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Physical mapping of the *Vrn-A1* and *Fr1* genes on chromosome 5A of wheat using deletion lines

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Abstract Homozygous deletion lines of wheat for 5AL, generated in the variety 'Chinese Spring', were tested for flowering time without vernalization and for frost resistance after cold hardening. It was found that the *Vrn-A1* gene for vernalization requirement mapped between breakpoints 0.68 and 0.78, whilst the frost resistance gene *Fr1* was flanked by deletion breakpoints 0.67 and 0.68. This confirms previous evidence that these genes are linked but are not the pleiotropic effect of a single gene. A comparison between the physical and genetic maps for *Vrn-A1* and *Fr1* shows that the linear order is identical. These results indicate that cytogenetically based physical maps of *Vrn-A1* and *Fr1* loci, together with genetic maps, could be useful in the further study of genome synteny and in elaborating a gene cloning strategy.

Key words Wheat · Frost resistance · Vernalization · Physical map · Deletion lines

Introduction

Low winter temperatures are one of the major abiotic stress factors limiting bread wheat (*Triticum aestivum* L., 2n = 6x = 42) growth, productivity and distribution. Wheat varieties are well known to differ in their responses to low temperatures, and low-temperature hardening allows winter wheats to protect critical cell structures and physiological processes during periods of freezing temperature. Genetic studies have shown that the genetic control of frost resistance is complex and can be regarded as a polygenic trait (Sutka 1981, 1984, 1994). Nevertheless, major genes have been mapped on chromosomes 5A and 5D using molecular markers (Galiba et al. 1995; Snape et al. 1997).

Wheat varieties have a further genetic system for successful adaptation to winter sowing, namely a vernalization requirement. Spring-sown wheats either have no vernalization requirement or have only a weak response. Again the major loci for vernalization requirement in wheat are located on chromosomes 5A (*Vrn-A1*, formerly *Vrn1*) and 5D (*Vrn-D1*, formerly *Vrn3*) (Law et al. 1976; Snape et al. 1985; Law et al. 1991).

The intrachromosomal location of *Vrn-A1* has been studied using RFLP (restriction fragment length polymorphism) markers (Galiba et al. 1995). The *Vrn-A1* and *Fr1* loci were located closely linked on the distal portion of the long arm of 5A, but recombination between them was found (cM = 2). The RFLP markers *Xpsr426* and *Xwg644* were tightly linked to the *Vrn-A1* locus (Galiba et al. 1995). The intrachromosomal location of *Vrn-D1* and *Fr2* on chromosome 5D was also established recently using RFLP, AFLP (amplified fragment length polymorphism) and microsatellite markers (Snape et al. 1997, 1998). This showed that *Vrn-D1* and *Fr2* are linked, but separable, on the long arm of 5D and that *Fr2* appears to be further away, genetically, from *Vrn-D1* than *Vrn-A1* is from *Fr1* (Snape et al. 1998). On the basis of results from using cross-hybridizing RFLP probes, it is clear that the long arms of 5A and 5D are homoeologous. This analysis fits in with other comparative studies of this region, where *Sh2* on chromosome 5H of barley (Laurie et al. 1995) and *Sp1* on chromosome 5R of rye (Plaschke

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et al. 1993) are homoeologous loci to *Vrn-A1*/*Vrn-D1*. Loci *Fr1* and *Fr2* are probably homoeologous to a frost tolerance quantitative trait locus (QTL) detected on barley 5H by Pan et al. (1994).

Recently, 436 wheat chromosome deletion lines were generated in the variety 'Chinese Spring' using the gametocidal chromosome of *Aegilops cylindrica* (Endo and Gill 1996). These deletions are powerful tools for the physical mapping of wheat chromosomes. The vernalization gene *Vrn-A1* was physically mapped using a set of deletion lines which located it to the region of chromosome 5A flanked by deletion breakpoints 0.68 and 0.78 (Sarma et al. 1998). This interval was shown to be homoeologous to a region of rice chromosome 3 that contains the flowering-time QTL *Hd-6* (Sarma et al. 1998). Here we report the physical map of the *Vrn-A1* locus relative to *Fr1* and a comparison of the physical maps with the genetic maps on a segment of the long arm of wheat chromosome 5A.

Materials and methods

Deletion lines

For the long arm of 5A there are 22 deletion lines including homozygous, heterozygous and hemizygous plants available for analysis. Seven homozygous deletion lines were used for the present study. Plants carrying deleted chromosomes were identified by C-banding. The breakage point for each deleted chromosome was calculated as a fraction length (FL) of the distance from the centromere to the total length of the arm.

Freezing tests

Tests for frost resistance on each of the homozygous 5AL deletion lines and the control variety 'Chinese Spring' were carried out in the Martonvásár phytotron using the procedures described previously by Sutka (1981) and Tischner et al. (1997). After freezing, the leaves were cut off with scissors a few centimetres above the soil. This was done in order that regrowth could be more accurately evaluated and to avoid the risk of infection by fungal diseases. Frost resistance was assessed in terms of regrowth on a 0 (dead) to 5 (undamaged) scale and also as percentage survival (Sutka 1981). For each deletion line 75 plants were available for freezing evaluation using a randomized block design of three replications.

Flowering time

Ten plants of each of the 5AL deletion lines were grown in pots in a controlled environment cabinet without vernalization under a regime of 16-h light at a temperature of 20°C using a randomized block design. Ear emergence time was recorded as the time to heading of the first tiller of each plant.

Results and discussion

The removal of the 5A chromosome from 'Chinese Spring' under unvernalized conditions, as in the monosomic and nulllisomic, leads to a delay in flowering (Flood and Halloran 1986). therefore, any deletion line lacking the *Vrn-A*1 locus would be expected to exhibit a similar late flowering. Analysis of variance for flowering time revealed significant differences between the deletion lines, which could be divided into two groups (Fig. 1). The mean difference in flowering between groups I and II was 14 days in this experiment. The division between the groups of deletion lines for flowering time occurred between breakpoints 0.68 and 0.78. This indicates that the *Vrn-A1* locus can be mapped between breakpoints 0.68 and 0.78 (Fig. 3). A similar result was revealed by Sarma et al. (1998).

From Fig. 2 it can be seen that the frost resistance of the deletion lines could also be divided into two groups. Group I had an average survival of 40%, while for group II the average survival was 27%. This 13% difference in survival between groups was statistically

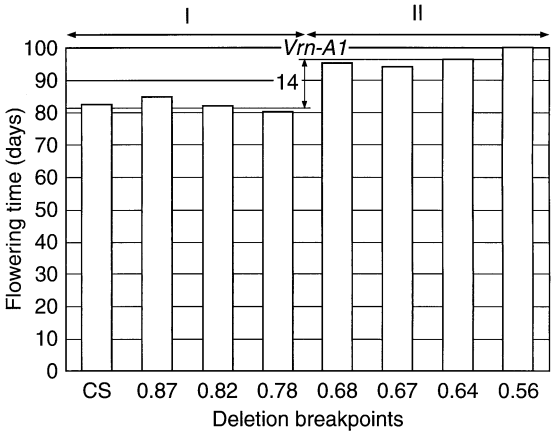

Fig. 1 Flowering times of 'Chinese Spring' and deletion lines for the 5AL chromosome arm

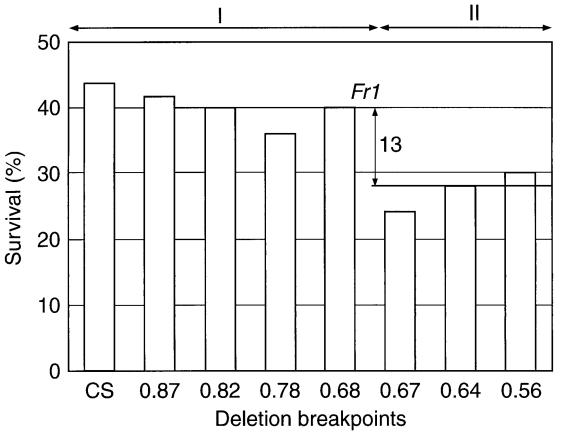

Fig. 2 Frost resistance of 'Chinese Spring' and deletion lines for the 5AL chromosome arm. Mean survival percentage at freezing temperatures of -9°C, -10°C and -11°C based on 75 plants for each deletion line

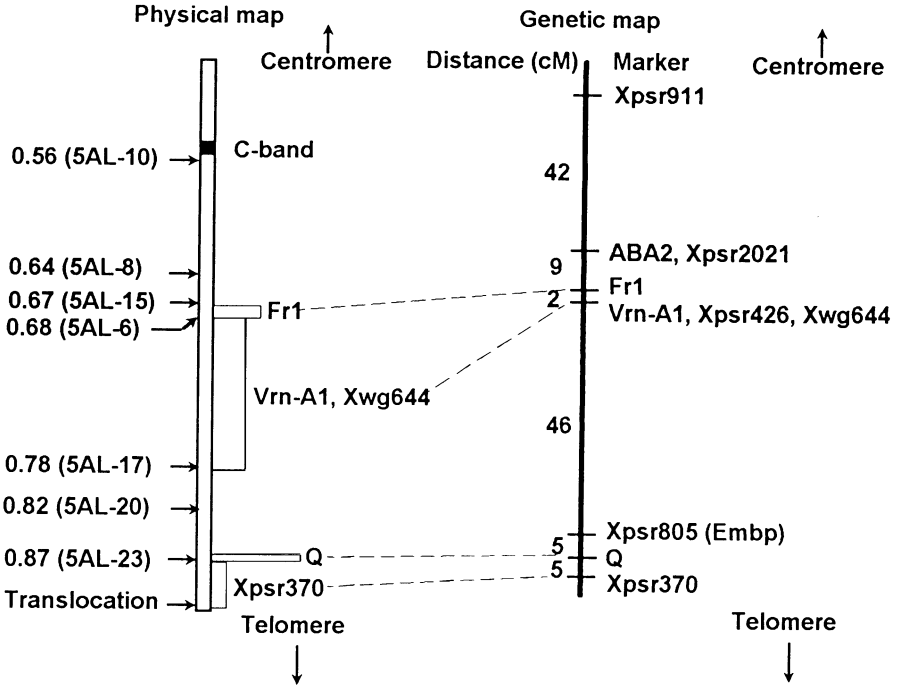

Fig. 3 Comparison between the physical and genetic maps of 5AL. Fraction lengths (FL) and deletion stock numbers are indicated on the *left* of the physical map. Marker names are indicated on the *right* of the chromosomes. The genetic map is based on Galiba et al. (1995) and Snape et al. (1997)

significant. This clearly indicates that the difference in frost resistance observed between groups I and II of the deletion lines was due to the deletion of *Fr1*, located in the 0.67 and 0.68 breakpoint interval. Thus, the physical mapping of *Vrn-A1* and *Fr1* shows that these two loci are linked, but separable. *Fr1* is more proximal to the centromere than *Vrn-A1*, confirming the results recently published by Snape et al. (1997).

The comparison of loci *Fr1*, *Vrn-A1*, *Xwg644*, *Q* and *Xpsr370* on both the genetic and physical maps (Fig. 3) shows that the linear order is identical (Gill et al. 1996; Snape et al. 1997). Additionally, the loci *Q* and *Xpsr370* are close to each other on both the maps, and the distance betwen *Q* and *Fr1* is large on both the physical and genetic maps. It is evident from both maps that *Vrn-A1* is close to molecular marker *Xwg*644. For this reason *Xwg644* can be recommended as a molecular marker in selection for the characters vernalization insensitivity and frost resistance.

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