Genetic mapping of *Vrn-D4* in hexaploid wheat

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ABSTRACT

Natural variation in vernalization requirement in wheat is mainly controlled by four loci, *Vrn-1*, *Vrn-2*, *Vrn-3*, and *Vrn-4*. The genes at the first three loci have been cloned and a model for their epistatic interactions was proposed. To clone the remaining *Vrn-4* locus, , we constructed a high-density map of *Vrn-4*. We show that natural variation of *Vrn-4* is only detected in the D genome of wheat, designated as *Vrn-D4* formerly known as *Vrn4* or *Vrn-D5*. The genetic stock for the dominant *Vrn-D4* allele is Triple Dirk F (TDF, hereafter), but there has been some controversy around the *vrn-1* alleles present in TDF. Therefore, we analysed TDF seed stocks from Japan and the US using molecular markers for known *Vrn* genes. The TDF stock from Japan showed recessive alleles for all three homoeologous *vrn-1* loci, and segregated only for *Vrn-D4*. In contrast, the TDF stock from WSU of USA showed dominant *Vrn-A1* and *Vrn-B1* alleles, and different SSR markers in the *Vrn-D4* region. These differences may explain previous inconsistencies. We crossed the TDF stock from Japan with the Japanese winter cultivar Hayakomugi carrying *vrn-D4* and generated a mapping population of 258 F_2 plants. Unvernalized plants grown under long day conditions (16-h light) showed a 3:1 ratio between spring and winter lines, confirming that *Vrn-D4* is the only growth habit gene segregating in this population. *Vrn-D4* was mapped on the centromeric region of chromosome 5D completely linked to SSR locus *Xcfd67,* and was flanked by *Xcfd*81 (8.4 cM) on the short arm and *Xbarc205* (1.1 cM) on the long arm. The arm location of *Vrn-D4* is still unknown. We are currently adding SNPs markers to the region in order to generate a comparative map with rice and saturate the *Vrn-D4* region with additional markers, while increasing the size of the mapping population.

INTRODUCTION

Flowering at an optimal time is very important for successful reproduction in higher plants. To achieve this, plants monitor seasonal changes using environmental cues, like photoperiod and temperature and also internal cues.

In Arabidopsis, the genetic regulation of flowering time has been shown to involve four major pathways[1]: vernalization pathway, photoperiod pathway, autonomous pathway, and gibberellin-mediated pathway. The former two are mainly determined by environmental signals, while the latter two are not. Plants integrate signals from these different pathways to determine flowering time. Among these pathways, the vernalization pathway,(a requirement for extended period of low temperatures, is critical for winter cereals.

In Arabidopsis, the *FLOWERING LOCUS C* (*FLC*), which encodes a MADS box transcription factor, plays a central role in the vernalization requirement pathway[2]. It represses downstream flowering promoters, such as *Flowering Locus T* (*FT*) and *SUPPRESSOR OF CO1* (*SOC1*)[3, 4]. *FT* encodes a Raf kinase inhibitor-like protein that is transmitted from leaf to shoot apex, while*SOC1* encodes a MADS box transcription factor up-regulated by *FT* in the shoot apex. When Arabidopsis plants are vernalized, *FLC* transcription is epigenetically down-regulated. This results in the up-regulation of *FT* and *SOC1* and promotes flowering. The epigenetic down-regulation of *FLC* requires the cooperative action of *VERNALIZATION INSENSITIVE 3* (*VIN3*) and *VIN3-like 1* (*VIL1*)[5, 6].

In wheat there are no homologues of *FLC*. Natural variation in vernalization genes is known for *Vrn-1*, *Vrn-2*, *Vrn-3*, and *Vrn-4*. The first three genes have already been cloned[7, 8, 9]: *Vrn-1* encodes an homologue of Arabidopsis *APETALA1*, a meristem identity gene required for the transition between the vegetative and reproductive stage. *Vrn-2* encodes a transcription factor with zinc finger and CCT domains (ZCCT), which islikely to repress *Vrn-1* and *Vrn-3* (the homologue of Arabidopsis *FT*). In winter-type plants, *Vrn-2* transcription is down-regulated by cold temperature and short-day photoperiod, thereby releasing the *Vrn-1* and *Vrn-3* transcription and inducing flowering. *Vrn-3* transcription is induced by long photoperiod and up-regulates *Vrn-1*. In contrast to the abundant information for the first three vernalization genes little is known for *Vrn-4*. Natural variation controlled by *Vrn-4* is found only in the D genome (*Vrn-D4,* formerly named as *Vrn4* or *Vrn-D5*). Kato et al. (2003) mapped *Vrn-D4* on chromosome 5D linked to SSR marker *Xgdm3*[10]. However there are contradictory reports on *Vrn-D4*[11, 12]. In this paper, we confirmed the existence of *Vrn-D4* and established more precise map localization.

MATERIALS AND METHODS

A near isogenic line of Triple Dirk (TD), TDF has been proposed to carry a dominant *Vrn-D4* allele for spring growth habit[10, 12]. We checked two TDF stocks maintained at two different places, Japan (designated as TDF-J, hereafter) and USA (designated as TDF-US, hereafter). TDF-J is the same line as that used by Kato et al. (2003) for the preliminary map of *Vrn-D4*[10].

A total of 258 F_2 plants from a cross between TDF-J and Hayakomug, a Japanese winter-type cultivar were used for genetic mapping. Plants were grown at constant 20ºC (non vernalizing conditions) and long-day (16-h) photoperiod. Their genomic DNA was extracted from young leaf tissue by CTAB method.

Six SSR markers, *Xcfd81*, *Xcfd78*, *Xcfd67*, *Xbarc205*, *Xwmc318*, and *Xgdm3*, were used for genetic mapping. Their location on chromosome 5D was confirmed by nulli-tetrasomic analysis, and they were assigned to chromosome bins using deletion lines for chromosome 5D (six lines for break point 5DS2, 5DS5, 5DS1, 5DL1, 5DL9, and 5DL5) and ditelosomic line (DT5L), all in a Chinese Spring background.

PCR conditions included a 95°C denaturing step for 3 min, followed by 35 cycles of 95°C for 30 s, 58°C to 60°C annealing (depending on SSR) for 30 s, and 72°C for 1 min, and a final extension step at 72°C for 10 min. PCR products were run in 6-18% polyacrylamide gels at a constant voltage (21 V/cm).

The genetic map was constructed using MAPMAKER/EXP3.0.

RESULTS

Differences between TDF stocks: The original cultivar Triple Dirk is known to carry dominant *Vrn-A1* and *Vrn-B1* alleles, and this was confirmed using markers for these genes [13, 14]. The TDF-J was found to carry the expected recessive winter alleles *vrn-A1* and *vrn-B1* genes, and segregated only for *Vrn-D4*[10] (Fig. 1). In contrast, the TDF-US was found to carry the dominant *Vrn-A1* with a 140-bp insertion in the promoter region and the dominant *Vrn-B1* allele with a large deletion in the intron 1 region (Fig. 1). These results suggest that TDF-US stock was incorrect and was likely the original TD variety. This was further confirmed by different haplotypes from TDF-J in the *Vrn-D1* region, and no segregation for flowering time associated with this region in a cross between TDF-US and winter line TDC. Therefore, TDF-J was used for all further studies.**Precise mapping of *Vrn-D4***: The 258 F_2 from the cross TDF-J x Hayakomugi showed clear differences in flowering time. The F_2 population showed a segregation of 184 spring-type plants and 74 winter-type plants. This segregation fits a 3:1 ration for a single dominant gene segregation (χ^2=1.866, P=0.172).

Six SSR markers were confirmed to be on chromosome 5D and were assigned to different chromosome bins. The *Xcfd81* locus was assigned to the 5DS1 bin, while the *Xcfd78* locus was assigned to the centromeric bin in the short arm. The other markers, *Xcfd67*, *Xbarc205*, *Xwmc318*, and *Xgdm3*, were all assigned to the centromeric region of the long arm. The marker order in this region is presented in Fig. 2. In this population *Vrn-D4* was completely linked with *Xcfd67* and flanked by *Xcfd78* on short arm and by *Xbarc205* on long arm (Fig. 2). The position of *Xcfd78* relative to *Vrn-D4* is based only on two critical recombination events so it will require further validation. The arm location of *Vrn-D4* is still unknown.

DISCUSSION

Pugsley (1972) identified *Vrn-D4* as a different vernalization gene from *Vrn-D1* in cultivar Gabo and transferred it to the isogenic stock TDF[15]. Since then, there have been contradictory reports for the presence of *Vrn-D4*. Stelmakh (1987) suggested that Gabo and TDF do not carry *Vrn-D4* but carry *Vrn-A1* and *Vrn-B1*[11]. Consistent with this suggestion, we found that the TDF-US seed stock carries *Vrn-A1* and *Vrn-B1* alleles. On the contrary, TDF-J has recessive *vrn-A1* and *vrn-B1* and segregates for a dominant spring growth habit gene on the centromeric region of chromosome 5D. Based on its map position this gene is clearly non-allelic to *Vrn-D1*. These results suggest that TDF-J carries *Vrn-D4* and is most likely to correspond with Pugsley's original description of TDF. The heterogeneity of TDF stocks reported here may explain the contradictory results found in the literature about this line. Pugsley (1972) pointed out that Gabo (the *Vrn-D4* donor) is also heterogenous[15].

The *Vrn-D4* gene was mapped in the centromeric region of chromosome 5D, which is colinear with rice chromosomes 12[16] where some flowering time QTLs have been found[17, 18, 19, 20, 21]. However, it is currently not possible to determine whether *Vrn-D4* corresponds to any of these QTLs, because a more precise *Vrn-D4* map including more common markers with rice is still missing.

To refine the current *Vrn-D4* map we are expanding the mapping population and conducting progeny tests for critical recombination events. We are also adding SNPs markers to the region to generate a more detailed comparative map with rice.

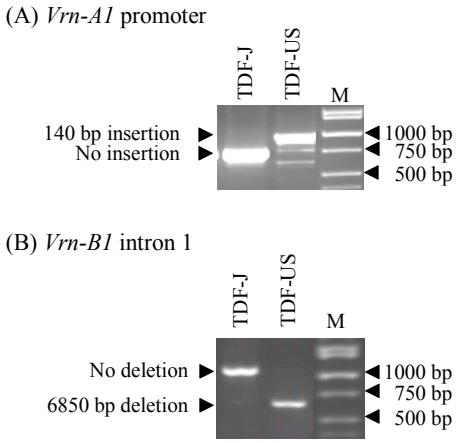

Fig. 1 Heterogeneity of TDF stocks. (A) PCR analysis of *Vrn-A1* promoter (presence / absence of 140 bp insertion). (B) PCR analysis of *Vrn-B1* intron 1 (presence / absence of 6,850-bp deletion).

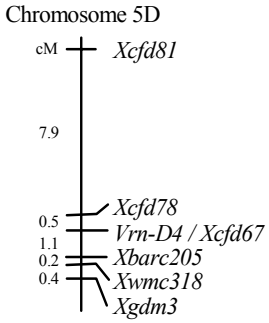

Fig. 2 Genetic map of *Vrn-D4* region on chromosome 5D. based on the F_2 population from a cross between TDF-J and Hayakomugi.

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