RESEARCH PAPER

Isolation and characterization of Viviparous-1 genes in wheat cultivars with distinct ABA sensitivity and pre-harvest sprouting tolerance

Y. Yang1,2, Y. Z. Ma1, Z. S. Xu1, X. M. Chen1, Z. H. He1,3,*, Z. Yu2, M. Wilkinson4, H. D. Jones4, P. R. Shewry4 and L. Q. Xia1,*

1 Institute of Crop Science/National Wheat Improvement Center/The National Key Facility for Crop Gene Resources and Genetic Improvement, Chinese Academy of Agricultural Sciences (CAAS), No. 12 Zhongguancun South Street, Beijing 100081, China
2 Agronomy College, Inner Mongolia Agricultural University, No. 306 Zhaowuda Road, Hohhot 010018, Inner Mongolia, China
3 CIMMYT China Office C/O CAAS, CAAS, Beijing 100081, China
4 Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

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Abstract

Pre-harvest sprouting (PHS) of wheat reduces the quality and economic value of grain, and increasing PHS tolerance is one of the most important traits in wheat breeding. Two new Vp-1B alleles related to PHS tolerance were identified on the 3BL chromosome of bread wheat and were designated Vp-1Bb and Vp-1Bc. Sequence analysis showed that Vp-1Bb has a 193 bp insertion and Vp-1Bc has a 83 bp deletion located in the third intron region of the Vp-1B gene, and that they shared 95.43% and 97.89% similarity, respectively, with the sequence of AJ400713 (Vp-1Ba) at the nucleotide level. Their sequences were deposited in the GenBank under the accession numbers DQ517493 and DQ517494. Semi-quantitative RT-PCR analysis showed that alternatively spliced transcripts of the Vp-1A, Vp-1B, and Vp-1D homologues were present and there were no differences in the splicing patterns or abundances of Vp-1A and Vp-1D from embryos 35 d after pollination between PHS-tolerant and -susceptible cultivars. Although Vp-1Ba, Vp-1Bb, and Vp-1Bc could each produce a set of transcripts, only one was correctly spliced and had the capacity to encode the full-length VP1 protein and was more highly expressed with Vp-1Bb and Vp-1Bc than with Vp-1Ba. Comparison of the expression patterns of Vp-1Ba, Vp-1Bb, and Vp-1Bc on different days after pollination also revealed that the expression of these genes was developmentally regulated. Furthermore, genotypes with different levels of tolerance to PHS respond differently to ABA exposure and differences in transcript levels of Vp-1Ba, Vp-1Bb, and Vp-1Bc were observed after ABA treatment. The results indicated that insertion or deletion in the third intron region might affect the expression of the Vp-1B gene and its sensitivity to ABA, and thus resistance to PHS.

Key words: ABA responsiveness, allele, expression, pre-harvest sprouting, RT-PCR, Triticum aestivum L., Vp-1.

Introduction

Pre-harvest sprouting (PHS) of grain while it is still in the ear, usually in response to damp conditions, is due to an early disruption of seed dormancy (Bewley, 1997; Lenton, 2001; Appels et al., 2003; Finch-Savage and Leubner-Metzger, 2006). The main effects of PHS on wheat are a lower yield due to harvest losses and, more importantly, a reduction in end-product quality. Flour obtained from sprouted grains loses its viscosity due to starch breakdown, and baked goods from such flour are smaller in volume and have a compact, sticky crumb structure.

* To whom correspondence should be addressed. E-mail: zhhe@public3.bta.net.cn and xialq@mail.caas.net.cn

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(Humphreys and Noll, 2002). Producers suffer great losses when wheat damaged by sprouting is sold at a discount; millers are faced with reduced flour yields and bakers encounter problems in processing and quality due to starch damage (Derera et al., 1977; Lenton, 2001).

A number of genes and QTLs involved in PHS tolerance or seed dormancy have been found and mapped in wheat (Flintham et al., 2002; Appels et al., 2003; Lohwasser et al., 2005). Among them, Viviparous-1 (Vp-1) homologues play an important role in seed maturation, dormancy, and desiccation (McCarty et al., 1989, 1991; Giraudat et al., 1992; Bailey et al., 1999). The Vp-1 gene is a major regulator of late embryo development in maize and inactivation of this locus leads to disruption of embryo maturation, resulting in the promotion of germination of embryos while still attached to the cob (vivipary) (McCarty et al., 1991). The phenotypes of vp-1 mutants show that this locus performs two distinct functions: to promote embryo maturation and embryo dormancy, and, simultaneously, to repress germination (McCarty et al., 1991; Hoecker et al., 1995). The Arabidopsis ABI3 gene encodes a transcriptional factor homologous to maize Vp-1 (McCarty et al., 1991; Giraudat et al., 1992) and acts with ABI5 to control embryonic gene expression and seed sensitivity to ABA (Lopez-Molina et al., 2002; Nakashima et al., 2006). Mutants in the Arabidopsis ABI3 gene result in a similar phenotype to that observed in maize vp-1 embryos; the apical meristem is activated prematurely during embryo maturation, storage products do not accumulate, and the promoter of the germination-related CAB3 gene is activated before seed shedding (Nambara et al., 1994). The Arabidopsis ABI3 and maize Vp-1 mutants do not have altered ABA levels in the embryo, but show reduced sensitivity to ABA, leading to precocious germination (Zhang et al., 2006b).

Orthologues of Vp-1 have been cloned from rice (OsVp1; Hattori et al., 1994), wild oat (AfVp1; Jones et al., 1997), and several dicotyledonous species such as Arabidopsis (ABI3; Giraudat et al., 1992) and poplar (PtABI3; Rohde et al., 2002). Biochemical analyses have shown that the Vp1 protein functions as a transcription factor, containing both transcriptional activation and DNA binding domains (McCarty et al., 1991; Suzuki et al., 1997). Comparisons with orthologues from other species identified four highly conserved amino acid domains: A1, which is an acidic region at the N-terminus of the protein, and three basic domains designated B1, B2, and B3 (Giraudat et al., 1992). The B1 and B2 domains play important roles in nuclear location and non-specific interactions with other proteins and enhance the DNA binding activity of other transcription factors (Giraudat et al., 1992; Ezcurra et al., 2000; Nakamura and Toyama, 2001), whereas the C-terminal B3 domain shows highly cooperative sequence-specific DNA binding to the RY/

Sph cis elements present in the promoter regions of related genes (Suzuki et al., 1997). The multiple domains of ABI3 enable it to function either as an activator or a repressor depending on the promoter context (Zhang et al., 2006b). The N-terminal A1 domain is responsible for ABA-dependent co-activation and repression activities (Hoecker et al., 1995; Carson et al., 1997), whereas the C-terminal B3 domain is essential for activation of a subset of genes (Carson et al., 1997).

Vp-1 homologues were mapped in wheat to the long arms of group 3 chromosomes at a location which is conserved relative to the Vp-1 gene in maize and the OsVp1 gene in rice (Bailey et al., 1999). The Vp-1 homologues from wheat show the same four highly conserved domains that are present in the Avena fatua, rice, and maize proteins (Nakamura and Toyama, 2001). The structure and expression of the three Vp-1 homologues in common wheat have been determined, showing that each has the potential to encode a full-length functional protein. However, alternative splicing of pre-mRNA leads to a diverse population of RNAs, which in most cases encode aberrant translation products (McKibbin et al., 2002). Comparison of the transcript structures of wheat and closely related wild and ancestral species suggest that mis-splicing of Vp-1 genes might occur before the evolution and domestication of common wheat (McKibbin et al., 2002). Further studies of Vp-1 transcript structure within the Triticeae tribe revealed that alternative splicing is also present in barley, rye, Brachypodium pinnatum, Agropyron repens, Elymus repens, and Thino- pyrum scripeum, with Triticum and Aegilops having similar alternative spliced forms with highly conserved exon/intron borders for intron 1 (Wilkinson et al., 2005). However, only one transcript exists for Vp-1, and its level in mature embryos of dormant and non-dormant wheat cultivars showed positive correlations with seed dormancy and embryo sensitivity to ABA (Nakamura and Toyama, 2001). In Avena fatua, expression of AfVp1 is controlled by the interaction between the environment and genotype, with a close correlation observed between AfVp1 mRNA levels and seed dormancy (Jones et al., 1997). Transgenic wheat seeds expressing the AfVp1 cDNA also showed increased dormancy and tolerance to PHS (McKibbin et al., 2002).

In order to exploit novel genetic resources of Vp-1 for PHS tolerance in bread wheat, the Vp-1 homologues were isolated from three common wheat cultivars which respond differently to ABA and tolerate PHS differently using genome-specific primers. Sequence analyses indicated that two new Vp-1 alleles associated with PHS tolerance were explored on the 3BL chromosome. The expression patterns of all three Vp-1B alleles at different stages of seed development were also compared and the responses of the three cultivars to ABA treatment were determined.
Materials and methods

Plant materials

Three common wheat cultivars, Yongchuanbaimai (a typical PHS-tolerant Chinese landrace, with a germination index of 0.14 averaged from three locations, i.e. Beijing, Anyang, and Zhenzhou in the 2005–2006 wheat season), Xinong 979 (a PHS-tolerant cultivar with a germination index of 0.50), and Zhongyou 9507 (a PHS-susceptible cultivar with a germination index of 0.84), were used in this study. In Beijing, they were planted at the CAAS experimental station, each in three rows, in late September 2005. The trials were kept free of weeds and diseases by two applications of PHS-susceptible cultivar with a germination index of 0.50), and Zhongyou 9507 (a PHS-tolerant Chinese landrace, with a germination index of 0.14

The primer sets used for amplification of the genomic sequence and semi-quantitative RT-PCR analysis of Vp-1A, Vp-1B, and Vp-1D available in GenBank (http://www.ncbi.nlm.nih.gov) under the accession numbers AJ400712, AJ400713, and AJ400714, respectively. Twelve pairs of primers, Vp-1AF1/R1, Vp-1AF2/R2, Vp-1AF3/R3, Vp-1AF4/R4, Vp-1BF1/R1, Vp-1BF2/R2, Vp-1BF3/R3, Vp-1BF4/R4, Vp-1DF1/R1, Vp-1DF2/R2, Vp-1DF3/R3, and Vp-1DF4/R4, were designed to amplify fragments of the Vp-1A, Vp-1B, and Vp-1D genes (Table 1). Another three pairs of genome-specific primers, RTVp-1AF/R, RTVp-1BF/R, and RTVp-1DF/R (Table 1), were designed to perform RT-PCR of the Vp-1A, Vp-1B, and Vp-1D genes, respectively. The wheat ACTIN gene was included as an internal control in each reaction in order to normalize the expression level of Vp-1 genes using a primer pair designed to amplify a 410 bp product (Table 1).

DNA extraction and PCR amplification

Genomic DNA was isolated from kernels using the method described by Gale et al. (2001). For each genotype, three samples (two from individual seeds and one from a composite sample of three to six seeds) were amplified in order to verify the purity of the sample. PCR reactions were performed in an MJ Research PTC-200 thermal cycler in a total volume of 50 μl including 10× PCR buffer, 125 μM each of dNTP, 8 pmol of each primer, 2.0 units of rTaq polymerase, and 100 ng of template DNA. The following conditions for PCR amplification were 94 °C for 5 min, followed by 36 cycles of 94 °C for 1 min, 53–66 °C for 1 min, and 72 °C for 1 min, with a final extension of 72 °C for 10 min. Amplified PCR fragments were separated on a 1.5% agarose gel, stained with ethidium bromide, and visualized using UV light.

RNA isolation and semi-quantitative RT-PCR analysis

Total RNA was extracted from embryos at different developmental stages as described by Chang et al. (1993). RNA concentration and quality were determined by spectrophotometer at 260 nm and by the A260/A280 ratio, respectively. RNA integrity was assessed by comparing the relative intensities of the 28S and 18S rRNA bands in 1.2% (w/v) agarose gels containing 2.2 M formaldehyde. cDNA was synthesized from 5 μg of the total RNA using M-MLV reverse transcriptase (TakaRa) with random hexamer primer oligo d(T)18 according to the manufacturer’s instructions.

Semi-quantitative RT-PCR reactions were performed in an MJ Research PTC-200 thermal cycler in a total volume of 25 μl, using the protocol in the instruction manual of the GC PCR kit (Clontech), including 1 μl of the cDNA template. The reaction conditions were 94 °C for 5 min, followed by 36 cycles of 94 °C for 1 min, 60–68 °C for 1 min, and 72 °C for 1 min, with a final extension of 72 °C for 10 min. The RT-PCR products were separated on a 2.0% agarose gel. Values were normalized with the amplification rate of the ACTIN gene as a constitutively expressed internal control. Three replicates were performed for each sample.

DNA sequencing and analysis

The PCR and cloned products were sequenced from both strands by Shanghai Sangon Biological Technology Co. Ltd (http://www.sangon.com). Sequence analysis and characterization were performed using software DNAMAN (http://www.lynon.com).

| Primer set          | Upstream (5′−3′)   | Downstream (5′−3′)   | Annealing (°C) | Fragment size (bp) |
|---------------------|--------------------|----------------------|-----------------|--------------------|
| Vp-1AF1/R1          | ATCCAAACCGCGGCTTCCCTCAAGA | CAAAATCGATCGATGGAGATCAG | 56              | 1108               |
| Vp-1AF2/R2          | AGGCAGATCCGGCACTTCTCA   | CTGTTGATTTTGGCAACCAGCAA | 53              | 912                |
| Vp-1AF3/R3          | TGGAGATCCGGCACTTCTCA   | CCAGAGCTTCTCCCGAGCA   | 67              | 1253               |
| Vp-1AF4/R4          | GAAATGCTGACGGGGGTTGA   | GCAATGCTAGCTAACTAGG   | 58              | 1207               |
| Vp-1BF1/R1          | ATCCAAACCGCGGCTTCCCTCAAGA | CAAAATCGATCGATGGAGATCAG | 55              | 960, 1153, 877     |
| Vp-1BF2/R2          | AGGCAGATCCGGCACTTCTCA   | CTGTTGATTTTGGCAACCAGCAA | 65              | 1227               |
| Vp-1BF3/R3          | GAAATGCTGACGGGGGTTGA   | GCAATGCTAGCTAACTAGG   | 66              | 911                |
| Vp-1BF4/R4          | ATCCAAACCGCGGCTTCCCTCAAGA | CAAAATCGATCGATGGAGATCAG | 60              | 1025               |
| Vp-1DF1/R1          | ATCCAAACCGCGGCTTCCCTCAAGA | CAAAATCGATCGATGGAGATCAG | 67              | 1282               |
| Vp-1DF2/R2          | AGGCAGATCCGGCACTTCTCA   | CTGTTGATTTTGGCAACCAGCAA | 60              | 1033               |
| Vp-1DF3/R3          | GAAATGCTGACGGGGGTTGA   | GCAATGCTAGCTAACTAGG   | 58              | 960, 1153, 877     |
| RTVp-1AF/R          | ATCCAAACCGCGGCTTCCCTCAAGA | CAAAATCGATCGATGGAGATCAG | 68              | 672                |
| RTVp-1BF/R          | ATCCAAACCGCGGCTTCCCTCAAGA | CAAAATCGATCGATGGAGATCAG | 60              | 672                |
| RTVp-1DF/R          | ATCCAAACCGCGGCTTCCCTCAAGA | CAAAATCGATCGATGGAGATCAG | 62              | 672                |
| ACTIN up/down       | GTTTCCTGGAAATTCGGATAGCA   | CATTATTTCTACAGCAGAGCAAGC | 62              | 410                |
Results

Isolation and sequence analysis of the three Vp-1 homologues in cultivars differing in PHS tolerance

Full sequences of the three Vp-1 homologues were isolated using genome-specific primers (Table 1). Sequence alignment showed that only the Vp-1B gene gave polymorphic fragments in the three cultivars with the primer set Vp-1BF2/R2, whereas no polymorphism was detected in the Vp-1A and Vp-1D genes (data not shown). The Vp-1B gene in Yongchuanbaimai (which showed consistently higher PHS resistance over years and locations) was 4227 bp with a 193 bp insertion in the third intron, whereas that in the newly released PHS-resistant cultivar Xinong 979 was 3942 bp with an 83 bp deletion in the same intron. Neither of these mutations was present in Zhongyou 9507 which is susceptible to PHS (Fig. 1).

comparison with the TIGR plant repeat database (http://

Fig. 1. Alignment of partial wheat Vp-1B genomic sequence in PHS-resistant cultivars Yongchuanbaimai (Y) and Xinong 979 (X), and PHS-susceptible cultivar Zhongyou 9507 (Z). Sequence alignment indicated that, compared with Z, Y has a 193 bp insertion, while X has an 83 bp deletion. Sequences underlined indicated the postulated exon–intron boundaries.
tigrblast.tigr.org) revealed that the 193 bp insertion was homologous with the maize and barley gypsy/Ty3 retrotransposon Tekay (AF050455 and AY040832) with a probability of 0.92, whereas the 83 bp deleted region had high similarity to a transposon-like sequence in rice (AY090462.1), with a probability of 0.9999. These two new Vp-1B alleles from the PHS-tolerant lines were designated Vp-1Bb and Vp-1Bc, respectively, according to the 2005 Supplement of the Wheat Gene Catalogue (McIntosh et al., 2005). Sequence analysis showed that the Vp-1Bb and Vp-1Bc alleles had 95.43% and 97.89% similarity to the sequence of AJ400713 (Vp-1Ba) at the nucleotide level. These two sequences were deposited in the GenBank under accession numbers of DQ517493 and DQ517494.

**Expression characterization of Vp-1A, Vp-1B, and Vp-1D in three cultivars differing in PHS tolerance**

In order to define the expression patterns of the three Vp-1 homologues and their relationship with PHS tolerance, semi-quantitative RT-PCR analysis was carried out to determine the expression levels of Vp-1A, Vp-1B, and Vp-1D in the three wheat cultivars differing in PHS tolerance, using the ACTIN gene as an internal control. A set of transcripts for Vp-1A, Vp-1B, and Vp-1D was detected in 35 DAP embryos but only one of these transcripts had the capacity to encode the correct protein product (data not shown). Furthermore, no differences were observed in the transcript abundances of Vp-1A and Vp-1D between the PHS-susceptible and -resistant cultivars (Fig. 2). Although all the three Vp-1B alleles tested in this study had alternatively spliced transcripts, different expression levels of the correctly spliced transcripts for Vp-1Ba, Vp-1Bb, and Vp-1Bc were observed, with Vp-1Bb being the most abundant and Vp-1Ba the least abundant (Fig. 3).

Semi-quantitative RT-PCR was also employed to determine the expression patterns of the Vp-1B alleles at different seed developmental stages (25, 30, and 35 DAP). As shown in Fig. 3, no transcripts of the three Vp-1B alleles were detected in 25 DAP embryos and only single and presumably correctly spliced transcripts of each were present in 30 DAP embryos, with Vp-1Bc having the highest transcript abundance and Vp-1Ba the lowest. Although mis-spliced transcripts were present in 35 DAP embryos, the majority of the transcripts were correctly spliced. The Vp-1Bb and Vp-1Bc alleles had higher abundances of correctly spliced transcripts than Vp-1Ba, with the level being highest in the Vp-1Bb allele and lowest in Vp-1Ba, as observed previously by Nakamura and Toyama (2001). It was also observed that mis-spliced transcripts were present only at the late stage of seed development with the correctly spliced transcripts accumulating during the 25–35 DAP period which corresponds to the end of the exponential accumulation of kernel components. This was consistent with the development of seed dormancy and indicated that expression of the Vp-1B gene and its alleles might be developmentally regulated.

**Expression characterization of three Vp-1B alleles upon ABA treatment**

Semi-quantitative RT-PCR was used to determine the expression of the three Vp-1B alleles in 35 DAP embryos stratified with or without 30 μM ABA solution in order to investigate the effects of insertion or deletion in the third intron on ABA responsiveness. As shown in Fig. 4, soaking in water for 2 d in the absence of ABA was sufficient to reduce the expression of all the three Vp-1B alleles to below detectable levels, indicating that the embryos developed into autotrophic seedlings. By contrast, in the presence of 30 μM ABA, all three alleles showed detectable transcripts in 2-d-old germinating embryos with the expression level of Vp-1Bb higher than that of the other two. However, the transcript levels of both Vp-1Bb and Vp-1Bc then decreased, falling to below detectable levels after 4 d of treatment with 30 μM ABA, whereas the level of Vp-1Ba transcripts remained unchanged, suggesting that the three alleles differed in their response to ABA exposure.

**Discussion**

The effect of insertion and deletion on expression of Vp-1B and PHS tolerance

Compared with Vp-1Ba, the new allele Vp-1Bb had a 193 bp insertion in the third intron at position 2496 bp of AJ400713 (Vp-1Ba), whereas Vp-1Bc had an 83 bp

![Fig. 2. Semi-quantitative RT-PCR analysis of Vp-1A and Vp-1D in 35 DAP embryos of three cultivars differing in PHS tolerance. Lanes: M, D-2000 DNA marker; 1, Zhongyou 9507; 2, Xiong 979; 3, Yongchuanbaizai. (I) Comparison of the transcript levels of Vp-1A in three cultivars; (II) comparison of the transcript levels of Vp-1D in three cultivars.](https://academic.oup.com/jxb/article/58/11/2863/609057/pdf)
deletion at position 2712–2795 within the same intron. The inserted sequence had several putative exon–intron boundaries (GT–AG), while the deletion had characteristics typical of an intron, starting at 5' GT and ending at 3' AG (Fig. 1) (Yan et al., 2000; Alexei et al., 2003). The precise origin of the alternatively spliced forms is not known, but they may arise from inefficient splicing of potential intron–exon boundary sequences (Wilkinson et al., 2005). In addition, as discussed previously, the 193 bp insertion is highly homologous with the maize and barley gypsy/Ty3 retrotransposon Tekay (AF050455 and AY040832), whereas the 83 bp deletion is similar to a transposon-like sequence in rice (AY090462.1). In maize, many spontaneous waxy mutations are caused by transposable elements (Fedoroff et al., 1983; Wessler and Varagona, 1985; Wessler et al., 1987). It is therefore possible that the insertion and deletion may have occurred separately during the evolution of the alleles. Furthermore, a reverse repeat structure is present at a position from 2705 bp to 2801 bp in Vp-1Ba (Fig. 5), meaning that the insertion and deletion might affect the pre-mRNA structure and the expression level of the correctly spliced Vp-1 transcripts. The insertion and deletion in the third intron region of Vp-1B was indeed associated with increased levels of correctly spliced Vp-1B transcript in 35 DAP mature embryos. Moreover, further studies have shown that most of PHS-resistant genotypes have either the Vp-1Bb or the Vp-1Bc allele compared with PHS-susceptible genotypes, and that genotypes with the insertion are more tolerant of PHS than those with the deletion. The majority of genotypes with the 193 bp insertion were PHS-resistant landraces with germination rates below 8% (Y Yang, XL Zhao, LQ Xia, XM Chen, XC Xia, Z Yu, ZH He, unpublished results). In recent years, it has become clear that introns are functionally active participants of gene and genome function (Erkkila and Ahokas, 2001; Fiume et al., 2004; Fu et al., 2005; Sjakste et al., 2006), they encode for regulatory elements with autocatalytic or alternative splicing activity, controlling gene transcription, and are sponsors of mobility by acting as either endonucleases or reverse transcriptases (Lewin, 2004). For example, in barley, a 126 bp insertion/deletion event (indel) in the 5′-region of intron III in the β-amylase gene is associated with allelic variants of the gene-encoding enzymes of correspondingly low or high thermo-stability (Erkkilä and Ahokas, 2001), and deletions in the promoter region and first intron of the waxy gene similarly result in decreased gene expression and reduced amylose levels (Domon et al., 2002; Patron et al., 2002).

McKibbin et al. (2002) reported that correctly spliced transcripts represented a small proportion of the wheat Vp-1 mRNA population with only three of the seven...
TaVp-1 cDNA clones studied in detail having the potential to encode full-length VP1 proteins. Furthermore, of the 84 random RT-PCR clones sequenced, 69% were shown to be properly spliced and from the B genome (Wilkinson et al., 2005). In this study, genome-specific primers were used to show that mis-spliced transcripts from the B genome are present only at the late seed developmental stage (35 DAP), with the majority of the transcripts being correctly spliced. Furthermore, the levels of correctly spliced transcripts increased during seed development, suggesting that alternative splicing might also be developmentally regulated. It was also shown that the genotype with higher PHS tolerance had higher transcript abundance than a PHS susceptible genotype, which is consistent with the close correlation between transcript abundance and the level of seed dormancy as reported by Nakamura and Toyama (2001). Moreover, no differences in expression between Vp-1A and Vp-1D were observed in 35 DAP embryos of PHS-tolerant and -susceptible genotypes although alternative splicing also existed for Vp-1A and Vp-1D transcripts.

**ABA sensitivity of the three Vp-1B alleles**

ABA plays an important role in the adaptation of vegetative tissue to abiotic environmental stresses such as drought and high salinity as well as in seed maturation and dormancy (Gubler et al., 2005; Nakashima et al., 2006). Maize VP1 and Arabidopsis ABI3 are orthologous transcription factors that regulate key aspects of plant seed development and ABA signalling (Suzuki et al., 2003). Null alleles of ABI3 and Vp-1 resulted in loss of ABA sensitivity, leading to non-dormancy or vivipary in Arabidopsis and maize (McCarty et al., 1989; Nambara et al., 1994). Moreover, there is evidence that ABA levels in mature wheat embryos are similar in both PHS-sensitive and -resistant cultivars and that sprouting behaviour is related more to the extent of ABA responsiveness of embryos than to ABA levels (Walker-Simmons and Sesing, 1990). A positive correlation between the degree of seed dormancy, ABA sensitivity, and the level of Vp-1 transcripts in wheat mature embryos was also observed by Nakamura and Toyama (2001). In the present study, a genotype with the Vp-1Bb allele was most sensitive to ABA treatment with transcript levels in mature embryos after 2 d exposure to ABA being higher than the other two genotypes. The transcript levels in this genotype then fell and were undetectable after an additional 2 d of treatment. Although the genotype with Vp-1Ba was less sensitive to ABA, it still showed comparable transcript levels and remained unchanged after the additional 2 d treatment. The ABA responsiveness of the genotype with the Vp-1Bc allele fell between those with the Vp-1Ba and Vp-1Bb alleles, consistent with its resistance to PHS. It is not known why the levels of Vp-1Bb fell dramatically at 4 d of treatment with ABA, but it could indicate that other regulators or

**Fig. 5.** Reverse repeat structures at position 2705–2801 bp of Vp-1Ba.
microRNAs could degrade the Vp-1 mRNA when it reached certain levels. Notably, ABI3 protein in germinating Arabidopsis seeds is known to be labile and an ABI3-interacting protein (AIP2) negatively regulates ABA signalling by targeting this protein for post-translational degradation (Zhang et al., 2006b). It has also been observed that ABA induces the accumulation of microRNA159 (miR159) in an ABI3-dependent fashion and that miRNA159 mediates the cleavage of MYB101 and MYB33 transcripts in vitro and in vivo. These two MYB transcription factors function as positive regulators of ABA responses as null mutants of them show hyposensitivity to ABA, suggesting that ABA-induced accumulation of miR159 is a homeostatic mechanism which directs MYB101 and MYB33 transcript degradation to desensitize hormone signalling during seed stress responses (Zhang et al., 2006b). In addition, the expression level of the Vp-1Ba genotype treated with ABA differed little at 2 d from that at 4 d, showing that Vp-1Bb and Vp-1Bc differ from Vp-B1a in the way they responded to ABA. While null mutants of ABI3 and Vp-1 in Arabidopsis and maize result in complete loss of ABA sensitivity, Vp-1Ba in wheat seems to retain partial responsiveness to ABA, although it is less sensitive to ABA than Vp-1Bb and Vp-1Bc. Embryos of Yongchuanbaimai (Vp-1Bb) were more sensitive to ABA than those of Zhongyou 9507 (Vp-B1a) (Zhang et al., 2006a). Considering that the 5′-UTR negatively regulates quantitative and spatial expression of ABI3 (Ng et al., 2004), it would be interesting to investigate if any differences in the promoter region of these Vp-1B alleles exist which could affect their expression level. Nevertheless, the present study clearly demonstrates that the insertion and deletion in the Vp-1B gene could enhance its expression and ABA sensitivity, and thus resistance to PHS.

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