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Using a wheat-rye amphihaploid population to map a rye gene responsible for dwarfness

Sen Yang · Hongjing Zhu · Jianjun Yu · YuYang Zhong · LaiBin Zhao · YunFeng Jiang · Ming Hao · Lianquan Zhang · Shunzong Ning · XueJiao Chen · Dengcai Liu · Zhongwei Yuan

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Abstract Gene identification in cross-pollinating plants such as rye can be arduous and time consuming because of the difficulties involved with genetic population construction. Here, we provide an alternative approach for the construction of mapping populations to rapidly map genes in cross-pollinated cereal rye. The aim of the present experiments was to genetically analyze the dwarf stature expressed by a germplasm accession of rye. The dwarf phenotype was reversible when the seedlings were exposed to gibberellic acid; the reductions in plant height occurred via reductions in cell size. A mapping population was constructed by generating a set of wheat-rye amphihaploids bred from a single rye plant heterozygous for the dwarfing gene(s). The dwarfness phenotype was expressed in the amphihaploid background, and segregation in the mapping population was consistent with the presence of a single gene. Using rye SSR markers, the gene responsible was located on chromosome arm 1RL, which is also the location of the known rye dwarfing gene *Ddw3*. This gene is valuable for dwarf breeding of wheat as well as rye.

Keywords Plant height · Dwarf gene · Wheat-rye amphihaploid · Gene mapping

S. Yang · H. Zhu · J. Yu · Y. Zhong · L. Zhao · Y. Jiang · M. Hao · L. Zhang · S. Ning · X. Chen · D. Liu (✉) · Z. Yuan (✉)
Triticeae Research Institute, Sichuan Agricultural University, 211 Huimin Road, Wenjiang, Chengdu 611130, Sichuan, People's Republic of China
e-mail: dcliu7@yahoo.com

Z. Yuan
e-mail: yzwyvon2004@hotmail.com

Introduction

Rye (*Secale cereale* L., 2n = 2x = 14, RR) is a cross-pollinated crop species. Rye is used as a source of food, feed and forage as well as a raw material for ethanol and vodka (Weipert 1997; Geiger and Miedaner 2009). The human-made crop species triticale (× *Triticosecale* Wittmack), derived crosses with wheat, constitutes an important forage and energy crop because of its high biomass and grain yield (Oettler 2005; Davis-Knight and Weightman 2008). Rye has also played a significant role as a source of alien genes for bread wheat improvement. Many important traits such as disease resistance and environmental adaptability have been transferred into wheat via wheat-rye translocation or substitution lines (Friebe et al. 1996). A particularly important example of this transfer is the 1RS.1BL translocation, which is present in many wheat cultivars (Zeller et al. 1973; Lelley et al. 2004).

Identifying genes or quantitative trait loci (QTL) is a key step in crop genetic improvement. Hybrid breeding opens up a powerful opportunity for gene

(Hackauf et al. 2017a) and QTL mapping in winter rye (Miedaner et al. 2012, 2014). Gene or QTL mapping in rye has traditionally relied primarily on mapping populations, such as F_2 (Börner et al. 2000; Milczarski and Masojć 2003), backcross (BC) (Causse et al. 1994), recombination inbred line (RIL), and introgression line libraries (Falke et al. 2008, 2009), constructed from self-fertile inbred lines. Recently, the testcross population (Miedaner et al. 2012, 2014) and a $F_{2:3}$ design (Hackauf et al. 2017b) have enabled a rapid approach for QTL mapping and, thus, appear to be particularly attractive for unraveling the genetic architecture of complex inherited traits. Many valuable genes or QTLs, including those related to plant height (Melz 1989; Korzun et al. 1996; Miedaner et al. 2012; Stojałowski et al. 2015), pre-harvest sprouting (Masojć et al. 2007; Tenhola-Roininen et al. 2011), male fertility restoration (Miedaner et al. 2000; Hackauf et al. 2017b), and in vitro response (Bolibok et al. 2007), morphological traits (Börner et al. 2000; Milczarski and Masojć 2003; Miedaner et al. 2012; Myśków et al. 2014; Hackauf et al. 2017b), have been identified. However, many rye germplasms are cross-pollinating species because of the self-incompatibility and inbreeding depression feature, and the development of inbred lines and the construction of mapping populations can be arduous and time consuming, which slows the process of mapping rye genes and QTLs.

As in most cereals used for grain production, tallness is not favored by breeders because this trait diverts assimilates away from grain and increases the risk of lodging. After the successful incorporation of the semi-dwarf habit to generate Green Revolution rice and wheat varieties (Evans 1998; Khush 1999), interest in using dwarfing genes to improve both rye and the wheat-rye hybrids has increased. At least three major dwarfing genes have been characterized in rye, namely, *Ddw1*, *2* and *3*: *Ddw1* maps to a location on chromosome arm 5RL (Korzun et al. 1996), *Ddw2* to a location on chromosome 7R (Melz 1989), and *Ddw3* to a location on chromosome arm 1RL (Stojałowski et al. 2015). All three genes induce a phenotype that is reversible when those plants are exposed to gibberellic acid (GA_3) (Börner and Melz 1988; Stojałowski et al. 2015).

In this study, using a wheat-rye amphihaploid population, we established a novel strategy to map a rye dwarf gene. The population was constructed in two steps: (1) crossing between dwarf and tall rye genotypes and (2) choosing an F_1 plant as a male for crossing with common wheat. This population was effective for mapping the target dwarf gene.

Materials and methods

Plant materials

The rye germplasm accession PI613129 was obtained from the U.S. National Plant Germplasm System (www.ars-grin.gov/npgs/). After undergoing multiple rounds of phenotypic recurrent selection, this line was derived from a cross between a dwarf line of unknown parentage and a standard-height cultivar (Pfahler et al. 2001). The accession segregates for height, so the initial step was to select for non-segregating derivatives (both tall and dwarf) by enforcing selfing. A contrasting pair of non-segregating selections was then intercrossed to form an F_1 hybrid that was therefore heterozygous for the gene(s) determining height. This hybrid was then crossed as a male with the bread wheat cultivar Shinchunaga to form a population of haploid triticale plants. The procedures used for emasculation and pollination were in accordance with those described by Liu et al. (1999). For validation purposes, a second mapping population was generated by enforcing the self-pollination of the dwarf × tall PI613129 F_1 hybrid (Fig. 1).

Field experiments and trait measurements

The two mapping populations were grown in the field at the Wenjiang Triticeae Research Institute (Sichuan Province, China). There were 204 and 52 individuals within the wheat-rye amphiploid population and F_2 population, respectively. This F_2 population came from an F_1 single-plant. For the other F_2 population from 14 F_1 single-plants open pollination, 709 individuals were used in the genetic analysis. Each plant was placed 10 cm from its neighbor within a 2-m row; adjacent rows were separated from one another at a distance of 30 cm. At maturity, both plant height and internode length were measured. The former was represented by the mean height from the soil surface to the tip of the three tallest spikes (Tenhola-Roininen and Tanhuanpää 2010), while the latter involved measuring the lengths of the internodes, starting from

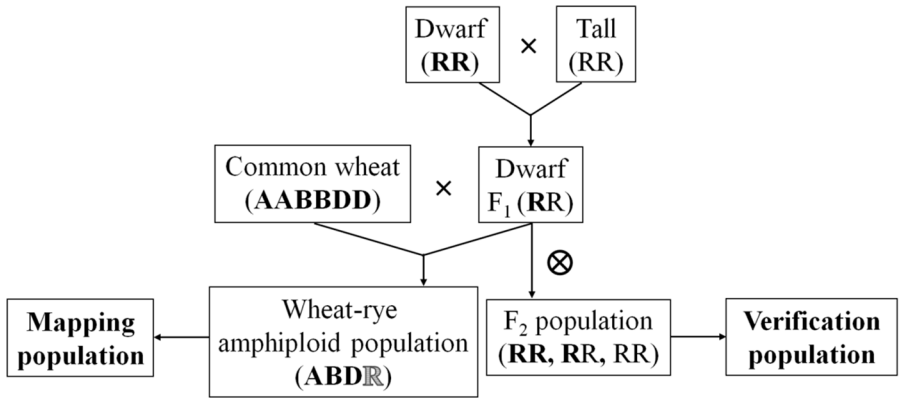

Fig. 1 The development of the mapping population

the topmost node to the sixth node below the spike. Significant differences were assessed using the non-parametric Kruskal–Wallis test, which was performed by routines implemented using the SPSS Statistics v21.0 software package (www.ibm.com). In addition, χ^2 tests were performed to test segregation ratios.

The dwarf seedling responses to exogenous GA_3

The GA_3 sensitivity of dwarf seedlings was tested in accordance with the methods described by Börner (1991). Small changes are shown below. Mature grains were germinated on moistened filter paper at 4 °C for 48 h, and grains at an equivalent stage of sprouting were then transferred to trays kept in darkness at room temperature for 13 days. Three replicate sets of 30 selected grains were fertilized with a standard nutrition solution, and an additional three sets were fertilized with the same solution supplemented with 50 μg/mL GA_3. The plant height and coleoptile length were measured in the GA_3 treatment group (90 individuals) and CK group (78 individuals), respectively.

Parenchyma cell morphology

Paraffin sections of the second internode elongation zone of both rye and haploid triticale plants (three replicates per entry) sampled at the heading stage were prepared. The sectioning procedures were in accordance with those of Sun et al. (2010). Longitudinal sections were stained with 0.5% (v/v) fast green in 95% ethanol, after which the preparations were dehydrated by immersion in absolute ethanol for 4 min. The length and width of parenchyma cells were estimated using CellSens Dimension v1.3 software (Olympus Corp., Tokyo, Japan). In parental lines, eighty-seven cells in tall rye and 107 cells in dwarf rye were measured. In hybrids (haploid triticale), 100 cells were measured in both tall and dwarf segregants.

Linkage mapping

In two mapping populations, 204 and 52 genomic DNA samples were extracted from young leaves using a Plant Genomic DNA kit (TianGen, Chengdu, China). The DNA served as a template for a series of PCRs that were based on a set of 106 rye microsatellites (Saal and Wricke 1999; Hackauf and Wehling 2002, 2003; Hackauf et al. 2009) of known chromosomal location. The PCR conditions were identical to those reported by Hackauf and Wehling (2002). Amplicons were separated by denaturing polyacrylamide electrophoresis and were visualized by silver staining (Shevchenko et al. 1996). The resulting genetic map was constructed using QTL IciMapping v4.1.2.0 software (Li et al. 2007); a logarithm of odds (LOD) threshold of 3 was applied.

Results

Dwarf stature of PI613129

The height difference was quite marked between the tall and dwarf rye of PI613129 (Fig. 2a; Table 1): the mean height of the former was 191.5 cm, while that of the latter was only 71.6 cm. The length of each of the six measured internodes also differed significantly (Fig. 2b), as did the parenchyma cell length and width

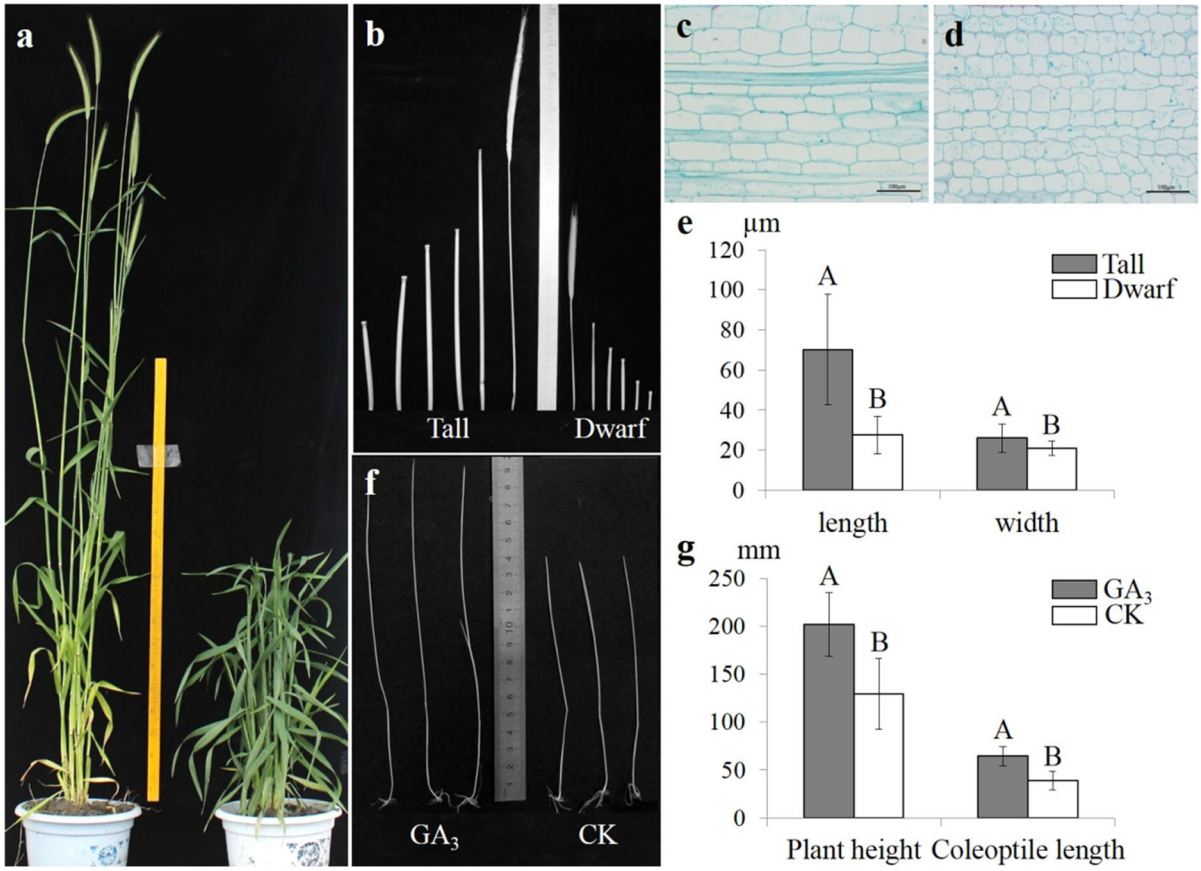

Fig. 2 Variation in plant height between tall and dwarf derivatives of rye accession PI613129. **a** Plant height (bar: 1 m); **b** length of each of the first six internodes ordered from the base of the plant to its apex (bar: 50 cm); **c**, **d** parenchyma cells of **c** tall and **d** dwarf plants (bar: 0.1 mm); **e** variation in parenchyma cell size. Columns marked with a different letter indicate that the means differed significantly ($P < 0.01$), and the whiskers represent standard deviations (SDs) (n = 3); **f**, **g** the response of dwarf rye seedlings to exogenous GA_3 treatment (CK: no GA_3): **f** images of the seedlings, **g** quantification of the treatment effect. Columns marked with a different letter indicate that the means differed significantly ($P < 0.01$), and the whiskers represent the SDs (n = 3)

Table 1 Variation in plant height and internode length between tall and dwarf segregants of rye accession PI613129

Genotype	Plant height (cm)		Internode length (cm)					
			1st	2nd	3rd	4th	5th	6th
Tall	Mean	191.46**	57.92**	48.85**	27.1**	20.6**	14.83**	7.61**
	SD	26.97	4.62	3.76	2	1.96	2.33	3.47
Dwarf	Mean	71.61**	24.98**	15.25**	8.64**	5.99**	3.99**	2.61**
	SD	12.30	5.14	3.67	2.55	1.22	1.06	1.27

**Means differed significantly from one another ($P < 0.01$)

(Fig. 2c, d). The mean parenchyma cell length of the tall derivative was 70.3 ± 27.44 µm, which was approximately 2.5-fold that of the dwarf derivative (27.6 ± 7.02 µm); the respective mean widths were 26.1 ± 9.26 µm and 21.0 ± 3.37 µm (Fig. 2e). After the dwarf seedlings were treated with GA_3, their

height increased by 56.1% and their coleoptile length by 56.4% (Fig. 2f, g), showing that exogenously supplied GA_3 could abolish the dwarf phenotype.

Genetic analysis of the dwarf trait

The height of the dwarf × tall PI613129 F_1 hybrid was more similar that of the dwarf form than to that of the tall form (Fig. 3a). The distribution of plant height within the rye F_2 population was discontinuous and centered on ~ 133.5 cm. The data showed that 538 individual plants were short, and 171 were tall (Fig. 3b); this ratio fits a monogenic 3:1 ratio ($\chi^2 = 0.07$, $P > 0.9$). Thus, the phenotype is governed by a single gene. The wheat-rye amphihaploid mapping population was composed of 204 individuals, and the plant height distribution of the population also was bimodally distributed. The discontinuity was centered on ~ 152 cm; there were 106 short plants and 98 tall plants, thereby fitting the expected 1:1 ratio ($\chi^2 = 0.24$, $P > 0.9$) (Fig. 4). The gene was therefore expressed in the haploid triticale background. The mean plant height of these tall segregants (hereafter TS) was 167.8 cm, while that of these dwarf segregants (hereafter DS) was only 134.6 cm (Fig. 5a; Table 2). Each of the six internodes also significantly

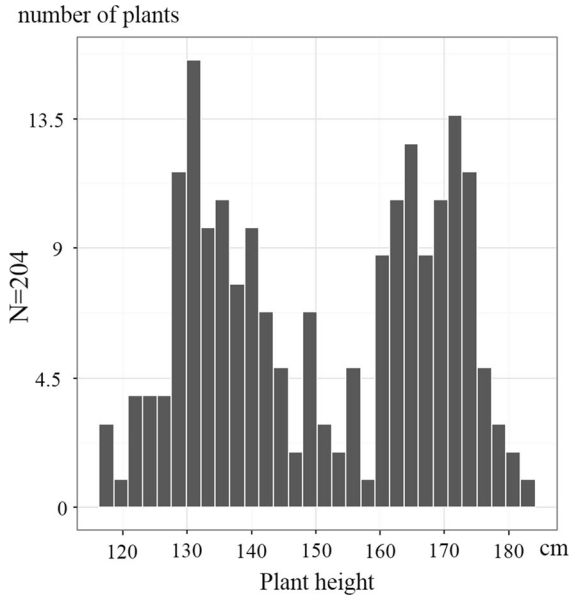

Fig. 4 The distribution of plant height in the wheat-rye amphiploid population

differed between the TS and DS plants ($P < 0.01$). The parenchyma cell sizes between the TS and DS plants were distinct (Fig. 5b, c): the mean parenchyma cell lengths were 133.4 ± 38.62 µm and 93.9 ± 23.18 µm, respectively, and their mean widths

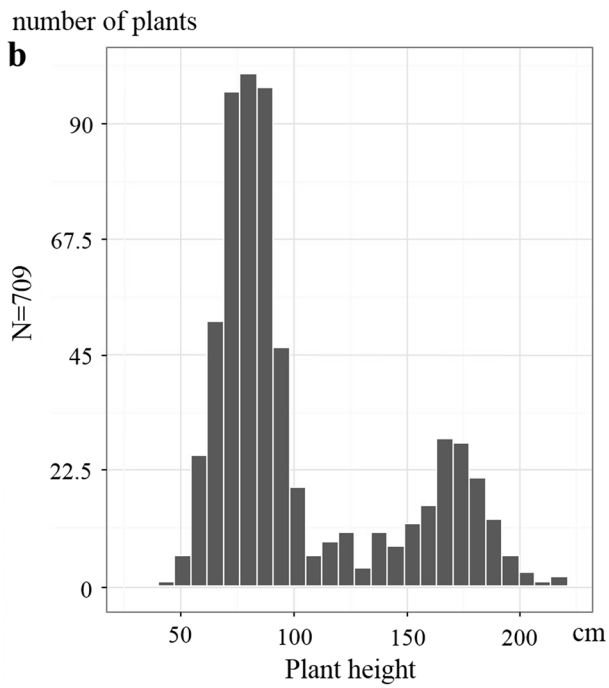

Fig. 3 The effect of the dwarfing gene in rye. **a** The phenotype of the two rye parents and their F_1 hybrid (bar: 2 m); **b** the distribution of plant height in the F_2 population

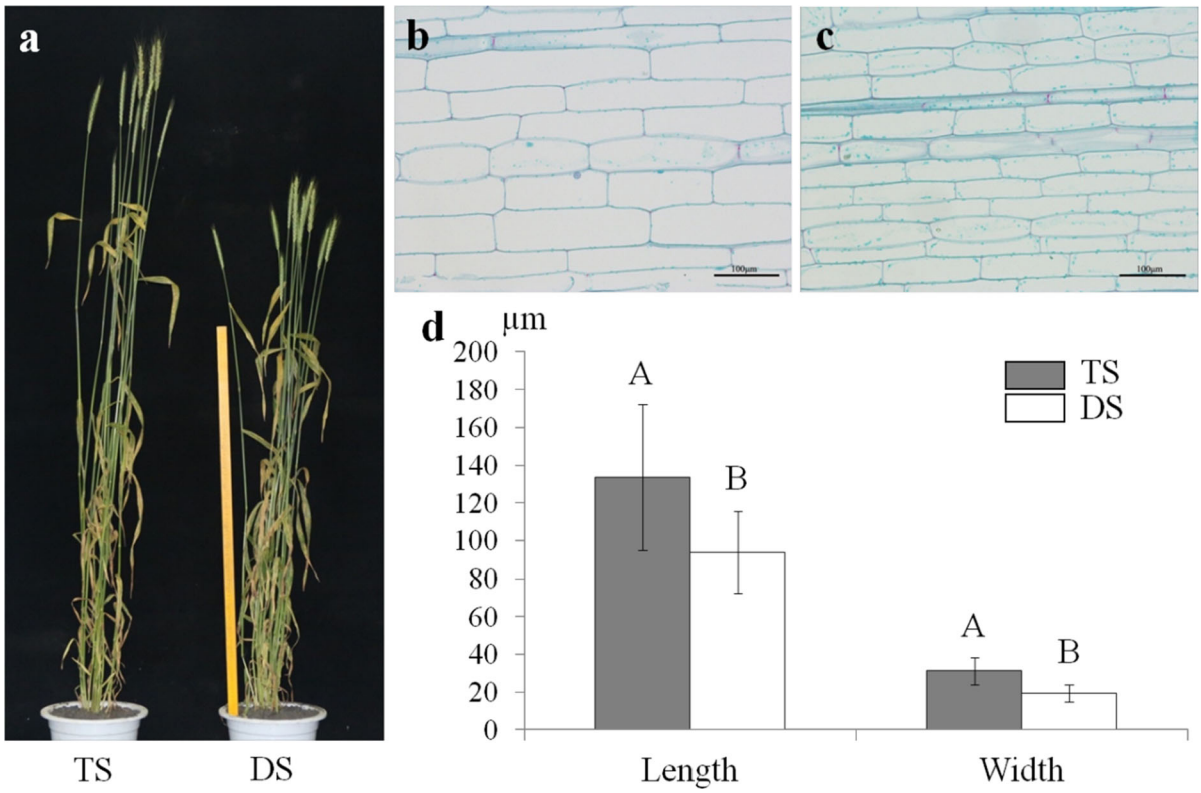

Fig. 5 Trait segregation in the wheat-rye amphiploid population. **a** Plant height (bar: 1 m); **b**, **c** parenchyma cells of the **b**TS and **c** DS plants (bar: 0.1 mm); **d** quantification of parenchyma cell size. Columns marked with a different letter indicate that the means differed significantly ($P < 0.01$), and the whiskers represent the SDs (n = 3)

Table 2 Variation in plant height and internode length between tall and dwarf segregants in the wheat-rye amphiploid population

Material	Plant height (cm)		Internode length (cm)				
			1st	2nd	3rd	4th	5th
TS	Mean	167.84**	56.31**	40.83**	25.65**	18.13**	12.51**
	SD	6.56	5.06	2.71	2.35	1.93	3.07
DS	Mean	134.63**	44.74**	31.83**	20.08**	15.61**	9.54**
	SD	8.36	5.95	3.33	2.13	2.03	3.02

**Means differed significantly from one another ($P < 0.01$)

were 31.2 ± 7.12 μm and 19.4 ± 4.64 μm, respectively (Fig. 5d).

Mapping the dwarf gene

Of the 106 rye microsatellites surveyed, 48 were informative in the wheat-rye amphihaploid mapping population. When these 48 markers were applied to the ten tallest and ten shortest individual plants, five (SCM1, SCM4, SCM21, SCM127 and SCM269), all of which mapped to a locus on chromosome 1R, showed evidence of linkage to the dwarfing gene; these five markers were therefore then tested within the full mapping population (N = 204) to generate a linkage map of chromosome 1R. The dwarfing gene mapped to a point 8.7 cM proximal to SCM4 and 9.3 cM distal to SCM21 (Fig. 6a). The application of the same five microsatellites to a subset of 52

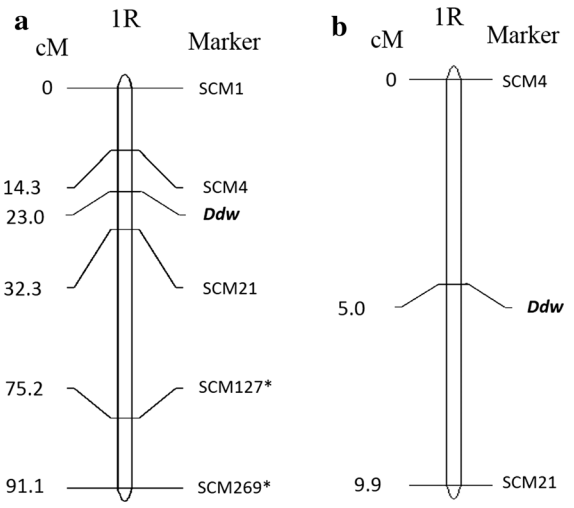

Fig. 6 Local linkage map of chromosome 1R. **a** Map derived from the wheat-rye amphiploid population; **b** Map derived from the rye F_2 population. Genetic distances are given in centimorgans (cM), *most favored map location (LOD < 3.0)

dwarf × high F_2 individuals confirmed that the dwarfing gene lay between SCM4 and SCM21 (Fig. 6b).

Discussion

Traditional gene or QTL mapping requires (1) construction of suitable mapping populations; (2) selection of appropriate molecular markers and (3) construction of genetic linkage maps using statistical programs. Along with the development of rye whole genome sequencing (Martis et al. 2013; Bauer et al. 2017), the quality and quantity of molecular markers have been improved. One major limiting factor for rye gene or QTL mapping is the construction of mapping population. The self-incompatibility and inbreeding depression because of its cross-pollination feature hampers the construction of traditional mapping population, such as large F_2, BC or RIL populations. In addition, tissue culture recalcitrance has prohibited the efficient generation of DH populations (Tenhola-Roininen 2009). Thus, the construction design for mapping populations will directly influence the effectiveness of gene or QTL mapping. In this study, a wheat-rye amphihaploid population was employed for mapping dwarf gene in rye material PI613129 (Fig. 1). Our results indicated that the dwarf gene can be rapidly and effectively located using this mapping population. The wheat-rye amphihaploid population is similar to a DH population but does not need tissue culture and chromosome doubling, which are tedious procedures (Tenhola-Roininen 2009). Thus, such a population can be constructed quickly and easily by just two manual crosses. In this population, every individual contained only one set of haploid R genome, which had been recombined via meiosis in the F_1 generation. In theory, the segregation ratio will be 1:1, and the difference of molecular marker linked to plant height trait will directly reflect phenotype variation in the population. Furthermore, heterozygous molecular markers are nonexistent in individuals. These advantages will help increase the efficiency for mapping. Unlike double haploids, however, the amphihaploid triticale plants were usually sterile or had very low fertility; these plants represent only an ephemeral population useful for a one-time identification of the genetic basis of phenotypic variation. This restricts its application for related traits of seeds, and it cannot be evaluated several times under different environmental conditions or in different years or locations. This led to the wheat-rye amphihaploid population being not suitable for QTL mapping. Objectively, this strategy can be an additional option for mapping of major rye genes.

To be practicable, the amphihaploid triticale strategy requires that a wide cross is readily made. Here, the choice of wheat parent was the cultivar Shinchunaga, as this cultivar exhibits a high level of crossability with rye (Yuan et al. 2011), as do several other lines (Riley and Chapman 1967; Zheng et al. 1993). A second requirement is that a single heterozygous rye × rye F_1 plant was used as the source of male gametes for the triticale population; the use of a single plant was advantageous because it reduced the complexity of the subsequent segregation. Furthermore, rye is a prolific pollen producer. A single plant has enough pollen for building large sample mapping population. In addition, The strategy relied on expression of the target phenotype (in this case dwarfness) in both amphihaploid triticale and rye, which may not always be the case, as some non-wheat genes are silenced when introduced into the wheat genome (Zeller and Hsam 1996; Houchins et al. 1997). Therefore, a phenotype survey of the population is important before use this mapping population. Ongoing research involving whole-genome sequencing of

rye (Bauer et al. 2017) is rapidly expanding the number of markers that can be applied to wheat-rye amphiploid populations to target rye genes. Thus, the present wheat-rye amphihaploid population serves as a base for the effective and rapid mapping of genes in out-crossing rye.

To date, three dominant dwarfing genes (*Ddw1*, *2* and *3*) have been mapped in rye (Korzun et al. 1996; Melz 1989; Stojałowski et al. 2015). Here, the dwarfing gene carried by germplasm accession PI613129 was mapped to chromosome arm 1RL (Fig. 6). Inspection of the consensus linkage map of rye based on SSRs (Hackauf et al. 2009) and DArT markers (Milczarski et al. 2011; Stojałowski et al. 2015) showed that, similar to the PI613129 gene, *Ddw3* is linked to the microsatellite locus SCM21. The morphological effects of the PI613129 gene also resembled those of *Ddw3*. As such, it is probably exactly the same gene with *Ddw3*. In this study, we found that the dwarf gene in PI613129 can reduce the height of haploid triticale plants in the same manner. This suggested that the dwarf gene could be used for dwarf breeding of wheat. To date, only a handful of dwarf genes have been successfully applied to wheat genetic improvement, such as *Rht-B1*, *Rht-D1*, *Rht24* (Würschum et al. 2018) and *Rht8* (Borojevic and Borojevic 2005). The rye 1R chromosome is one of the most intensively used sources of alien chromosomes in bread wheat (Baum and Appels 1991). In many commercial wheat cultivars, 1R provides some valuable disease resistance genes, such as *Lr26*, *Sr31*, *Yr9* and *Pm8* (Mago et al. 2005; Ren et al. 2009). Moreover, it can improve the yield and environmental adaptability of wheat (Kumlay et al. 2003; Ren et al. 2012). Nonetheless, wheat-rye F_1 haploid hybrids are usually have very low fertility, yet those few offspring can easily create wheat-rye 1R addition, substitution and translocation lines (Mettin et al. 1973; Darvey and Gustafson 1975). This will enable exploitation of this dwarf gene for wheat bread. Our results may open novel options for breeders to improve lodging resistance in wheat using dwarfing genes independent of the genes of the Green Revolution.

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Authors' contributions SY, ZY and DL designed the study. SY, HZ, JY, YZ, LZ and XC created the mapping and verification population. SY, HZ and JY carried out molecular genotyping. SY, YJ and MH analyzed the data. SY, ZY and DL drafted the manuscript; LZ and SN participated in planning the study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors declare that all experiments complied with the current laws of China.

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