenic lines that were homozygous or heterozygous for *dwf2* had 47.4% or 30.0% reduction in plant height, respectively, compared to the tall wild-type line, and the reduction was due to reduced internode length and number of nodes. The *dwf2* homozygous and heterozygous lines had the same or significantly higher numbers of primary branches than the wild-type line, but did not differ in flowering time. The *DWF2* gene was mapped to the bottom of linkage group R6, in a region having homology to the top of *Arabidopsis thaliana* chromosome 2. The map position of *DWF2* in comparison to markers in *A. thaliana* suggests it is a homolog of *RGA* (*repressor of ga1-3*), which is a homolog of the wheat "Green Revolution" gene. This dwarf gene could be used to gain more insight on the gibberellin pathway and to reduce lodging problems in hybrid oilseed *Brassica* cultivars.

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Introduction

Dwarf genes have been utilized extensively in plant breeding to improve lodging resistance. Their use has been associated with increased yields, higher fertility, early maturity, and high tillering capacity, and the introduction of dwarf cultivars was a major factor in the success of the "Green Revolution" in wheat and rice (Hedden 2003; Khush 2001). Dwarf genes also may be useful for improving oilseed *Brassica napus* because many current cultivars are prone to lodging, which can lead to yield loss and difficulty harvesting.

The important dwarf genes that have been used in agriculture are mutations of genes in the gibberellin biosynthesis or response pathways. Gibberellins (GAs) are essential endogenous regulators of plant growth and development that affect many aspects of a plant life cycle, including seed germination, leaf expansion, stem elongation, flower initiation, and flower and fruit development (Harberd et al. 1998). Mutations of genes in the biosynthesis pathway cause GA deficiency and dwarf phenotypes, and exogenous GA application can restore wild-type phenotype in these mutants (Phillips 1998). The predominant dwarf gene in rice cultivars, *semidwarf1* (*sd1*), which was an important component of the Green Revolution, encodes a mutant form of the biosynthetic enzyme GA 20-oxidase (Monna et al. 2002; Sasaki et al. 2002; Spielmeyer et al. 2002).

Dwarf mutants in the GA response pathway display a similar phenotype to the GA biosynthesis mutants, although they fail to respond to exogenous GA treatment (Sun 2000). Recent studies have shown that GA response is likely to be repressive, and GA seems to activate the response pathway by de-repression to allow GA-stimulated growth and development (Olszewski et al. 2002; Sun 2000). The dwarf genes used in the Green Revolution of wheat, *Rht-B1b*, and *Rht-D1b* are mutations in the GA

response pathway, and most modern commercial wheat cultivars contain one of these *Rht* mutant alleles (Silverstone and Sun 2000).

Arabidopsis thaliana has been an important model for understanding the function and components of GA pathways. Information obtained from studying *A. thaliana* provided the means to identify and clone the Green Revolution dwarf genes of wheat and rice (Peng et al. 1999; Sasaki et al. 2002; Spielmeyer et al. 2002). The wheat *Rht-B1* and *Rht-D1* genes and maize *d8* gene are, in fact, orthologs of *GAI* and *RGA* in *Arabidopsis* (Peng et al. 1999; Sun 2000). Recently, the *GAI*/*RGA* orthologs in rice, barley, and grapevine also have been isolated (Boss and Thomas 2002; Chandler et al. 2002; Ikeda et al. 2001; Ogawa et al. 2000). Transgenic rice plants containing a mutant *GAI* allele are dwarf, indicating that mutant *GAI* orthologs could be used to induce dwarf phenotypes in a wide range of crop species (Peng et al. 1999).

Arabidopsis thaliana is a close relative of *Brassica* species, and comparative genome analysis between *A. thaliana* and *Brassica* species can be used to transfer information and resources from the widely studied model organism to this important group of crop plants. Coding regions in *A. thaliana* and *Brassica* are highly conserved (85% average sequence identity, Cavell et al. 1998). Gene order is also highly conserved between these species for large segments of chromosomes (Parkin et al. 2002; Schmidt et al. 2001), allowing the possibility to identify candidates for agronomically important genes in *Brassica* species.

In this paper, we report on the characterization of *dwf2*, a potentially useful dwarf gene identified in *B. rapa*. We determine the effects of exogenous GA on plant height and flowering time of the mutant, genetic control of the dwarf phenotype, and the effects of the dwarf gene on plant height, branching, and flowering time in field experiments. We also identify a candidate gene for *dwf2* using comparative mapping with *A. thaliana*.

Materials and methods

Plant materials

Rapid cycling *B. rapa* stocks CrGC1-21 (dwarf, genotype *dwf2*/*dwf2*; Zanewich et al. 1991) and CrGC1-33 (wild type, +/+) were obtained from Paul Williams through the Crucifer Genetics Cooperative. CrGC1-21 was crossed with R500, a non-dwarf, yellow sarson cultivar of *B. rapa*. The resulting F_1 plants were self-pollinated to create F_2 seeds and backcrossed to R500 for two generations to generate BC_2 seeds. The *dwf2* allele was backcrossed for three additional generations to R500 to produce BC_5 seeds and a BC_5 *dwf2*/+ plant was self-pollinated to produce BC_5S_1 seeds. BC_5S_1 plants were selected, self-pollinated, and two +/+ and three *dwf2*/*dwf2* BC_5S_2 lines were selected and bulked within genotypes. BC_5S_2 +/*dwf2* seeds were obtained by crossing bud emasculated BC_5S_2 *dwf2*/*dwf2* plants with pollen from the BC_5S_2 +/+ plants.

Gibberellin treatment

CrGC1-21 (*dwf2*/*dwf2*) and CrGC1-33 (+/+) seeds were planted in a Com Pack plastic flat (TO Plastics, Minneapolis, Minn.), containing 4×6×5.5-cm receptacles filled with Readi-Earth (Scotts-Sierra Horticultural Products Company, Marysville, Ohio). The flats were kept in a growth chamber under short-day photoperiod (9 h) (Fridborg et al. 1999) at 22±2°C with light intensity of 350±50 $\mu molm^{-2}s^{-1}$ as measured with a LI-COR (model LI-189) light meter. The flats were watered daily to soil saturation and fertilized weekly with Peter's 20-20-20 (Scotts-Sierra Horticultural Products). Beginning 1 week after planting, 0, 0.001, 0.1, 10 or 100 μg GA_3 (Sigma) in 5 μl of 50:50 (v/v) ethanol:water was applied to the shoot tip of 8–10 seedlings for each treatment once a week until flowering. Plant heights were measured from the cotyledon to the first flower on the day the first flower opened. Flowering time was recorded as the number of days from planting to the day that the first flower opened for each plant. Analysis of variance was performed using generalized linear models in SAS (SAS 2001).

Genetic segregation analysis

F_2 and BC_2 plants segregating for *dwf2* were grown in a greenhouse at 22–24°C under a long day photoperiod (16 h) with supplemental lighting produced by sodium and metal halogen lights. Plant height from the cotyledon to first open flower was measured on the day the first flower opened.

Field trial and trait measurements

Experiment I was conducted at the Arlington Agriculture Research Station (Columbia County, Wis.) on a non-irrigated field. Seeds were planted in early May 2002 in plots consisting of five rows 2 m long with 0.24 m between rows. The seeding rate was 27 seeds/m of row. The treatment plots were interspaced with *B. napus* cv. Hyola 402 to produce a homogeneous border effect. The experimental design was a completely randomized design with six replications for the BC_5S_2 +/+ and BC_5S_2 *dwf2*/*dwf2* genotypes, and three replications for the BC_5S_2 +/*dwf2* genotype. For each plot, the dates when 10% of the plants had at least one open flower and when 90% of the plants had finished flowering were recorded. At maturity, plant height in each plot was recorded by straightening a few plants randomly chosen from the middle of the plot, and then a single measurement was taken from the ground to the highest point. For node and branching measurements, three individual plants were randomly selected from each plot, and the number of nodes, number of primary branches, height of last branch, height of first branch, and internode length were recorded. Number of nodes and primary branches were counted for all the nodes and branches of the main stem. Height of last branch was measured to the highest primary branch. Height of first branch was measured to the lowest primary branch. All plant heights were measured from the ground. Internode length was calculated by dividing the last branch height by number of nodes.

Experiment II was conducted in Madison, Dane County, Wis. in cold frames. Each seed was planted in a plastic pot (20-cm diameter) in the beginning of May 2002. The experiment design was a completely randomized design with five replications for all genotypes and pots were spaced sufficiently far apart to avoid any interplant competition. For each plant, the date that the first flower opened was recorded as flowering time. All other traits were measure as in experiment I, unless specified.

Data from both experiments were analyzed using the generalized linear models in SAS (SAS 2001). For node, internode length, and branching measurements in experiment I, data were analyzed as a completely randomized design with subsamples. All other measurements were analyzed as a completely randomized design.

Comparative mapping with A. thaliana

To identify molecular markers linked to the dwarf gene, bulked segregant analysis (Michelmore et al. 1991) was performed using 18 plants of each phenotypic class (short, tall, or intermediate) from a BC_1S_1 population and 92 *Brassica* RFLP probes. These RFLP probes were previously used to construct an RFLP genetic map for *B. rapa* (Kole et al. 1997). RFLP analysis was carried out as described previously (Teutonico and Osborn 1994). *Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I restriction endonucleases were used for DNA digestion. After hybridization, membranes were washed twice in 2× SSC for 5 min each at room temperature, 2× SSC, 1%SDS, and 0.2× SSC for 20 min, each at 60°C.

Sequences of the *Brassica* RFLP probes (Lukens et al. 2003) that showed polymorphism among the bulked DNA samples were used to identify putatively homologous sequences within the *A. thaliana* genome. *Arabidopsis thaliana* sequences putatively homologous to the *Brassica* species query sequences were identified using BLASTN searches against the *A. thaliana* genome database [The Arabidopsis Information Resource (TAIR), http://www.arabidopsis.org, accessed July 2001].

Arabidopsis thaliana genes surrounding the sequences homologous to *Brassica* RFLP probes were selected from the *A. thaliana* physical map obtained from TAIR using SeqViewer and MATDB database (http://mips.gsf.de/proj/thal/db/index.html, as accessed during 2001–2002). *A. thaliana* gene-specific primers were identified using the Primer 3 program in Biology WorkBench (http://biowb.sdsc.edu/CGI/BW.cgi). The primers were used in PCRs to amplify *A. thaliana* DNA, and amplification products were gel-purified. These products were used as probes on Southern blots containing the bulked DNA samples, and probes that showed polymorphism among the samples were used for fine mapping the *DWF2* locus by screening 410 BC_5 individuals for plant height and marker segregation. A linkage map was constructed using the software package JoinMap (Stam 1993) with a maximum recombination fraction of 0.4 and a LOD threshold of 10 for linkage.

Results

GA response and genetic analysis

CrGC1-21 (*dwf2*/*dwf2*) showed no significant response ($P>0.05$) at all tested levels of GA_3 for both plant height (Fig. 1a) and flowering time (Fig. 1b). CrGC1-33 (+/+) showed a significant response for plant height at high levels of GA_3 (Fig. 1a) but showed no significant response at all tested levels of GA_3 for flowering time (Fig. 1b).

The F_2 population did not segregate into discrete height classes, although the skewed distribution suggested that the *dwf2* allele had some degree of dominance (Fig. 2a). After backcrossing the *dwf2* allele into R500 for two generations, a BC_2 population segregated into discrete height classes of 36 short and 34 tall (Fig. 2b), suggesting that a single locus controlled the dwarf phenotype.

Effect of the *dwf2* gene in the field experiments

Plant heights of the BC_5S_2 *dwf2*/*dwf2* and BC_5S_2 +/*dwf2* were significantly reduced, compared to the BC_5S_2 +/+ line (Table 1). The BC_5S_2 *dwf2*/*dwf2* line was 52.5% and 52.8% the height of the BC_5S_2 +/+ line grown in field plots and in cold frames, respectively. Plant height of the BC_5S_2 +/*dwf2* line was intermediate between the two parental lines and slightly closer to the *dwf2*/*dwf2* parent in both experiments. The reduced height of BC_5S_2 *dwf2*/*dwf2* and BC_5S_2 +/*dwf2* plants was primarily due to shorter internodes, although they also had slightly fewer nodes than BC_5S_2 +/+ plants (Table 1, experiment I).

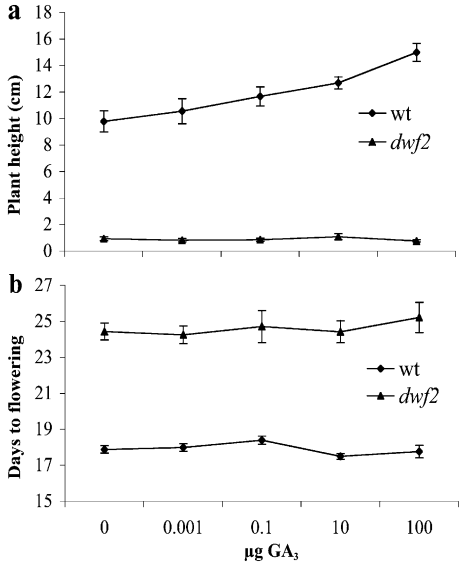

Fig. 1 Effects of gibberellin (GA) on plant height (**a**) and flowering time (**b**) of *Brassica rapa* CrGC1-33 (*wt*) and CrGC1-21 (*dwf2*). Plants were grown under short days in a growth chamber at 22±2°C with light intensity of 350±50μmolm^{-2}s^{-1}. Zero to 100 μg GA_3 in 5 μl of 50:50 (v/v) ethanol:water were applied once a week after planting. On the day the first flower opened, plant heights were measured in centimeters from the cotyledon to first open flower, and days-to-flowering were recorded (mean±SE, n=10±2)

There was no significant difference in the number of primary branches among these three genotypes in experiment I, but in experiment II, the BC_5S_2 *dwf2*/*dwf2* and the BC_5S_2 +/*dwf2* lines had a significantly higher number of primary branches than the BC_5S_2 +/+ line (Table 1). The BC_5S_2 *dwf2*/*dwf2* and the BC_5S_2 +/*dwf2* plants had their first and last branches at significantly lower positions on the main stems than did the BC_5S_2 +/+ line. The branching patterns of BC_5S_2 +/*dwf2* plants were closer to the *dwf2*/*dwf2* plants than the +/+ plants (Table 1).

There was no significant difference among the three genotypes in time-to-10% flowering (experiment I) or in days-to-flowering (experiment II), but the BC_5S_2 *dwf2*/*dwf2* and the BC_5S_2 +/*dwf2* lines were about 1 day later for 90% of the plants finished flowering (Table 1).

Comparative mapping with *A. thaliana*

Three out of the 92 *Brassica* RFLP probes that were screened showed polymorphism among the bulked-DNA samples. Results from the BLASTN search against the *A. thaliana* database showed that two of these *Brassica*

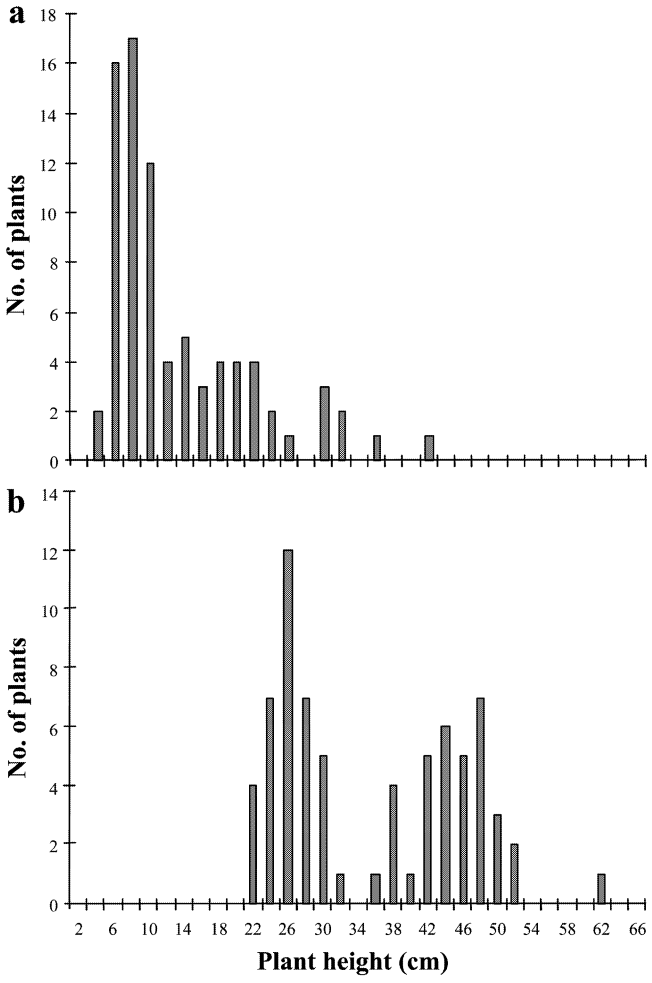

Fig. 2 Plant height distribution of F_2 (**a**) and BC_2 (**b**) generations derived from *Brassica rapa* CrGC1-21 (*dwf2*) × R500 (*wt*) and backcrossed to R500. On the day the first flower opened, plant height was measured in centimeters from the cotyledon to the first open flower

probes (pW257=WG9E9 and pX110=EC2C12) had the highest homology to sequences in bacterial artificial chromosome clone T23K3 (score of 200, E-value of 4E-050) and part of gene *At2g02250* (score of 98, E-value of 1E-019), respectively. These two sequences are at 352 kb and 594 kb at the top of *A. thaliana* chromosome 2 (At 2). The third *Brassica* probe (pX104=EC2B3) showed the highest homology to part of *At5g66910* (score of 242, E-value of 2E-063), which is at 26.3 mb in the bottom of *A. thaliana* chromosome 5 (At 5).

A total of 68 *A. thaliana* genes were selected from these two regions to screen for polymorphism among the bulked-DNA samples from the BC_1S_1 segregating population. None of the five *A. thaliana* genes selected from the bottom of At 5 showed polymorphisms among the bulked-DNA samples; however, 12 genes from At 2 detected polymorphism. These loci, along with those detected by the three *Brassica* probes, and the phenotypic markers *dwf2* and *Pub1*, which controls pubescence (Kole et al. 2002), were mapped in a population of 410 BC_5 individuals. All loci mapped to the end of *B. rapa* linkage group 6 (R6, previously labeled "Br4" by Kole et al. 2002). Thirteen of the RFLP loci showed high collinearity with a 784-kb region of At 2; only two loci (*At2g02580* and *pX110*) were not in collinear positions, although they still occurred in this chromosomal region (Fig. 3). *DWF2* was mapped at the end of R6, 0.5 cM from the *At2g01810* locus. In *A. thaliana*, this gene is 93 kb below *RGA*, a homolog of the Green Revolution dwarf gene from wheat. *RGA* and nine *A. thaliana* genes above *At2g01810* were tested for polymorphism using the bulked-DNA samples, but none showed polymorphism.

Discussion

Gibberellin dwarf mutants can be classified into two main categories: mutants that are defective in the GA biosynthesis pathway and mutants that are defective in the GA response pathway. The growth response of dwarf mutants

Table 1 Effects of *dwf2* on plant height, nodes, branching, and flowering time (LS means from ANOVA) in field experiments I and II

Genotypes[a]	Total height (cm)	Internode length (cm)	No. of nodes	No. of primary branches	Height of first branch	Height of last branch	10% flowering (days)	90% finished flowering (days)	Days-to-flowering
Experiment I[b]									
BC_5S_2 (+/+)	91.2	4.5	11.5	6.8	18.2	51.4	42	55	nd
BC_5S_2 (+/*dwf2*)	63.3	3.5	8.4	7.6	1.7	29.7	42	56	nd
BC_5S_2 (*dwf2*/*dwf2*)	47.8	2.9	8.3	7.7	1.2	23.9	42.3	55.8	nd
$LSD_{0.05}$	5.6	0.5	1.1	ns	6.6	5.9	ns	0.4	–
Experiment II[b]									
BC_5S_2 (+/+)	82.7	nd	nd	7	nd	37.6	nd	nd	43.4
BC_5S_2 (+/*dwf2*)	58.3	nd	nd	9.2	nd	20.6	nd	nd	43.8
BC_5S_2 (*dwf2*/*dwf2*)	43.7	nd	nd	8.8	nd	13.3	nd	nd	43.8
$LSD_{0.05}$	8.6	–	–	1.4	–	2.4	–	–	ns

[a] Nearisogenic BC_5S_2 lines were derived as described in Materials and methods: homozygous for wild type (+/+), or dwarf alleles (*dwf2*/*dwf2*), or heterozygous (+/*dwf2*)
[b] Values are plot averages in experiment I and individual plant values in experiment II. *nd* Not determined, *ns* not significant

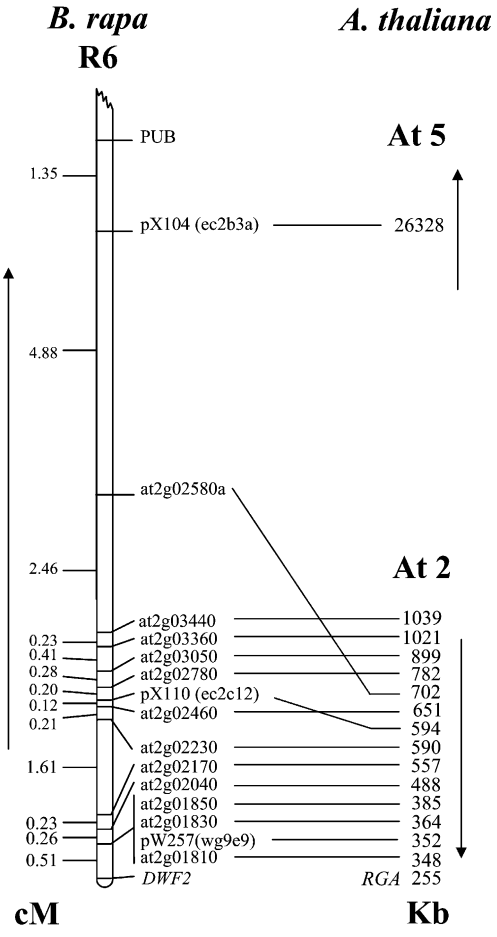

Fig. 3 Comparison of the physical map for segments of *Arabidopsis thaliana* chromosome 2 (At 2) and chromosome 5 (At 5), and the genetic map for a segment of *Brassica rapa* linkage group 6 (R6) containing *DWF2*. *Horizontal lines* showed the same markers in *B. rapa* and *A. thaliana* chromosome segments. *Arrows* indicate the direction to the top of chromosomes. Physical distances are shown in kilo bases for *A. thaliana* genes as estimated from SeqViewer in the *Arabidopsis* genome database. Genetic distances are shown in centiMorgans for *B. rapa* as estimated from analysis of 410 BC_5 plants segregating for *DWF2*

after exogenous GA application is an important first screen because it allows the potential mutant pathway to be identified. The *dwf2* mutant we analyzed was insensitive to exogenous GA application in both plant height and flowering time (Fig. 1). The effect of applied GA on plant height is in agreement with a previous study (Zanewich et al. 1991); however, the previous study did not report effects of applied GA on flowering time.

Genetic segregation analysis indicated that the *dwf2* phenotype is controlled by an allele with some degree of dominance at a single locus, and the analyses of BC_5S_2 genotypes confirmed that the *dwf2* allele was semidominant. In *A. thaliana*, only *gai* (Peng et al. 1997) and *shi* (Fridborg et al. 1999) have been reported to be GA-insensitive dwarfs that are controlled by semidominant alleles. The *dwf2* mutant resembles the *gai* mutant of *A. thaliana* in that both mutants are insensitive to GA for plant height (Koornneef et al. 1985) and flowering time (Fridborg et al. 1999), and the dwarf phenotypes are caused by semidominant alleles. The *shi* mutant, unlike *dwf2*, responds to exogenous GA_3 by flowering earlier (Fridborg et al. 1999). In wheat, the Green Revolution dwarfs *Rht-B1b* and *Rht-D1b* are also semidominant (Peng et al. 1999) and GA insensitive (Ellis et al. 2002). These dwarf mutants are defective in the GA response pathway (Fridborg et al. 1999; Peng et al. 1999). Interestingly, wheat *Rht-B1* and *Rht-D1* genes are homologs of *GAI* and *RGA* in *Arabidopsis* (Peng et al. 1999; Sun 2000). Since the *dwf2* mutant was not rescued by GA treatment, it is most likely defective in the GA response pathway. Alternatively, it may contain a mutation in a target gene of the GA response pathway.

Mutants defective in the brassinosteriod (BR) pathway show dwarf phenotypes similar to those of GA-deficiency mutants. Gibberellin mutants such as *ga1* to *ga5*, *gai* and *shi* also respond to BR application. However, when germinated in the dark, these GA mutants respond to darkness in a similar manner to that of wild type, while *A. thaliana* BR mutants showed de-etiolated growth habit with short hypocotyls, open cotyledon and no hook (Fridborg et al. 1999). The *dwf2* mutant was germinated in darkness and showed etiolated growth similar to that of the wild type (data not shown), suggesting that it is not defective in the BR pathway.

The BC_5S_2 *dwf2*/*dwf2* and the BC_5S_2 +/*dwf2* lines showed significant height reduction compared to the near-isogenic BC_5S_2 +/+ line (Table 1). This height reduction was due to decreases in internode length and number of nodes. A recent study in oats also showed that dwarf genes cause height reduction due to the reduction in internode length and/or node number (Milach et al. 2002). Since the heights of BC_5S_2 *dwf2*/*dwf2* and the BC_5S_2 +/*dwf2* plants were greater than 50% that of the BC_5S_2 +/+ plants, they were classified as semidwarf (Hedden 2003). The Green Revolution dwarfs in wheat and rice are classified as semidwarfs, and they have been associated with increased seed yields through partitioning a greater proportion of assimilate into the grain and by reducing yield losses due to lodging (Hedden 2003). In addition, the BC_5S_2 *dwf2*/*dwf2* and the BC_5S_2 +/*dwf2* lines produce branches lower on the plants than the near-isogenic BC_5S_2 +/+ line (Table 1). This branching structure may help to decrease lodging due to more even weight distribution throughout the length of the plant stem. Moreover, the three near-isogenic lines did not differ in flowering time, suggesting that this dwarf gene did not delay flowering under these test conditions. Thus, the *dwf2* mutant gene has potential to be a useful allele for height reduction in crop plants, which could lead to yield improvement due to lodging resistance.

The results from comparative mapping showed that the *B. rapa* and *A. thaliana* genomes had high collinearity for a 784-kb segment of At 2 (Fig. 3). Other studies have reported high degrees of collinearity between regions of *A. thaliana* and *Brassica* genomes (Parkin et al. 2002; Ryder et al. 2001; Schranz et al. 2002). Kole et al. (2002)

detected collinearity between the bottom of Br4 (equivalent to R6) and At 5, including pX104 (=EC2B3) and *Pub1* used in this study, but they did not test markers below pX104. We mapped markers below pX104 that showed high collinearity with the top of At 2, but we could not map markers below the *DWF2* locus. It is possible that the collinearity with the At 2 terminates below *DWF2*, or that the R6 chromosome terminates just below the *DWF2* locus. Alternatively, the At 2 collinearity may continue, but polymorphism could not be detected for the tested probes.

Several lines of evidence suggest that *DWF2* is homologous to *A. thaliana RGA (repressor of ga1-3)*. First, *DWF2* was mapped below *At2g01810*, which is the approximate position of *RGA* in the *A. thaliana* genome (Fig. 3). Second, *RGA* is homologous to *A. thaliana GAI* (Peng et al. 1999; Silverstone et al. 1998), and *gai* mutants and mutants of *RGA*/*GAI* orthologs from wheat (*Rht-B1b*, *Rht-D1b*), maize (*D8*), and grapevine (*Vvgai*) are dwarf (Boss and Thomas 2002). Although there are no reports of dwarfing due to natural or mutagenized alleles of the *RGA* locus, transgenic plants expressing an *RGA* gene having an identical mutation as in *gai* (*rga-Δ17*) are dwarf (Dill et al. 2001). Third, the *dwf2* mutant is GA insensitive, similar to *rga-Δ17*, *gai*, *Rht-B1b*, *Rht-D1b, D8*, and *Vvgai* (Boss and Thomas 2002; Dill et al. 2001; Peng et al. 1999). Lastly, results from the inheritance studies and field experiments demonstrated that *dwf2* is semidominant to the *wt* allele, similar to dwarf mutations in the *gai*, *Rht-B1b*, *Rht-D1b, D8*, and *Vvgai* loci.

Recent studies have reported that RGA/GAI proteins and their orthologs in wheat (Rht), maize (d8), grapevine (VvGAI), barley (SLN, Fu et al. 2002), and rice (SLR, Ikeda et al. 2001) function as GA-derepressible repressors of plant growth, indicating that RGA/GAI function is conserved among dicots and monocots (Olszewski et al. 2002; Peng et al. 1999). The *RGA* and *GAI* genes share a high degree of sequence similarity (82% amino acid identity, Dill and Sun 2001). Therefore, it seems likely that they have overlapping functions. However, RGA and GAI are not completely redundant. Although RGA and GAI interact synergistically to repress plant growth, RGA has a stronger effect than GAI (Dill and Sun 2001; King et al. 2001). In *A. thaliana*, these two genes are major repressors of stem growth but not of seed germination or flower development (Dill and Sun 2001; King et al. 2001). Based on our results, *dwf2* also showed a strong effect on the reduction of plant height (Table 1), but no effect on seed germination or flower development (data not shown).

Brassica napus is the major *Brassica* oilseed crop grown worldwide in temperate climates. Dwarf genes from *B. oleracea* and *B. rapa* can be utilized in *B. napus* because these diploid species are the hypothesized progenitors of the amphidiploid *B. napus*, and they can be hybridized to resynthesize a cross-fertile *B. napus*. It will be important to evaluate the effects of *dwf2* in cultivated *B. napus* to determine its potential use in agriculture. *dwf2*, or other *Brassica* dwarf mutants, could be used to improve hybrid oilseed *B. napus,* which is often very tall, making it more susceptible to lodging and more difficult to harvest mechanically. The *dwf2* gene is especially promising for use in hybrid cultivars because it is controlled by a semidominant allele at single locus. This would allow for phenotypic selection in backcross progeny, and only one parent of a hybrid may need to contain the dwarf gene to produce a desirable dwarf hybrid. This is especially true if homozygous dwarf plants are too small to produce a high yield, and the dwarf allele behaves as a semidominant in *B. napus*.

The present study demonstrates the application of the completed sequence of *A. thaliana* as a tool for marker development and candidate gene identification. The work on cloning the *DWF2* gene and its use in oilseed *B. napus* breeding is under way. This dwarf gene could enhance our understanding of the GA pathway, and could improve lodging resistance in hybrid oilseed *Brassica* cultivars.

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