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Genetic variation and relationships among spring camelina (*Camelina sativa,* Brassicaceae) accessions of different origin

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Summary: *Camelina sativa* L. is one of the oldest crops of the Brassicaceae family, first domesticated in the region of south-eastern Europe. It has regained interest as a very promising alternative oilseed crop with broad adaptability, a wide range of tolerances to pests and diseases, and low-input requirements. The genetic diversity in spring camelina proved to be limited, so the identification and characterization of genetic variations in germplasm originating from different sources is considered very useful for development of efficient breeding programmes. The aim of the study was to use SSR markers in order to investigate genetic variation of twenty spring camelina accessions of different origin and estimate their genetic relatedness. Forty-five individual samples were taken from each accession and used for amplification of SSR markers P4C11, P6E4 and LIB19. Percentage of polymorphic loci, number of alleles, effective number of alleles, expected heterozygosity and Shannon's information index were used to estimate genetic variation. The accessions expressed different levels of genetic variation. The highest variability was found in cultivar Zavolzskij, breeding line CK2X-7, cultivar NS Zlatka and breeding line CK2X-9. The most uniform were cultivar Pernice, and population Maslomania. AMOVA (analysis of molecular variance) showed that 64% of the total genetic variation was attributed to variance within accessions and 36% to variance among them. IBased on genetic distance, accessions were divided in two clades, which both were further divided in two subclades. Genetic distance analysis indicated that there was overlapping in certain breeding programs and exchange of breeding germplasm.
Key words: accessions, breeding, camelina, genetic variation, spring camelina, SSR markers

Introduction

Camelina sativa (L.) Crantz, also known as "false flax" or "gold of pleasure", is a self-pollinated, annual oilseed that belongs to the *Brassicaceae* family. Over the last twenty years, it has re-emerged as a very promising multipurpose oilseed crop, with a wide range of positive attributes that make it more adaptable to diverse environmental conditions than most other oilseed crops (Kuzmanović et al., 2021). It possesses a high seed oil content which is rich in antioxidants and essential fatty acids, particularly the OMEGA-3 fatty acid, α-linolenic acid (Vollmann et al., 2007; Berti et al., 2016). Additionally, camelina is more tolerant to most diseases and pests that generally attack oilseed crops. The hexaploid spring *C. sativa* has limited genetic diversity, encouraging the exploration of germplasm originating from different sources, for novel allelic variation for traits

of interest, such as seed yield, oil content, pathogen or pest resistance, etc. The previous studies concluded that there were low levels of genetic diversity available within spring type *C. sativa* compared with other oilseed crops (Vollmann et al., 2005; Ghamkhar et al., 2010; Singh et al., 2015; Luo et al., 2019; Blume et al., 2020). Since the identification and incorporation of new variation is essential for further crop improvement, the aim of this study was to assess the genetic diversity of spring camelina accessions of different origin and estimate their genetic relationship through SSR (simple sequence repeats) markers.

Material and methods

From each of twenty spring camelina accessions, originating from Austria, Ukraine, Croatia and Serbia, 45 individual samples were taken and analysed with three polymorphic SSR markers, P4C11, P6E4 and LIB19 (Manca et al., 2013; Kurasiak-Popowska et al., 2018). A total of ten polymorphic bands were generated. Each amplified fragment was treated as binary unit character and scored "0" for absence and "1" for presence. Estimation of genetic variation was carried out by using the GenAlEx (Genetic Analysis in Excel) software package, version 6.502 (Peakall and Smouse 2006; 2012). The following parameters were calculated: percentage of polymorphic loci P (%), number of alleles. effective number of alleles per loci and expected heterozygosity, based on allelic frequencies (Nei, 1987) and Shanon´s information index (I) of phenotypic diversity (Lewontin, 1972) based on marker frequencies. For estimation of variance components among and within the groups of tested populations analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was performed, also using the GenAlEx software package. Nei´s coefficient of similarity was used for grouping of the populations by unweighted pair group arithmetic mean (UPGMA) cluster method, using POPGENE software package (Yeh et al., 1997). Dendrogram, based on genetic distance was drawn using SAHN clustering method as available in NTSYSpc software package, version 2.11a (Rohlf, 2000) and generated by using TREE display option.

Results and discussion

The highest percentage of polymorphic loci and number of alleles were found in the cultivar Zavolzskij and the breeding line, CK2X-7, while the effective number of alleles, Shannon's information index and expected heterozygosity were the highest in the cultivar NS Zlatka and the breeding line CK2X-9 (Table 1.). The lowest values of most variation parameters were surveyed in the cultivar Pernice, while the population Maslomania had the lowest values for effective number of alleles and expected heterozygosity. The most heterogeneous were breeding line CK2X-9 and cultivars NS Zlatka and Zavolzskij, with highest values for expected heterozygosity and Shannon's information index. The analysis of molecular variance (AMOVA) showed that 64% of the total genetic variation was attributed to variance within accessions (Table 2).

According to dendrogram based on genetic distance, accessions were divided in two clades (Figure 1). The first clade was divided in two subclades, the first with cultivar NS Zlatka and breeding line CK2X-7, and the second with cultivars Maksimir and Zavolzskij. The second clade was divided in two subclades. The first subclade consisted of two groups, one with cultivar NS Slatka, Pernice, Iwan and population Maslomania, while in the second group were all remaining CK breeding lines, CJ11X-79, populations Irkutskij, Omskij, Calena and TYP Klagenfurt. Cultivars Calena and Omskij showed high level of similarity, which is not surprising, having in mind that Omskij was used in the breeding program for development of Calena cultivar. The second subclade consisted of breeding line CJ11X, populations Leindotter Korneuburg, Unkrautsform Rollsdorf and cultivar Gomholka. The grouping of accession indicates that there was overlapping in certain breeding programs and exchange of breeding germplasm.

Table 1. Estimates of genetic variation in camelina populations using SSR markers P4C11, P6E4 and LIB19

Accessions	%P	Na	Ne	I	He
NS Zlatka	80.00%	1.600 ± 0.267	1.626 ± 0.126	0.489 ± 0.089	0.340 ± 0.064
NS Slatka	70.00%	1,600 ± 0.221	1.321 ± 0.117	0.294 ± 0.091	0.192 ± 0.064
Maximir	70.00%	1.500 ± 0.269	1.507 ± 0.136	0.407 ± 0.098	0.280 ± 0.070
Maslomania	60.00%	1.500 ± 0.224	1.170 ± 0.062	0.214 ± 0.066	0.126 ± 0.042
CK2X-7	90.00%	1.800 ± 0.200	1.445 ± 0.091	0.437 ± 0.064	0.282 ± 0.047
CK3X-7	80.00%	1.700 ± 0.213	1.302 ± 0.110	0.311 ± 0.073	0.192 ± 0.054
CK2X-9	80.00%	1.700 ± 0.213	1.530 ± 0.112	0.456 ± 0.082	0.309 ± 0.058
CK1X-25	80.00%	1.700 ± 0.213	1.449 ± 0.127	0.395 ± 0.084	0.261 ± 0.062
CJ11X-43	80.00%	1.700 ± 0.213	1.334 ± 0.093	0.351 ± 0.071	0.220 ± 0.050
CJ11X-79	80.00%	1.700 ± 0.213	1.322 ± 0.119	0.311 ± 0.081	0.196 ± 0.059
Zavolzskij	90.00%	1.900 ± 0.100	1.508 ± 0.110	0.452 ± 0.076	0.302 ± 0.055
Omskij	80.00%	1.700 ± 0.213	1.462 ± 0.128	0.401 ± 0.086	0.267 ± 0.064
Irkutskij	60.00%	1.300 ± 0.300	1.226 ± 0.102	0.226 ± 0.082	0.142 ± 0.057
Pernice	40.00%	1.100 ± 0.277	1.219 ± 0.118	0.190 ± 0.089	0.126 ± 0.062
Typ Klagenfurt	70.00%	1.500 ± 0.269	1.413 ± 0.130	0.360 ± 0.090	0.239 ± 0.065
Leindotter (LK)	60.00%	1.300 ± 0.300	1.204 ± 0.091	0.220 ± 0.077	0.135 ± 0.052
Unkrauftform RS	60.00%	1.400 ± 0.267	1.299 ± 0.109	0.208 ± 0.091	0.184 ± 0.062
Gomholka	50.00%	1.200 ± 0.291	1.308 ± 0.129	0.263 ± 0.096	0.176 ± 0.068
Iwan	50.00%	1.200 ± 0.291	1.221 ± 0.110	0.206 ± 0.086	0.134 ± 0.060
Calena	80.00%	1.700 ± 0.213	1.371 ± 0.117	0.347 ± 0.084	0.225 ± 0.061
Total	70.50 ± 3.12%	1.540 ± 0.054	1.362 ± 0.026	0.331 ± 0.019	0.216 ± 0.013

P (%) – percentage of polymorphic loci, Na – number of alleles Ne – effective number of alleles, He – expected heterozygosity, I – Shannon's information index

Table 2. Analysis of molecular variance (AMOVA) of camelina populations

Source	df	SS	MS	Est. Var.	%
Among Pops	19	510.491	26.868	0.574	36%
Within Pops	880	907.156	1.031	1.031	64%
Total	899	1417.647		1.605	100%

Fixation index = 0.358, P<0.001

The assessment of genetic diversity and estimation of genetic relationship, combined with phenotypic studies and the performance evaluated in the field trials can provide the useful information for further development of camelina breeding programs.

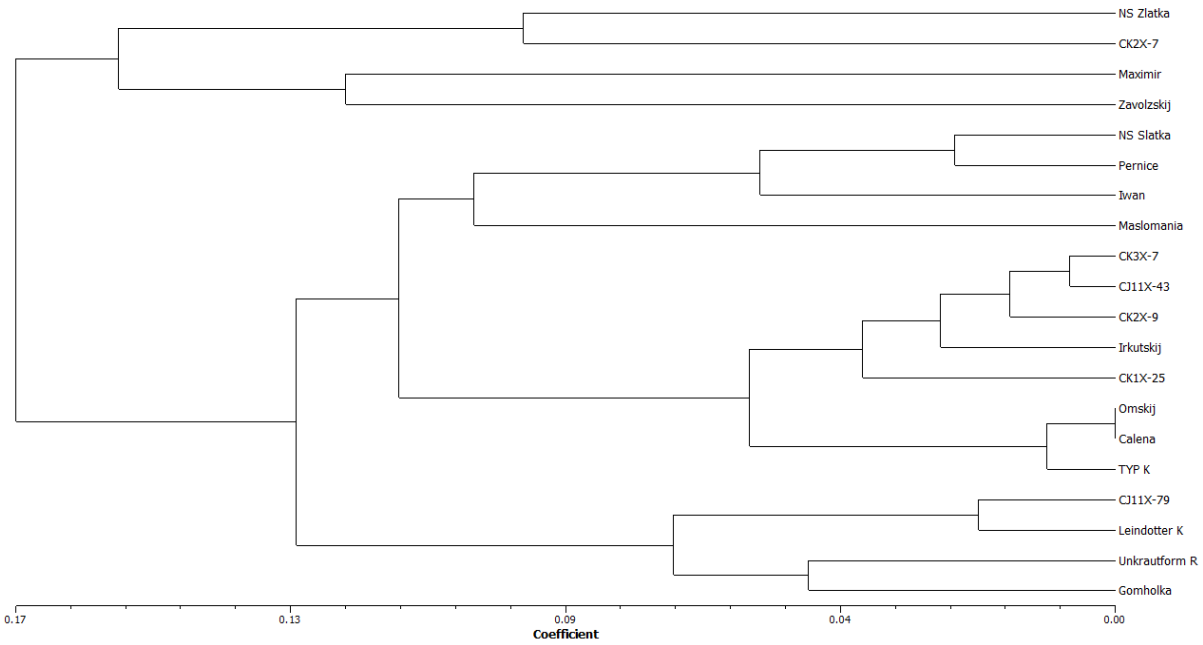

Figure 1. Dendrogram generated by UPMGA cluster analysis showing relationships between twenty camelina accessions, based on P4C11, P6E4 and LIB19 SSR markers

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References

Berti, M., Gesch, R., Eynck, C., Anderson, J., & Cermak, S. (2016). Camelina uses, genetics, genomics, production, and management. *Industrial Crops and Products, 94*, 690–710. http://dx.doi.org/10.1016/j.indcrop.2016.09.034

Blume, R. Y., Rabokon, A. M., Postovoitova, A. S., Demkovich, A. Ye., Pirko, Ya. V., Yemets, A. I., Rakhmetov, D. B., & Blume, Ya. B. (2020). Evaluating the diversity and breeding prospects of Ukrainian spring camelina genotypes. *Cytology and Genetics, 54*(5), 420-36. http://dx.doi.org/10.3103/S0095452720050084

Excoffier, L., Smouse, P., & Quattro, G. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics 131*, 479-491 distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, 131(2): 479–491. https://doi.org/10.1093/genetics/131.2.479

Ghamkhar, K., Croser, J., Aryamanesh, N., Campbell, M., Kon'kova N., et al. (2010). Camelina (*Camelina sativa* (L.) Crantz) as an alternative oilseed: molecular and ecogeographic analyses. *Genome, 53*, 558–567. http://dx.doi.org/10.1139/G10-034

Kurasiak-Popowska, D., Tomkowiak, A., Człopińska, M., Bocianowski, J., Weigt, D., & Nawracał, J. (2018). Analysis of yield and genetic similarity of Polish and Ukrainian *Camelina sativa* genotypes. *Industrial Crops and Products, 123*, 667–675. http://dx.doi.org/10.1016/j.indcrop.2018.07.001

Kuzmanović, B., Petrović, S., Nagl, N., Mladenov, V., Grahovac, N., Zanetti, F., Eynck, C., Vollmann, J., & Marjanović Jeromela, A. (2021). Yield-Related Traits of 20 Spring Camelina Genotypes Grown in a Multi-Environment Study in Serbia. *Agronomy, 11*, 858. https://doi.org/10.3390/agronomy11050858

Lewontin, R.C. (1972). The Apportionment of Human Diversity. *Evolut. Biol. 6*, 381-398. https://doi.org/10.1007/978-1-4684-9063-3_14

Luo, Z., Brock, J., Dyer, J. M., Kutchan, T. M., Augustin, M. et al. (2019). Genetic diversity and population structure of a Camelina sativa spring panel. *Front. Plant Sci., 10*, 184. https://doi.org/10.3389/fpls.2019.00184

Manca, A., Pecchia, P., Mapelli, S., Masella, P., & Galasso, I. (2013). Evaluation of genetic diversity in a *Camelina sativa* (L.) Crantz collection using microsatellite markers and biochemical traits. *Genet Resour Crop Evol, 60*, 1223–1236. https://doi.org/10.1007/s10722-012-9913-8

Nei, M. (1987). Molecular Evolutionary Genetics, Columbia University Press, New York, USA.

Peakall, R., & Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics, 28*, 2537-2539. https://doi.org/10.1093/bioinformatics/bts460

Peakall, R., & Smouse, P.E. (2006). GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes, 6*, 288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Rohlf, F.J. (2000). *NTSYSpc: Numerical Taxonomy and Multivariate Analysis System*, Version 2.1a; Exeter Software: Setauket, NY, USA, p. 44.

Singh, R., Bollina, V., Higgins, E. E., Clarke, W. E., & Eynck, C. (2015). Single-nucleotide polymorphism identification and genotyping in *Camelina sativa*. *Mol. Breed., 35*, 35. https://doi.org/10.1007/s11032-015-0224-6 0224-6

Vollmann, J., Grausgruber, H., Stift, G., Dryzhyruk, V., & Lelley, T. (2005). Genetic diversity in camelina germplasm as revealed by seed quality characteristics and RAPD polymorphism. *Plant Breed., 124*, 446-453. https://doi.org/10.1111/j.1439-0523.2005.01134.x

Vollmann, J., Moritz, T., Kargl, C., Baumgartner, S., & Wagentristl, H. (2007). Agronomic evaluation of camelina genotypes selected for seed quality characteristics. *Industrial Crops and Products, 26*, 270–277. https://doi.org/10.1016/j.indcrop.2007.03.017

Yeh, F. C., Yang, R-C., Boyle, T., Ye, Mao, Z-H., & Judy, X. (1997). *POPGENE, the User-Friendly Shareware for Population Genetic Analysis*. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Genetička varijabilnost i srodnost genotipova jarog lanika različitog porekla

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Sažetak: *Camelina sativa* L., ili lanik, je jedna od najstarijih gajenih kupusnjača, koja je prvo bila domestifikovana na prostorima Jugoistočne Evrope. U poslednjih dvadeset godina, obnovljen je interes za ovu, skoro zaboravljenu biljnu vrstu, kao potencijalnu alternativnu uljanu kulturu. Razlozi za to su visok stepen adaptabilnosti, tolerantnost na mnoge štetočine i bolesti i nizak stepen potrebnih ulaganja za njeno gajenje. Genetička varijabilnost jarog lanika je relativno ograničena, tako da se njeno ispitivanje smatra izuzetno važnim za razvoj efikasnih programa oplemenjivanja ove kulture. Cilj istraživanja je bio ispitivanje genetičke varijabilnosti i srodnosti dvadeset genotipova jarog lanika, pomoću mikrosatelitskih (SSR) markera. Od svakog genotipa uzeto je 45 individualnih uzoraka, koji su korišćeni za amplifikaciju SSR prajmerima P4C11, P6E4 i LIB19. Ispitivani genotipovi su ispoljili različite stepene genetičke varijabilnosti, a AMOV (analiza molekularne varijanse) je ukazala da je 64% od ukupne genetičke varijabilnosti pripadalo varijabilnosti unutar genotipova. Analizom genetičke distance se može videti da je došlo do preklapanja različitih programa oplemenjivanja, kao i da je dolazilo do razmene germplazme između njih.

Ključne reči: genetička varijabilnost, genotipovi, jari lanik, lanik, oplemenjivanje, SSR markeri