

The use of CAPS marker to study the influence of mutated alleles of the *BnaA.FAD2* gene on the oleic acid content in seeds of winter rapeseed

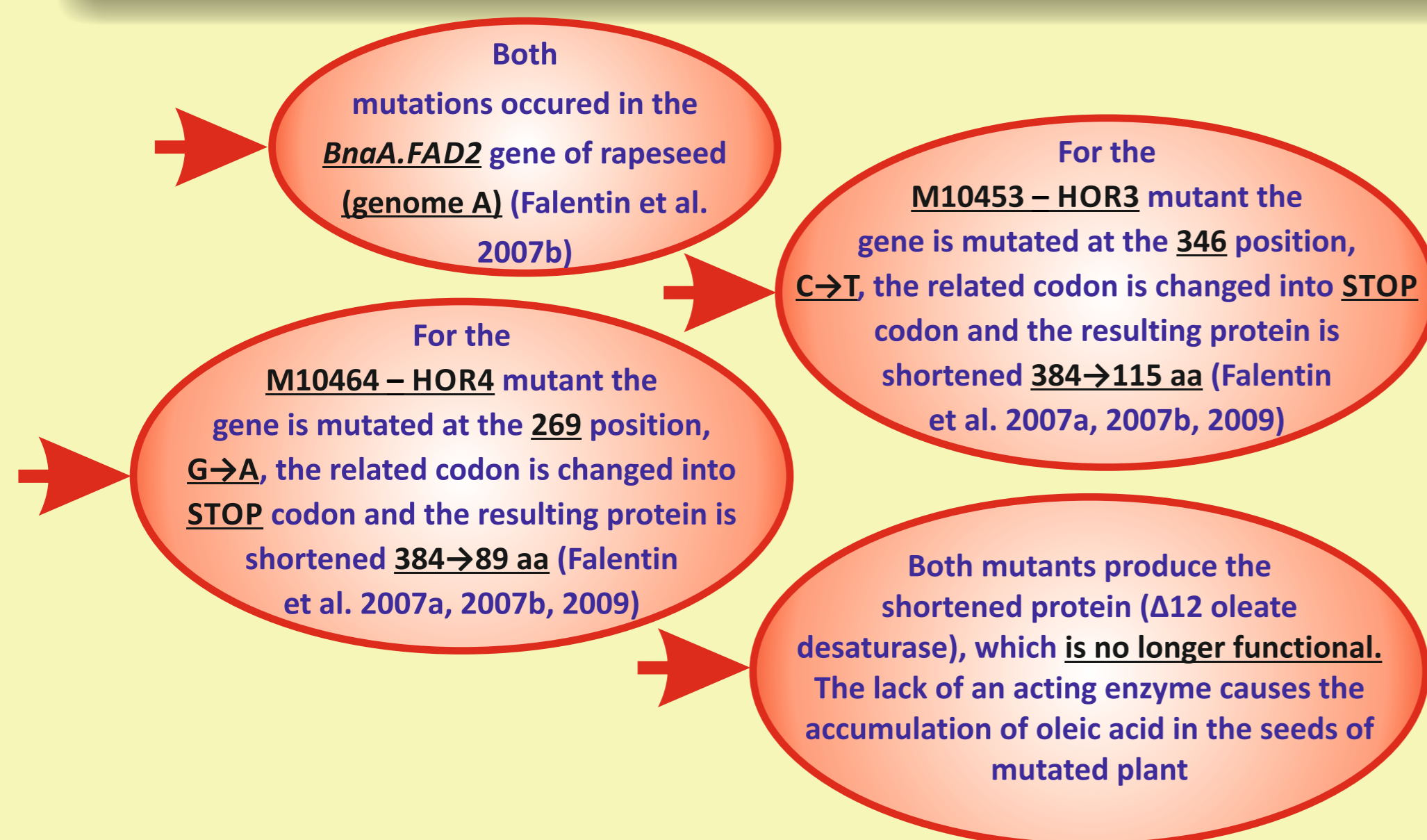


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1 The mutants

Two mutants of rapeseed (*Brassica napus* L. var *oleifera*) having increased amount of oleic acid in seeds, have been described (M10453 – HOR3 and M10464 – HOR4) (Spasibionek 2006). The mutations have been identified and patented (WO 2007/138444, Falentin et al. 2007a) (fig. 1).



2 The CAPS marker

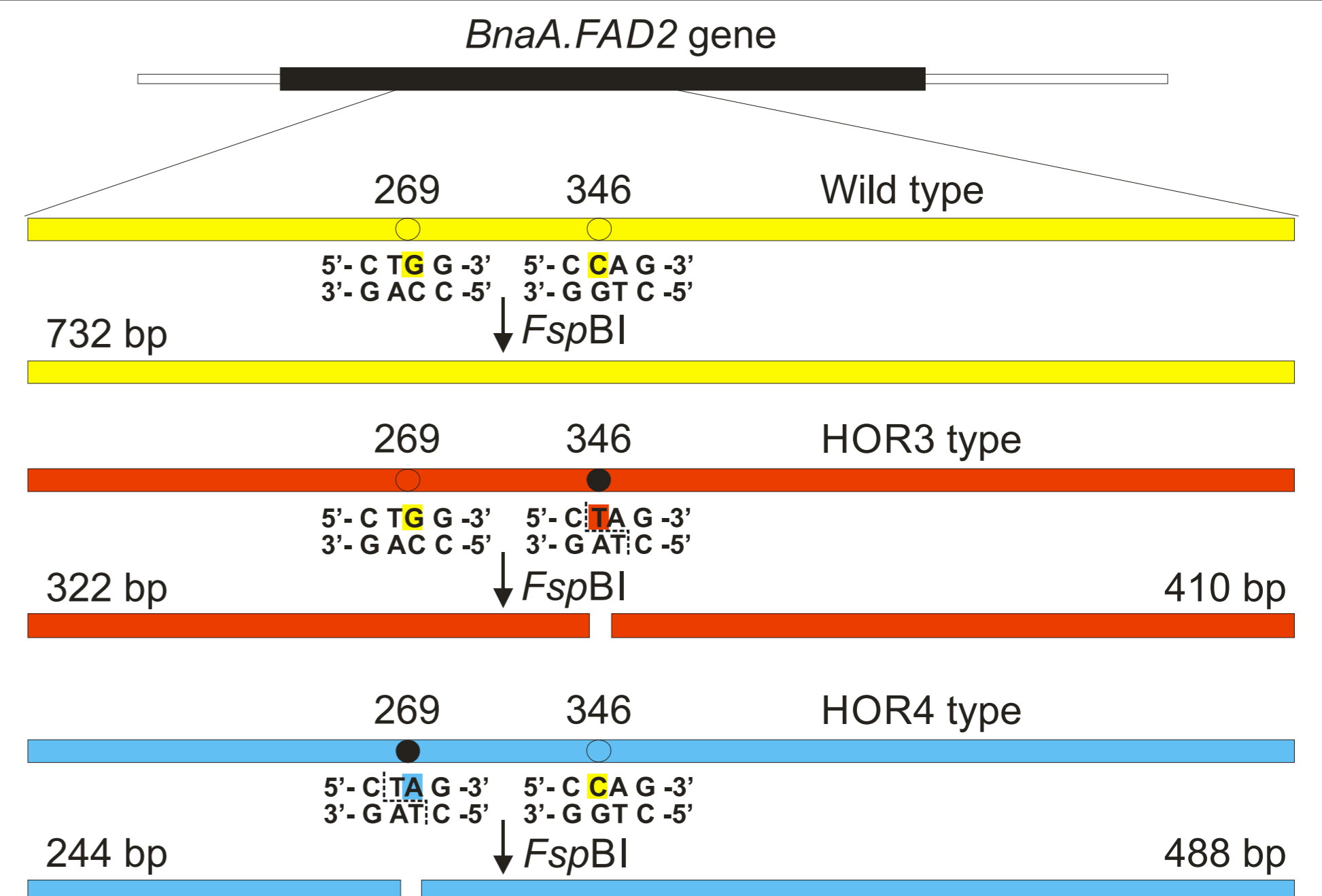


Fig. 1. The mutations in *BnaA.FAD2* gene of rapeseed resulting in the increased amount of oleic acid in seeds and the method to obtain the codominant CAPS marker specific for these mutations.

3 The GxE interaction experiment

The designed CAPS marker has been used to study correlation between the mutated forms of the *BnaA.FAD2* gene and the level of oleic acid in seeds. The HOR4 type mutation was studied (fig. 2 and 3).

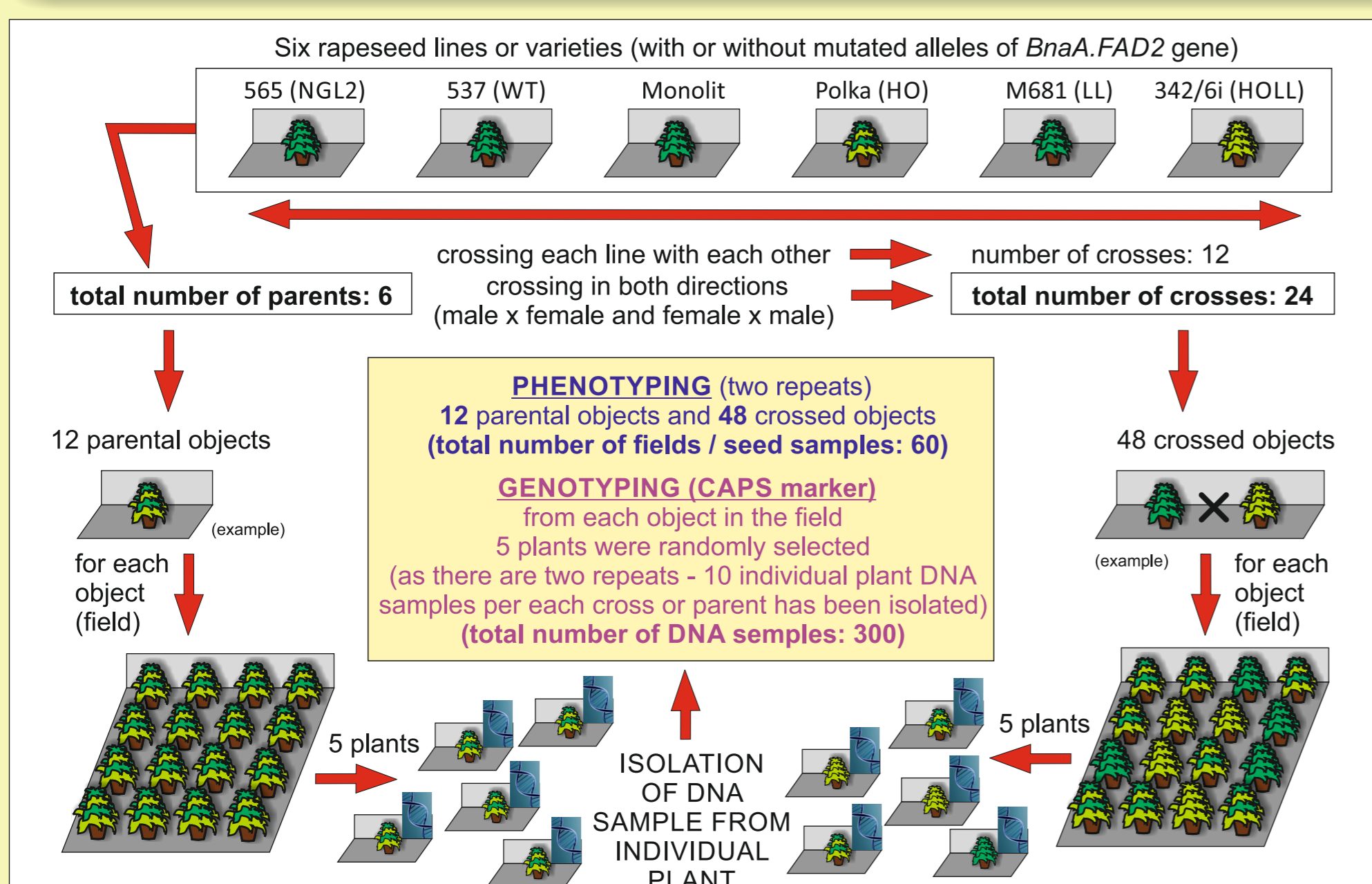


Fig. 2. The scheme of the GxE experiment.

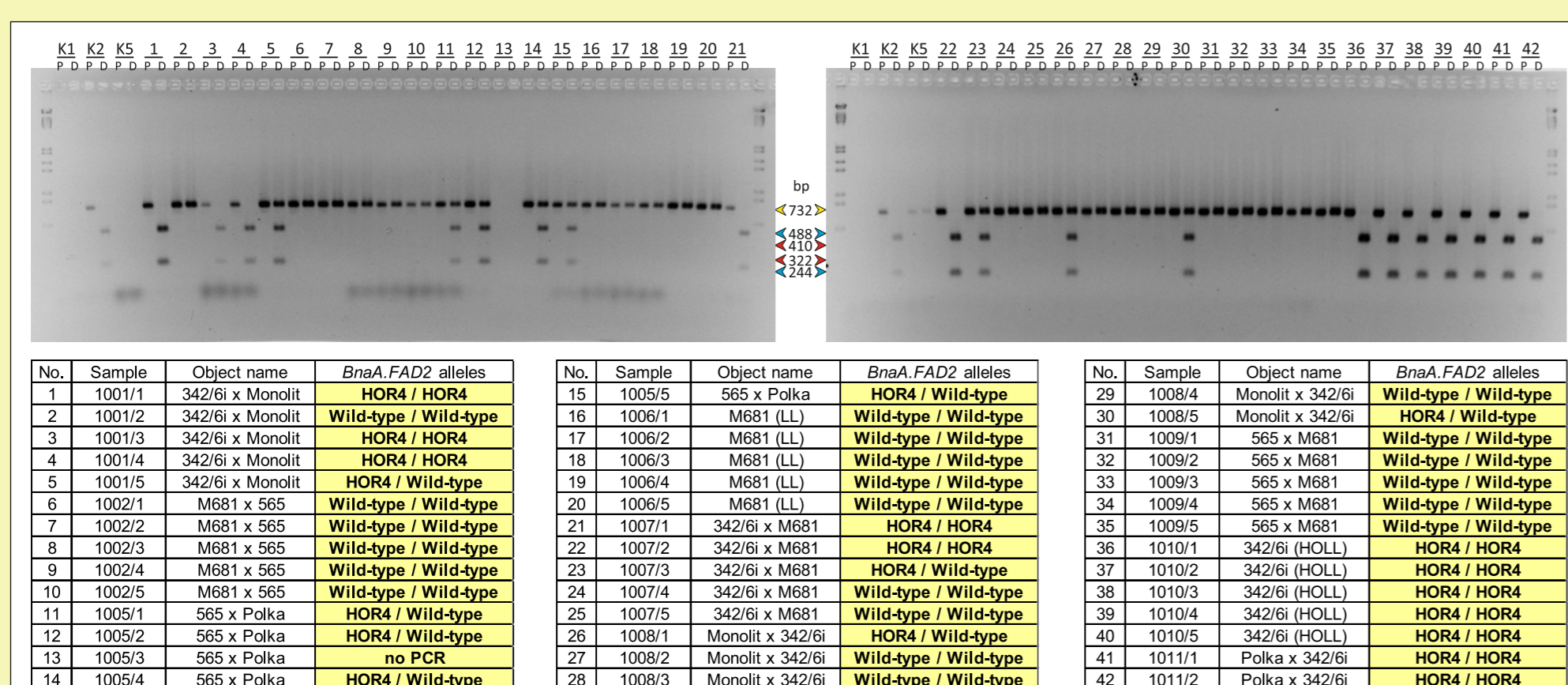


Fig. 3. The results of CAPS analyses. K1, K2, K5 - controls (HOR3, HOR4, Wild-type). P - PCR product, D - PCR product digested with *FspBI* enzyme. The colors of the arrows correspond to these on fig. 1.

Based on the patented sequence of the *BnaA.FAD2* gene from *B. napus* genome A, the allele-specific codominant CAPS (Cleaved Amplified Polymorphic Sequences) marker has been designed (Falentin et al. 2007a, 2007b, 2009). The marker has been thoroughly tested on various rapeseed breeding lines, showing its ability to distinguish between two types of mutated and wild-type alleles. Even more important is that the marker has the ability to distinguish between heterozygotes and homozygotes of both kinds, which makes it a perfect tool for breeders (Falentin et al. 2007a, 2009, Matuszczak et al. 2013, Matuszczak and Tokarczuk 2014, Matuszczak et al. 2018) (fig. 1, table 1).

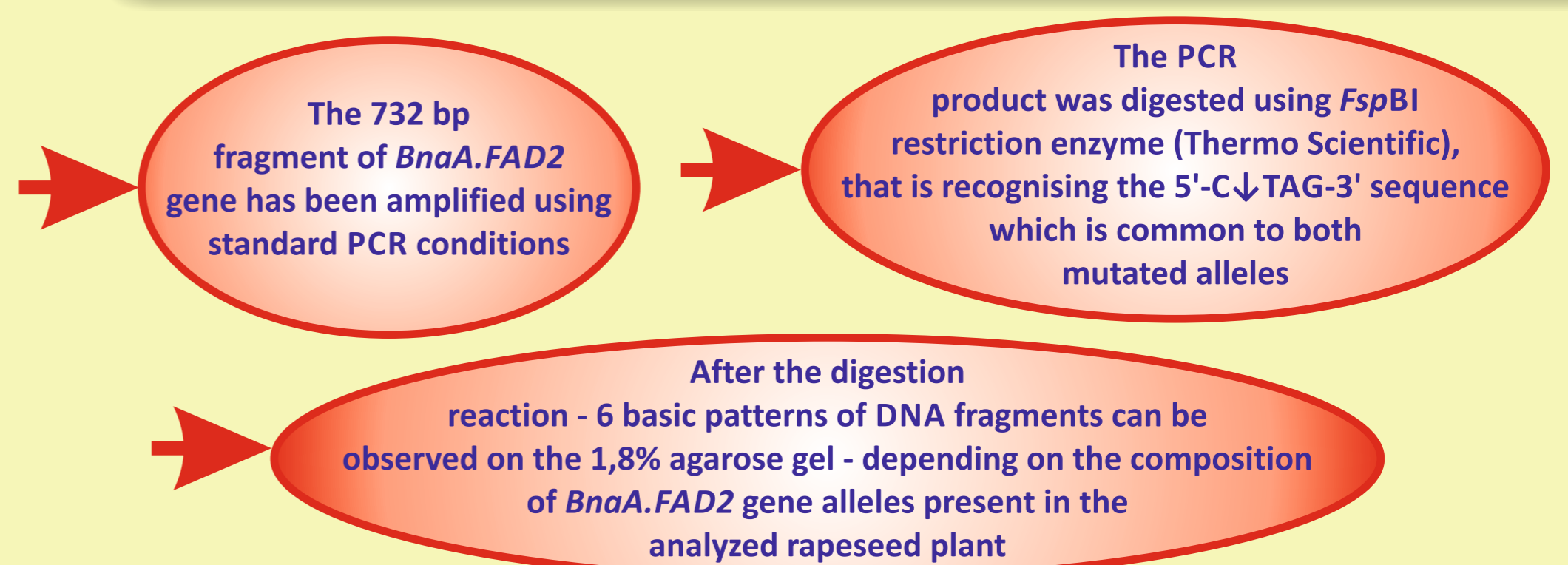


Table 1. The band patterns that can be obtained using the universal, codominant CAPS marker for detection of HOR3 and HOR4 type mutations in *BnaA.FAD2* gene of rapeseed.

No.	HOR3 type mutation	HOR4 type mutation	DNA fragments (pattern)
1.	wild type homozygote	wild type homozygote	732 bp
2.	mutated homozygote	wild type homozygote	410 bp, 322 bp
3.	heterozygote	wild type homozygote	732 bp, 410 bp, 322 bp
4.	wild type homozygote	mutated homozygote	488 bp, 244 bp
5.	wild type homozygote	heterozygote	732 bp, 488 bp, 244 bp
6.	heterozygote ¹	heterozygote ¹	488 bp, 410 bp, 322 bp, 244 bp

¹ - HOR3 and HOR4 type mutations are in repulsion phase

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Acknowledgments

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