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Rapd-Analysis of Flax Varieties of the Ukrainian National Collection

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ABSTRACT

The article presents the results of research on the use of RAPD (Random Amplified Polymorphic DNA) molecular primers, linked to the sign of fiber content in the stems of flax. The main purpose of the research is to identify genetic polymorphism based on the fiber content, inter- and intraspecific genetic relationships between domestic and foreign flax varieties and hybrids. Realization of this aim will allow the number of tasks: 1) to increase the possibilities for successfully identify flax varieties and hybrids, 2) group the breeding material according to its genetic affinity, which will optimize the selection of pairs for crossing, 3) it is better to integrate traditional breeding methods (hybridization, selection, mutagenesis) with molecular biology methods for creation flax varieties with specified parameters of valuable economic traits and, as a consequence, to make selection most successful. 24 varieties and hybrids of flax were investigated using the method of polymerase chain reaction and separation of DNA fragments by gel electrophoresis. A small number of loci on electrophoregrams were detected, which indicates a small affinity of the selection material, which was also confirmed by the use of cluster analysis.

Keywords: *Linum usitatissimum* L., variety sample, RAPD analysis, primer, locus, cluster

INTRODUCTION

In the early 90s of the last century, the RAPD method was actively used in plant genetics – for some of the plant species, it was used to make genetic maps. As a rule, studies using RAPD are performed as follows: from a large number of decanucleotides, those are empirically selected that give in this research object the most easily typed amplification products, which are reproduced during the repeated DNA studies [Montaldo Hugo, 1998; Khlestkina, 2013; Syvolap, 2013; Chesnokov, 2018; Sukharevab & Kuluev, 2018; Litke et al. 2019; Nan et al. 2019; Rieznik et al. 2021; Lys et al. 2021].

The RAPD analysis was used to assess the genetic diversity within and between flax varieties and landraces, as well as the genetic diversity and geographical distribution of Canadian flax that enables to understand the process of domestication of the crop [Vromans, 2006; Guzenko et al. 2008; Ludvikova & Griga, 2015; Woźniak, 2019; Novák et al. 2020; Hryhoriv et al. 2021; Yakupoglu et al. 2021; Karbivska et al. 2022a]. It is shown that fibre flax varieties are very similar in genetic markers and form a homogeneous group, while oil flax forms several groups together with nine landraces. It is also noted that in Canada, flax breeding has led to a greater loss of genetic diversity than in the United States. These conclusions have applied to most loci under Canadian breeding programs. When analyzing geographically distant samples, it has turned out that the variability between forms from different countries reaches 84.2%, while within one country it is amounted to 15.8%. It has also turned out that the samples from East Asia and Europe are characterized by the greatest genetic diversity, and the samples from Africa and India are more genetically homogeneous [Porokhovinova, 2012; Bayer, 2014; Uschapovskii et al. 2016; Postovoitova et al. 2016; Cheţan et al. 2021; Demydas et al. 2021; Tanchyk et al. 2021].

The biological and economic features of flax varieties of the Ukrainian national collection have been studied thoroughly, but the phylogeny of the initial forms, as well as the molecular determination of economic traits, require a more detailed study [Diederichsen & Yong-Bi, 2008; Loginov et al. 2014; Kvitko et al. 2021; Tonkha et al. 2021; Karbivska et al. 2022b]. The goal of our research is to determine the genetic polymorphism of varieties of the Ukrainian national collection based on the fiber content of the stem.

MATERIAL AND METHODS

As the object of research, we used shadow seedlings of 24 varieties of flax and oilseed flax from the collection of the Institute of Bast Crops of the National Academy of Agrarian Sciences of Ukraine, in particular 18 varieties of flax of Ukrainian origin (Hlazur, Hlukhivskyi Yuvileinyi, Charivnyi, Siverskyi, Hlobus, Hladiator, Esman, Rushnychok, Nadiia, Tomskyi 10/Viking, Zoria 87//Hermes/Electra, Ukrainian Rannii/Hermes/Charivnyi, Tomskyi-16/Prizyv-81//Prizyv-81, M-8, Vruchyi, LKS 8, Blakutnyi, 2406), two varieties of fibre flax of the Russian selection: Tost 2, Tomskyi 5, one variety sample Lida (Belarus), one variety sample Bonet (Czech Republic). Intermedia flax – Agatha (Netherlands), oil flax – Lirina (Germany).

Flax seeds were pre-germinated in a thermostat on filter paper without access to light for 3–5 days at a temperature of +26 °C. DNA was isolated by extraction with the use of ready-made sets of solutions for lysis and washing (manufacturer – Neogene Leading Biotechnology Laboratory (Ukraine). The subsequent DNA isolation was performed automatically using a magnetic sorbent. The sprouts were ground by hand in a porcelain mortar until smooth. 50 mg of plant homogenate were placed in 1.5 ml tubes, and added by 800 μL of lysing solution, and the contents of the tubes were mixed by turning up to 10 times. The test tubes were thermalized for 30 minutes at a temperature of 65 °c, then centrifuged on a vortex for 30 seconds at 3,500 revolutions per minute. After cell lysis, DNA was washed three times with salt buffer. 100 μL was added to each tube. ExtraDNA solution was thoroughly mixed on a vortex and transferred to a magnetic tripod. 5 μL of DNA extract were used for the reaction.

The DNA amplification was performed in a Bio-Rad T100 thermal cycler (USA) with a ready-made reaction mixture (ArtTaq DNA polymerase, 10-fold buffer and 50 mm $MgCl_2$). The final volume of the reaction mixture was amounted to 20 μL. The RAPD primers of random sequence developed by Eurofins Genomics, Ver_1 AATCGGGCTG and Ver_2 GTTGCGATCC, linked with the fiber trait, were used for amplification. The polymerase chain reaction (hereinafter referred to as PCR) was performed in the following mode: initial denaturation – for 12 min at a temperature of 95°C, the next 30 cycles in this mode: denaturation – for 30 sec at a temperature of 95°C, annealing of primers – for 1 min at a temperature of 32°C, elongation – for 30 sec at a temperature of 72°C.

The separation of amplification products was performed by horizontal electrophoresis in 2% agarose gel subject to the existence of ethidium bromide. A 1.0% TBE buffer was used as the electrode buffer. The visualization of amplification products was performed using a Bio-Rad UV Uviev Mini transilluminator, followed by gel photographing. pUC19 DNA/Kzo9I manufactured by SibEnzyme Ltd. Scientific-Production Association (Russian Federation) was used as a molecular weight marker. The marker is a plasmid hydrolyzed by an enzyme to form 15 fragments, and includes from 955 to 8 pairs of nucleotides.

The analysis of genetic diversity was performed by calculating the genetic distance according to Nei and Li [Masatoshi & Wen-Hsiung, 1979]. Statistical processing was performed using the PHYLIP-3.69 and TotalLabquant programs.

RESULTS AND DISCUSSION

When studying 15 flax varieties with two arbitrary oligomeric RAPD primers, a small number of amplification products were detected – only 6 fragments distributed relatively evenly and located within 341 pairs of nucleotides (hereinafter referred to as n.p.), with the exception of a single locus corresponding to 258 n.p. The variety Charivnyi has oligomeric loci that simultaneously correspond to both primers. No fragments were found for most of the presented varieties and interspecies hybrids. The Ver_2 primer is common to such varieties as Charivnyi, Hlobus, Esman, Agatha and variety sample Tomskyi-16/Prizyv-81//Prizyv-81. The most part of the studied material has no locus that is obviously related to the structure of the primers themselves (Fig.1 A, B).

In addition, a large number of amplification products were not detected among the 9 flax samples. Three fragments within 341 n.p. were reliably identified. The Ver_2 primer is also common to Bonet and 2406 variety samples; the latter variety sample had a locus according to the Ver_1 primer (Fig. 2).

For a more multifaceted assessment and clarity of the results, we have performed the cluster analysis, which involves identifying genetic distances between variety samples. The smallest genetic distance was recorded between such varieties as Charivnyi and Agatha (0.141). The congeniality of the remaining samples is proved by the distance between them, which is 0.9 – Hlobus, Esman and Tomskyi-16/Prizyv-81// Prizyv-81.

In order to display the patterns of genetic congeniality of flax samples, a dendrogram has been developed based on the nearest neighbor algorithm (Fig. 3). The analysis has resulted in the identification of two clusters.

The first cluster includes such varieties as Charivnyi and Agatha, the second – Hlobus, Esman and Tomskyi-16//Prizyv-81//Prizyv-81. Such features of distribution are explained by the common origin of the studied variety samples themselves. The variety Charivnyi is created from a variety of fibre flax of mutant origin Stodolyshchenskyi, treated with dimethyl urea (DCMU) with the repeated selection towards increasing the fiber content in the stems. The second cluster consisted of hybrids and varieties of hybrid origin selected by the Institute of Bast Crops of the National Academy of Sciences of Ukraine (Hlukhiv). The variety Hlobus was created by the hybridization of Mohyliovskyi-2 and Natasja varieties with selection based on fiber content. The variety Esman is the result of hybridization of foreign varieties Argos and Bertelin with multiple selection for seed yield and simultaneous preservation of fiber content. Tomskyi-16/Prizyv-81// Prizyv-81 is a promising hybrid with a high fiber content and seed productivity.

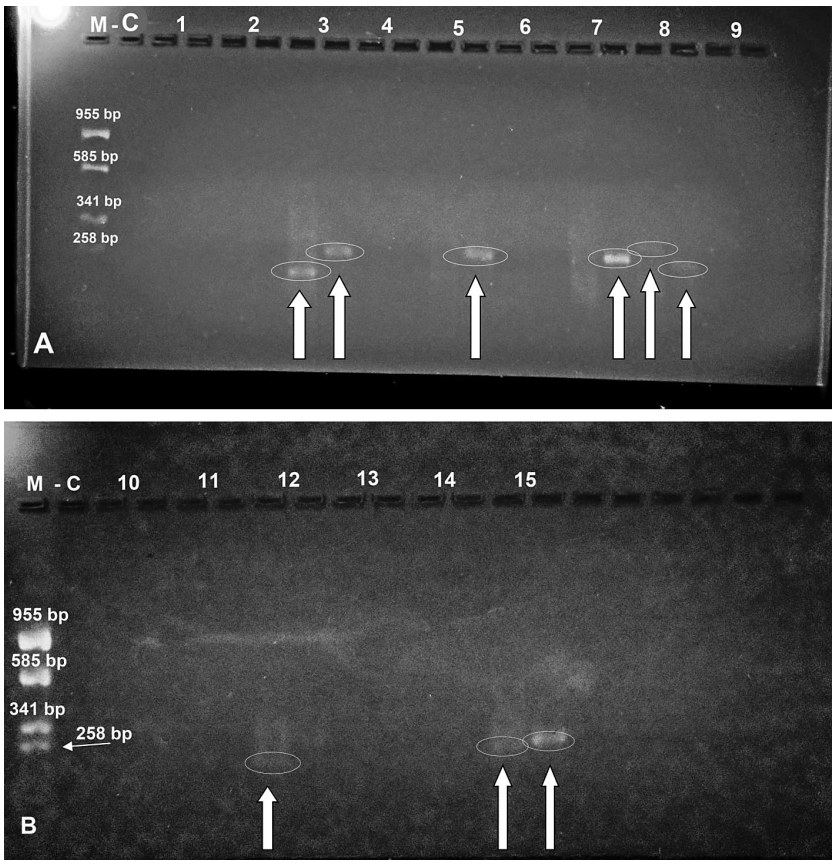

Fig. 1. Electrophoretic spectra of RAPD fragments of 15 flax samples (Ver_1 and Ver_2 primers): M – molecular weight marker, C – negative control, 1 – Hlazur, 2 – Hlukhivskyi Yuvileinyi, 3 – Charivnyi, 4 – Siverskyi, 5 – Hlobus, 6 – Hladiator, 7 – Esman, 8 – Agatha, 9 – Rushnychok, 10 – Lirina, 11 – Nadiia, 12 – Tomskyi 10Niking, 13 – Zaria 87//Hermes/ Electra, 14 – Ukrainian Rannii /Hermes//Charivnyi, 15 – Tomskyi-16/Prizyv-81//Prizyv-81.

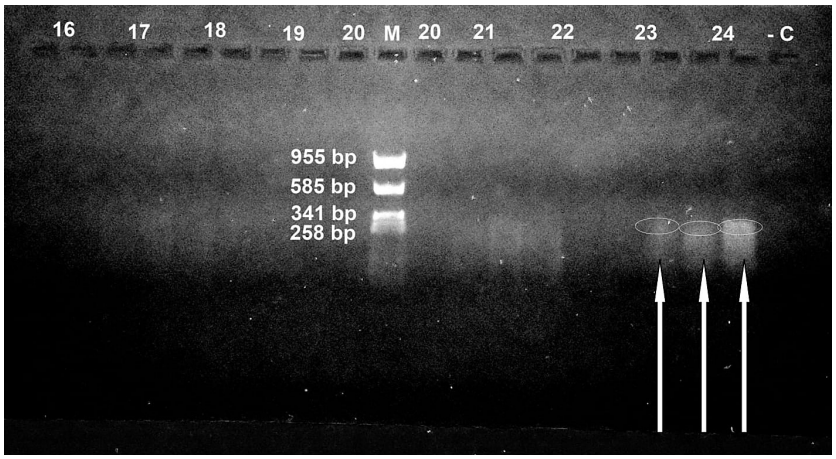

Fig. 2. Electrophoretic spectra of RAPD fragments of 9 flax samples (Ver_1 and Ver_2 primers): M – molecular weight marker, C – negative control, 16 – Lida, 17 – M-8, 18 – Vruchyi, 19 – Tost 2, 20 – LKS 8, 21 – Blakytnyi, 22 – Tomskyi 5, 23 – Bonet, 24 – 2406.

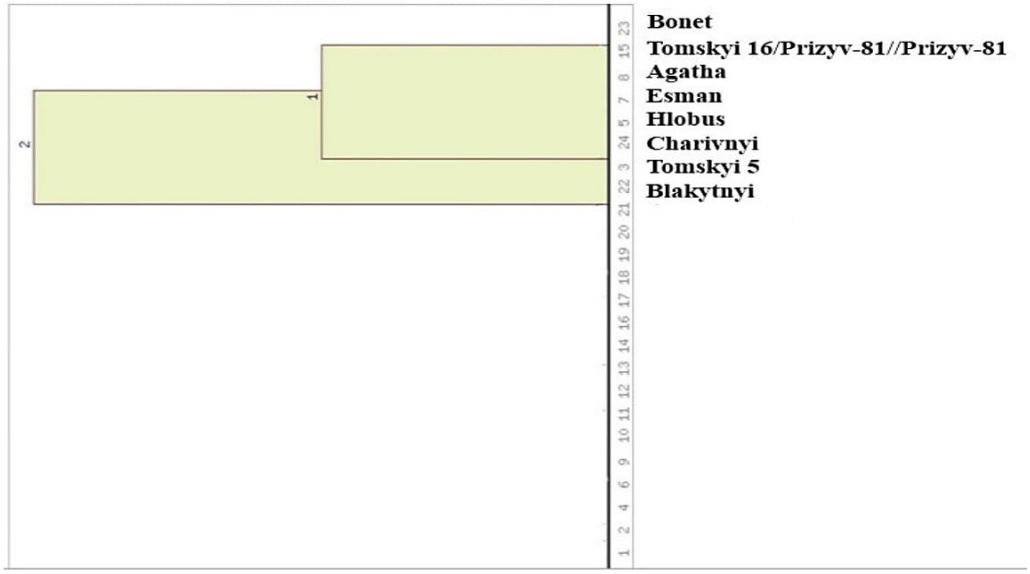

Fig. 3. Dendrogram of genetic congeniality of flax samples of different ecological and geographical origin based on the results of RAPD analysis

CONCLUSIONS

The RAPD analysis of 24 varieties and interspecies hybrids of flax using two oligonucleotide primers shows a small number of loci. The cluster analysis shows the relationship of such varieties as Charivnyi and Agatha, as well as Hlobus, Esman, sample 2406 and hybrid Tomskyi-16/Prizyv-81//Prizyv-81 varieties. In general, we have obtained the results similar to those described in the literature, namely: the most genetically close relationship has been recorded between variety samples of fibre flax, as well as mainly between samples of domestic breeding.

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