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Original Article

The Assessment of Genetic Polymorphism in Triticale SC1 Somaclones using RAPD-PCR Markers

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Abstract *- The triticale varieties are becoming more and more widespread crops as human and animal feed. To carry out genotyping of the valuable varieties, molecular markers are important in identification and polymorphism analysis. In the proposed investigation, triticale somaclones derived from three triticale cultivars (Ingen 35, Ingen 93, 188TR5021) were evaluated and compared to the forms obtained from seeds exposed to gamma irradiation (150 Gy). Molecular genetic variability was evaluated based on twelve arbitrary primers using the RAPD method. The results of genotyping with RAPD primers found the most informative oligomers in discriminating the triticale forms. According to the higher weight showed a pronounced polymorphism between somaclones, irradiated and recurrent cultivars. A higher genetic diversity has been identified in forms derived from cultivar Ingen 93 (25% polymorphism) compared to Ingen 35 (15%) or 188TR5027 (19%), especially in somaclone obtained from Ingen 93, confirmed by the number of specific fragments (5) and the highest dissimilarity index (0.26). The significant variability in the frame of somaclones was confirmed by the genetic distance based on the Jaccard index, differentiating them from the control and irradiated treatments.*

Keywords *- Triticale, Genetic polymorphism, RAPD primers, Somaclone.*

1. Introduction

The development of new varieties of agricultural cultures is based on the use of natural or induced genetic variability. Genetic marker methods are used to identify and characterise various valuable genotypes for breeding. These can be morphological, biochemical or molecular (Cifci and Yagdi, 2012).

2. Literature Review

Morphological markers define the shape, colour or specific biochemical components, and their number is relatively small. With the development of molecular genetics, a new class of molecular markers - DNA fragments corresponding to nucleotide sequences that are part of or correlate with the structure of the valuable gene - has emerged. Identifying a new class of genetic markers, i.e. molecular markers, represents a revolutionary event in plant breeding methodology. The strategy of using molecular markers is based on highlighting molecular polymorphism, which in plant genomes is mainly due to variation in the amount of repetitive DNA. The most important applications of molecular markers in plant breeding are marker-assisted selection, conducting the backcross acceleration, and detection of genetic diversity and differences between various populations (Milczarski et al., 2001; Sharma et al., 2008; Patra et al., 2011; Hafashjani, 2012; DimahAli Zreikah et al., 2019).

The application of markers at the level of radical DNA has thus modified the methods of assessing the genetic variability of plants, the passporting and classification of varieties, and the mapping and determination of certain loci responsible for valuable qualities in breeding (Winter and Kahl, 1995; Cichy and Goates, 2009; Petrovičová et al., 2015; Chesnokov, Artemyeva, 2015; Mayavel et al., 2020). DNA-level markers can be used directly for molecular analysis of the genome. Polymorphism of DNA sequences can be detected due to differences in hybridisation sites with an arbitrary primer by MAAP techniques (Multiple Arbitrary Amplicon Profiling DNA): RAPD (Random-Amplified Polymorphic DNA), AP-PCR (Arbitrarily Primed PCR), DAF (DNA Amplification Fingerprinting). These methods consist of performing a PCR reaction on the DNA under study using an arbitrary sequence primer. Techniques using arbitrary primers, in particular RAPD, have become widespread because they are fast, require minimum DNA and being PCR based are easier to be automated (Ganea et al., 2009; Szućko and Mądrach, 2019).

The RAPD technique is successfully used in constructing genetic maps, which allow the marking of valuable qualitative and quantitative traits and the identification of markers associated with plant resistance to various diseases and pests (Orlovskaya et al., 2012; Khaled et al., 2015). However, mostly, this technique is used to detect a genomic DNA sequence polymorphism (Williams et al., 1990; Tonk et al., 2014).

So, molecular markers are an effective tool in the genetic study of plants, greatly simplifying research on the genes structure, quantitative trait loci, gene mapping, and gene transfer to other forms of plants with the creation of new varieties of cereal, vegetable and fruit crops. However, the practical use of molecular markers for creating new highly productive varieties of cereal crops is not yet widely applied (Badea et al., 2011; Kumar et al., 2009; Trebichalský et al., 2013).

Due to its increased production capacity, wide adaptability, agronomic characteristics and high-quality properties, triticale cultivation in the Republic of Moldova has gained a recognized economic significance in human food, industry and animal feed. Nowadays, triticale is gaining an increasingly important role in food, having considerable agricultural potential and a high protein content with a balanced level of amino acids. It plays an important role in increasing the productivity of cereal crops. Research in recent years has been important in breeding and studying new varieties of triticale, which are much more productive and resistant than the parent forms. These varieties are becoming more and more widespread in our country's agriculture and are successfully used in both human and animal feed. It dictates the need to carry out genetic studies on passport genotypes, identifying molecular markers associated with valuable traits. A current task remains to increase cereal crops' genotype pool to diversify forms and obtain new genotypes. Therefore, as an important objective, it aims to assess the genetic diversity of some triticale genotypes using RAPD markers. So, the purpose of this scientific research comprises the identification and polymorphism analysis of amplified DNA fragments of triticale SC_1 somaclones.

3. Materials and Methods

In this investigation, three triticale genotypes: Ingen 35, Ingen 93 (as standard), and 188TR5021, were evaluated.

Table 1. List of microsatellite primers used for the RAPD analysis.

No.	Primer	Sequence (5′ → 3′)
1	OPB-12	5’-CCTTGACGCA-3’
2	OPB-14	5’-TCCGCTCTGG-3’
3	OPC-05	5’-GATGACCGCC-3’
4	OPC-07	5’-GTCCCGACGA-3’
5	OPF-08	5’-GGGATATCGG-3’
6	OPD -07	5’-TTGGCACGGG-3’
7	OPA-11	5’- GTGCCTAACC-3’
8	OPG-6	5’-AGGGCCGTCT-3’
9	OPG-10	5’-CTACTGCCGT-3’
10	OPE-17	5’-TCTCCGCCCT-3’
11	OPI-16	5’-AATGGCGCAG-3’
12	OPH-15	5’-CAATCGCCGT-3’

In the study was used the seed material collected from triticale somaclones with valuable agronomic traits selected from SC1 progeny, which were obtained from *in vitro* regenerated plants of mature embryos of 3 triticale cultivars (Ingen 35, Ingen 93), 188TR5021 and seeds exposed to gamma irradiation (150 Gy).

Twelve arbitrary primers were used to estimate the molecular genetic variability of triticale somaclones by RAPD analysis shown in Table 1.

3.1. Isolation and Purification of Genomic DNA

Samples for analysis were collected for each genotype from plants at the 2-leaf stage grown in laboratory conditions, fixed in liquid nitrogen and stored at -80^{0}C till isolation and processing. Gene JET Plant Genomic DNA Purification Mini Kit #K0791 was used for genomic DNA isolation according to the protocol provided by the manufacturer. Qualitative quantification of total DNA was determined by electrophoretic analysis in 1% agarose gel (Sambrook, 2001).

To exclude the contamination of the DNA isolated from the plant samples, purification with 12M $LiCl_2$ is used:

- 12M $LiCl_2$ is added so that the final concentration in the DNA is 4M. Samples are put on ice for 20-30 min, then centrifuged for 10 minutes at 10000 rpm.
- The supernatant is collected in Epindorf, and the specimen with the precleaned impurities is discarded. The collected supernatant is processed with formalin chloride and isoamyl alcohol solution in 24: 1 ratio, equal volume is added, tubes are inverted; centrifuge for 5 min at 10000 r/min, collect the supernatant.
- Add 5M NaCl so that the final concentration in the DNA solute is 0.2 M. Then add an equal volume of isopropanol and centrifuge for 2 min.
- Precipitate is washed with 70% ethyl alcohol with a volume of 1ml, centrifuge 2 min at 10000 rot/min. This step is repeated twice. The precipitate is dried thoroughly when dissolved in 50-100 µl distilled H_2O and filtered through a millipore.

To perform the amplification reactions, several dilutions (between 1-15 times) of each DNA sample were made to select an ideal amount of DNA and avoid blocking amplification.

3.2. Analysis with RAPD Primers

Amplification reactions were performed in a volume of 15 µl per sample with the following components: 50-70 ng DNA, dNTP 200 µM of each type (dATP, dCTP, dGTP, dTTP), primer 0.4µM, 1U/µl DreamTaq Green DNA polymerase in buffer solution (1x); sterile water; 2.5 mM $MgCl_2$ (Thermo Scientific). The amplification process was carried out in the Applied BiosystemGeneAmp PCR System 9700 (Singapore) automated thermocycler according to the

following conditions: 95ºC - 5 min, 35 cycles: 95ºC - 1 min, 36ºC - 1 min, 72ºC - 1 min, final elongation 72ºC - 5 min, for primers with 60% G/C ratio and for primers with 70% G/C ratio: 95ºC - 5 min, 35 cycles: 95ºC - 1 min, 38ºC - 1 min, 72ºC - 1 min, final elongation 72ºC - 5 min.

Amplicons were analysed by electrophoresis in 2% agarose gel (2 g agarose, 2 ml TAE 10x, at 100 ml volume) in the presence of 0.5 μg/ml ethidium bromide (Sambrook, 2001). RAPD analysis was performed with 12 primer primers (Table 1). Amplification products were visualized using the UV transluminator and the DOC gel documentation system - PRINT-VX2.

4. Results and Discussion

In this study, genetic variability analysis was performed on 3 triticale genotypes (Ingen 35, Ingen 93 and 188TR5027) using 12 RAPD primers. Based on these genotypes, 3 different treatments were created: control (M), in vitro (IV) and irradiated (IR).

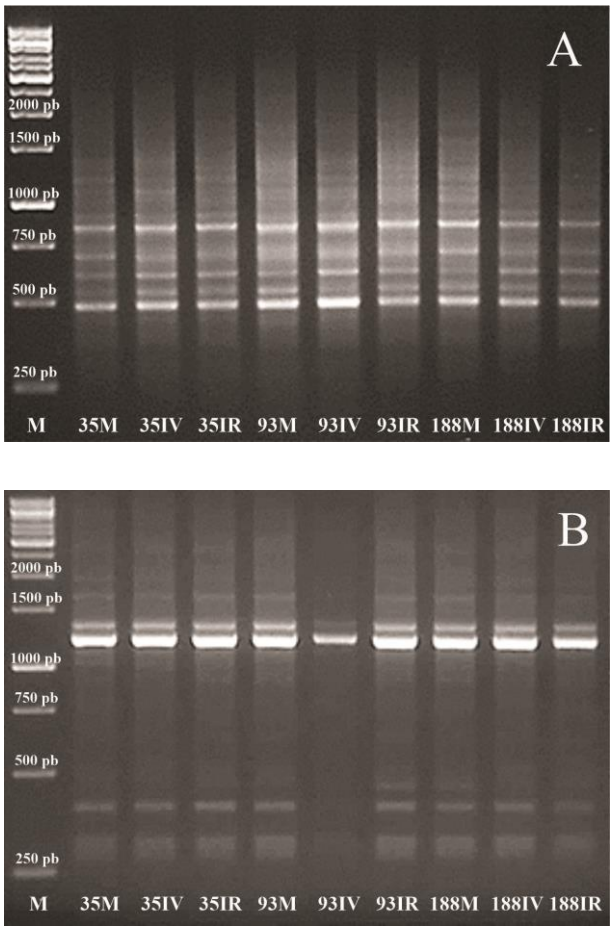


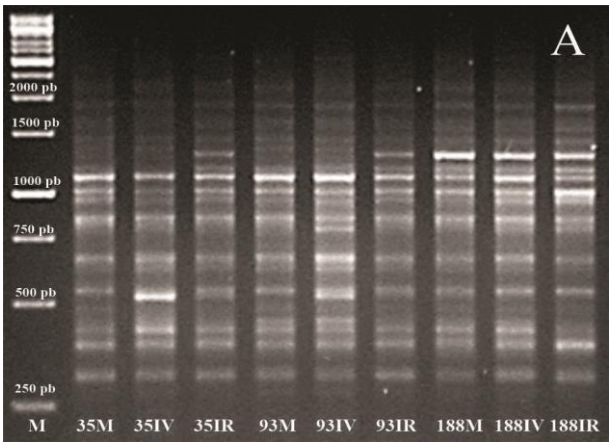
Fig. 1 Electrophoretic spectrum of DNA fragments amplified with primer OPA11 (A) and OPB14 (B). M – molecular marker (GeneRuler 1 kb DNA Ladder, pb – base pairs); genotypes: Ingen 35, Ingen 93, 188 TR5027; treatments: control variant (M), in vitro (IV), irradiated (IR).

The results revealed the diversity of molecular-genetic fingerprints depending on the genotype and primers used. Analysis of the amplification products with the OPA11 primer revealed 5 nucleotide fragments ranging in size from 477-855 bp (base pairs) (Fig. 1, A). The genotypes investigated and their forms showed the same molecular spectrum (of 5 bands) except 35, M, which was characterized by a different spectrum (4 bands). Thus, the primer generated 4 fragments common to all genotypes and one polymorphic fragment (546 bp) missing in 35M.

In triticale genotypes, 6 DNA fragments were detected by RAPD genotyping with OPB14 (Figure 1, B). Sequence length ranged from 320 bp to 1671 bp. The amplicons of 320, 407, 464 and 1671 bp were missing in the electrophoretic fingerprint of the in vitro form 93. Therefore, OPB14 identified 2 common fragments, which also occur with the highest frequency being present in all samples studied, and 4 - polymorphic.

Similar results were observed for the OPC05 oligomer (Fig. 2, B), which resulted in the amplification of a single molecular profile for most genotypes, except for 93IV, which showed a different profile. In total, for this primer, 9 nucleotide sequences were identified with an amplification range between 372 - 1251 bp. Among the bands obtained, 3 common and 4 polymorphic bands were detected according to the spread frequency. The lowest number of amplicons was observed in 93IV (5) compared to the other genotypes and forms, all recording the same molecular profile of 7 amplicons. Thus, 93IV was missing fragments: 372, 543, 617 and 737 bp. The same genotype, 93IV, was also distinguished by the presence of 2 specific amplicons: 692 and 863 bp.

OPB12 is among the primers used in the present study with the most amplified bands and molecular profiles (Fig. 2, A).



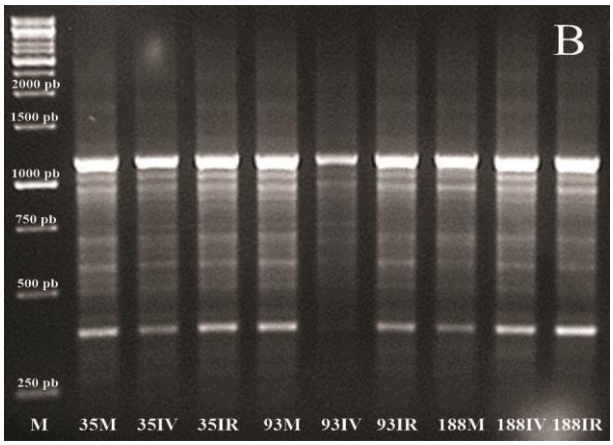


Fig. 2 Electrophoretic spectrum of DNA fragments amplified with primer OPB12 (A) and OPC05 (B). M – molecular marker (GeneRuler 1 kb DNA Ladder, pb – base pairs); genotypes: Ingen 35, Ingen 93, 188 TR5027; treatments: control variant (M), in vitro (IV), irradiated (IR).

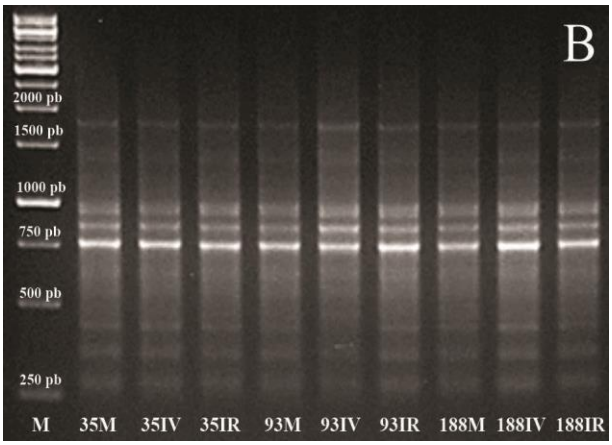
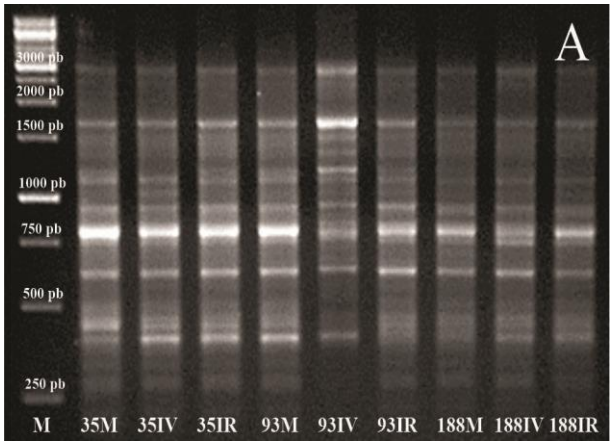
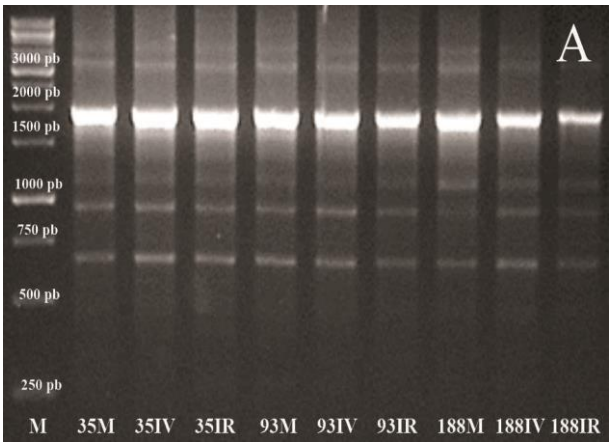


Fig. 3 Electrophoretic spectrum of DNA fragments amplified with primer OPC07 (A) and OPD07 (B). M – molecular marker (GeneRuler 1 kb DNA Ladder, pb – base pairs); genotypes: Ingen 35, Ingen 93, 188 TR5027; treatments: control variant (M), in vitro (IV), irradiated (IR).

Analysing the 5 molecular profiles identified, the similarity between the irradiated forms 35IR and 93IR, 188IV and 188IR can be observed. Also, based on genotyping with OPB12, a pronounced differentiation between the molecular fingerprints of the control and in vitro/irradiated treatments was observed. A total of 16 amplicons with molecular mass ranging from 320 - 1764 bp were obtained, of which 7 were common, and 9 were polymorphic. Amplicons specific to one triticale sample were not identified, but a fragment of 1150 bp was detected and amplified in genotype 188 (control and irradiated forms), differentiating it from the other genotypes. OPB12 showed the most bands in the electrophoretic spectra at 188M and 188IR (15) and the fewest at 35IR (11). Thus, at 188M and 188IR, the 606 bp size fragment was missing from the RAPD molecular profile.

Analysis of RAPD fragments amplified with the OPC07 oligomer (Fig. 3, A) revealed results similar to those described for OPB12. OPC07 generated 16 amplicons with sizes falling within a wide amplification range, such as 295 - 2891 bp.





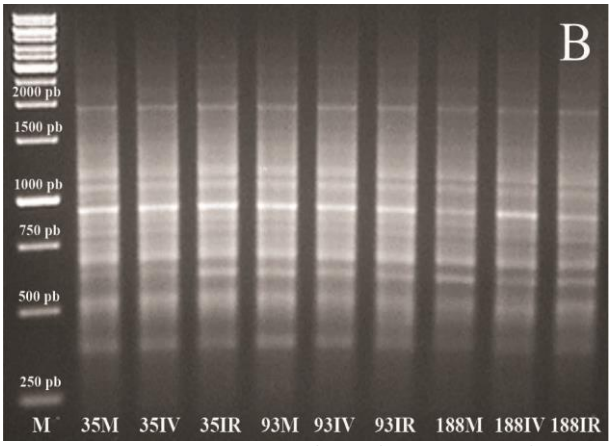


Fig. 4 Electrophoretic spectrum of DNA fragments amplified with OPE17 (A) and OPF08 (B). M – molecular marker (GeneRuler 1 kb DNA Ladder, pb – base pairs); genotypes: Ingen 35, Ingen 93, 188 TR5027; treatments: control variant (M), in vitro (IV), irradiated (IR).

The study of the molecular fingerprints obtained with OPC07 established 4 different molecular profiles. Based on these profiles, discrimination between the control and in vitro/irradiated forms was found, but the genotype 188

TR5027 forms were found to be identical. Genotype 188 TR5027 was also distinguished by the fewest amplified fragments (11) for all forms. Among the DNA fragments identified with OPC07, 7 commons, 7 polymorphic and 2 specific fragments were detected. The in vitro form of the Ingen 93 genotype was characterized by the presence of a different molecular spectrum from all other forms studied (Figure 3, A) and by the presence of 2 specific bands (1353 and 1500 bp). Thus, this primer contributed majorly to the genetic differentiation of form 93IV.

The OPD07 primer amplified 8 DNA fragments (Fig. 3, B) with different concentrations (intensity in the electrophoretic spectrum), most of which were detected in all PCR samples, resulting in low polymorphism in the investigated triticale. Amplicon sizes ranged from 272 - 1730 bp. Thus, 6 common fragments, one polymorphic and one specific for 93IV (1443 bp), were identified. OPD07 determined the presence of 2 molecular profiles, one common for all genotypes and one characteristic for 93IV, with the same number of amplicons (7) but different by molecular mass. Thus, in the electrophoretic fingerprint of 93IV, the 430 bp fragment was missing, but a unique fragment was detected.

Analysis of amplification products with the OPE17 primer revealed 6 nucleotide sequences with molecular mass ranging from 656 to 4000 bp (Fig. 4, A). The investigated genotypes showed the same molecular profile (out of 6 fragments) except 188IR, which was characterized by a different profile (5). Thus, the primer identified 5 fragments common to all genotypes and one polymorphic fragment (4000 bp) missing in 188IR.

OPF08 resulted in the amplification of a higher number of fragments compared to other primers (Fig. 4, B). Eleven DNA fragments were detected with a length ranging from 409 - 1962 bp. Their frequency within the analysed genomes is high, with 9 fragments being common and 2 polymorphic. A study of each electrophoretic spectrum separately revealed similar molecular profiles with minor exceptions. The maximum number of amplicons (11) was observed in triticale treatments 35IV and 35IR showing an identical profile. A different profile is found in the Ingen 93 and 188M genotype forms, 188IV, where 10 amplicons are missing the 754 bp amplicon. In genotype 35M, the 907 bp fragment is missing. The minimum number of amplicons (9) was found in 188IR, in which the 754 and 907 bp sequences were not amplified.

The same number of electrophoretic bands as OPF08 was established with primer OPG06 (Figure 5, A). Among the 11 identified fragments, 9 were shown to be common, one polymorphic fragment (778 bp) and one specific for 188M (961 bp). The molecular mass of the nucleotide fragments ranged from 429 to 1513 bp. The 778 bp amplicon was not detected in the spectrum of irradiated variants in all investigated genotypes and 188M. Thus, the irradiated forms of triticale genotypes, based on OPG06 genotyping, were characterized by the fewest DNA fragments (9) in their electrophoretic profile compared to the control and *in vitro* variants (10).

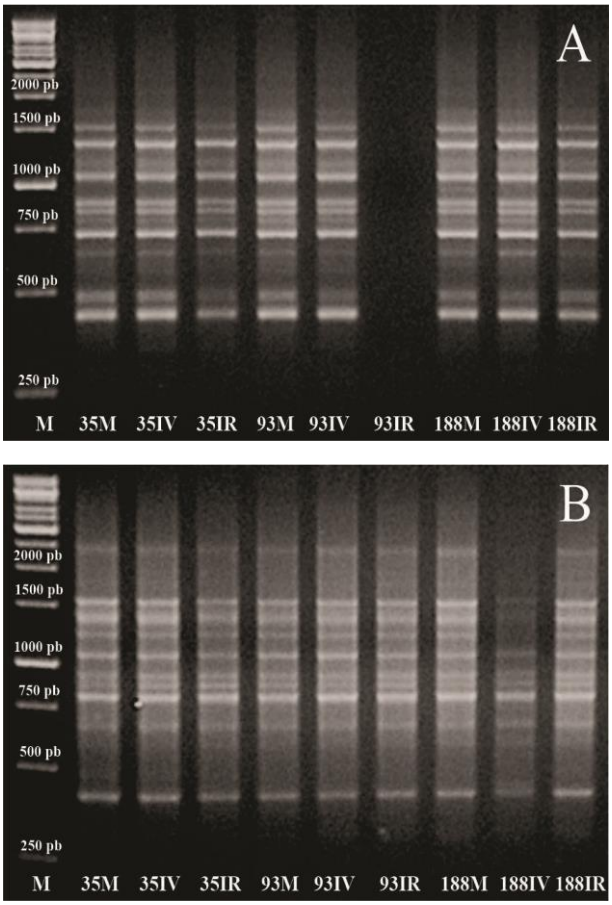


Fig. 5 Electrophoretic spectrum of DNA fragments amplified with primer OPG06 (A) and OPG10 (B). M – molecular marker (GeneRuler 1 kb DNA Ladder, pb – base pairs); genotypes: Ingen 35, Ingen 93, 188 TR5027; treatments: control variant (M), *in vitro* (IV), irradiated (IR).

OPG10 is the next primer that generated a total of 11 amplicons with their length ranging from 418 to 2420 bp (Fig. 5, B). Molecular profiles are similar between genotypes, with some exceptions for 188. In the electrophoretic profile of treatments for the 188TR5027 genotype, the 578 bp fragment was identified, which is missing in the other samples. Thus, amplification results with this primer differentiated this genotype from Ingen 35 and Ingen 93. At the same time, differences were observed in the molecular profile of form 188IV in which 1237, 1366 and 2420 bp fragments were not detected. The spread frequency of amplicons generated by OPG10 revealed 7 common and 4 polymorphic amplicons, showing moderate polymorphism.

The electrophoregram obtained following amplification with the OPH15 primer (Fig. 6, A) revealed 7 fragments, 5 of

which were common and 2 polymorphic. The size of the fragments is in the range of 704 - 1674 bp. Investigation of the individual molecular profiles revealed genotype 93IV with the highest number of amplicons (7). In the other genotypes, the number of amplicons varies between 5-6 nucleotide fragments.

The OPI16 oligomer showed a pronounced polymorphism among the investigated triticale genotypes. Only 3 fragments out of 8 identified in total (**Fig. 6**, B) resulted in a high frequency in the genome of the studied forms, 5 being polymorphic. The length of the amplicons obtained ranged from 455 to 2478 bp. Both single-form and genotype-specific fragments were observed. In the molecular fingerprint of 188IV, the 455 bp specific fragment was amplified. Also, genotype 35IV and 35IR forms were distinguished by the presence of 1342 bp fragments, while 188IV and 188IR - 1834 bp. The number of electrophoretic bands in the molecular profiles ranged from 3 (in 93IV and 188M) to 6 (in 35IV and 188IV).

The study of the variability of triticale genotypes based on 12 RAPD primers revealed the presence of 114 amplified fragments (Table 2), of which 67 were found to be common and 41 polymorphic, revealing a rather pronounced polymorphism by 39%. The size of the PCR samples ranged from 272 to 4000 bp. Primer OPE17 had the widest molecular mass range (3344 bp) and OPA11 the narrowest (378 bp), but both oligomers amplified a small number of fragments (6 and 5, respectively) with a moderate polymorphism of 17 and 20%, respectively.

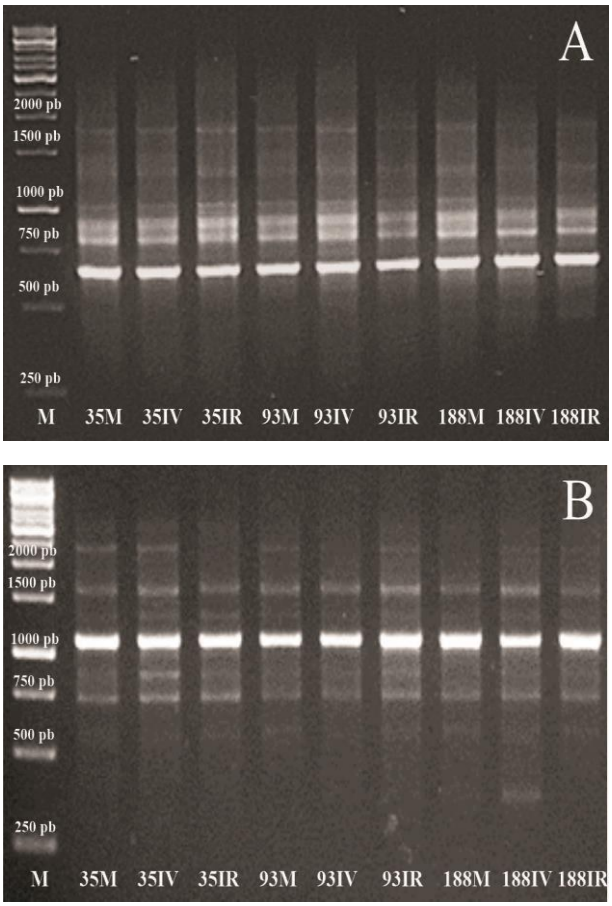


Fig. 6 Electrophoretic spectrum of DNA fragments amplified with primer OPH15 (A) and OPI16 (B). M – molecular marker (GeneRuler 1 kb DNA Ladder, pb – base pairs); genotypes: Ingen 35, Ingen 93, 188 TR5027; treatments: control variant (M), in vitro (IV), irradiated (IR).

Table 2. Features of RAPD primers analysed in triticale forms.

Primer	Length (bp)	Number of fragments				Polymorphism (%)	PIC
		Total	Common	Polymorph	Specific		
OPA11	477-855	5	4	1	-	20.00	0.04
OPB14	320-1671	6	2	4	-	66.67	0.25
OPC05	372-1251	9	3	4	2	66.67	0.37
OPB12	320-1764	16	7	9	-	56.25	0.30
OPC07	295-2891	16	7	7	2	56.25	0.39
OPD07	272-1730	8	6	1	1	25.00	0.22
OPE17	656-4000	6	5	1	-	16.67	0.04
OPF08	409-1962	11	9	2	-	18.18	0.15
OPG06	429-1513	11	9	1	1	18.18	0.21
OPG10	418-2420	11	7	4	-	36.36	0.17
OPH15	704-1674	7	5	2	-	28.57	0.33
OPI16	455-2478	8	3	5	-	62.50	0.49
Total Media	272-4000	114	67	41	6	39.28	0.25

The same 8 primers listed above are also characterised by a discriminatory power of the triticale forms analysed based on the PIC index (polymorphic information content), which ranged from 0.17 (OPG10) to 0.49 (OPI16). Polymorphism analysis of nucleotide sequences also revealed the OPG06 primer with a PIC index of 0.21. For the primer system used, the PIC index ranged from a minimum of 0.04 for OPA11 and OPE17 to a maximum of 0.49 for OPI16.

Table 3. Specific nucleotide sequences identified with RAPD primer analysis in triticale genotypes.

Genotype/ treatment	Specific fragment (bp)		Total
	form	genotype	
35M	-	$OPF08_{754}$, $OPI16_{1342}$	2
35IV	-		
35IR	-		
93M	-	-	5
93IV	$OPC05_{692}$, $OPC05_{863}$, $OPC07_{1353}$, $OPC07_{1500}$, $OPD07_{1443}$		
93IR	-		
188M	$OPG06_{961}$	$OPB12_{1150}$, $OPG10_{578}$, $OPI16_{1834}$	5

Also, the used primers allowed the differentiation of the genotypes according to the specific amplicons identified. Out of the total of 6 fragments found in electrophoretic profiles analysed with 12 RAPD primers, most of them were revealed by primers OPC05 (2) and OPC07 (2) followed by the OPD07 (1) and OPG06 (1). Generalizing the results of genotyping with 12 RAPD primers, 9 out of 12 oligomers were revealed to be the most informative in discriminating the triticale studied forms.

Analyzing the molecular-genetic fingerprints based on RAPD primers for each genotype separately revealed a higher number of fragments in genotype Ingen 93 (107), of which 80 - were monomorphic and 27 - polymorphic, also indicating the highest genetic polymorphism of 25%. A proximal number of fragments (101) was also detected in genotype 188TR5027 forms but were characterized by the presence of more common bands - 86, compared to polymorphic ones - 15, also identifying the lowest level of polymorphism among the investigated genotypes, 15%. Comparative analysis of electrophoretic spectra obtained from Ingen 35 genotype forms determined the presence of 104 fragments, among which the majority are common - 84, and less than half are polymorphic - 20, with a polymorphism of 19%.

At the same time, individual form analysis detected the most specific fragments in Ingen genotype 93 (12), followed by Ingen 35 (7) and the fewest in 188TR5027 (5). Thus, primers that generated amplicons specific to a form or genotype also resulted in a high level of genetic differentiation: OPB12, OPB14, OPC05, OPC07, OPD07, OPG06, OPH15 and OPI16. From the set of decamers used in this study in genotyping triticale forms, 3 groups were distinguished that have a higher weight in discriminating and showing a pronounced polymorphism for Ingen 35 genotype forms (OPB12, OPB14, OPC07, OPH15 and OPI16), Ingen 93 (OPB12, OPB14, OPC05, OPC07, OPD07, OPH15 and OPI16) and 188TR5027 (OPB14, OPG06, OPG10, OPH15 and OPI16).

Based on the whole dataset generated by 12 RAPD primers, analysing the forms of the genotypes studied, the most varied electrophoretic profiles were found in the Ingen 93 genotype *in vitro* form. Also, this form of triticale showed 5 specific fragments with different sizes depending on the oligomer. OPC05 and OPC07 resulted in the presence of two specific fragments (692, 863 bp, and 1353, 1500 bp, respectively), followed by OPD07 with a single specific amplicon (1443 bp) (Table 3).

Specific fragments were also identified in Ingen 35 genotype forms, revealed by primers OPF08 (754 bp) and OPI16 (1342 bp). Genotype 188TR5027 forms show the presence of 5 specific amplicons, 3 of which were characteristic of the genotype being highlighted by OPB12 (1150 bp), OPG10 (578 bp) and OPI16 (1834 bp). One fragment was identified only in the 188M and 188IV form profiles generated by OPG06 (961 bp) and OPI16 (455 bp), respectively (Table 3).

Cluster analysis based on the dendogram (Fig. 7) generated by the UPGMA method (Jaccard dissimilarity index) determined the delineation of triticale genotypes and their forms into two main clusters. It was observed that the forms of genotypes Ingen 35 and Ingen 93 clustered together, with the exception of form 93IV, which also recorded the highest genetic distance index (0.26), confirming the marked diversity in its genetic profile and the presence of specific fragments.

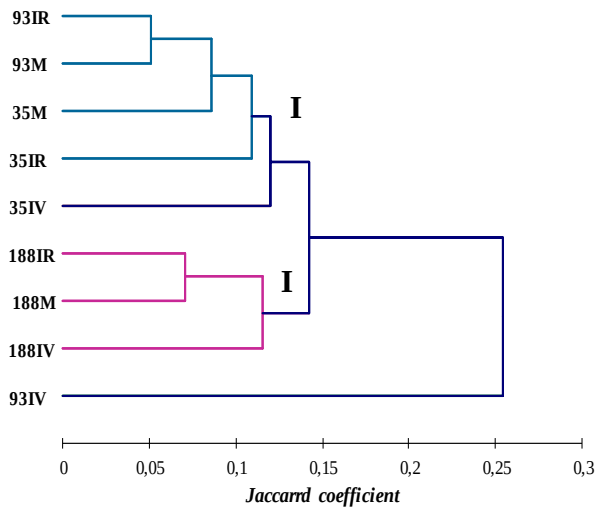


Fig. 7 The clustering of triticale forms based on RAPD genotyping by the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean). Note: genotypes: Ingen 35, Ingen 93, 188 TR5027; treatments: control (M), in vitro (IV), irradiated (IR).

The data from molecular analyses demonstrate the common origin of these two cultivars, Ingen 35 and Ingen 93 and the importance of initial materials in breeding programs (Veverita, Buiucli, 2008). In the case of the forms of genotype 188TR5027, which form another cluster (II), a higher similarity was found, especially between the control and irradiated forms. The lowest genetic distance (0.05) was found between the control and irradiated forms of genotype Ingen 93, indicating a high degree of similarity of the electrophoretic profiles after most RAPD primers.

A pronounced diversity was evident based on UPGMA analysis between the Ingen 35 forms included in group I. Among these forms, the most genetically different based on the RAPD primers were found to be the in vitro form (35IV) with a distance index of 0.11.

5. Conclusion

Summarizing the results of genotyping with arbitrary RAPD primers, 9 oligomers (OPD07, OPH15, OPG06, OPG10, OPB12, OPC07, OPI16, OPB14 and OPC05) were found to be the most informative in discriminating the triticale forms, according to all analysed parameters (total amplicon number - 6-16, polymorphs - 1-9, PIC - 0.17-0.49).

From the total set of primers, those with a higher weight in discriminating and showing a pronounced polymorphism for the forms of cultivar Ingen 93 (OPB12, OPB14, OPC05, OPC07, OPD07, OPH15 and OPI16), Ingen 35 (OPB12, OPB14, OPC07, OPH15 and OPI16) and 188TR5027 (OPB14, OPG06, OPG10, OPH15 and OPI16) were identified and classified.

Analysis of genetic polymorphism based on the used primers identified higher genetic diversity between forms derived from cultivar Ingen 93 (25% polymorphism) compared to Ingen 35 (15%) or 188TR5027 (19%).

Pronounced genetic differentiation was found in the electrophoretic spectrum of somaclone obtained from Ingen 93, confirmed by the number of specific fragments (5) and the highest dissimilarity index (0.26). Comparative analysis of the genetic fingerprints revealed significant variability in the frame of somaclones obtained from all three studied genotypes, demonstrated by the genetic distance based on the Jaccard index: 35IV - 0.11; 93IV - 0.26; 188IV - 0.12, differentiating them from the control and irradiated treatments.

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