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Chemical and molecular characteristics of winter oilseed rape (*Brassica napus* L.) volunteers from the soil seed bank

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ABSTRACT

Oilseed rape (OSR) has one of the highest potential for gene flow through seed and pollen. Volunteer plants are one of the most important sources of contamination of OSR crop of different quality cultivars. The aim of this study was to estimate the abundance of soil seed bank after the harvest of high erucic (HE) cultivar Maplus and to determine biochemical and molecular characteristics of OSR volunteers. The investigation comprised volunteers of oilseed rape obtained from the soil seed bank in two localities in Poland: Dlon and Zielecin (Greater Poland Voivodeship). The seeds of volunteers and reference cultivars were analyzed using biochemical (erucic acid and glucosinolates [GLS]) and molecular biology methods. In seeds of volunteers, erucic acid content ranged from 0 to 57.4% of all fatty acids and glucosinolate content ranged from 5.2 to 105.0 μmol g^{-1} of seeds. In sowing seeds of the cultivar Maplus, the erucic acid content was 57% and total glucosinolates were 12.6 μmol g^{-1} of seeds. RAPD markers were used to determine genetic similarity coefficient and relationship between polymorphic RAPD markers and erucic acid and total GLS content. Twenty RAPD markers showed statistically significant association with erucic acid and GLS content in seeds. Volunteers originating from the investigated soil seed bank were characterized by different content of erucic acid and GLS in seeds typical for different types of oilseed rape cultivars: double low (00, canola type), cultivars with zero erucic acid and high GLS content (0HG) and traditional cultivars with high erucic acid and high GLS content (HEHG). The results of this study, especially the presence in soil seed bank the seeds of very old cultivars, confirm that seeds of rapeseed of different origin may persist for a long time at the stage of secondary dormancy.

Introduction

Oilseed rape (*Brassica napus* L.) is the most important oil crop in the world after soybean, especially in countries with moderate climate. Oilseed rape oil is high-quality oil for human

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nutrition and can also be used as an important renewable resource for nonfood purposes such as biofuels (biodiesel). Moreover, after oil extraction from oilseed rape, the protein-rich meal is used as fodder for livestock (Wittkop et al. 2009). Because of its economic importance, rapeseed is one of the principal GM crops, along with soybean, maize, and cotton.

Oilseed rape is the plant species with the highest risk for coexistence of different types of cultivars including genetically modified (GM) cultivars. The gene transfer may occur through pollen-mediated gene flow from GM crops to non-GM crops, between cultivars with different quality traits, or between other plants from the family Brassicaceae; the gene transfer can also occur by seeds dropped before and during harvest as well as during transport. OSR is both self- and cross-pollinated plant (facultative pollination). Each cultivar can show different levels of outcrossing depending on environmental conditions, pollen quantity, and selfing rate (Mesquida et al. 1988; Pierre et al. 2003; Poplawska et al. 2012). Hüsken and Dietz-Pfeilstetter (2007) indicated that the range of cross-fertilization is determined by weather conditions, local topography around the field trial site, and the number of pollinators such as honey bees, bumble bees, and other insects that are likely to increase the amount of pollen transfer. However the most efficient gene transfer is through seeds. Oilseed rape is characterized by a high frequency of seed shattering before and during harvest. The quantity of seed spilled to the soil is between 5% and 10% of the total seed set. Usually, seed losses during harvest can amount to several thousand seeds per square meter (Gruber and Claupein, 2007), but under adverse climatic conditions (storm, heavy rain, hail), this loss can even reach up to 15 000 seeds/m^2 (Gruber et al. 2005). The number of seeds dropped per square meter of the field surface depends on the variety, method, and time of harvesting and environmental conditions. Seeds can be transported over long distances during transport by the farm equipment as well as by animals, mainly birds. The seeds of oilseed rape falling on the soil achieve a state of secondary dormancy that can be induced by particular conditions: dryness, low temperature, light, and certain gases (Gruber and Claupein, 2004; Soltani et al. 2019). In the soil, the seeds in the stage of secondary dormancy often persist for a long time, and they are often not released from dormancy for more than 10 years (Lutman et al. 2005). These seeds after emerging as volunteer plants in the subsequent crops can contribute to unwanted gene dispersal by pollen flow and by seeds (Gruber and Claupein, 2007; Bartkowiak-Broda et al. 2008; Gruber et al. 2012).

In Poland, cultivars characterized by high erucic acid (~50% of all fatty acids in oil) and high glucosinolate content (~160 μmol·g^{-1} seeds) (HEHG) were replaced over 25 years ago by double low '00' winter OSR cultivars (erucic acid <2%; glucosinolate content <15.0 μmol·g^{-1} seeds). Thus far, in some fields in different regions of Poland, there were some plants whose seeds were

characterized by high erucic acid or glucosinolate content or both (Bartkowiak-Broda et al. 2008). This phenomenon is especially important when cultivars coexist in space and/or in time, e.g., genetically modified (GM) cultivars, non-GM cultivars, and cultivars with specific fatty acid content-high oleic (HO), high oleic and low linolenic (HOLL), or yellow seeded. In some cases, the number of volunteers originating from seeds of old cultivars (HEHG) is high enough to reduce the quality of harvested seeds and thus quality of raw material for oil industry, which may cause financial loss to farmers (Colbach, 2011). The objectives of the present study were: estimation of soil seed bank abundance after the harvest of high erucic acid cultivar Maplus (about 57%); biochemical characterization of volunteers to determine erucic acid and glucosinolate content; and molecular analysis of volunteers.

Material and Methods

In the growing season of 2008/2009, two experimental oilseed rape fields were located in different environmental conditions in Poland: Dlon in Rawicki district (N51º46′, E17 º14′) and Zielecin in Grodzisk district (N52º10′, E16º14′). The localities are characterized by various soil complexes: Dlon-typical heavy soil of quality class III/IV and Zielecin-light soil of quality class IVa. Acreage of the fields in both localities was approximately 9 ha; 1 ha of high erucic acid winter OSR cultivar Maplus (~57% erucic acid; total glucosinolate content 12.6 μmol g^{-1} of seeds—HE0) was surrounded by 8 ha of double low (00) winter OSR cultivar Monolit (0% erucic acid; total glucosinolate content 14.5 μmol g^{-1} of seeds).

To determine the number and origin of seeds in the soil seed bank, in September 2009 after the harvest of the trials from 1 ha of HE oilseed rape cultivar Maplus and first ploughing, 32 soil samples—soil cores with 2.5 cm diameter from the depth of 10–20 cm were taken in Dlon and Zielecin. The soil samples (2 × 32) were placed in a greenhouse under conditions that allowed the germination and growth of volunteers. After vernalization, OSR plants during the flowering period were isolated with bags and harvested at the maturity stage. The seeds from 66 volunteers (13 volunteers from Zielecin; 53 volunteers from Dlon) were analyzed using biochemical and molecular methods. Erucic acid content was measured using the method of fatty acid methyl esters (Byczynska and Krzymanski), and glucosinolate (GLS) content was measured using the gas chromatography method for silyl derivatives of desulfoglucosinolates (Michalski et al. 1995).

To determine the genetic similarity of 66 volunteers, a study was performed using RAPD molecular markers. The following cultivars were used as reference cultivars for this study: double low (00) WOSR cultivar Monolit (erucic acid content < 2.0%, total glucosinolate content < 25.0 μmol

g^{-1} of seeds); HE0 cultivar Maplus (erucic acid content > 2.0%, total glucosinolate content < 25.0 μmol g^{-1} of seeds); F_1 hybrid 'Monolit × Maplus', doubled haploid (DH) WOSR lines: DH ER (HE0), DH WG (0HG) and DH JN (0HG); and cultivar of turnip rape (*Brassica campestris*) Ludowy (HEHG erucic acid content > 2.0%, total glucosinolate content > 25.0 μmol g^{-1} of seeds). Genomic DNA was extracted from 6-day-old leaves of volunteers and reference cultivars by using a modified CTAB procedure according to Doyle and Doyle (1990). The RAPD reactions were performed using 25 primers (Table 1) (Operon Technologies, Alameda, USA) in accordance with the standard method described by Williams et al. (1990). DNA profiles of 66 volunteers were compared with those of reference cultivars. The mean values and coefficients of variation for erucic acid content and total glucosinolate content were calculated (Kozak et al. 2013). One-way analysis of variance (ANOVA) was carried out to determine the effects of group according to the chemical composition on the variability of erucic acid content and total glucosinolate content. The mean values and coefficients of variation were calculated for each group. The least significant differences (LSDs) were estimated and used for multiple comparison testing. The coefficients of genetic similarity (GS) of the investigated genotypes were calculated using the following formula (Nei and Li, 1979):

$$GSij = 2Nij\frac{\mathrm{N}i}{\mathrm{N}j}$$

where N_{ij} is the number of alleles present at the *i*-th and *j*-th genotype, N_i—the number of alleles present at the *i*-th genotype, N_j, the number of alleles present at the *j*-th genotype, and $i, j = 1, 2, \ldots, 73$.

Table 1. Polymorphic RAPD primers used in the analysis of winter oilseed rape volunteers.

Primers	(a)	(b)	(c)	Primers	(a)	(b)	(c)
OPA 07	9	4	44,44	OPN 20	11	3	27,27
OPA 08	10	5	50,00	OPO 13	12	4	33,33
OPA 14	13	4	30,77	OPP 03	10	7	70,00
OPA 15	14	6	42,86	OPP 05	9	3	33,33
OPC 18	14	6	42,86	OPP 11	13	7	53,85
OPD 08	13	11	84,62	OPP 14	13	6	46,15
OPF 01	11	7	63,64	OPW 05	14	11	78,57
OPF 04	11	10	90,90	OPW 09	13	5	38,46
OPG 03	12	5	41,67	OPY 01	9	3	33,33
OPG 04	13	2	15,38	OPY 02	11	6	54,55
OPL 12	17	7	41,18	OPY 13	15	11	73,33
OPN 13	9	6	66,67	OPY 15	14	11	78,57
OPN 18	16	3	18,75				

(a): number of all bands; (b): number of polymorphic bands; (c): (b/a × 100)– present of polymorphism.

According to the calculated coefficients, genotypes were grouped hierarchically using the unweighted pair group method of arithmetic means (UPGMA), and the relationship among them

was presented in the form of a dendrogram. By using the same coefficients, principal coordinate analysis (PCA) was performed. The association between molecular markers and erucic acid content and total glucosinolate content was determined using regression analysis (Bocianowski et al. 2011). The marker observations were tested as independent variables and considered in individual models. Moreover, analysis of molecular variance (AMOVA) was used to compute the distribution of genetic variability among and within groups according to the chemical composition. The relationship between erucic acid content and total glucosinolate content was calculated using Pearson's correlation coefficient.

Results and Discussion

The investigated volunteers showed phenotypic variation in erucic acid and total glucosinolate content. Erucic acid content ranged from 0% (GM 95, GM 121) to 57.4% (GM 92) of all fatty acids, with the mean value of 43.58% and coefficient of variation of 42.56%; the variation in total glucosinolate content was very large, ranging from 5.2 (GM 125) to 105.5 μmol g^{-1} of seeds (GM 66), with the mean value of 25.27 μmol g^{-1} of seeds and coefficient of variability of 89.84% (Table 2). In reference cultivars, erucic acid content was as follows: 0.0% for double low (00) WOSR Monolit and DH WG (0HG), 2.1% for DH JN (0HG), 39.5% for *B. campestris* cultivar Ludowy, 55.6% for DH ER (HE0), and 57.0% for WOSR cultivar Maplus. Total glucosinolate content varied from 5.3 μmol g^{-1} of seeds in DH ER to 12.6 μmol g^{-1} of seeds in cultivar Maplus, 14.5 μmol g^{-1} of seeds in Monolit, 44.3 in *B. campestris*, 82.2 in DH WG, and 92.1 μmol g^{-1} of seeds in DH line JN. The F_1 hybrid 'Monolit × Maplus' showed 26.4% erucic acid of all fatty acids in seeds and the total GLS content of 16.5 μmol g^{-1} of seeds (Table 2).

On the basis of chemical composition of seeds as the grouping criterion, 66 volunteers were divided into four groups (A–D). The characteristics of groups A, B, C, and D are shown in Tables 3–5 and Figures 1 and 2. Group A consists of double low WOSR cultivar Monolit and six volunteers with low erucic acid and low glucosinolate contents. Group B has one volunteer and two doubled haploid DH WG and DH JN with the chemical composition of seeds that is characteristic for 0HG rapeseed. The third largest group *C comprises* 49 volunteers and HE0 reference genotypes: Maplus, F_1 hybrid 'Monolit × Maplus', and DH ER. The last group D includes ten HEHG volunteers and *B. campestris* cultivar Ludowy. ANOVA test indicated a statistically significant ($P<0.001$) difference between groups formed on the basis of the chemical composition of seeds - erucic acid and GLS content (Table 5). Groups C and D were characterized by the highest content of erucic acid, and groups A and B had the lowest content of this seed compound (Table 3, Figure. 1). In terms of total GLS content, group B stands out because of the observed highest average value of this trait (86.43 μmol

g^{-1} of seeds) with the lowest variability, CV = 5.90 (Table 4, Figure. 2). The lowest total GLS content is characteristic for groups A and C (16.09 and 16.35 μmol g^{-1} of seeds, respectively). The greatest variation, CV = 53.88, for the observed trait was noted in group D (Table 4, Figure. 2).

Table 2. Chemical composition of seeds of volunteers originating from soil seed bank.

Sample	Erucic acid content (%)	Total GLS content (μmol g^{-1} of seeds)	Group according to the chemical composition
GM62- 1/4.1.Z	2.0	18.6	A
GM63- 1/4.2.Z	55.5	27.3	D
GM64- 2/4.1.Z	54.3	15.9	C
GM65- 2/4.2.Z	55.4	26.8	D
GM66- 2/4.4.Z	48.6	105.5	D
GM67- 2/4.5.Z	53.2	93.7	D
GM68- 2/4.6.Z	51.8	23.3	C
GM69- 2/4.8.Z	36.7	87.7	D
GM70- 3/2.1.Z	3.8	19.0	A
GM71- 3/2.2.Z	53.9	22.8	C
GM72- 7/3.1.Z	54.7	15.8	C
GM73- 7/3.2.Z	2.4	18.4	A
GM74- 7/4.1.Z	56.0	17.6	C
GM75- 1/1.1.D	44.4	23.8	C
GM76- 1/3.1.D	54.5	23.4	C
GM77- 1/3.2.D	53.6	18.7	C
GM78- 1/3.3.D	39.5	12.7	C
GM79- 1/3.4.D	51.6	17.2	C
GM80- 1/4.1.D	51.5	17.3	C
GM81- 1/4.3.D	52.6	9.5	C
GM82- 1/4.5.D	51.1	7.8	C
GM83- 1/4.6.D	55.0	14.4	C
GM84- 1/4.7.D	48.9	17.2	C
GM85- 1/4.8.D	52.3	16.4	C
GM86- 1/4.9.D	51.2	19.1	C
GM87- 1/4.10.D	48.9	12.7	C
GM88-1/4.11.D	50.7	17.5	C
GM89- 1/4.12.D	49.1	11.8	C
GM90- 2/2.1.D	55.7	13.4	C
GM91- 3/3.1.D	50.5	15.1	C
GM92- 3/2.2.D	57.4	16.8	C
GM93- 3/2.3.D	46.5	12.1	C
GM94- 3/2.4.D	56.3	15.1	C
GM95- 4/3.1.D	0.0	13.3	A
GM96- 4/3.2.D	27.6	15.7	C
GM97- 4/3.3.D	49.6	86.5	D
GM98- 4/3.4.D	53.7	17.4	C
GM99- 4/3.5.D	53.2	17.2	C
GM100- 5/1.2.2	50.6	23.3	C
GM101- 5/2.1.D	52.8	48.9	D
GM102- 5/3.1.D	52.4	28.5	D
GM103- 5/3.2.D	49.9	24.3	C
GM104- 6/1.1.D	50.4	17.7	C
GM105- 6/4.1.D	54.2	16.1	C
GM106- 6/4.2.D	56.7	17.6	C
GM107- 6/4.3.D	54.2	13.3	C

GM108- 7/2.1.D	53.6	16.0	C
GM109- 7/2.2.D	15.4	15.7	C
GM110- 7/3.1.D	53.6	16.0	C
GM111- 7/3.2.D	36.2	23.5	C
GM112- 7/3.3.D	55.7	13.7	C
GM113- 7/3.4.D	48.5	13.1	C
GM114- 7/4.1.D	54.9	45.6	D
GM115- 7/4.2.D	54.4	18.9	C
GM116- 7/4.3.D	55.8	20.1	C
GM117- 8/2.2.D	55.6	18.2	C
GM118- 8/3.1.D	54.4	28.1	D
GM119- 8/3.2.D	52.1	12.5	C
GM120- 8/3.3.D	47.9	20.0	C
GM121- 8/4.1.D	0.0	85.0	B
GM122- 8/4.2.D	42.9	18.4	C
GM123- 8/4.3.D	55.9	14.1	C
GM124- 8/4.4.D	55.4	20.3	C
GM125- 223/1.D	53.3	5.2	C
GM126 – "00"	0.0	14.5	A
GM127 – "00"	0.0	14.3	A
MONOLIT	0.0	14.5	A
MAPLUS	57.0	12.6	C
MON/MAP	26.4	16.5	C
DH ER	55.6	5.3	C
LUDOWY	39.5	44.3	D
DH WG	0.0	82.2	B
DH JN	2.1	92.1	B
Mean	43.58	25.27	
Coefficient of variation	42.56%	89.84%	

A: "00" double low OSR; B: "0HG"—low erucic acid, high GLS OSR.
C: "HE0"—high erucic acid, low GLS OSR; D: "HEHG"—high erucic acid, high GLS OSR.
DH ER: doubled haploid line high erucic acid, low GLS; DH WG: doubled haploid line low erucic. acid, high GLS; DH JN: doubled haploid line from cultivar Jet Neuf low erucic acid, high GLS.

Table 3. Characteristics of the groups formed on the basis of chemical composition—erucic acid content.

Class/Group	Mean	Minimum	Maximum	Median	Skewness	Kurtosis	s.d.	cv
A	1.17 b	0	3.8	0	0.672	−1.093	1.56	133.33
B	0.70 b	0	2.1	0	0.707	−1.5	1.212	173.14
C	50.34 a	15.4	57.4	52.9	−2.584	6.948	8.119	16.13
D	50.27 a	36.7	55.5	52.8	−1.278	0.176	6.443	12.82

In the mean column, mean values followed by the same letters are not significantly different.

These studies have shown that volunteers come from very diverse sources. The investigated volunteers indicate the presence in the soil seed bank seeds derived from the cultivar Maplus, 00, WOSR, 0HG, HE0, and traditional cultivars HEHG. A particularly interesting observation was the presence of volunteers with high erucic acid and high glucosinolate content in seeds, despite the fact that this traditional type of cultivars had not been grown in these fields in the last 25 years. Similar results were obtained in studies on volunteers conducted in Poland in the EU SIGMEA

project covered a wide geographical area in the country: Western Pomerania, Pomerania, and Warmia-Masuria. High erucic acid rapeseed (HEAR) cultivars were not grown in Poland after 1985, and high glucosinolate content cultivars after 1995. However seeds from 93 oilseed rape volunteers collected from winter oilseed rape fields in the growing season 2005/2006 were of different quality: double low (2.1% of all plants), HE0 (6.5%), 0HG (39.8%) and HEHG (51.6%). These results and those obtained in the SIGMEA project indicate that the seeds of traditional HEHG, HE0 or 0HG cultivars persist for a long time (almost 20 years) in the soil seed bank at the stage of secondary dormancy (SIGMEA, 2007).

Table 4. Characteristics of the groups formed on the basis of chemical composition glucosinolate content.

Class /Group	Mean	Minimum	Maximum	Median	Skewness	Kurtosis	s.d.	cv
A	16.09 c	13.3	19	14.5	0.2161	−1.801	2.45	15.23
B	86.43 a	82.2	92.1	85	0.4753	−1.5	5.1	5.90
C	16.35 c	5.2	24.3	16.45	−0.339	0.418	4.31	26.36
D	56.63 b	26.8	105.5	45.6	0.4558	−1.453	30.51	53.88

In the mean column, mean values followed by the same letters are not significantly different.

Table 5. Mean squares analysis of variance according to the distribution of the chemical composition for erucic acid and total glucosinolate content.

Source of variation	Number of degrees of freedom	Erucic acid content [%]	Total GLS content [μmol g^{-1} of seeds]
Class/Group according to the chemical composition	3	6992.04***	8922.9***
Residual	69	54.99	149.9

*** P<0.001

Many studies have shown that germination of seeds spilled to the soil can be prevented by natural induction of secondary dormancy (Gruber et al. 2011; Sausse et al. 2011; Schatzki et al. 2011; Stockmann et al. 2011). Secondary seed dormancy in oilseed rape allows the seeds to survive in the soil seed bank for many years without germination, where in the dormancy period could be 10 years or even longer (D'Hertefeldt et al. 2008; Lutman et al. 2005). The presence of rapeseed with high erucic acid content, high glucosinolate content (or both) or double low volunteers can be detected by a biochemical method and by genetic markers (PCR). RAPD molecular markers were used to determine the level of polymorphism and genetic similarity of investigated OSR volunteers and to search for relationship between molecular markers and erucic acid and GLS content. Twenty five RAPD primers were used in this study. A total of 306 fragments were amplified, of which 153 (50.0%) were polymorphic. Table 1 shows the RAPD markers, number of polymorphic bands, and

polymorphism level. The similarity data matrix based on Nei and Li (1979) coefficient showed the lowest similarity value of 0.06 between *B. campestris* cv. Ludowy and GM 71 and the highest value of 1.0 for GM 126 and GM 127 (Table 6).

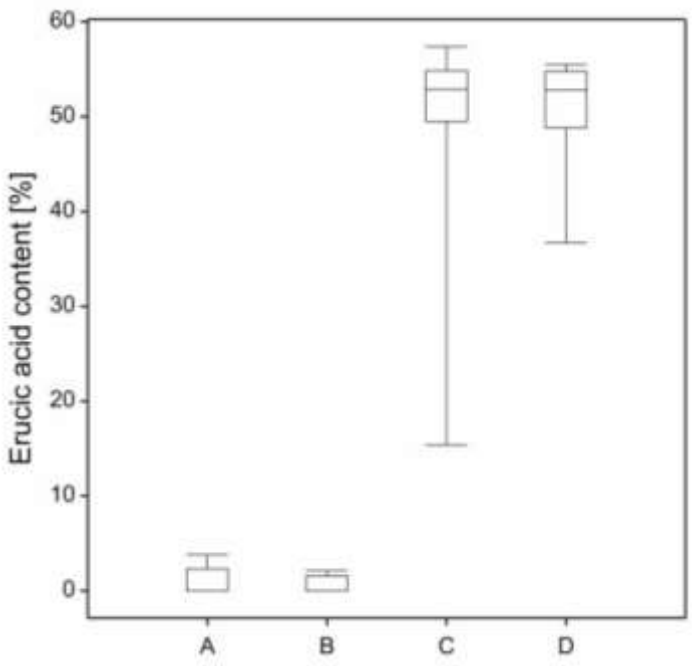

Figure 1. Boxplot of erucic acid content in the seeds of investigated volunteers and reference genotypes divided into groups according to the chemical composition of the seeds.

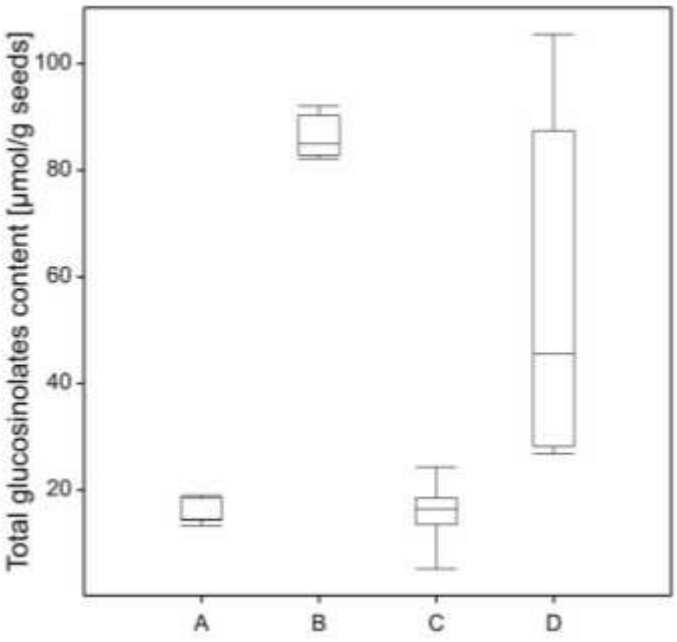

Figure 2. Boxplot of GLS content in the seeds of investigated volunteers and reference genotypes divided into groups according to the chemical composition of the seeds.

Dendrogram formed on the basis of cluster analysis of the Nei and Li coefficient of similarity divided the investigated OSR genotypes into two mains clusters: I and II; *B. campestris* cv. Ludowy formed a separate branch with respect to other genotypes (Figure. 3). Cluster I included the six 00 volunteers, Monolit (group A), one 0HG (GM 121—group B), one HE0 (GM 125—group C), references genotypes (DH ER, DH WG, and DH JN) and three volunteers HEHG (GM 66, GM67, and GM69—group D). Cluster II represents 48 volunteers HE0 (group C), F_1 hybrid Monolit × Maplus, Maplus, and seven HEHG volunteers (group D) (Figure. 3).

Table 6. Minimum and maximum genetic similarity values calculated using the measure of genetic similarity reported by Nei and Li (1979).

Minimum genetic similarity values			Maximum genetic similarity values		
Ludowy	GM 71	0.06	GM 127	GM 126	1
Ludowy	GM 104	0.08	GM 87	GM 82	0.98
Ludowy	GM 64	0.09	GM 88	GM 80	0.98
Ludowy	GM 66	0.09	GM 81	GM 80	0.97
Ludowy	GM 67	0.09	GM 82	GM 81	0.97
Ludowy	GM 68	0.09	GM 83	GM 80	0.97
Ludowy	GM 69	0.09	GM 85	GM 84	0.97
Ludowy	GM 75	0.09	GM 87	GM 81	0.97
Ludowy	GM 79	0.09	GM 87	GM 84	0.97
Ludowy	GM 89	0.09	GM 89	GM 82	0.97
Ludowy	GM 90	0.09	GM 89	GM 87	0.97
Ludowy	GM 91	0.09	GM 113	GM 112	0.97
Ludowy	GM 92	0.09	GM 82	GM 80	0.96
Ludowy	GM 103	0.09	GM 83	GM 81	0.96
Ludowy	GM 107	0.09	GM 87	GM 80	0.96
Ludowy	GM 109	0.09	GM 89	GM 81	0.96
Ludowy	GM 110	0.09	GM 89	GM 84	0.96
Ludowy	GM 112	0.09	MONOLIT	GM 126	0.96
Ludowy	GM 113	0.09	MONOLIT	GM 127	0.96
Ludowy	GM 116	0.09	GM 83	GM 82	0.95
Ludowy	GM 118	0.09	GM 84	GM 82	0.95
Ludowy	GM 123	0.09	GM 85	GM 83	0.95
Ludowy	GM 124	0.09	GM 87	GM 83	0.95

Analysis of molecular variance (AMOVA) using erucic acid and GLS content as a grouping criterion (four groups) revealed that the within-class variance was 83% of the total variation, while the variation between groups was only 17% (Table 7, Fig. 4). Molecular variability within the individual groups varied considerably: group A, 98.000; group B, 45.333; group C, 663.019; and group D, 256.909 (Table 8). A total of 153 polymorphic markers were used to determine the relationship between RAPD markers and erucic acid and total GLS content in volunteers of rapeseed.

Table 7. Analysis of molecular variance (AMOVA) of 153 polymorphic AFLP markers from volunteers and reference genotypes.

Source of variation	Number of degrees of freedom	Sum of squares	Mean squares	Estimated variance	Percentage of variation
Among groups	3	148.793	49.598	3.061	17%
Within groups	69	1063.262	15.410	15.410	83%
Total	72	1212.055		18.471	100%

Table 8. Results of the analysis of molecular variance for the particular groups of populations.

Population	Number of genotype in the group/N	Sum of squares within group/SSWP	Mean square within the group/MSWP
Group A	7	98.000	14.00
Group B	3	45.333	15.11
Group C	52	663.019	12.75
Group D	11	256.909	23.36

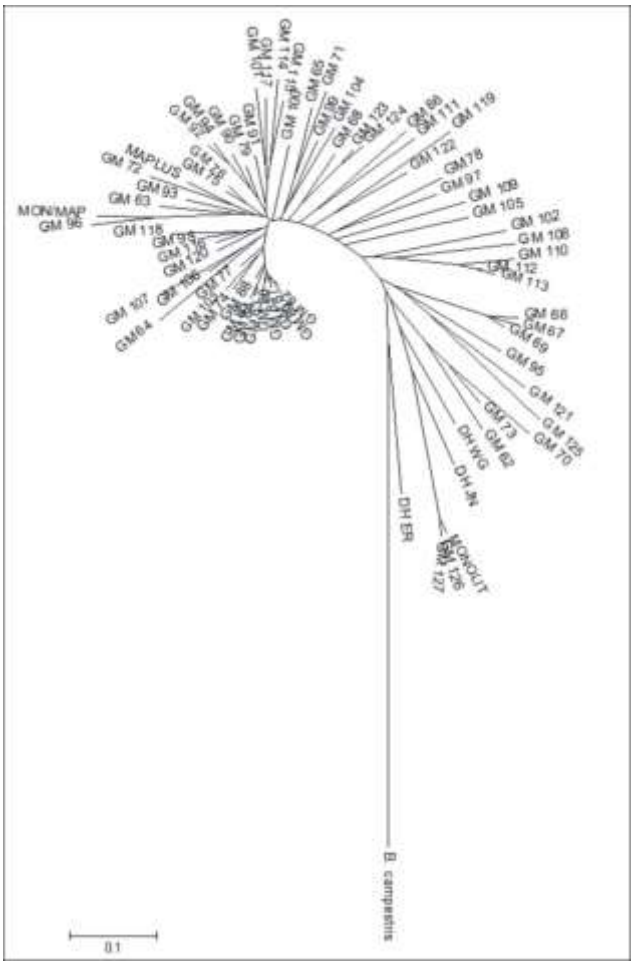

Figure 3. Dendrogram of winter oilseed rape volunteers from the soil seed bank created on the basis of RAPD markers.

Table 9 lists the RAPD markers significantly associated with erucic acid, and Table 10 - markers associated with the total glucosinolate content in OSR seeds. A total of 53 RAPD markers were associated with the erucic acid content. Twenty-nine markers are associated with a decrease in the content of this acid and 24 showed an increase in erucic acid content in seeds. The percentage of total phenotypic variability explained by the particular markers ranged from 5% to 71.1% (Table 9).

Table 9. Markers associated with erucic acid content in volunteers and reference genotypes of WOSR.

Marker	Estimates of regression coefficients	P-value	Percentage variation accounted	Standard error of observations
OPA 08~1160	14.83	<0.001	13.7	17.2
OPA 08~1000	−42.04	<0.001	71.1	9.97
OPA 14~1900	13.74	0.012	7.3	17.9
OPA 14~1660	−32.35	<0.001	35.6	14.9
OPA 14~630	−27.53	<0.001	27.6	15.8
OPA 14~690	−23.4	0.006	9	17.7
OPA 15~1160	−20.75	0.008	8.3	17.8
OPA 15~1054	−16.62	0.016	6.7	17.9
OPA 15~1100	−12.46	0.011	7.5	17.8
OPG 04~1260	14.33	0.017	6.4	17.9
OPN 13~1260	24.47	<0.001	35.3	14.9
OPN 13~960	−22.32	<0.001	28.2	15.7
OPN 13~955	20.72	<0.001	18	15.7
OPN 18~2100	−24.08	<0.001	20.8	16.5
OPN 18~1270	−14.11	0.004	10.1	17.6
OPN 18~1160	20.62	0.008	8.2	17.8
OPO 13~500	22.3	<0.001	33.7	15.1
OPW 09~1500	22.08	<0.001	14.3	17.2
OPW 09~1960	−20.25	0.003	10.5	17.5
OPY 01~1750	12.73	0.003	10.6	17.5
OPY 01~1160	14.13	0.002	12	17.4
OPA 07~1500	11.56	0.009	7.9	17.8
OPA 07~1480	−14.65	0.002	10.9	17.5
OPF 01~2400	13.8	0.004	9.9	17.6
OPF 01~2300	−19.93	<0.001	16	17
OPF 01~1300	−30.44	<0.001	53.7	12.6
OPF 01~890	−14.22	0.001	12.7	17.3
OPF 01~500	−21.64	0.005	9.1	17.7
OPF 01~450	17.97	<0.001	18.9	16.7
OPN 20~2000	17.53	0.001	12.8	17.3
OPW 05~2120	−35.76	<0.001	18.4	16.8
OPW 05~800	15.98	<0.001	17.1	16.9
OPW 05~760	−25.24	<0.001	29.7	15.6
OPY 15~1830	−15.59	<0.001	14.3	17.2
OPY 15~1800	13.27	0.004	9.7	17.6
OPY 15~1270	−29.72	<0.001	41.7	14.2
OPY 15~650	−26.82	<0.001	38.5	14.5
OPC 18~1050	16.72	<0.001	16.2	17
OPC 18~730	−21.85	<0.001	30.1	15.5
OPC 18~690	−24.55	<0.001	23.3	16.2

OPF 04~1600	25.66	<0.001	33.7	15.1
OPF 04~1050	16.67	<0.001	15.1	17.1
OPD 08~1375	−15.66	<0.001	16.5	16.9
OPL 12~700	−37.33	<0.001	30	15.5
OPP 03~1260	14.14	0.003	10.8	17.5
OPP 03~1230	−33.01	<0.001	30.4	15.5
OPP 03~690	20.26	0.002	11.9	17.4
OPP 14~1160	−33.52	<0.001	44.7	13.8
OPP 14~1120	33.52	<0.001	44.7	13.8
OPY 13~1750	−12.96	0.032	5	18.1
OPY 13~1280	16.54	<0.001	13.8	17.2
OPY 13~1580	−26.52	<0.001	19.1	16.7
OPD 08~830	19.68	0.001	12.3	17.4

Bold: markers common to both traits.

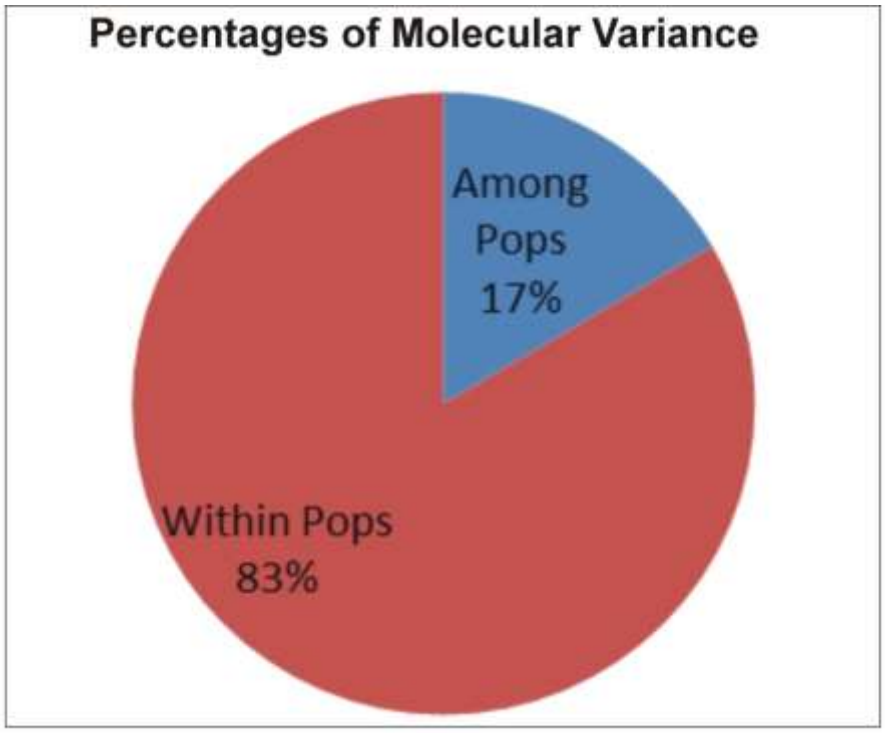

Figure 4. Pie chart summarizing the outcome of analysis of molecular variance (AMOVA), showing the partitioning of molecular variance within and among populations.

In these studies 29 RAPD markers were associated with the total glucosinolate content in seeds of rapeseed volunteers. Among them, 17 markers were linked with the highest level of GLS content. The presence of 12 markers indicated a decrease in glucosinolate content. The markers explained 5.1–29.8% of phenotypic variation in total glucosinolate content in seeds of rapeseed volunteers (Table 10). Among the total of 53 RAPD markers, 20 markers showed a significant association with both traits. Eleven markers were linked with low level of erucic acid content and highest level of total GLS content in seeds of volunteers (Tables 9 and 10). The traits of erucic acid and

glucosinolate content observed in this study were not significantly correlated (r = −0.2277, P = 0.0527) (Figure. 5).

Table 10. Markers associated with total GLS content in volunteers and reference genotypes of WOSR.

Marker	Estimates of regression coefficients	P-value	Percentage variation accounted	Standard error of observations
OPA 08~450	18.93	0.001	12.8	21.2
OPA 14~1900	−17.6	0.008	8.2	21.8
OPN 13~1260	−19.63	<0.001	14.3	21.0
OPN 13~960	15.61	0.008	8.3	21.7
OPN 13~955	−15.02	0.006	8.9	21.7
OPO 13~500	−16.94	0.001	12.1	21.3
OPW 09~1960	33.43	<0.001	20.3	20.3
OPY 01~1160	−17.17	0.002	11.8	21.3
OPA 07~1500	−13.55	0.013	7.1	21.9
OPA 07~1480	15.18	0.011	7.4	21.8
OPF 01~2400	−24.86	<0.001	23.1	19.9
OPF O1~2300	22.98	<0.001	14	21.1
OPF 01~500	45.57	<0.001	29.8	19.0
OPF 01~450	−15.85	0.005	9.1	21.6
OPW 05~1850	13.24	0.015	6.7	21.9
OPW 05~800	−12.28	0.022	5.9	22.0
OPC 18~730	18.94	<0.001	14.4	21.0
OPF 04~890	17.33	0.006	8.8	21.7
OPF 04~500	−11.89	0.031	5.1	22.1
OPD 08~1375	16.33	0.002	11.6	21.3
OPD 08~1480	17.53	0.002	11.5	21.4
OPL 12~650	12.56	0.018	6.3	22.0
OPP 03~690	−31.42	<0.001	19.9	20.3
OPP 11~700	31.05	<0.001	26.7	19.4
OPY 13~1750	21.98	0.003	10.9	21.4
OPY 13~1280	−22.22	<0.001	16.9	20.7
OPY 13~1100	12.35	0.019	6.2	22
OPY 13~1580	32.78	<0.001	19.5	20.4
OPA 07~610	44.1	0.006	8.9	21.7

Bold: markers common to both traits

The principal coordinate analysis (PCA) was performed to confirm the structure and acquire more information about the 66 volunteers (Figure 6). The first two principal components explained a total of 28.43% variation (20.26% and 8.17%, respectively) revealed by RAPD markers. The PCA analysis (Figure 6) showed a tendency toward clustering in three groups. The genotypes double low OSR were clustered together, and most of the genotypes with high erucic acid content in seeds HE0 such as cultivar Maplus were also clustered together; three genotypes HEHG, GM 66, GM 67, and GM 69 (traditional OSR), formed a small well-defined group. All 66 volunteer plants from seed bank in Dlon and Zielecin have typical morphotype of oilseed rape plants. Genetic studies of the 66 volunteers and reference genotypes indicated two major clusters representing rapeseed volunteers

and oilseed reference genotypes and a separate *B. campestris* cultivar Ludowy. Molecular markers that revealed an association with erucic acid content and high glucosinolate content may thus be useful to monitor volunteers growing in plantations of rapeseed and feral populations growing along roads. Genetic study of 31 volunteers from the three voivodeships of Western Pomerania, Pomerania, and Warmia-Masuria using RAPD markers and flow cytometry divided investigated volunteers according of morphotype, erucic acid and glucosinolate content (Bocianowski et al. 2008; Liersch et al. 2008). Jørgensen et al. (2007) investigated the purity of certified seed lots, the abundance and origin of volunteers, and the longevity and origin of seeds in the soil seed bank by using molecular ISSR markers. They reported that the volunteer plants or seeds belonged to varieties cultivated at the site 4–17 years earlier; this finding suggests the long persistence of volunteers in soil seed bank. Moreover, ISSR analysis of 14 reference varieties showed that three of the certified seed lots contained the seeds of other varieties (Jørgensen et al. 2007). Molecular marker method and flow cytometry were used to monitor genetically modified (GM) oilseed rape and feral GM plants growing along railway lines and port areas at four sites in Switzerland. The plant samples were screened for molecular markers of genetic modification and analyzed by flow cytometry to measure nuclear DNA contents of putative hybrid species. Both methods allow to effectively control feral GM plants identified as glyphosate-resistant GM plants during the growing season after regular glyphosate treatments (Schulze et al. 2014).

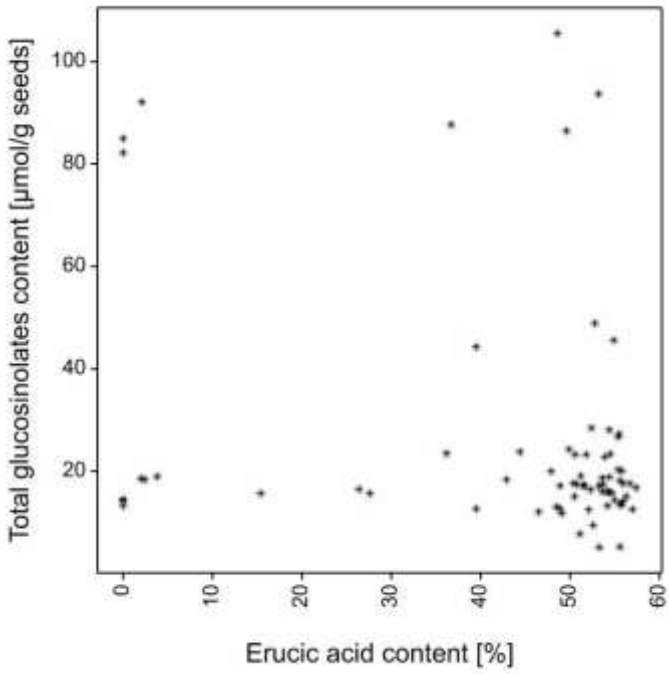

Figure 5. The interdependence of the observed traits.

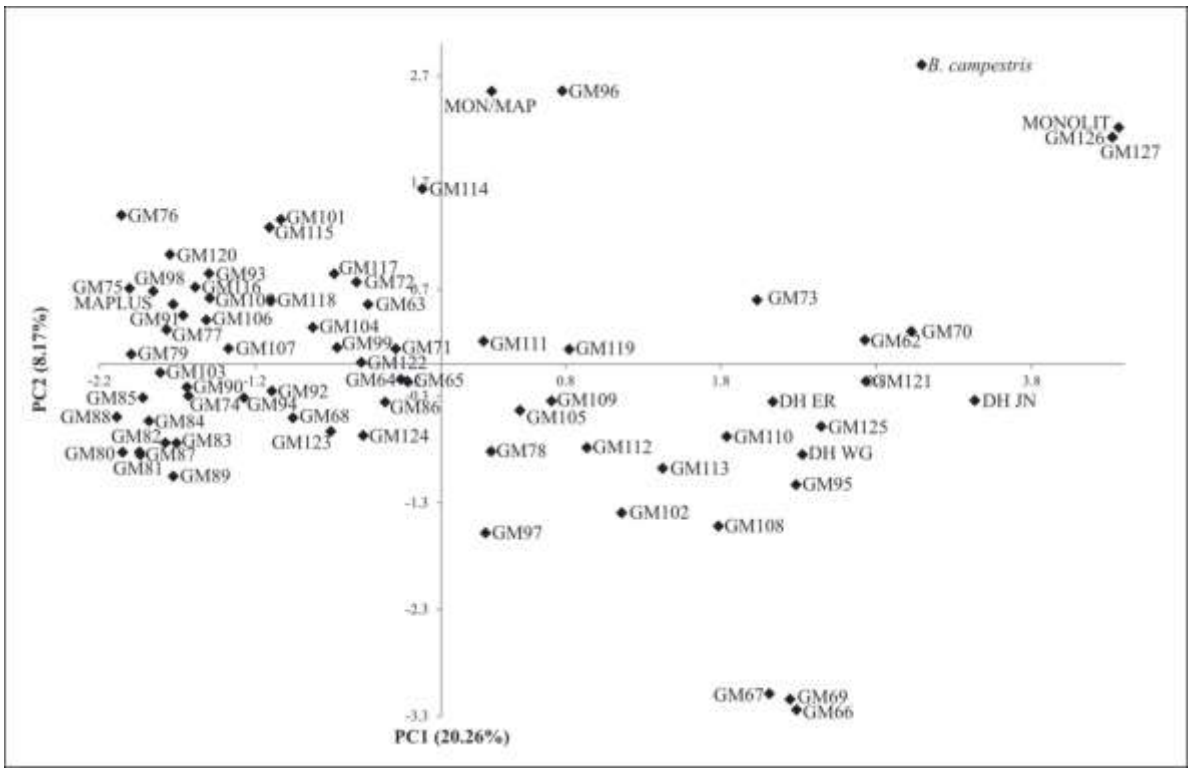

Figure 6. Scatterplot of principal coordinates 1 and 2 of 66 volunteers according to molecular marker observations.

Oilseed rape cultivars and genotypes show a large variation in secondary seed dormancy. OSR cultivars can differ significantly in their predisposition to dormancy (Gruber et al. 2009), ranging from almost 0% to nearly 100% (Weber et al. 2011). Secondary seed dormancy potential in *Brasssica* crops was primarily controlled by genotype with some influence of pre- and postharvest environmental conditions and soil texture (Garbutt and Witcombe 1986; Gulden et al. 2004; Schatzki et al. 2011, Weber et al. 2011). The results from the analysis of several genotypes obtained by Gruber et al. (2012) and Schatzki et al. (2011) show the positive correlation of seed dormancy and NDF (neutral detergent fiber), ADF (acid detergent fiber), and ADL (acid detergent lignin) content, it means fiber and lignin in the seeds of OSR. The type of soil and soil environmental conditions specific to soil texture, such as temperature, aeration, humidity, pathogens, and soil physical structure, may also influence the different conditions for maintaining or breaking dormancy (SIGMEA, 2007). In our research the number of volunteers obtained from the soil seedbank was much higher at Dlon (80.3%) that has more heavy soils than at Zielecin (19.7%), which is characterized by a light soil. Lutman et al. (2002; 2005) showed that weed seeds persist for a shorter time in light soils than in heavy soils. If the seeds from soil seed bank germinate and develop to volunteers, they grow in the same crop rotation and become the source of several agricultural problems in the subsequent crops, such as harvest problems due to different stages of

maturity or gene dispersal by pollen flow and direct seed admixture (Bartkowiak-Broda et al. 2008, Stockmann et al. 2011). Moreover, volunteers compete with current oilseed rape plantation for water, nutrients, and light (for increased crop densities) and also act as a gateway for pest infestation and diseases for this crop. Volunteers reduce the potential of seed yield, which can not only cause financial loss for farmers but also change the quality of harvested seeds. Some of the seeds from the seed bank in Dlon and Zielecin can be a potential source of volunteer populations in the next oilseed rape plantation with negative influence on yield quality. OSR volunteers in newly sown oilseed rape plantation may develop well and cannot be identified or controlled chemically before seed production.

Conclusion

The results of this study confirm that seeds of rapeseed of different origin may persist for a long time at the stage of secondary dormancy. These seeds are the source of volunteers that contaminate next plantation of winter oilseed rape. This is the most important problem concerning the coexistence of different qualities of conventional oilseed rape cultivars and modern GM varieties. The best approach to minimize the high frequency of volunteers in the context of the coexistence of OSR with different qualities or in the case of coexistence of GM and non-GM OSR involves careful harvesting; large-scale reduction in seed loss at harvest; minimizing the amount of seeds incorporated into the soil seed bank; controlling OSR seed bank dynamics; choosing appropriate timing and type of postharvest cultivation and controlling of certified seeds and monitoring of volunteers and feral plants by using biochemical, morphological, and DNA markers (SIGMEA, 2007; Gruber et al. 2012; Jørgensen et al. 2007). However seeds of any type OSR cultivars and in any environment can be the source of volunteers because of that in addition to proper post-harvest tillage the best solution is selection of genotypes with the seed's low ability to undergo to secondary dormancy (Gruber et al. 2018).

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Conflicts of Interest

Authors declare no conflict of interest.

References

Bartkowiak-Broda I, Walkowski T, Poplawska W, Ogrodowczyk M, Liersch A. 2008. Influence of volunteers and oilseed rape-like plants on the quality of winter oilseed rape yield. Oilseed Crops. XXIX (2): 185-198 (in Polish).

Bocianowski J, Kozak M, Liersch A, Bartkowiak-Broda I. 2011. A heuristic method of searching for interesting markers in terms of quantitative traits. Euphytica. 181: 89-100.

Bocianowski J, Liersch A, Bartkowiak-Broda I, Poplawska W. 2008. Characteristics of winter oilseed rape (*Brassica napus* L.) volunteers with the use of RAPD markers. Bulletin of Plant Breeding and Acclimatization Institute. 249: 183-192 (in Polish).

Byczynska B, Krzymanski J. 1969. Quick method to obtain fatty acids methyl esters for gas chromatography analysis. Tłuszcze Jadalne. 108-114 (in Polish).

Colbach N. 2011. Evaluation of prospective cropping system scenarios for managing oilseed rape volunteers and harvest purity using the GENESYS model. In: Proceedings 13th International Rapeseed Congress, 5-9 June, Prague, Czech Republic, Abstract book, 6-9.

Doyle J.J, Doyle J.L. 1990. Isolation of plant DNA from fresh tissue. Focus. 12: 13-15.

D'Hertefeldt T, Jørgensen R.B, Pettersson L.B. 2008. Long-term persistence of GM oilseed rape in the seedbank. Biology letters. 4: 314-317.

FAOSTAT 2015, hhtp://www.faostat3.fao.org

Felsenstein J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Garbutt K, Witcombe J.R. 1986. The inheritance of seed dormancy in *Sinapis arvensis* L. Heredity. 56: 25-31.

Gruber S, Claupein W. 2004. Secondary dormancy of oilseed rape: first aspects of heredity. In: Proceedings of the 4th International Crop Science Congress, Brisbane, Australia.

Gruber S, Claupein W. 2007. Fecundity of volunteer oilseed rape and estimation of potential gene dispersal by a practice-related model. Agric Ecosys Environ. 119: 401-408.

Gruber S, Emrich K, Claupein W. 2009. Classification of canola (*Brassica napus*) winter cultivars by secondary dormancy. Can J Plant Sci. 89: 613-619.

Gruber S, Feike T, Weber E.A, Claupein W. 2011. Following the trace of harvesting seed losses - a model to predict the soil seed bank and oilseed rape volunteers. In: Proceedings of 13th International Rapeseed Congress, June 5-9, Prague, Czech Republic, Abstract book, 168-171.

Gruber S, Hüsken A, Dietz-Pfeilstetter A, Möllers C, Weber E.A, Stockmann F, Thöle H, Schatzki J, Dowideit K, Renard M, Becker H.C, Schiemann J, Claupein W. 2012. Biological confinement strategies for seed-and pollen-mediated gene flow of GM Canola (*Brassica napus* L.). AgBioForum. 15(1): 44-53.

Gruber S, Lutman P, Squire G, Roller A, Albrecht H, Lecomte J. 2007. Using the SIGMEA database to provide an overview of the persistence of oilseed rape in the context of the coexistence of GM and conventional crops. In: Proceedings of GMCC. 7: 261-262.

Gruber S, Pekrun C, Claupein W. 2005. Life cycle and potential gene flow of volunteer oilseed rape in different tillage systems. Weed Res. 45: 83-93.

Gruber S, Weber E.A, Thöle H, Möhring J, Dietz-Pfeilstetter A, Clupein W. 2018. Impact of cultivar on survival of volunteer oilseed rape populations in fields is more important than field management. Weed Res. 58: 89-98.

Gulden R.H, Thomas A.G, Shirtliffe S.J. 2004. Relative contribution of genotype, seed size and environment to secondary seed dormancy potential in Canadian spring oilseed rape (*Brassica napus*). Weed Res. 44: 97-106.

Hüsken A, Dietz-Pfeilstetter A. 2007. Pollen-mediated gene flow from herbicide-resistant oilseed rape (*Brassica napus* L.). Transgenic Res. 16: 557-569.

Jørgensen T, Hauser T.P, Jørgensen R.B. 2007. Adventitious presence of other varieties in oilseed rape (*Brassica napus*) from seed banks and certified seed. Seed Sci Res. 17: 115-125.

Kozak M, Bocianowski J, Rybiński W. 2013. Note on the use of coefficient of variation for data from agricultural factorial experiments. Bulgarian J Agric Sci. 19(4): 644-646.

Liersch A, Poplawska W, Ogrodowczyk M, Bartkowiak-Broda I, Bocianowski J. 2008. Phenotypic characteristics of winter oilseed rape volunteers (*Brassica napus* L.) occurring in northern regions of Poland. Bulletin of Plant Breeding and Acclimatization Institute. 250: 249-260 (in Polish).

Lutman P.J.W, Berry K, Payne R.W, Simpson E, Sweet J.B, Champion G.T, May M.J, Wightman P, Walker K, Lainsbury M. 2005. Persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (*Brassica napus*). In: Proc. R. Soc. B. 272: 1909-1915.

Lutman P.J.W, Cussans G.W, Wright K.J, Wilson B.J, Mcn Wright G, Lawson H.M. 2002. The persistence of seeds of sixteen weed species over six years in two arable fields. Weed Res. 42: 231-241.

Michalski K, Kołodziej K, Krzymański J. 1995. Quantitative analysis of glucosinolates in seeds of oilseed rape – effect of sample preparation on analytical results. In: Proceedings 9th International Rapeseed Congress, 4-7 July 1995, Cambridge, UK 3 911-913.

Mesquida J, Renard M, Pierre J.S. 1988. Rapeseed (*Brassica napus* L.) productivity; the effect of honeybees (*Apis mellifera* L.) and different pollination conditions in cage and field tests. Apidologie. 19: 51-72.

Nei M, Li W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. In: Proc. Natl. Acad. Sci. 76: 5269-5273.

Pierre J, Marsault D, Genecque E, Renard M, Champolivier J, Pham Delegue M.H. 2003. Effects of herbicide-tolerant transgenic rape genotypes on honey bees and other pollinating insects under field conditions. Entomologia Experimentalis et Apllicata. 108: 159-168.

Poplawska W, Liersch A, Jedryczka M, Kaczmarek J, Wolko J, Ogrodowczyk M, Bartkowiak-Broda I. 2012. Wind-mediated pollen dispersal of oilseed rape-an estimation using pollen traps. In. Breckling, B. & Verhoeven, R. GM-Crop Cultivation – Ecological Effects on a Landscape Scale. Theorie in der Ökologie 17. Frankfurt, Peter Lang. 17: 26-33.

Sausse C, Lucas J.L, Wagner D. (2011. Estimation of oilseed rape losses before and during harvest in France in various conditions. In: Proc. 13th International Rapeseed Congress, June 5-9: 2011 Prague, Czech Republic, Abstract book: 151-154.

Schatzki J, Klöppel C, Becker H.C, Möller C. 2011. Genetic variation for secondary seed dormancy in a set of current European winter oilseed rape cultivars. In: Proceedings th. International Rapeseed Congress, June 5-9, 2011 Prague, Czech Republic, Abstract book: 680-682.

Schulze J, Frauenknecht T, Brodmann P, Bagutti C. 2014. Unexpected diversity of feral genetically modified oilseed rape (*Brassica napus* L.) despite a cultivation and import ban in Switzerland. PLOS ONE. 1-18.

SIGMEA. 2007. Sustainable Introduction of GM Crops into European agriculture. Project no. 501986. Final Activity Report. 1-31.

Soltani E, Baskin J.M, Baskin C.C. 2019. A review of the relationship between primary and secondary dormancy, with reference to the volunteer crop weed oilseed rape (*Brassica napus*). Weed Research. 59: 5-14.

Stockmann F, Weber E.A, Frick K, Gruber S, Calupein W. 2011. Pre-harvest sprouting – a risk for low-dormancy oilseed rape? Development of simple test and first results. In: Proc. 13th International Rapeseed Congress, June 5-9, Prague, Czech Republic, Abstract book: 266-267.

Weber E.A, Frick K, Stockmann F, Gruber S, Claupein W. 2011. Seed persistence of novel-trait oilseed rape (*Brassica napus* L.) genotypes in different soils. In: Proc. 13th International Rapeseed Congress, June 5-9, Prague, Czech Republic, Abstract book: 299-303.

Williams J.G.K, Kubelik A.R, Livak K.J, Rafalski J.A, Tingey S.V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18: 6531-6535.

Wittkop B, Snowdon R.J, Friedt W. 2009. Status and perspectives of breeding for enhanced yield and quality of oilseed crops for Europe. Euphytica. 170: 131-140.

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