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Genetic Mapping of Gibberellic Acid-sensitive Genes for Semi-dwarfism in Durum Wheat

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Semi-dwarf varieties in wheat associated with gibberellic acid (GA_3)-insensitive height reducing genes have led to significant increases in yield but often fall below this potential because of poor seedling emergence after deep showing. Alternative semi-dwarf genes may have the potential to reduce plant height without compromising early plant growth. In durum wheat, bulk segregant analysis was used to screen microsatellite markers linked with the GA_3-sensitive genes *Rht14* in cv. Castelporziano, *Rht16* in cv. Edmore M1 and *Rht18* in cv. Icaro. Molecular marker *Xbarc3-6A* for *Rht14, Rht16* and *Rht18* showed significant polymorphic differences among DNA bulks for height classes. The genes *Rht14, Rht16* and *Rht18* were linked with *Xbarc3* (11.7–28.0 cM) on the short arm of chromosome 6A and they appear to be allelic. Semi-dwarf genes on chromosome 6AS may potentially be used in breeding for improved establishment.

Keywords: *Triticum durum*, GA_3-sensitive, plant height, reduced-height

Introduction

The introduction of stem-shortening genes into commercial bread wheat (Perry and D'Antuono 1989) and durum wheat (Waddington et al. 1987) has led to substantial yield increases per unit area. The dwarfing genes *Rht-B1b (Rht1)* and *Rht-D1b (Rht2)* from *Triticum aestivum* cv. Norin 10 resulted in an increased harvest index and reduced risk of lodging under high nitrogen fertilization (Evans 1993). Although these genes can lead to yield increases in favorable environments, they have been associated with yield reduction in unfavorable environments (Laing and Fisher 1977; Richards 1992). For successful crop establishment, it is necessary to achieve a high proportion of emerged plants relative to the number of seeds sown. This has become an important issue in wheat because crop establishment is poor when seeds of varieties containing the gibberellic acid (GA_3)-insensitive dwarfing genes *Rht-B1b* and *Rht-D1b* are sown deep to gain early access to deep soil moisture and when conservation farming systems and stubble retention practices have

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been adopted (Schillinger et al. 1998). These GA_3-insensitive varieties have short coleoptiles and will not establish well if sown too deep. Addisu et al. (2009) reported that the GA_3-sensitive *Rht12* and *Rht8* were not associated with reduced coleoptile length. It has been suggested that lines containing dwarfing genes that are responsive to applied GA_3 and have longer coleoptiles will emerge more successfully when sown deep or when used in conservation farming systems (Schillinger et al. 1998; Rebetzke et al. 2005, 2007).

A number of accessions containing alternative dwarfing genes (i.e. unrelated to *Rht1* and *Rht2*) are known in wheat. They reduce plant height to varying degrees, and are sensitive to GA_3. Semi-dwarf wheats containing these genes produce long coleoptiles, which assist rapid germination and plant establishment. Therefore, there is potential to utilize these semi-dwarf genes in wheat breeding programs (Rebetzke et al. 1999). Ellis et al. (2004) assessed the effect of different height-reducing genes on the early growth of wheat, and Ellis et al. (2005) mapped GA_3-sensitive dwarfing genes in bread wheat *(T. aestivum)* on chromosomes 3BS *(Rht5),* 5AL *(Rht12),* 7BS *(Rht13),* 2BL *(Rht4),* 2DS *(Rht8)* and 5AL *(Rht9)*. Semi-dwarf durum wheat accessions, var. Castelporziano, Edmore M1 and Icaro, were identified as GA_3-sensitive (Ellis et al. 2004). These accessions were expected to have the potential to produce long coleoptiles and to be utilized in breeding tetraploid and hexaploid wheats. However, no map locations were reported for the genes conferring semi-dwarf stature in these accessions.

Molecular markers are widely used for mapping genes of agricultural importance. Microsatellite markers have been widely used in such applications in wheat. Numerous polymorphic microsatellite markers have been integrated into genetic framework maps (Röder et al. 1998; Song et al. 2005; Torada et al. 2006). The aim of the present study was to map GA_3-sensitive genes for semi-dwarfism in three durum wheat accessions.

Materials and Methods

The three reduced height sources studied here were GA_3-sensitive. *T. durum* mutant line Edmore M1 (PI 499362) was derived from cv. Edmore by mutagenesis with methylnitrourea (C.F. Konzak, Washington State University, USA). *T. durum* cv. Castelporziano (PI 347731) was derived from mutagenesis of cv. Capelli, whereas cv. Icaro (PI 503555) was obtained by fast-neutron treatment of cv. Anhinga. The semi-dwarf genes in these materials were designated as *Rht14* for Castelporziano (Gale et al. 1985), *Rht16* for Edmore M1 (Konzak et al. 1984), and *Rht18* for Icaro (Konzak 1988). The mean culm lengths of mutant lines Castelporziano, Edmore M1 and Icaro were 73.8 ± 4.2 cm, 76.8 ± 4.5 cm, and 63.5 ± 3.4 cm, respectively (measured after ripening). The culm length of *T. durum* cv. LD222, which dose not carry any known reduced height alleles, was 150 ± 8.1 cm.

All materials for study were grown in the experimental field at the College of Agriculture, Ibaraki University, Japan. Culm lengths of individual genotypes were measured after ripening. F_2 hybrid populations of Castelporziano × LD222 and Icaro × LD222 for genetic analysis were sown in October 2006 and those of Edmore M1 × LD222 in October 2007.

Bulk segregant analysis

Bulk segregant analysis (BSA) (Michelmore et al. 1991) was used to identify markers linked to the GA_3-sensitive reduced-height genes in specific chromosomal regions. Pooled DNA samples of the semi-dwarf and tall progeny from the F_2 mapping populations were screened for polymorphisms using 87 microsatellite markers known to be located on A or B genome chromosomes (*Triticum aestivum* is a hexaploid containing genomes A, B and D, whereas *T. durum* is tetraploid and contains only genomes A and B). DNA samples from the parents, six individual semi-dwarf F_2 plants, and six individual tall F_2 plants with heights similar to the respective parents were also used to check the amplification characteristics of the linked markers. Markers close to a semi-dwarfing gene should be polymorphic between both parents and bulks. If unlinked, a similar banding pattern will be seen in each of the two bulks.

Allelic relationships of the Rht *genes*

Castelporziano, Edmore M1 and Icaro were crossed with each other to test for allelism among the *Rht14, Rht16* and *Rht18* genes using the F_2 populations.

Microsatellite mapping of the GA_3-sensitive Rht *genes*

Genomic DNA was extracted from seedlings of 94 individuals per F_2 hybrid population according to the method of Dellaporta et al. (1983). The microsatellite markers *Xgwm, Xbarc* and *Xhbg* located on chromosomes 6A were chosen based on the results of BSA and the potentially allelic relationship detected between the semi-dwarf genes under study. These markers were obtained from Röder et al. (1998), Song et al. (2005), and Torada et al. (2006), respectively. PCR analysis was performed as described by Kosuge et al. (2008). Linkage values in centiMorgans (cM) were calculated using Map Manager QTX (http://mapmgr.roswellpark.org/). Minimum LOD scores of at least 3.0 were used to develop the linkage map. The software calculated genetic distances in cM using the Kosambi (1944) mapping function.

Results

Allelism test

No tall plants were observed in F_2 populations derived from the three crosses between Castelporziano, Edmore M1 and Icaro (n = 152–158 for each F_2 population), thereby indicating that *Rht14, Rht16* and *Rht18* are allelic.

Chromosomal location of GA_3-sensitive genes

Figure 1 shows the segregation of culm length in F_2 populations of Castelporziano × LD222, Edmore M1 × LD222, and Icaro × LD222. The culm lengths of three F_2 hybrid populations were classified 94 semi-dwarf: 25 tall for the F_2 of Castelporziano × LD222, 94 semi-dwarf: 40 tall for the F_2 of Edmore M1 × LD222 and 108 semi-dwarf : 26 tall for

the F_2 of Icaro × LD222. The observed segregation ratios in all three F_2 populations fit a model of 3 semi-dwarf: 1 tall (χ^2 values of 1.011, 1.681 and 2.239, respectively; df = 1). The semi-dwarf mutant lines were, on average, 50% shorter than the LD222.

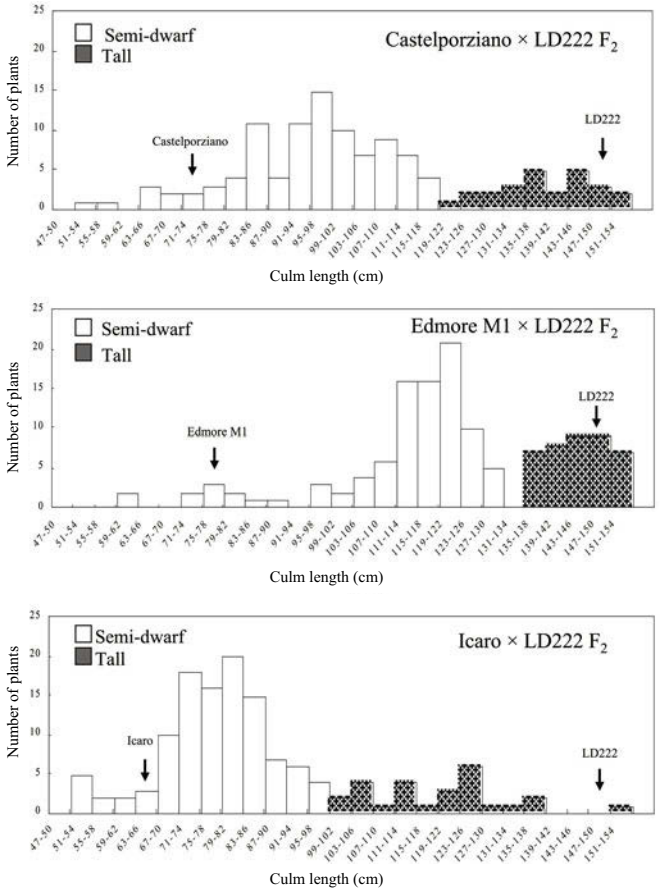

Figure 1. Segregation of culm length in F_2 populations of Castelporziano × LD222, Edmore M1 × LD222 and Icaro × LD222. Plant height was categorized as semi-dwarf (white bars) or tall (grey bars). Arrows indicate mean culm length of the parents

The distributions in culm length shown in Figure 1 indicate that the semi-dwarf phenotypes were incompletely dominant. Selection of more extreme phenotypes for BSA resulted in plants homozygous for the contrasting alleles being chosen.

Amplification using microsatellite marker *Xbarc3,* located on chromosome 6A, showed polymorphism between the parents and bulks as well as individuals in BSA (Fig. 2). Based on the results of the BSA, 22 microsatellite markers on chromosome 6A were

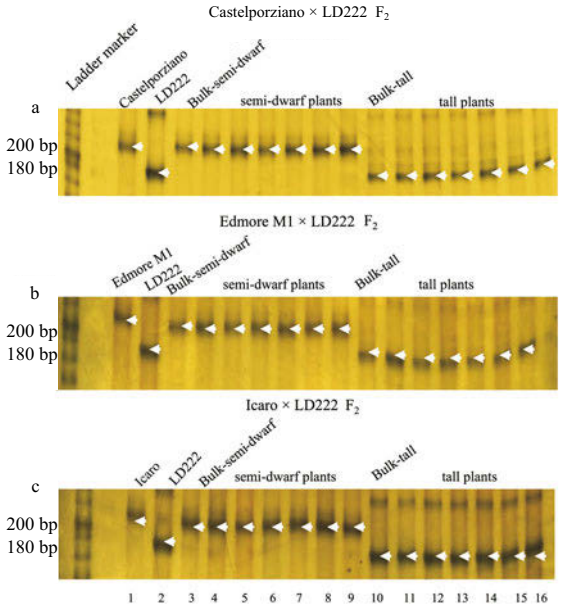

Figure 2. Amplification profiles of microsatellite marker *Xbarc3-6A* by bulk segregant analysis in F_2 populations of (a) Castelporziano × LD222, (b) Edmore M1 × LD222 and (c) Icaro × LD222.
Left to right: Semi-dwarf parent (lane 1), LD222 (lane 2), bulk of semi-dwarf plants (lane 3), individual semi-dwarf F_2 plants (lanes 4 to 9), bulk of tall plants (lane 10), and individual tall F_2 plants (lanes 11 to 16). Arrows indicate bands in common between a given parent, individual homozygous progeny with the same phenotype as that parent and the corresponding bulk

used to map the *Rht14, Rht16* and *Rht18* genes. Markers *Xbarc3, Xbarc171* and *Xgwm256* were polymorphic in all three combinations.

Rht14, Rht16 and *Rht18* each mapped distal to marker *Xbarc3* on the short arm of chromosome 6A (Fig. 3). These mapping data provide further evidence for an allelic relationship between *Rht14, Rht16* and *Rht18*.

Discussion

The GA_3-insensitive semi-dwarf genes *Rht-B1b* and *Rht-D1b* produce short coleoptiles, which may reduce seedling vigour and crop yield stability (Richards 1992; Rebetzke et al. 1999). It was proposed that GA_3-sensitive semi-dwarf genes could resolve these problems in durum wheat (Giorgi et al. 1984). Rebetzke et al. (2004) reported that coleoptile lengths of progeny derived from GA_3-sensitive *Rht8, Rht9* and *Rht12* semi-dwarf genes were an average 47% longer coleoptiles than comparative materials with *Rht-B1b* and *Rht-Dlb,* thus offering the potential of longer coleoptiles as well as reduced height for deep sowing. The present study focused on mapping three GA_3-sensitive semi-dwarfing genes that came from different mutation events. *Rht14, Rht16* and *Rht18* were located to similar positions on chromosome 6AS and tests of allelism based on relatively small populations of

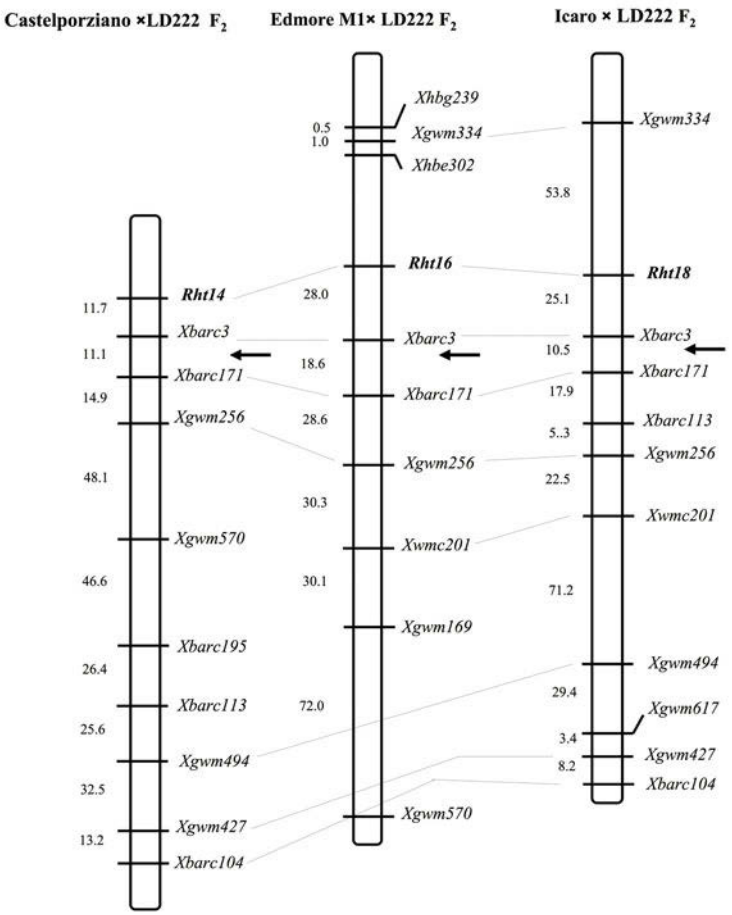

Figure 3. Linkage maps for *Rht14, Rht16* and *Rht18* on chromosome 6A. Arrows indicate the supposed position of the centromere. Distances between markers are shown in cM

F_2 plants indicated the possibility of allelism. Spielmeyer et al. (2007) located a QTL for coleoptile length on chromosome 6AS, and they indicated that the QTL on this region promoted coleoptile length during early plant growth. Further investigation is needed to assess the potential of these semi-dwarf genes in dryland wheat production.

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