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## Genetic map locations for orthologous *Vp1* genes in wheat and rice

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**Abstract** Chromosome locations for gene orthologues of the dormancy-related maize transcription factor VIVIPAROUS-1, encoded by the *Vp1* locus on maize chromosome 3, were determined in wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) via linkage to markers on existing molecular maps using a cDNA of a wheat *Vp1* orthologue as a probe in genomic Southern analyses. *Vp1*-orthologous loci were detected on the long arms of wheat chromosomes 3A, 3B and 3D [*Xlars10* (*taVp1*) loci] and rice chromosome 1 (*osVp1*), in line with previous evidence of synteny between these regions of the rice and wheat genomes and chromosome 3 of maize. The wheat loci mapped some 30 cM from the centromeres and some 30 cM proximal to the red grain (*R*) loci that control seed colour and coat-imposed dormancy. This unequivocal, genetic separation of the *Vp1* and *R* loci may offer an opportunity for improving resistance to pre-harvest sprouting in wheat by combining the coat-imposed dormancy associated with red seed colour and true embryo dormancy regulated by *Vp1*.

**Key words** Maize · Vivipary · Grain colour · Dormancy

### Introduction

Pre-harvest sprouting (PHS) in bread wheat is detrimental to grain quality because  $\alpha$ -amylase in flour,

produced in the early stages of germination, causes dextrin production during baking and a sticky crumb structure (Buchanan and Nicholas 1980). PHS can be particularly serious in susceptible varieties if rain falls just prior to, and causes a delay in, harvest. Attempts to breed wheats with a higher degree of seed dormancy have been hampered because of the unpredictable nature of the environmental factors that interact with genotype and affect this trait (Derera 1989). Relatively little is known about the molecular events underpinning the expression of dormancy in cereals, however the transcription factor VIVIPAROUS-1 (encoded by the *Vp1* gene) plays a critical role in the induction and maintenance of dormancy in maize (McCarty et al. 1991). Maize mutants lacking VP1 activity are viviparous, i.e. the immature embryos germinate precociously on the cob. The action of a closely related gene, *Abi3* from *Arabidopsis thaliana*, also confers dormancy in maturing seeds (Giraudat et al. 1992). Genes orthologous to *Vp1* and *Abi3* have been cloned from tobacco (Phillips and Conrad 1994), rice (Hattori et al. 1994), bean (Bobb et al. 1995), resurrection plant *Craterostigma plantagineum* (Chandler and Bartels 1997) and the wild oat *Avena fatua* (the *afVp1* cDNA, Jones et al. 1997). A significant role for *afVp1* in the control of dormancy was indicated by the finding that steady-state levels of *afVp1* mRNA in the grains of four inbred lines of *A. fatua* correlated with the depth of dormancy, as assessed by the length of after-ripening time required to break dormancy, being high in grains from the two dormant lines and low in grains from the two non-dormant lines (Jones et al. 1997).

In maize, *Vp1* has been mapped to the long arm of chromosome 3 (Burr et al. 1993). Many molecular markers that map to this distal region of maize chromosome 3 also map to rice chromosome 1, and their relative positions along the chromosomes are conserved (Ahn and Tanksley 1993). Restriction fragment length polymorphism (RFLP) markers that map to wheat group 3 chromosomes have also been shown to

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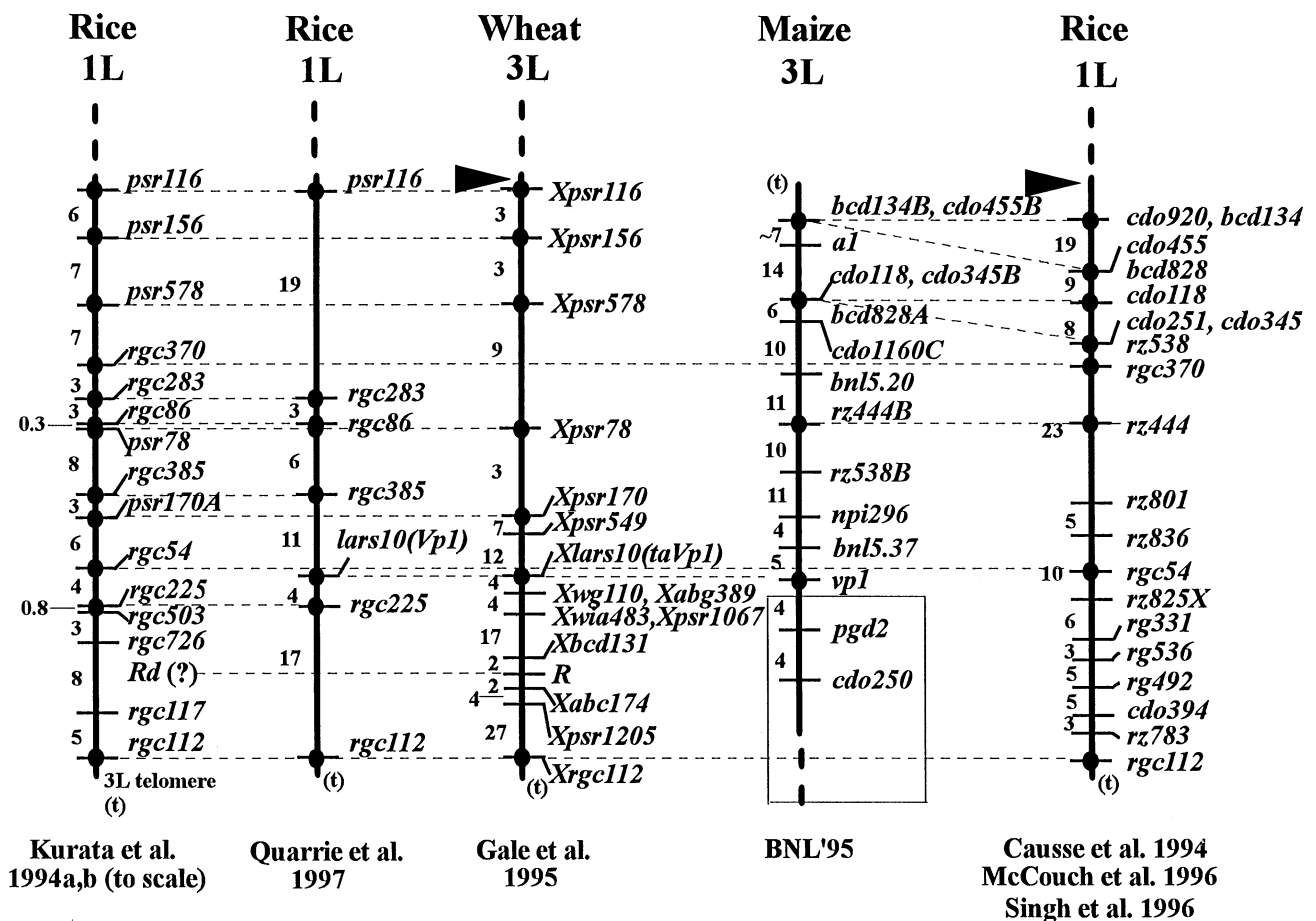
map to similar relative positions on rice chromosome 1 (Kurata et al. 1994a; Van Deynze et al. 1995). The available evidence thus suggests that orthologues of genes present on most of the long arm of maize chromosome 3, for example *Vp1*, are located on the long arm of wheat group 3 chromosomes. Orthologues of *Vp1* have been detected on chromosome arms 3EL and 7E $\beta$  of *Lophopyrum elongatum*, a wild relative of wheat, but mapping *Vp1* in wheat itself was not achieved using a maize cDNA clone as a Southern probe (Cadle et al. 1994).

**Fig. 1** Genetic maps of long arms of wheat group 3 chromosomes, rice chromosome 1 and maize chromosome 3. Locations of *Vp1* in maize and orthologues *taVp1* (wheat) and *osVp1* (rice) are shown relative to other markers with conserved locations across the genomes of these three cereal crops. From left to right, the rice maps are based on those of Kurata et al. (1994a,b) and Quarrie et al. (1997), wheat on the consensus map of Gale et al. (1995), maize (inverted relative to wheat and rice) on the BNL'95 map (Anon 1995) and rice on the map of Causse et al. (1994) with additional markers mapped by McCouch et al. (1996) and the centromere positioned proximal to *cdo920* as demonstrated by Singh et al. (1996). Homoeologous marker loci present on two or more maps are joined by dotted lines. Arrows indicate centromeres. Figures to the left of each map denote intervals in centiMorgans. Some markers present on the published maps have been omitted for the sake of clarity. The boxed region on the maize map indicates the region of uncertainty regarding collinearity between maize and the other grass genomes

Amongst wheat cultivars the red grain colour phenotype is associated with dormancy, with red-grained wheats tending to have greater resistance to PHS than white-grained varieties. The red pigment is deposited during the late stages of development in the testa layer and is thought to consist of phlobaphene (a polymerised flavonoid). Interestingly, the red grain colour (*R*) loci have also been mapped to the long arms of group 3 chromosomes (Flintham and Humphray 1993; Flintham and Gale 1996; Nelson et al. 1995). As *Vp1* is involved in flavonoid pigmentation in maize seed tissues, the aim of this study was to establish the genetic relationship between the *R* and *taVp1* loci.

### Map position for *Vp1* in wheat

An *afVp1* cDNA clone from wild oat (Jones et al. 1997) had sufficient sequence identity to allow the isolation of a wheat cDNA [*lars10(taVp1)*, R. S. McKibbin et al., unpublished data]. These clones share 81% DNA sequence identity over the coding region. In the present study the *lars10* clone was used to determine the chromosomal locations of *taVp1* loci in hexaploid wheat and to identify RFLPs for genetic mapping. In genomic Southern analysis only three bands were detected, indicating that the *taVp1* gene is present as a single copy



in each of the A, B and D genomes. The presence of one *taVp1* homoeolocus on each of the group 3 chromosomes was confirmed in Southern analysis of nullisomic-tetrasomic lines of 'Chinese Spring' wheat. Two RFLPs were identified in *EcoRI* digests of genomic DNA from 'Chinese Spring' and 'Synthetic' wheats, and the corresponding *Xlars10* (*taVp1*) loci were mapped on chromosomes 3A and 3D using the F<sub>2</sub> population from the 'Chinese Spring' × 'Synthetic' cross described by Devos et al. (1992). The 3B orthologue was not polymorphic in digests with *EcoRI*, *EcoRV*, *DraI* or *HindIII* and was not mapped. Mapping data were incorporated into the existing linkage map for this cross using MAPMAKER version 3.0. On chromosome 3A, *Xlars10* (*taVp1*) is positioned distal to marker *Xpsr549* by 12.1 cM and proximal to marker *Xabg389* by 3.9 cM. The 3D homoeolocus is distal by 12.1 cM to *Xpsr170* and proximal to *Xpsr1067* by 5.5 cM. These two gene locations are consistent with the location shown in Fig. 1, in the interval *Xpsr549*-*Xwg110* on the wheat group 3 consensus map of Gale et al. (1995), about 30 cM from the centromere. *Xlars10*(*taVp1*) shows clear recombination with the *R* loci which map about 60 cM from the centromere. Thus, although *Xlars10*(*taVp1*) and *R* are linked, they are clearly distinct genes.

#### Map position for *Vp1* in rice

Mapping in rice was carried out using a population of 123 F<sub>2</sub> plants from a cross between IR20 (*indica*) and 63–83 (*japonica*) lines and the data set of Quarrie et al. (1997). The region of the *taVp1* cDNA clone used for the probe in Southern analysis shared 74% identity at the DNA level to the *OsVp1* gene isolated by Hattori et al. (1994). In addition, as only one band was detected in Southern analysis it almost certainly represented *OsVp1*. The RFLP band mapped onto rice chromosome 1 in a position collinear to that in wheat. This is illustrated in Fig. 1, which shows the positions of *Vp1* orthologues relative to linked markers on the rice, wheat and maize maps. The order of these markers, including the relative positions of *Vp1* orthologues, are conserved in these monocot genomes (the main difference being in the long arm of maize chromosome 3, along which the marker order is a direct inversion relative to that of rice and wheat). Collinearity is less clear in the region proximal to *Vp1* on maize chromosome 3 (boxed region, Fig. 1). The *Rd* gene of rice has been depicted on the rice map as being orthologous to the *R* genes of wheat, mapping to a locus at least 8 cM distal to *osVp1* (Kurata et al. 1994a). However, a consideration of the RFLP map of Tsunematsu et al. (1996) and the integration of the rice morphological map with several of their RFLP markers (Ideta et al. 1996) suggests that the location of *Rd* may be too proximal for it to be an orthologue of the wheat *R* gene. More linkage information for markers common to both

species will be needed to clarify whether *R* and *Rd* are truly orthologous.

#### Conclusion

Physiological evidence from a range of plants indicates that seed dormancy may be controlled by two ontogenetically distinct systems. One level of control operates via maternal effects and is commonly referred to as 'coat-imposed' dormancy. In contrast, zygotic effects are termed 'embryo' or 'true' dormancy. The *Vp1* gene is known to exercise zygotic control of dormancy during seed maturation in maize, and *R* genes exert maternal control of dormancy loss during after-ripening of wheat. No obvious orthologue of the *R* genes has been mapped to the relevant area of maize chromosome 3, however we show that both wheat and rice carry *Vp1* orthologues in loose genetic linkage with previously mapped genes controlling seedcoat pigments (*R* loci in wheat, the *Rd* locus in rice). We propose that this genetic separation between the two loci reflects separate roles for *taVp1* and *R*, respectively, in zygotic and maternal dormancy mechanisms. To date no quantitative trait loci (QTLs) mapping in the proximity of *Xlars10* have been detected in wheat (Anderson et al. 1993). QTLs for malting quality that map to the long arm of chromosome 3 in barley vary in position between genetic crosses and different environments, and it is not clear whether these QTLs reflect effects on dormancy (compare, for example, Hayes et al. 1993 and Oberthur et al. 1995). Therefore, more direct information about the role of *taVp1* in wheat seed dormancy will be needed to test whether beneficial alleles at both of these loci can be combined to produce wheats with greater resistance to PHS.

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