

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

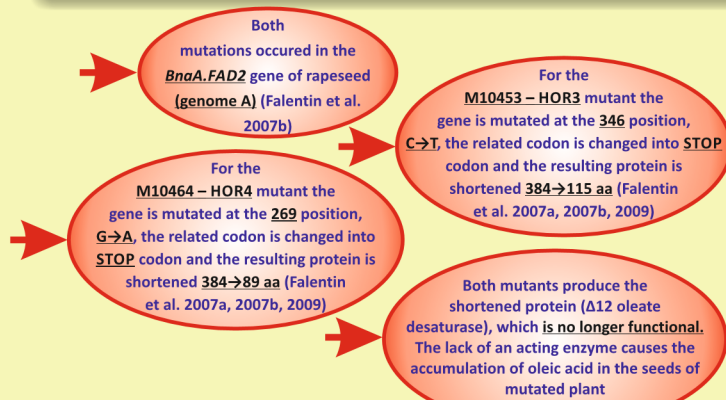


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1 The mutants

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) (Spasibonek 2006). The mutations have been identified and patented (WO 2007/138444, Falentin et al. 2007a) (fig. 1).



2 The CAPS marker

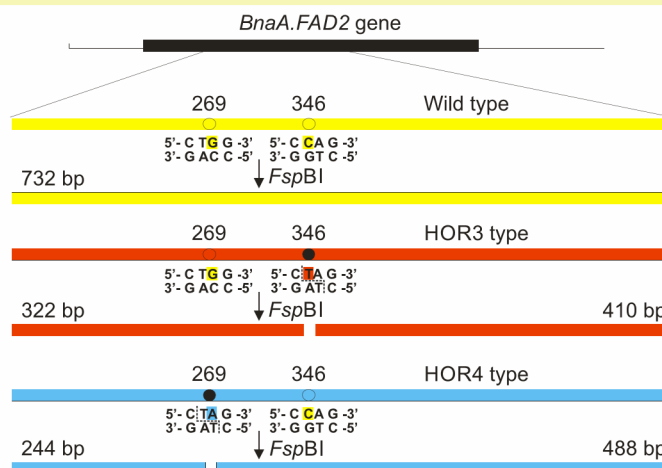


Fig. 1. The mutations in *BnaA.FAD2* gene of rapeseed resulting in the increased amount of oleic acid in seeds and the method to obtain the codominant CAPS marker specific for these mutations.

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).

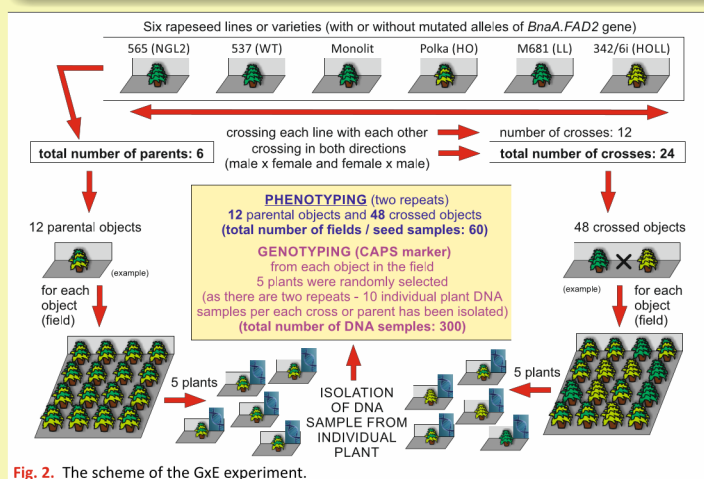


Fig. 2. The scheme of the GxE experiment.

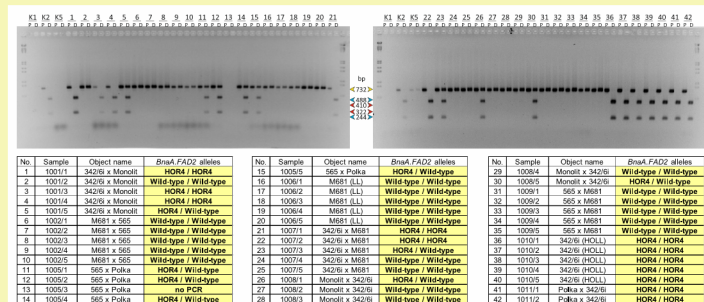


Fig. 3. The results of CAPS analyses. K1, K2, K5 – controls (HOR3, HOR4, Wild-type). P – PCR product, Δ – PCR product digested with *FspBI* enzyme. The colors of the arrows correspond to these on fig. 1.

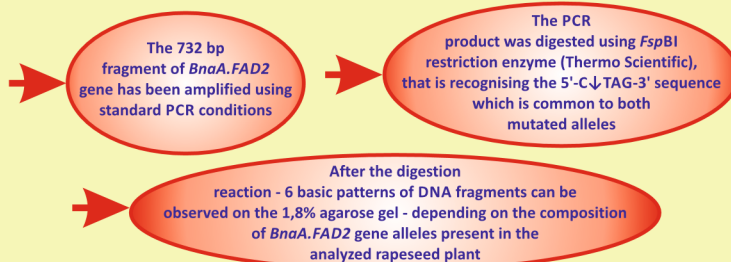


Table 1. The band patterns that can be obtained using the universal, codominant CAPS marker for detection of HOR3 and HOR4 type mutations in *BnaA.FAD2* gene of rapeseed.

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	heterozygote	488 bp, 410 bp, 322 bp, 244 bp

¹ – HOR3 and HOR4 type mutations are in repulsion phase

References

- Falentin C., Brégeon M., Lucas M.O., Renard M. 2007a. Genetic markers for high oleic content in plants. International Patent Application Publication, WO 2007/138444.
- Falentin C., Brégeon M., Lucas M.O., Deschamps M., Leprince F., Fournier M.T., Delourme R., Renard M. 2007b. Identification of *fad2* mutations and development of Allele-Specific Markers for High Oleic acid content in rapeseed (*Brassica napus* L.). In: Fu T, Guan C (ed.) Proceedings of the 12th International Rapeseed Congress, Wuhan, China, March 26–30, 2007: Sustainable Development in Cruciferous Oilseed Crops Production, vol. II Biotechnology. Science Press USA Inc., Princeton Junction, 117–119.
- Falentin C., Brégeon M., Lucas M.O., Renard M. 2009. Genetic markers for high oleic content in plants. United States Patent Application Publication, US 2009/307806.
- Matuszczak M., Spasibonek S., Tokarczuk I., Nowakowska J., Gacek K., Bartkowiak-Broda I. 2018. The use of CAPS marker for the selection of winter rapeseed lines with high oleic acid content in seeds. 34th Scientific Conference „Oilseed Crops – Advances in Genetics, Breeding, Technology and Analytics of Lipids”, Poznań, Poland, April 10–11, 2018, Abstracts: 53–56.
- Matuszczak M., Tokarczuk I., Spasibonek S., Bartkowiak-Broda I. 2013. Analysis of rapeseed DNA using the marker specific for the *fad2* gene mutation (in Polish). Scientific Conference „Nauka dla Hodowli i Nasiennictwa Roslin Uprawianych”, Zakopane, Poland, February 04–08, 2013, Abstracts: 147–148.
- Matuszczak M., Tokarczuk I. 2014. Testing of functionality and methods to obtain CAPS marker designed to detect two mutated forms of *BnaA.FAD2* gene in winter rapeseed. 32nd Scientific Conference „Oilseed Crops”, Poznań, Poland, May 19–20, 2014, Abstracts: 80–83.
- Spasibonek S. 2006. New mutants of winter rapeseed (*Brassica napus* L.) with changed fatty acid composition. Plant Breeding, 125: 259–267.

Acknowledgments

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