Selection for low seed dormancy in GM oilseed rape

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Abstract

Genetically modified (GM) oilseed rape is not considered suitable for co-existence because of its capacity for seed persistence in the soil. Varieties which do not become dormant and do not persist in the soil would be an appropriate completion of farmer's management measures to ensure co-existence and offer perspectives for growing GM oilseed rape. The aim of the study was to obtain non-dormant genotypes by a simple selection approach. Seed of the variety Smart (75% dormant seeds) was separated into dormant and non-dormant seed lots by dormancy induction in the laboratory. The sub-populations were grown in the greenhouse, self-pollinated and harvested. The plants of each population showed individual seed dormancy levels which ranged from 0 to 85%. A second generation was grown from dormant seeds of dormant plants and from non-dormant seeds of non-dormant plants These plants again showed individual seed dormancy levels. The mean dormancy levels of the segregated populations differed significantly. The sub-populations "dormant" had a mean of 75% or 77% dormant seeds, and the sub-populations "non-dormant" had a mean 17% or 34% dormant seeds. By selection over two generations a clear segregation of genotypes was being achieved and many low-dormancy plants were produced from an initially highly dormant variety. Using low-dormancy plants which can be probably found in many current varieties for future transformations, would support measures to make GM oilseed rape suitable for co-existence.

Key words: Oilseed rape, *Brassica napus*, dormancy, selection, genetically modified, co-existence

Introduction

The capacity of oilseed rape for seed dormancy and seed persistence is one of the objections to growing genetically modified (GM) oilseed rape. Seeds from harvesting loss can become secondarily dormant and then last in the soil seed bank for more than 10 years (Schlink, 1998; Lutman et al., 2003). Years after growing genetically modified (GM) oilseed rape, volunteers can emerge from the soil seed bank, flower, set seed, and result in seed impurities in a following conventional crop. Even a few GM volunteers in a conventional oilseed rape crop would lead to outcrossing and impurities in the harvested crop (Gruber & Claupein, 2006). Because of this concern, GM oilseed rape is often considered as not being suitable for co-existence between conventional, organic and GM crop. Varieties which do not become dormant and do not persist in the soil would be an appropriate completion of farmer's management measures to ensure co-existence and offer perspectives for growing GM oilseed rape.

As shown for many wild plants (Naylor & Jana, 1976; Garbutt & Witcombe, 1986; Foley & Fennymore, 1998) and field crops (Buraas & Skinnes, 1984; De Pauw & McCraig, 1991; Nyachiro et al., 2002), there also exist a wide range of seed dormancy levels in oilseed rape varieties (Pekrun et al., 1997, Momoh et al., 2002; Gulden et al., 2004; Gruber et al., 2004a). Low-dormancy varieties had a smaller soil seed bank and resulted in fewer number of volunteers (Gruber et al., 2004b). Maybe not all varieties with low seed dormancy available at present are suitable for genetically modification or any further breeding. Therefore, it would be an option to systematically select genotypes with low or no seed dormancy in varieties with a suitable crop performance but high seed dormancy. The aim of the study was to obtain non-dormant genotypes by a simple selection approach in the greenhouse. The hypotheses were: 1. The offspring of low-dormancy and high-dormancy plants differs in the level of seed dormancy; 2. The production of plants from seeds which were either dormant or non-dormant is a quick and easy method to support selection.

Material and Methods

The experiment was performed in the greenhouse of the Institute for Crop Production and Grassland Research of the University of Hohenheim in the years 2002–2005. The winter oilseed rape variety Smart was tested for secondary seed dormancy in the laboratory. The dormancy test for all experiments was as following:

Hohenheim Standard Dormancy Test (HSDT): 4 × 100 seeds were exposed to water stress (-15 bar, ~ permanent wilting point) and darkness over a period of 14 days. Water stress was induced by 8ml polyethylene glycol per Petri dish (90mm diameter, double layer of filter paper) at 20°C for each seed lot of 100 seeds. Non-dormant seeds were allowed to germinate in a following germination test on 6ml de-ionized water in Petri dishes (90 mm diameter, double layer of filter paper). The germination test also took place in darkness and 20°C. All seedlings were removed at certain times under a green safety light (500-600 nm), and the remaining seeds were exposed to alternating temperature and light conditions (12h/12h light/darkness, 30°C/3°C) over seven days to break dormancy. Seeds which germinated during the germination test were declared to be non-dormant, and seeds which germinated after dormancy breaking treatment were declared to be (formerly) dormant.

Greenhouse 2002/2003: The seeds derived from the harvest 2002 on an experimental field in south west Germany. Fifteen plants per variety were grown from seedlings in the year 2002/2003, deriving from dormant seeds, from non-dormant seeds and from a directly sown control (not selected), i.e. 45 plants per variety altogether. This group of plants was called the experiments first generation. After germination in October 2002, plants were grown under short day conditions and 20°C until 6-leaf-stage, and then vernalised at 3°C for 8 weeks in a climate chamber. Following vernalisation, plants were put back into the greenhouse in a row-column design at long day conditions (> 14 hrs light) and 20°C at least. All plants were isolated for self-pollination. A test for secondary dormancy was performed with the ripened and cleaned seed in the year 2004.

Greenhouse 2004/2005: Two plants with high number of dormant seeds and two plants with low number of dormant seeds were chosen for further experiments. Seeds were separated into dormant and non-dormant seeds by the Hohenheim Standard Dormancy Test. Sixteen plants were grown from the dormant seeds of each high-dormancy plant, and another 16 from seeds of each low-dormancy plant, i.e. 64 offspring plants altogether (experiments second generation). Plants were grown in the greenhouse in a block design under similar conditions as described for the first generation. The experiments were analysed by the statistical programme SAS by the procedure MIXED.

Results

Winter oilseed rape Smart has been characterized by the Hohenheim Standard Dormancy Test as a variety with high dormancy prior to the selection experiment. Dormancy was 75.7% for the original seed lot harvested in 2002 which was used for the segregation experiment. Dormancy was mainly secondary (i.e. induced) dormancy, but also included a small proportion of primary dormancy. Secondary and primary dormancy, if present, will be called "dormancy" in the following.

There were plants with different levels of dormancy in their seeds in the sub-populations of the first generation (Table 1). High-dormancy and low-dormancy plants were also found in the unselected seed lot of the control group.

Table 1. Seed dormancy (% dormant seeds/viable seeds) in plants deriving from dormant seeds, non-dormant seeds or the control (unselected seeds) of the winter oilseed rape Smart from greenhouse experiments; in brackets: standard deviation

Individual plant no.	% dormant seeds in plants deriving from		
	Dormant seeds	Non-dormant seeds	Control (seeds not selected)
1	85.0 *(4.24)*	89.7 *(1.70)*	98.0 *(1.98)*
2	71.1 *(1.76)*	69.6 *(10.13)*	95.7 *(0.51)*
3	53.5 *(5.28)*	54.9 *(11.36)*	94.0 *(2.14)*
4	39.1 *(8.77)*	52.8 *(3.76)*	91.7 *(4.50)*
5	37.7 *(3.70)*	40.8 *(6.65)*	86.0 *(5.92)*
6	36.0 *(2.94)*	21.8 *(11.35)*	82.3 *(6.80)*
7	31.3 *(5.42)*	3.8 *(1.70)*	82.2 *(4.77)*
8	21.9 *(4.83)*	1.8 *(0.50)*	81.6 *(2.97)*
9	14.5 *(4.65)*	1.3 *(0.96)*	79.3 *(9.67)*
10	8.5 *(5.69)*	0.5 *(1.00)*	69.7 *(6.37)*
11	8.2 *(5.21)*	0.3 *(0.50)*	43.4 *(5.87)*
12	5.7 *(1.35)*	0.3 *(0.52)*	34.4 *(11.76)*
13	4.5 *(2.57)*	0.3 *(0.50)*	18.3 *(9.25)*
14	0.0 *(0.00)*	0.0 *(0.00)*	17.6 *(4.08)*
15	-	-	7.5 *(4.36)*

In the second generation, deriving from dormant seeds of plant 1 and 2 of the dormant population (do 1 and do 2) and plant 13 and 14 of the non-dormant population (n-do 13 and n-do 14), individual dormancy levels were found again (Fig. 1). Dormancy ranged from 18–98% (offspring of plant do 1), 20–95% (offspring of plant 2 do), 0–79% (offspring of plant n-do 13) and 0–53% (offspring of plant n-do 14). There were more plants with relatively high dormancy in the offspring of high-dormancy plants than in the offspring of low-dormancy plants. Vice versa, dormancy was comparatively low in most plants which had low-dormancy parents.

The comparison of all four segregated populations showed significant differences between the mean dormancy level of the offspring of plant do 1 and do 2 on the one hand and n-do 13 or n-do 14 on the other hand (Fig..2). The two populations deriving from low-dormancy plants differed just significantly ($P > t = 0.047$).

The initial level of dormancy determined in the parental seed lot in the year 2002 was not reached in the mean of any population, but individual plants exceeded this level.

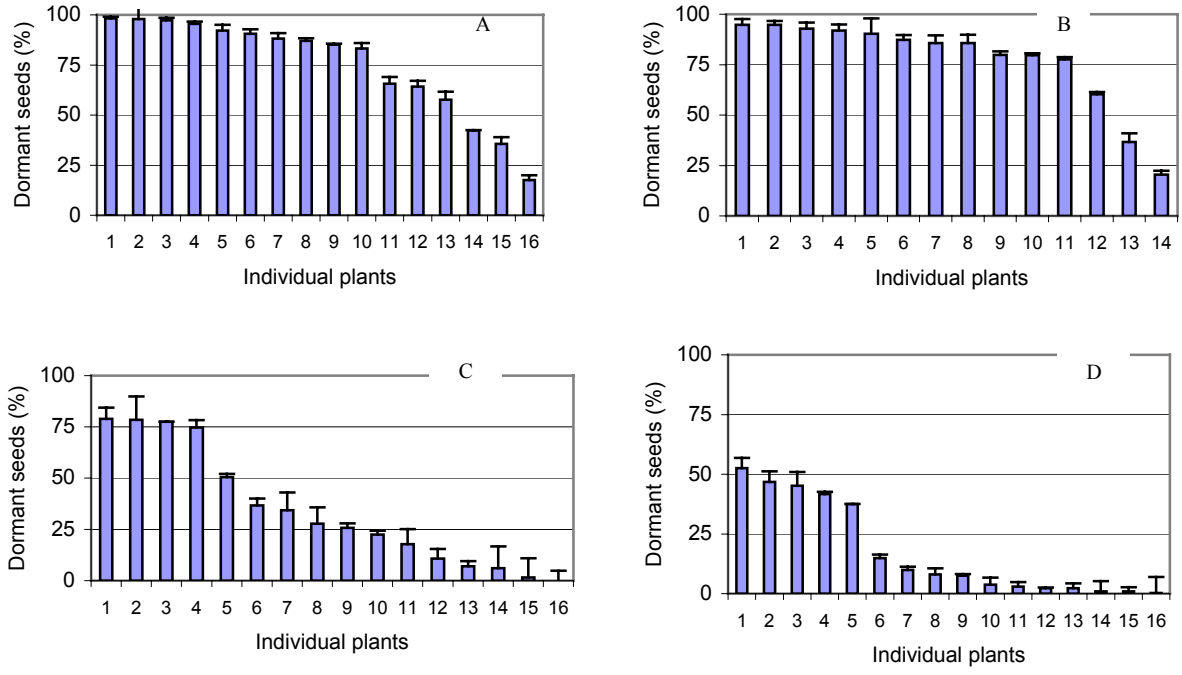

Fig. 1. Seed dormancy (% dormant seeds/viable seeds) of individual plants deriving from high-dormancy (A: offspring of 1 do, B: offspring of 2 do) or low-dormancy plants (C: offspring of 13 n-do, D: offspring of 14 n-do)

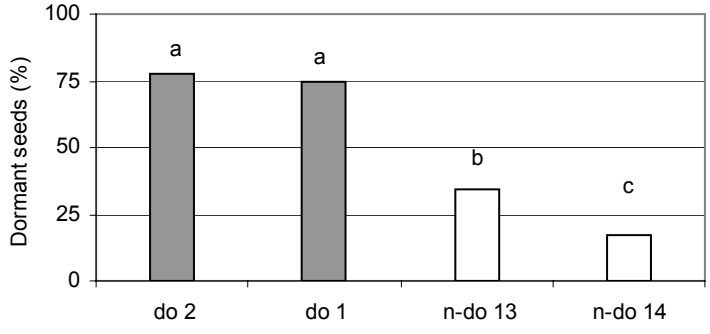

Fig. 2. Mean dormancy levels in the offspring of two highly dormant winter oilseed rape plants (do 1, do 2) and two lowly dormant plants (n-do 13, n-do 14); Fisher's test, $P < 0.05$, n (offspring) = 16 (14)

Discussion

Dormancy was determined and described for oilseed rape in several studies before (Pekrun et al., 1997; Momoh et al., 2002; Gulden et al., 2004; Gruber et al., 2004a). The current results suggest that all these values are the mean of the dormancy levels of individual plants in the population. Different, plant specific levels of dormancy were also found in the variety Zenith (Gruber & Claupein, 2004). Therefore, it can be concluded that several or all winter oilseed rape varieties show this pattern of dormancy, even if not tested. Since seed dormancy never was a criterion for selection in oilseed rape, and since oilseed rape shows about 30% cross-pollination, this heterogeneity within a variety is quite plausible. Future dormancy testing should rely on seed lots which represent a high number of single plants of a certain accession to avoid experimental errors.

Growing plants from non-dormant seeds (first generation) seemed to increase the number of low-dormancy plants in comparison with the control. Nevertheless, the number of low-dormancy plants was also higher than in the control when dormant seeds were selected for cultivation. This result may be due to an unknown experimental effect during the selection process or, most likely, due to the small number of plants used for the experiment. The selection of seeds from high-dormancy plants and low-dormancy plants led to a clear segregation of the dormancy levels in the sub-populations. The unwanted high-dormancy genotypes could be significantly reduced by one selection step. If the selection of dormant or non-dormant seeds for the next generation has an effect on the selection success is not quite clear.

Conclusions

Selection for low-dormancy types is possible also in initially high-dormancy varieties. This result offers the opportunity

to breed low-dormancy varieties and to use low-dormancy plants in high-dormancy for genetically modification without loosing the wanted agronomical quality of a variety. The next steps of research would be to 1. to include more varieties; 2. to use a higher number of plants; 3. to select pure lines for crossing experiments to identify the genetic background of dormancy in oilseed rape. If seed dormancy is a restriction for growing GM oilseed rape, low-dormancy varieties would support other agronomical measures, i.e. suitable soil tillage, to minimize the soil seed bank and to avoid exceeding the GM labelling threshold.

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