Genetic variation for secondary seed dormancy in a set of current European winter oilseed rape cultivars

Jörg Schatzki✉, Coretta Klöppel, Heiko C. Becker and Christian Möllers
Georg-August-Universität Göttingen, Department of Crop Sciences, Plant Breeding, Von-Siebold-Str. 8, 37075 Göttingen, Germany. Email: jschatz1@gwdg.de

ABSTRACT
Secondary seed dormancy in oilseed rape is a phenomenon that allows seeds to survive in the soil for many years without germination. Following soil cultivation, dormant seeds may germinate in subsequent years and they are the main reason for the occurrence of volunteer oilseed rape plants in successive crops. Genetic reduction of secondary dormancy in oilseed rape could provide a mean to reduce the frequency of volunteer plants and especially the dispersal of transgenic oilseed rape. However, little is known about the genetic variation for and the environmental influence on the secondary seed dormancy of current winter rapeseed cultivars. The aim of the present study was to analyse secondary seed dormancy in a set of 28 current winter rapeseed cultivars tested in 2008/2009 in field experiments at six different locations in Germany. Bulked seeds samples obtained from open pollinated plants from 4 replicates (yield plots) were used for the analysis of secondary seed dormancy, applying an in vitro laboratory test. In the ANOVA highly significant effects were found for the influence of the locations and for the variation among the genotypes. Among the genotypes, secondary seed dormancy ranged from 8% to 56% and the heritability was high (0.97). The means for the six locations ranged from 13% to 37%. The large genetic variation and the high heritability indicate that an effective breeding for reduced secondary seed dormancy could be performed.

INTRODUCTION
Following oilseed rape cultivation volunteer oilseed rape plants may occur in succeeding crops during several successive years. They may occur in high numbers requiring herbicide application. In winter oilseed rape fields volunteer oilseed rape plants may represent a problem, if the seed quality has been changed e.g. from high erucic acid to low erucic acid or from 00-quality to high oleic low linolenic quality (HOLL). Problems occur due to seeds harvested from those volunteer plants but also due to cross pollination. Volunteer oilseed rape plants occur due to the fact that seeds of oilseed rape may become secondary dormant (Gruber et al. 2004) and may survive in the soil for 10 years or even longer. The longevity of oilseed rape seeds in the soils is also a problem if transgenic oilseed rape is cultivated. Genetically reducing the capacity of oilseed rape cultivars to produce secondary dormant seeds provides a possibility to reduce seed and pollen dispersal of transgenic modified cultivars. The objective of the present study was to analyze the genetic variation for secondary seed dormancy in a set of current European winter oilseed rape cultivars.

MATERIALS AND METHODS
Plant material and field experiments: The seed material consisted of 28 double low quality winter oilseed rape cultivars (Fig. 1). The material was tested in 2008/09 at 15 locations in Germany (Bundes- und EU-Sortenversuch 1 Winterraps; Gronow et al. 2009). Field experiments were conducted as a RCB design with 4 replicates for each cultivar at each location. Seed samples were taken after combined harvesting of the yield plots. Samples from the 4 replicates of each cultivar at each location were equally mixed and used for Near-Infrared-Reflectance-Spectroscopy (NIRS) analysis. Based on the mean oil content of the seed samples of the locations, seed samples from locations with a low oil content (Langenstein, Ihinger Hof), an intermediate oil content (Hohenschulen, Futterkamp) and a high oil content (Mollenfelde, Sophienhof) were chosen for the analysis of secondary dormancy. For more details about the locations see Gronow et al. (2009).
Test for secondary seed dormancy: The test for secondary dormancy (SD) was performed with 2x 100 seeds per field replicate and essentially following the protocol described by Gruber et al. (2004). The germination rate (GR) was determined after two weeks incubation on moist filter paper. SD and GR rates are given in % seeds. Thousand kernel weight (TKW in g) was determined on 500 mg seed samples with the Contador (www.pfeuffer.com)
Analytical methods: Seed samples of about 3 g were scanned with a NIRS monochromator model 6500 (NIRSystems, Inc., Silversprings, MD, USA). Oil, protein and moisture content were determined using the calibration raps2009.eqa provided by VDLUFA Qualitätssicherung NIRS GmbH (Kassel, Germany). Oil and protein content are expressed in % on a seed dry matter basis.

Statistics: Analysis of variance and calculation of heritabilities (h^2) were performed by using PLABSTAT software (Utz 2008) considering the locations as random. For secondary dormancy and germination rates ArcSin-transformed data were used. Mean values of the genotypes across the locations were used to calculate Spearman's rank correlation coefficients between traits. $^{+, *, **}$ denotes significant at P=10, 5 and 1%, respectively.

RESULTS

The analysis of variance showed highly significant effects of the locations and the genotypes on secondary seed dormancy of 28 winter oilseed rape cultivars as determined by the in vitro test (Tab. 1). Highly significant effects of the locations and the genotypes on thousand kernel weight (TKW), seed oil and protein content, and protein content of the defatted meal were also found. Comparatively large variance components were detected for the effect of the genotypes on secondary dormancy and thousand kernel weight, whereas large effects of the locations on oil and protein content were observed. A large effect for the genotype x location interaction on germination rate was found. Heritability was high for all traits investigated, except for germination rate.

Tab.1: Variance components for secondary seed dormancy and for other seed quality traits in 28 current winter oilseed rape cultivars tested in field experiments at 6 locations

Source of variance	Secondary Dormancy	Germination rate	TKW	Oil	Protein
Location	76.8**	0.09	0.05**	6.9**	5.22**
Genotype	262.7**	0.98**	0.10**	0.9**	0.36**
G x L	59.2	3.27	0.04	0.6	0.33
Heritability	0.97	0.64	0.94	0.90	0.87

** Significant at P = 0.01 (F-test, ANOVA)

Among the 28 cultivars there was a large variation for secondary dormancy which ranged from 8.2% for cultivar Safran to 55.6% for cultivar DK Secure (Fig. 1). Between the locations the mean secondary dormancy varied considerably: 12.5% for Langenstein, 21.6% for Ihinger Hof, 22.8% for Hohenschulen, 30.1% for Futterkamp, 32.4% for Mollenfelde, and 37% for Sophienhof. There was an obvious relationship between mean seed oil content of the locations (see Materials and Methods) and the capacity to form secondary dormant seed.

Among the different traits recorded there was no significant correlation between secondary seed dormancy of the genotypes and their germination rate, thousand kernel weight, oil and protein content (data not shown). There was also no correlation to the seed fibre (NDF, ADF and ADL) content of those cultivars (data not shown; cf. Dimov and Möllers, this CD).

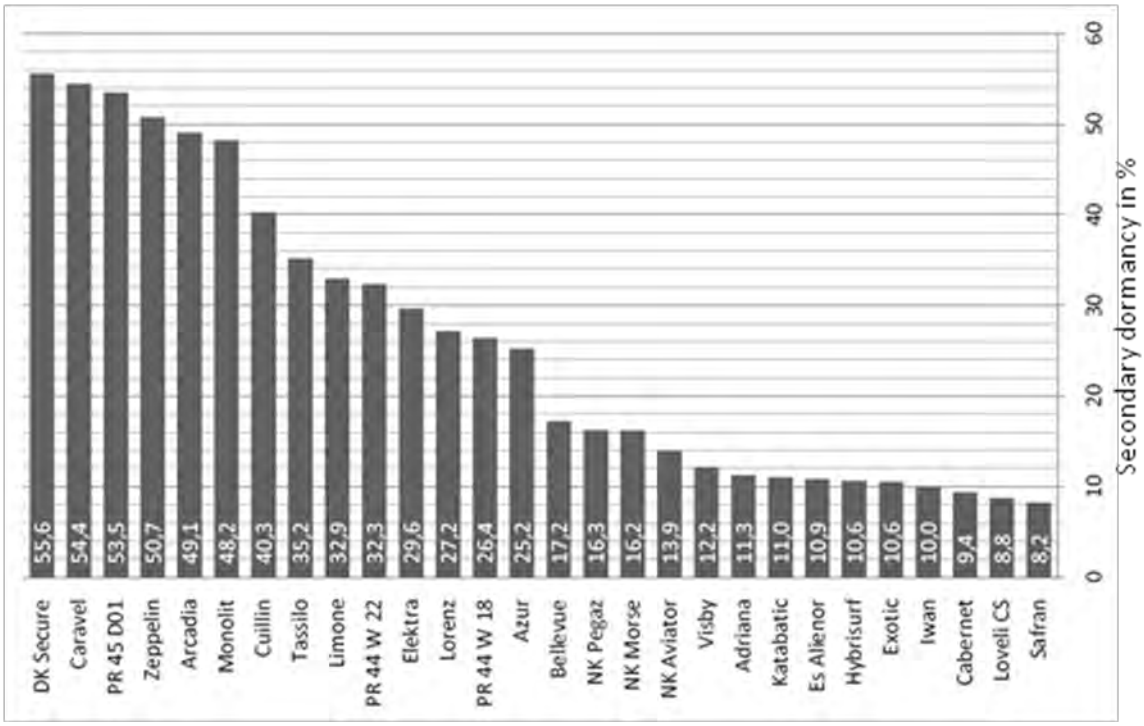

Fig. 1: Secondary seed dormancy of 28 winter oilseed rape cultivars as determined by an in vitro assay. Depicted are means over 6 locations

DISCUSSION

Results of this study show that there is a large variation for secondary seed dormancy among current winter oilseed rape cultivars and that the trait secondary seed dormancy has a high heritability. However, performing the in vitro test for secondary dormancy is laborious and time consuming and it appears unlikely that this test can be integrated in a breeding program. The results did not indicate that an indirect selection for high seed germination rate, large seed size or low seed fibre content could lead to genotypes with reduced secondary dormancy. These results are partly in contrast to the results of Schatzki *et al*. (see this CD) who found in a doubled haploid population a negative correlation between secondary dormancy and germination rate as well as seed size. They also reported a positive correlation between secondary dormancy and NDF, ADF and ADL content of the seeds. It can be concluded that for the generation of transgenic oilseed rape plants, cultivars with a low secondary dormancy should be used.

ACKNOWLEDGEMENTS

The technical assistance of Gunda Asselmeyer is greatly appreciated. Many thanks to Sabine Gruber (University of Hohenheim) for her help in establishing the secondary seed dormancy test. The authors express their gratitude to Sortenförderungsgesellschaft (SFG) in Bonn for providing seed sample material from the Bundes- und EU-Sortenversuch Winterraps 2008/2009 field experiments. We are grateful for the financial support given by the German Federal Ministry of Education and Research (BMBF) FKZ 0315211C.

REFERENCES

Gronow J, W Sauermann, G Barthelmes (2009) Sortenversuche 2009 – Mit Winterraps, Futtererbsen und Sonnenblumen. UFOP Schriften, Heft 37/Agrar (E-Book).
http://www.ufop.de/publikationen_schriften.php

Gruber S, C Pekrun, W Claupein (2004) Seed persistence of oilseed rape (Brassica napus): variation in transgenic and conventionally bred cultivars. The Journal of Agricultural Science 142:29-40. Cambridge University Press

Utz HF (2008) PLABSTAT (Version 2N), a computer program for the computation of variances and covariances. Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Stuttgart, Germany (http://www.uni-hohenheim.de/ipspwww/soft.html)