smo^{b641}

Nature of the mutation

The *b641* allele contains a single G-to-A point mutation that changes glycine to arginine at amino acid residue 242 (Varga et al., Development 128: 3497-3509, 2001).

Genotyping assay

Genotyping of the *b641* 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 *b641* allele, a mismatch (marked in red) has been introduced into the forward primer. During PCR, this mismatch and the *b641* mutation create a DdeI restriction enzyme site in the amplified product. The DdeI site is not present in the PCR product derived from the WT DNA template.

Primers:
SMT_04d: 5' CGT CAA CGC CTG TTT CTT CCT T 3'
SMT_05: 5' CTC CAG ACA TAA GCG AGT AAT ACA CAA TG 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: 346 bp

Digestion of the PCR product with the DdeI restriction enzyme:

Product type	Product digestion	DNA fragments after digestion (bp)
PCR product derived from the WT template	unaffected	346 bp
PCR product containing the mutation	cleaved	326 bp and 20 bp

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