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ASSESSING GENETIC DIVERSITY IN TRITICALE POPULATIONS USING iPBS MARKERS

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Abstract. *This article aimed to evaluate the efficacy of the iPBS (inter-Primer Binding Site) molecular marker system, leveraging mobile genetic elements, for genotyping diverse triticale accessions sourced from the collection of the Institute of Genetics, Physiology and Plant Protection. The objectives included the isolation and purification of genomic DNA from 7-day-old triticale seedlings of 'Ingen-54' and 'Ingen-93' genotypes, the empirical determination of optimal iPBS primer efficiency through a series of experimental amplifications, and the subsequent selection of highly informative primers for triticale genotyping. Standard polymerase chain reaction (PCR) amplifications were conducted utilizing specific iPBS primers (Pb13, Pb20, Pb21), followed by electrophoretic separation of amplification products on agarose gel. Amplification produced 33 clear, 100% polymorphic loci. POPGENE analysis revealed high informativeness (Nei's gene diversity h=0.1290, Shannon index I=0.2403) despite low genetic distance (0.0074–0.0169) between the closely related varieties. Primers Pb20 and Pb21 generated the most polymorphic loci. These results confirm the high utility of iPBS markers for genetic diversity assessment and cultivar identification in triticale breeding, even for closely related genotypes.*

***Keywords: genotyping, polymorphisms, retrotransposons molecular markers, triticale*.**

INTRODUCTION

Triticale (× Triticosecale Wittmack) is an artificial amphidiploid species derived from a cross between wheat (*Triticum aestivum*) and rye (Secale cereale). This hybrid was developed to combine the agronomically valuable traits of both progenitors: the high yield and grain quality of wheat with the cold tolerance, disease resistance, and environmental adaptability typical of rye.[1, 2] In terms of nutritional and feed value, triticale holds an intermediate position between wheat and rye. Triticale has high antioxidant activity due to its rich content of polyphenolic compounds and flavonoids, particularly phenolic acids such as 4-hydroxybenzoic acid, vanillic acid, caffeic acid, chlorogenic acid, p-coumaric acid, and rosmarinic acid [3]. Due to its combined traits, triticale isonsidered a promising cereal crop, especially in regions with harsh climatic conditions and marginal soils.

The world production of triticale currently exceeds 20 million tons per year and is mainly concentrated in Europe, accounting for over 80% of the global output. In the countries of Central and Eastern Europe, including Romania and Ukraine,

triticale is actively introduced into crop rotations as an alternative to winter wheat on low-productivity soils.[4] Its adaptability, resistance to abiotic stress, and relatively low input requirements make it an important candidate for sustainable agriculture and food security in the context of climate change. In Moldova, national agricultural research institutions have prioritized the development of high-performing triticale varieties. [2, 5]

Because of long-term efforts by the Institute of Genetics, Physiology and Plant Protection of the Republic of Moldova, was created a diverse collection of autochthonous triticale varieties, which has been developed to improve agronomic performance under Southeastern European conditions. Notable examples include Ingen 93 (1998), widely used as a reference standard in local research, as well as Ingen 33, Ingen 35, Ingen 40 (2015), Ingen 54 (2020), and Costel (2022) [2]. The existence of such a diverse gene pool within a relatively limited geographic area underscores the necessity for precise identification and differentiation of genetic variability among these cultivars.

Genetic diversity analysis plays a fundamental role in crop breeding, conservation, and genotype selection. Various marker systems have been employed to evaluate genetic polymorphisms, including phenotypic, biochemical, cytogenetic, and molecular markers. Among these, molecular markers are considered the most reliable due to their high reproducibility, independence from environmental factors, and the potential for automation and scalability. Techniques such as RFLP, SSR, RAPD, AFLP, SNP, and iPBS have been used to assess genetic variation in cereal crops [6]. The iPBS (inter-Primer Binding Site) marker system, based on the conserved regions of long terminal repeat (LTR) retrotransposons, offers several advantages, including high sensitivity to polymorphisms, especially among closely related genotypes. This makes it a powerful tool for cultivar identification and genetic diversity studies in breeding programs [7]. Current work focuses on using iPBS markers for triticale genotyping as well as assessing molecular diversity within populations with different genetic background.

MATERIALS AND METHODS

Plant material. Two triticale genotypes – Ingen-54 and Ingen-93 were used in this study. The Laboratory of genetic resources of Institute of Genetics, Physiology and Plant Protection provided plant material. Seeds of 2022 yield were germinated on Petri dish and filter paper under 27°C during 7 days. Eight seedling of each genotype were selected for population analysis. Approximately 2 cm of apical meristem were cut off and used for DNA extraction.

DNA extraction. DNA extraction was performed using the ISO 21571 method [8]. A sample of extracted DNA was subjected to a series of dilutions (1:4, 1:14). From the extracted samples, we randomly selected 16 genotypes, 8 genotypes

each of the Ingen-54 and Ingen-93 varieties, for PCR testing. Purified and diluted DNA was subsequently used for PCR tests.

Primers. Several primers (Pb13, Pb20, and Pb21) designed to universal iPBS markers and described in (Kalendar R. et al., 2010) were used for genotyping and population analysis (Tab.1).

Table 1. Primers used for qPCR analysis

Primers	Sequence	Tm (°C)
Pb13	CCATTGGGTCCA	45.7
Pb20	GGCTCAGATGCCA	50.5
Pb21	GCTCATCATGCCA	47.6

Amplification. PCR was performed in 25 µl of mix containing: Commercial buffer x10 (DreamTaq Buffer) – 2.5 µl, primer (forward + reverse) – 4.5 µl, dNTP mix – 0.5 µl, Taq polymerase – 0.3 µl, diluted DNA template – 10 µl, water – up to 25 µl volume. The amplification protocol was set up as follows: initial denaturation: 95°C, 3 minutes, followed by 45 cycles of denaturation at 95°C, 30 seconds, primer annealing at 55°C, 30 seconds and elongation at 72°C, 1 minute. Amplification concluded with final elongation: 72°C, 7 minutes.

Product analysis. PCR results was visualized on a 1.5% agarose gel stained with ethidium bromide. Molecular weight of the amplicons was assessed using 100 bp DNA Ladder (Thermo Fisher Scientific).

Data processing was carried out using the following software: GelAnalyzer, MS Excel, POPGENE.

RESULTS AND DISCUSSIONS

Total DNA quality test via agarose gel electrophoresis showed crisp and intense bands of high molecular weight without smears and low RNA content (Fig.1). This presumes high yield of integral DNA suitable for molecular genotyping.

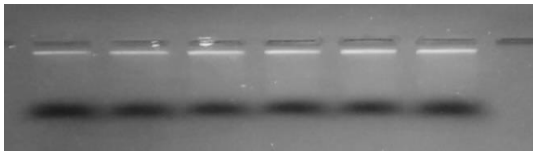


Figure 1. Electrophoregram of total triticale DNA

The amplification profiles obtained using three iPBS primers (Pb13, Pb20, and Pb21) are shown in Figures 2–4. The banding patterns reveal the presence of polymorphic loci and demonstrate the capacity of the chosen primers to

discriminate between triticale genotypes.

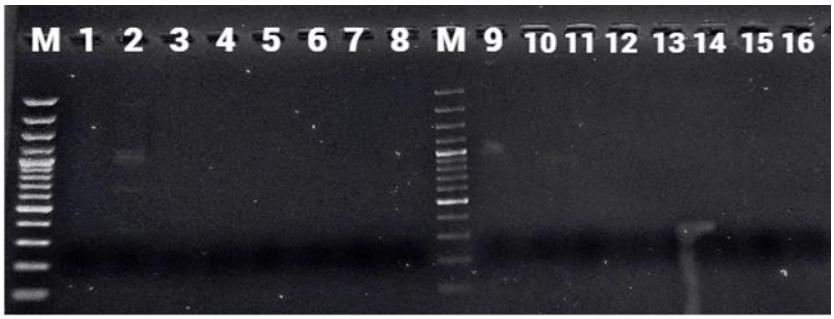


Figure 2. Electrophoregram of PCR products amplified with iPBS primer Pb13.
1-8 – Ingen-54; 9-16 – Ingen-93; M – 100 Plus Ladder (Thermo Fisher Scientific)

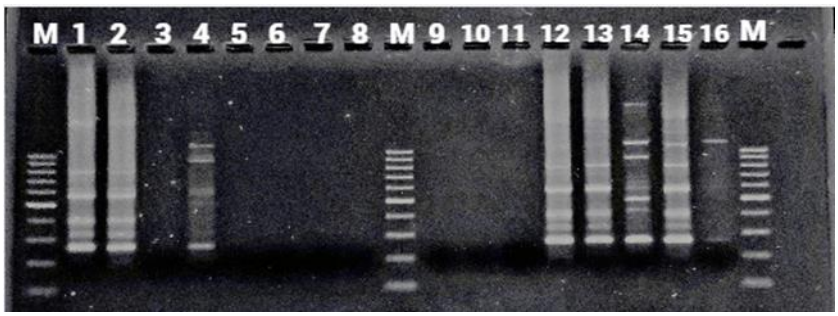


Figure 3. Electrophoregram of PCR products amplified with iPBS primer Pb20.
1-8 – Ingen-54; 9-16 – Ingen-93; M – 100 Plus Ladder (Thermo Fisher Scientific)

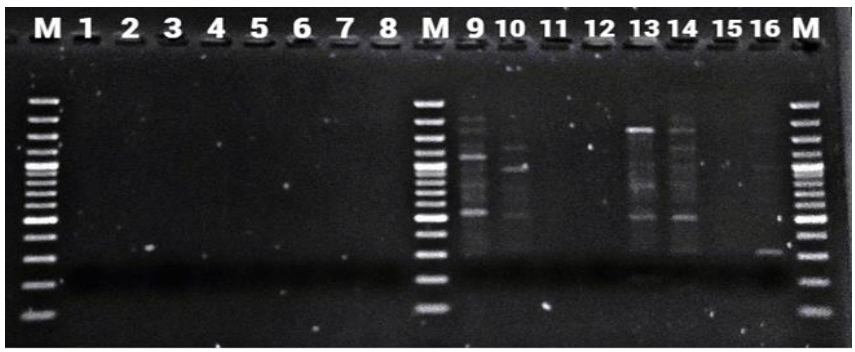


Figure 4. Electrophoregram of PCR products amplified with iPBS primer Pb21.
1-8 – Ingen-54; 9-16 – Ingen-93; M – 100 Plus Ladder (Thermo Fisher Scientific)

Amplification with these primers produced a total of 33 clear and reproducible loci (attested in three amplifications), ranging in size from approximately 243 to 2912 bp.

According to POPGENE statistical analysis, all 33 loci were polymorphic (100%), indicating the high informativeness of the chosen primer set. Nei's gene diversity (h) ranged from 0.0625 to 0.4188 with a mean of 0.1290, while Shannon's Information Index (I) varied from 0.1426 to 0.6096 (mean = 0.2403). The highest polymorphism was observed at locus 257 (h = 0.4188), suggesting it as a key marker for genotypic discrimination, which was detected when amplifying with Pb20 primer.

Nei's genetic distance between the two triticale varieties was low (0.0074–0.0169), with genetic identity ranging from 0.9832 to 0.9926, indicating a relatively close genetic relationship. Gst values (genetic differentiation) averaged 0.0642, and gene flow (Nm) was high (mean Nm = 7.29), reflecting limited genetic subdivision.

Primers Pb20 and Pb21 generated the greatest number of polymorphic loci and showed higher values of gene diversity (h), making them more suitable for distinguishing closely related triticale genotypes. Consequently, these specific primers are highly recommended as potent tools for prospective genotyping and breeding programs.

CONCLUSIONS

iPBS markers proved to be effective for both discriminating between triticale cultivars with different genetic background, as well was evaluating population diversity for this species. It has potential application in triticale breeding programs and supplying national Gene bank information on triticale.

PCR amplification using primers Pb13, Pb20, and Pb21 yielded a total of 33 clear and reproducible loci. All 33 loci demonstrated 100% polymorphism, confirming the high informativeness of the selected iPBS primer set.

The highest level of polymorphism (h=0.4188) was recorded for locus 257. Nei's genetic distance between the two triticale varieties ('Ingen-54' and 'Ingen-95') is low, while genetic identity is high.

Primers Pb20 and Pb21 generated the greatest number of polymorphic loci and exhibited higher gene diversity (h) values compared to Pb13, making them more suitable for distinguishing closely related triticale genotypes.

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