bmp2b^{ta72a}

Nature of the mutation

The *ta72a* allele contains a single A-to-G point mutation that converts the stop codon into a Trp residue. As a result, the mutation extends the bmp2b open reading frame by six amino acids (Kishimoto et al., Development 124: 4457-4466, 1997; Nguyen et al., Developmental Biology 199: 93-110, 1998).

Genotyping assay

Genotyping of the *ta72a* allele is based on the dCAPS assay (**d**erived **C**leaved **A**mplified **P**olymorphic **S**equence; Neff *et al.*, The Plant Journal 14(3): 387-392, 1998). In this assay, a restriction enzyme recognition site that includes the single nucleotide polymorphism (SNP) is introduced into the PCR product by a primer containing one or more mismatches to template DNA. The PCR product modified in this manner is then subjected to restriction enzyme digestion and the presence or absence of the SNP is determined by the resulting restriction pattern.

To genotype the *ta72a* allele, a mismatch (marked in red) has been introduced into the forward primer. During PCR, this mismatch and the *ta72a* mutation create a StyI restriction enzyme site in the amplified product. The StyI site is not present in the PCR product derived from the WT DNA template.

Primers:
SWI_10d: 5' AGG GCT GCG GTT GCC CAT G 3'
SWI_11: 5' TGC CAT TGC ACT TGT TTT GGA AT 3'

PCR program (55_30_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **55**ºC for **30** sec
4. 72ºC for **30** sec
5. Go to step 2 (above) for 39 cycles
6. 72ºC for 5 min
7. 8.0ºC hold
8. END

Product size: 227 bp

Digestion of the PCR product with the StyI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	227 bp
PCR product containing the mutation	cleaved	212 bp and 15 bp

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