BIOLOGIJA. 2008. Vol. 54. No. 4. P. 238–241
DOI: 10.2478/v10054-008-0049-x

Investigations of oilseed rape (*Brassica napus* L.) genetic diversity by molecular methods

M. Jodinskienė[1]*,

A. Paulauskas[2],

J. Žukauskienė[2]

[1] *Laboratory of Plant Physiology, Institute of Botany, Mokslininkų 12, LT-08406 Vilnius, Lithuania*

[2] *Department of Biology, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania*

Oilseed rape (*Brassica napus* L.) is grown all over the world, and in Lithuania its plots are expanding every year. It is used for oil, feed, fuel, etc. Genetic variability of oilseed rape in Lithuania was analysed employing RAPD molecular markers. Three different random Lithuanian regions in Kėdainiai, Vilkaviškis and Jurbarkas were selected for collecting oilseed rape leaf samples. '*Baldur*' (A) leaves were collected in the Kėdainiai region, '*Olano*' (O) in the Vilkaviškis region and '*SW Pastell*' (P), '*Remy*' (R), '*Banjo*' (B) in the Jurbarkas region. Nine primers were screened for their ability to produce polymorphic patterns. Eight primers which gave reproducible and distinct amplification products were selected for evaluation of diversity in five oilseed rape cultivars. The test primers generated different polymorphic fragments ranging from 1 to 10 per reaction, with an average of 6.75 bands per primer – in total 54 polymorphic amplification products in the range of 250–1750 bp. Dendrograms based on UPGMA cluster analysis confirmed the suitability of all the primers for the further analysis and showed a significant genetic variation among individuals of different cultivars.

Key words: oilseed rape (*Brassica napus* L.), PCR, RAPD method, genetic polymorphism

INTRODUCTION

Brassica napus was domesticated only about 400–500 years ago [1]. This crop is grown all over the world, and in Lithuania its plots are expanding every year. It is used for oil, feed, fuel, etc. In 2007, Lithuania has distinguished itself from the other EU countries by declaring a lot of energetic plants, and 70% of them were covered by oilseed rapes – 67 thousand ha of spring and 53 thousand ha of winter oilseed rape [2]. *Brassica napus* is a self-pollinating species that can outcross; for field-grown oilseed rape, it is estimated that at least 20% of seed will result from outcrossing [3]. *Brassica napus* cultivars have been produced by traditional and non-traditional means, so cultivars of various genetic complexity are available [4]. Variation may also arise during maintenance of a cultivar, out-crossing species in particular being at risk of increased heterogeneity in the next generation, if pollination is not carefully controlled [4].

In the past years, DNA-based molecular markers such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), single sequence repeats (SSRs), sequence related amplified polymorphism (SRAP) [5] and restriction fragment length polymorphism (RFLP) have been used to identify and characterize important molecular traits in *Brassica* species [6–8]. The RAPD markers are easier and quicker to use and preferred in applications where relationships between closely related breeding lines are of interest [9]. RAPD markers have been applied to taxonomic comparisons of many plant species to date [10–14] and have in general been found to generate results comparable to those obtained by other methods (i. e. morphological, isozyme or RFLP markers) [4]. DNA-based markers have an apparent advantage as cultivar descriptors in that they are unaffected by environmental or physiological factors [15]. They can reveal polymorphisms between closely related genotypes and are technologically less demanding in comparison to other DNA-based techniques [4]. RAPD markers can reveal variations between individuals within a cultivar [15]. Some authors [16, 17] observed a significant correlation between genetic distance and heterosis for seed yield in *Brassica* inbred lines. No such investigations have been made in Lithuania to show genetic polymorphism within different cultivars or in the same cultivar.

The main object of this work was to examine the genetic variability of different oilseed rape cultivars in Lithuania by RAPD molecular markers.

MATERIALS AND METHODS

Three different random Lithuanian regions (Kėdainiai, Vilkaviškis and Jurbarkas) were selected for collecting oilseed rape samples. The oilseed rape fields in Kėdainiai and Vilkaviškis regions belonged to two different individual farmers, and oilseed rapes from the Jurbarkas region were from a bigger joint corporation. The distance among the regions was more than 100 km. '*Baldur*' (A) samples were collected from the Kėdainiai region, '*Olano*' (O) from the Vilkaviškis region and '*SW Pastell*' (P), '*Remy*' (R), '*Banjo*' (B) from the Jurbarkas region.

* Corresponding author. E-mail: milda.jodinskiene@botanika.lt

DNA was extracted from oilseed rape leaves using a genomic DNA extraction kit (MBI Fermentas, Vilnius, Lithuania), with some modifications [14]. The concentration of DNA was estimated with a spectrophotometer (Eppendorf, Hamburg). DNA was diluted up to 100 ng/µl for use in PCR.

DNA amplification was carried out in PCR tubes; the total reaction volume was 25 µl. DNA amplification was performed using a thermocycler (Eppendorf, Hamburg) programmed to 1 cycle at 94 °C for 1 min 30 s, following 30 cycles at 94 °C for 30 s, at 36 °C for 35 s, at 72 °C for 1 min, final extension at 72 °C for 2 min, and hold at 4 °C [4]. The primers were synthesised by MBI Fermentas. After amplification, PCR products were separated by electrophoresis in 1.5% agarose gel. Agarose gel was stained with ethidium bromide and photographed under the UV light (EASY Win32, Herolab, Germany). The gene Ruler™ 100 bp DNA Ladder Plus (MBI Fermentas) was used as a marker.

For data analysis, a binary matrix reflecting the presence (1) or absence (0) of a specific RAPD band was generated. Genetic distance (GD) among individual trees in the study was estimated according to the Nei and Li formula [18]. The GD value was calculated using the TREECON for Windows [19], and the dendrogram was drawn using the UPGMA method [20].

RESULTS AND DISCUSSION

The main object of the work was to choose suitable primers for the future analysis of oilseed rape DNA samples – to screen RAPD primers used by other authors in *Brassica napus*, to evaluate their ability to produce polymorphic PGR products and to compare the results. Nine primers (210, 222, 250, 266, 268, 340, 474, 516, 563) used in experiments with *Brassica napus* [4, 21] were screened for their ability to produce polymorphic patterns using five winter rape cultivars, '*Baldur*' (A), '*SW Pastell*' (P), '*Remy*' (R), '*Banjo*' (B) and '*Olano*' (O), from three different regions of Lithuania.

Eight primers which gave reproducible and distinct amplification products were selected for diversity evaluation in five oilseed rape cultivars. Primer 210 gave one 470 bp amplification product and was discarded as non-informative. The remaining primers (88.8%) varied in the ability to produce different polymorphic fragments ranging from 1 to 10 per reaction, with an average of 6.75 bands per primer. The test primers generated 54 polymorphic amplification products in the range of 250–1750 bp (Table 1). Mailer et al. [22] used RAPDs to discriminate among 23 oilseed rape cultivars, 100 primers were tested, and the selected 6 primers produced 23 polymorphic bands between 300 to 2200 base pairs. Cartea et al. [23], for the evaluating the genetic diversity of 33 Spanish and 18 British oilseed rape cultivars, analysed 18 primers which produced 105 polymorphic amplification products in the range of 350 to 2500 bp. Other authors tested 60 to 90 primers for description of 6–22 oilseed rape cultivars, and only 14% [24] to 76% [9] of them were polymorphic; the number of polymorphic bands was only 3.36 per one polymorphic primer [24]. Our results showed a good choice of primers: eight of the nine screened primers were suitable.

Table. **Codes and sequences of primers used for analysis in '*Baldur*' (A), '*SW Pastell*' (P), '*Remy*' (R), '*Banjo*' (B) and '*Olano*' (O), total number of bands counted and DNA fragment size**

Primer	Sequence	Total number of bands	Fragment size range (bp)
222	5′-AAGCCTCCCC-3′	1–8	350–1500
250	5′-CGACAGTCCC-3′	2–4	550–850
268	5′-CGACAGTCCC-3′	4	250–850
269	5′-CCAGTTCGCC-3′	5–10	350–1100
340	5′-GAGAGGCACC-3′	4–8	200–1750
474	5′-AAGCCTCCCC-3′	3–9	280–1500
516	5′-AGCGCCGACG-3′	1–4	550–1200
563	5′-CGCCGCTCCT-3′	3–7	300–1350

Primer 222 generated 1 to 8 bands in all test cultivars, the strongest bands corresponding to 1500 and 1200 bp, and they varied among the individuals. Primer 250 produced 2 to 4 fragments, two of them, 580 and 850 bp, being present in all individuals and the other two, 600 and 700 bp, varying among the individuals. Dulson et al. [4] used bulked DNA samples from four oilseed rape cultivars and obtained invariant 825 and 800 bp bands with primer 250. These bands were not observed in our samples. These differences could be due to the different cultivars tested and to the bulked samples used in [4]. The authors failed to find the 800 bp fragment in bulked samples, but they found it in one individual from one group [4]. Primer 268 produced four fragments; 850, 550, 250 bp fragments were produced in all '*Remy*' and '*Baldur*' individuals, but they had no 500 and 600 bp fragments, some '*SW Pastell*' individuals had no 250 bp fragments, but had 600 bp fragment.

Primer 269 gave 5 to 10 amplification products ranging from 350 to 1100 bp; the latter was produced in all individuals, and 580 bp fragment was also present in all except two '*Olano*' individuals. Primer 340 produced 4 to 8 amplification products, 380 and 1031 bp fragments were present in all test individuals, 580 bp product was not found in P1 individual, and 1750 bp product was not found in P1, R2 and R3 individuals (Fig. 1).

Primer 474 produced 3 to 9 amplification products, 600 bp fragment was found in all individuals, and 800 bp fragment was

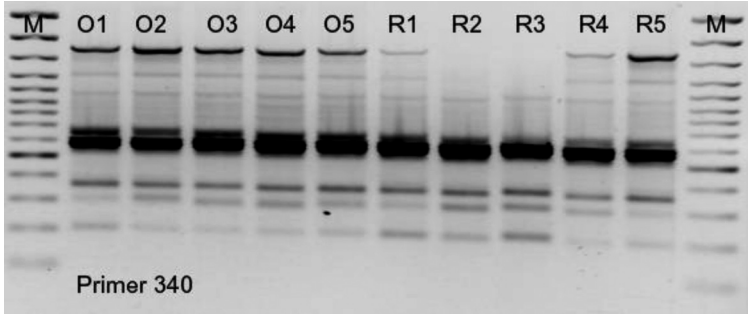

Fig. 1. DNA fingerprints from different samples of different oilseed rape cultivars obtained by PCR with primer 340. O1–O5 – '*Olano*', R1–R5 – '*Remy*'. M – Gene Ruler™ 100 bp DNA Ladder Plus (MBI Fermentas)

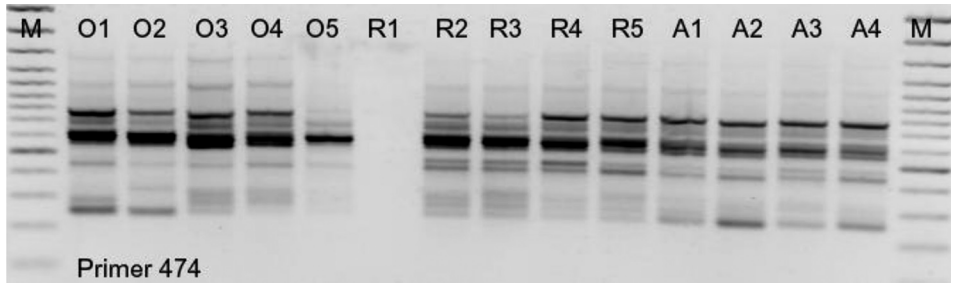

Fig. 2. DNA fingerprints from different samples of five different oilseed rape cultivars obtained by PCR with primer 474. O1–O5 – '*Olano*', R1–R5 – '*Remy*', A1–A4 – '*Baldur*'. M – Gene Ruler™ 100 bp DNA Ladder Plus (MBI Fermentas)

present in all except O5 (Fig. 2). Primer 516 produced 1 to 4 amplification products, 550 bp fragment was detected in all individuals, and 1000 bp in all R individuals. Primer 563 generated from 3 to 7 bands; 600 bp fragment was found in all individuals.

According to [4], 700 bp fragment varied among bulked DNA samples in RAPD results with primer 563; the same was observed in our samples. RAPD results show that there are bands common for all test individuals; some RAPD fragments were not found only in some of individuals, or these individuals had the fragments that were not found in the others. The primers that produced only four polymorphic products were also informative because some of these fragments varied strongly among the individuals.

To get a better view of the results and to test the suitability of every primer for the further analysis, genetic distances were analysed and genetic dendrograms were drawn for every primer (Fig. 3). Dendrograms based on UPGMA cluster analysis showed that clusters were formed differently by different primers, but not for all oilseed rape cultivars. '*Baldur*' was always in one cluster according to all examined primers. Some populations from different regions were located in the same clusters: '*Baldur*' from Kėdainiai and '*Banjo*' from Jurbarkas regions were present in the same or adjacent clusters according to four primers: 222, 268, 269 and 340. '*Remy*' from Jurbarkas region was located next to '*Baldur*' and '*Banjo*' according to primers 222, 268, 269 and 474. In most cases, two cultivars ('*SW Pastell*' and '*Olano*') were the

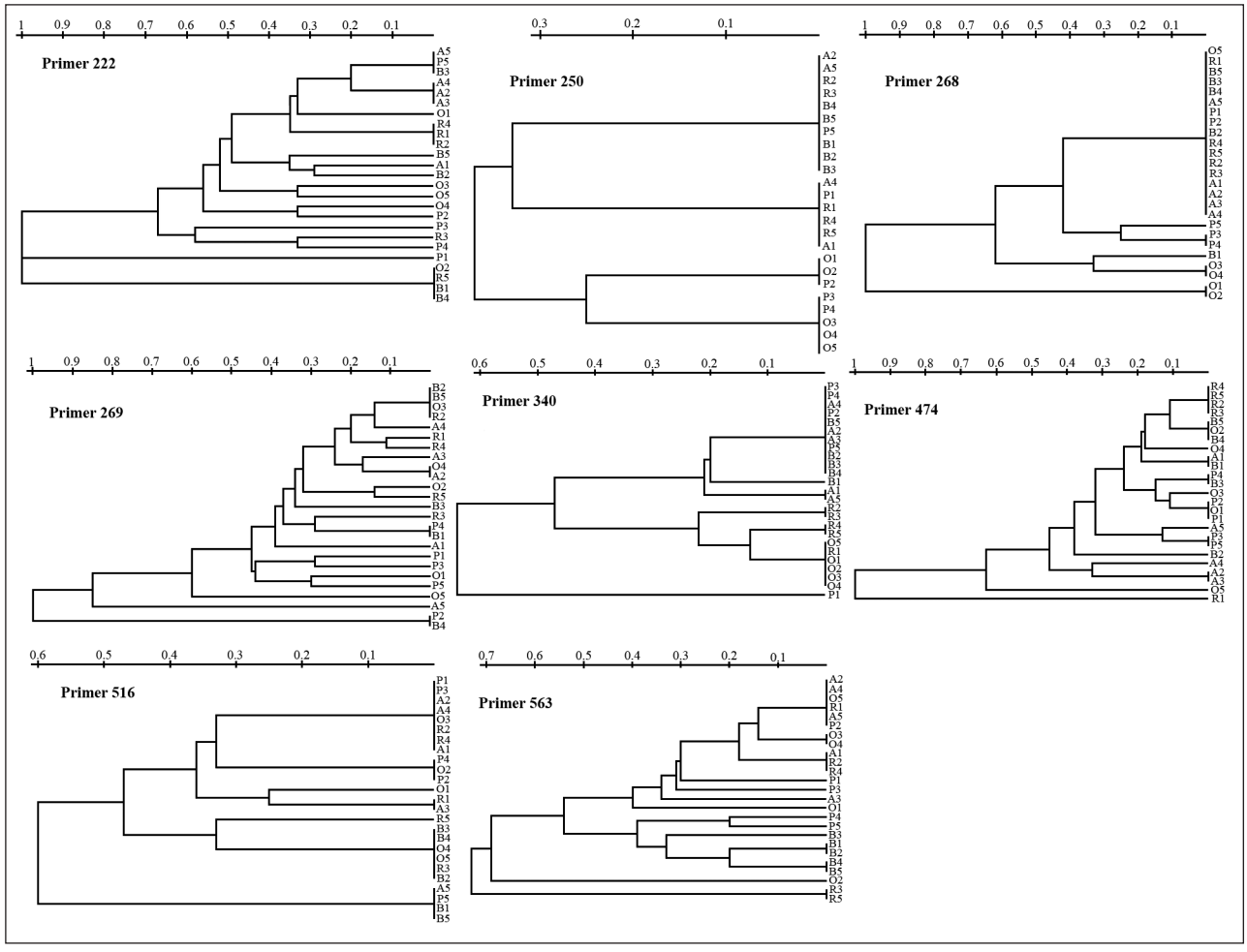

Fig. 3. Dendrograms based on Nei genetic distance [18], constructed for 25 analysed individuals of *Brassica napus* with eight primers: 250, 222, 268, 269, 340, 474, 516, 563. Individuals of *Brassica napus*: A1–A5 – '*Baldur*', P1–P5 – '*SW Pastell*', R1–R5 – '*Remy*', B1–B5 – '*Banjo*' and O1–O5 – '*Olano*'

most distant from the others. A significant part of genetic variance was due to an individual variation within the populations. Intra-cultivar polymorphism in oilseed rape was reported by Mailer et al. [22] in their RAPD analysis. A significant genetic variation existed among various combinations of rapeseed germplasm reported by [25].

In the present work, for the first time in Lithuania, investigations of oilseed rape genetic polymorphism in randomly selected farmer fields were carried out. The dendrograms confirmed the suitability of all the primers for the further analysis and showed a significant genetic variation among individuals of different cultivars.

AKNOWLEDGEMENT

This work was partially supported by a programme of the Lithuanian Ministry of Education and Science, the Lithuanian State Science and Studies Foundation and by the EU Postdoctoral Research project.

Received 19 June 2008
Accepted 26 November 2008

References

1. Gómez-Campo C. Biology of *Brassica* coenospecies. Elsevier, Netherlands, 1999: 33–58.
2. http://www.rapsai.lt/lt.php/rapsu-augintoju-zinios/
3. Lühs W, Friedt W. In: Designer Oil Crops: Breeding, Processing and Biotechnology. Murphy DJ, ed. New York: VCH Publishers, 1994: 5–71.
4. Dulson J, Kott LS, Ripley VL. Euphytica 1998; 102: 65–70.
5. Riaz A, Li G, Quresh Z et al. Plant Breed 2001; 120: 411–5.
6. Veldboom LR, Lee M. Crop Sci 1996; 36: 1310–9.
7. Chevre AM, Barret P, Eber F et al. Theor Appl Genet 1997; 95: 1104–11.
8. Pongam P, Osborn CT, Williams HP. Phytopathology 1998; 88: 1068–72.
9. Halldén C, Nilson N-O, Rading LM et al. Theor Appl Genet 1994; 88: 123–8.
10. Rafalski JA, Tingey SV, Williams JGK. AgBioTech News and Info 1991; 3: 645–8.
11. Williams JGK, Hanafey MK, Rafalski JA et al. Nucl Acids Res 1993; 218: 704–40.
12. Bachmann K. New Phytol 1994; 126: 403–18.
13. Žvingila D, Verbylaitė R, Baliuckas V et al. Biologija 2005; 3: 46–53.
14. Areškevičiūtė J, Paulauskas A, Česonienė L et al. Biologija 2006; 1: 5–7.
15. Bowditch BM, Albright DG, Williams JGK et al. Methods in Enz 1993; 224: 294–309.
16. Knaak C, Ecke W. Proc 9th Int Rapeseed Congr. Cambridge, 1995: 110–2.
17. Diers BW, Macvetty PBE, Osborn TC. Crop Sci 1996; 36: 79–83.
18. Nei M, Li W. Proc Natl Acad Sci USA 1976; 76: 5269–73.
19. Van de Peer Y, De Wachter R. Comput Appl Biosci 1994; 10: 569–70.
20. Yeh F, Boyle T. Belg J Bot 1997; 129: 157.
21. Asghari A, Mohammadi SA, Moghaddam M et al. Biotechnol & Biotechnol Eq 2007: 413–6.
22. Mailer RJ, Scarth R, Fristensky B. Theor Appl Genet 1994; 87: 697–704.
23. Cartea ME, Soengas P, Picoaga A et al. Genet Resour Crop Evol 2005; 52(6): 655–62.
24. Sobotka R, Dolanska L, Čurn V et al. J Appl Genet 2004; 45(2): 161–73.
25. Ahmad N, Munir I, Khan I et al. Biotechnology 2007; 6(3): 334–8.

M. Jodinskienė, A. Paulauskas, J. Žukauskienė

RAPSŲ ĮVAIROVĖS TYRIMAI MOLEKULINIAIS METODAIS

Santrauka

Žieminiai rapsai (*Brassica napus* L.) auginami visame pasaulyje, jų plotai kievienais metais didėja ir Lietuvoje. Iš jų gaminamas aliejus, pašarai, kuras ir t. t. Žieminių rapsų genetinė įvairovė Lietuvoje buvo tiriama atsitiktinai padaugintos polimorfinės DNR (APPD) metodu. Lapų pavyzdžiai analizei buvo surinkti iš trijų atsitiktinai parinktų Lietuvos rajonų – Kėdainių, Vilkaviškio ir Jurbarko: 'Baldur' (A) iš Kėdainių, 'Olano' (O) iš Vilkaviškio, 'SW Pastell' (P), 'Remy' (R), 'Banjo' (B) iš Jurbarko rajono. Iš devynių patikrintų pradmenų aštuoni buvo informatyvūs ir generavo polimorfinius amplifikacijos produktus, todėl buvo atrinkti tolimesnei penkių rapsų veislių analizei. Tirti pradmenys generavo nuo 1 iki 10 polimorfinių amplifikacijos produktų, kurių dydis siekė nuo 250 iki 1750 bp, iš viso 54 fragmentus, vidutiniškai 6,75 fragmento vienai reakcijai. Pagal UPGMA metodą nubraižytos dendrogramos patvirtino visų pradmenų tinkamumą tolimesniems tyrimams, taip pat atskleidė genetinę įvairovę tarp skirtingų veislių atskirų individų.

Raktažodžiai: žieminiai rapsai (*Brassica napus* L.), PGR, APPD metodas, genetinis polimorfizmas