



Figure S3 Analysis of potential alternative splicing in *P1P2* fusion alleles. **A.** Gene structures and possible transcript structures for *P1P2-6*, *P1P2-1 or 2*, and *P1P2-3* alleles. We predicted one possible transcript for *P1P2-6* and similar alleles; and two possible forms for *P1P2-1, 2* and *3* resulting from alternative splicing. The alternatively-spliced forms differ in the origin of exon 2 from either *p1* or *p2*. Unlike the other *P1P2* fusion alleles, *P1P2-3* contains a Composite Insertion at *p2* locus [40]. The Composite Insertion carries a part of flanking gene GRMZM2G073064 (orange box) that is expressed in pericarp; transcription initiated from this promoter may produce an additional chimeric transcript resulting in *p2* expression in pericarp.

B. Results of PCR using primers P5+Ac8 (left panel), and primers P4+P5 (right panel). For allele *P1P2-3*, a PCR product was amplified by P5+Ac8, indicating that *p2* is expressed in the pericarp driven by GRMZM2G073064 promoter. The PCR product cannot be from genomic DNA contamination due to the absence of *p1* intron 1 sequences in this PCR product (Figure 5).

C. The Chromas sequence files of RT-PCR products for *P1P2-6*, *P1P2-1*, *P1P2-2*, and *P1P2-3* from the experiment shown in Figure 5. The PCR products were aligned to determine whether a mixture of alternative transcript forms were present in the RT-PCR products. The polymorphic sites between *p1* and *p2* sequences in exon 2 are enclosed in grey boxes. For *P1P2-6* that can generate only one spliced product, we observed a single peak at each polymorphism site, both of which are from *p1*. For *P1P2-1*

and *P1P2-2*, which possess two possible spliced forms, we also observed only *p1* peaks at the polymorphic sites, indicating that no alternative splicing occurred. For *P1P2-3*, a mixture of peaks is present, indicating the inclusion of both *p1* and *p2* sequences. However, we cannot determine whether the observed *p2* signal derives from alternative splicing of the *P1P2* gene or the ectopic expression of *p2* driven by the Composite Insertion.