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The usage of the SCoT Molecular Markers for determining the genetical purity of the commercial hybrids of *Helianthus annuus* L..

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Abstract

The sunflower (*Helianthus annuus* L.), a globally significant oilseed crop, plays a vital role in agriculture, industry, and the economy. Ensuring the genetic purity of sunflower hybrids is critical for achieving superior agronomic traits such as high yield, resistance to diseases and pests, and adaptability to environmental stresses. This study explores the application of Start Codon Targeted (SCoT) molecular markers to assess the genetic purity of commercial sunflower hybrids, focusing on the hybrid AH1023, characterized by high oil content (50-51%), resistance to *Orobanche cumana*, and tolerance to drought and heat.

Leaf tissue samples were collected from 15 individual plants of the AH1023 hybrid, and DNA was extracted following the Doyle and Doyle method. Using SCoT primers, genomic DNA was amplified and analyzed through agarose gel electrophoresis. The presence or absence of polymorphic bands was scored, with statistical methods applied to determine genetic uniformity among the samples. The study highlights the high polymorphism detection capability of SCoT markers, underscoring their effectiveness in identifying genetic diversity within sunflower populations.

The findings demonstrate that SCoT markers provide a robust and cost-effective approach for genome mapping, enabling the detection of gene-associated polymorphisms linked to traits of agronomic interest. This study contributes to advancements in genetic analyses, supporting the development of sunflower breeding programs aimed at optimizing hybrid performance and ensuring genetic integrity. The methods and results presented here are also applicable to other crop species, marking a significant step forward in the use of molecular markers for plant genetic research and breeding.

Introduction

The sunflower (*Helianthus annuus*, $2n=34$) is an oilseed crop plant of great importance to agriculture, industry, and the economy. Its seeds, or achenes, are harvested to extract oils used for everyday food consumption as well as industrial purposes. The seeds are also used as animal feed, for human consumption in raw form, and as sources of fatty acids like oleic acid (omega-9), linoleic acid (omega-6), palmitic acid, and stearic acid, which are utilized in the food, pharmaceutical, cosmetic, and chemical industries.

The issue of genetic purity in creating sunflower hybrids is an important topic in plant genetics and agricultural sciences because it influences the quality, productivity, and characteristics of crop plants. The creation of sunflower hybrids aims to combine valuable hereditary traits, such as high yield, resistance to diseases, pests, and stress caused by soil and climatic conditions.

Maintaining the genetic purity in sunflowers involves spatial and reproductive isolation to prevent unintended cross-pollination from other varieties that could lead to genetic contamination, preserving the purity of parental lines (inbred lines) to ensure uniformity and stability in maintaining genetic traits, controlling pollination by using androsterile maternal lines and fertile paternal lines as parents, testing genetic purity through genetic analyses with molecular markers or by assessing phenotypic uniformity through observation of physical traits.

Genetic purity can be established by identifying genetic polymorphisms through biotechnology and molecular markers, which allow for the identification of DNA sequences associated with the desired traits. SCoT markers (Start Codon Targeted Polymorphism) are a type of dominant marker used for genetic analysis through genome mapping based on sequences surrounding the start codon (ATG) of genes. They are effective in detecting gene-related polymorphisms and may be particularly relevant for genetic studies of functional traits, such as disease resistance, resilience to soil and climatic stress, or high yield. The target DNA sequences are amplified through Polymerase Chain Reaction (PCR) using SCoT primers to recognize conserved regions around the start codon. SCoT markers have proven effective in genetic studies and plant and animal breeding, as well as evolutionary biology, due to their high polymorphism rate, providing valuable information about genetic diversity within populations and varieties. Other species tested with SCoT markers include *Brassica napus*, *Petroselinum crispum*, and *Sesamum indicum*.

In this experiment, we aimed to study SCoT molecular markers to determine genetic purity in commercial sunflower hybrids and to demonstrate a statistical method for assessing genetic purity based on the presence or absence of bands, using the correlation coefficient between plants of the same hybrid.

Material and method

Germplasm

The study focused on the genetic purity assessment of the pre-commercial sunflower hybrid AH1023. A total of 15 individual plants were sampled, with 100-150 mg of leaf tissue collected from each plant. The samples were obtained from the growth chambers of the Genetic Engineering Department at the University of Life Sciences "King Mihai I" in Timișoara. The hybrid AH1023 is mid-late maturing, with medium plant height, a large capitulum, long-ovoid seeds, an oil content of 50-51%, resistance to *Orobanche cumana*, drought and heat tolerance, and a potential yield of 4000-4200 kg/ha.

DNA Extraction

DNA was extracted from young leaf tissues at an early developmental stage using the Doyle and Doyle method. DNA concentration and quality were measured using a Nanodrop 8000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). High concentrations of DNA were detected, and samples were diluted to 100 ng/μL for subsequent analyses.

SCoT Primer Design and PCR Amplification

Genetic diversity was analyzed using Start Codon Targeted (SCoT) primers. Two primers were selected for amplification:

- SCoT 34: CAATGCACGAATCGTGTC
- SCoT 7: ACATGGCAAGCAGGTCGCA

The amplification mixture contained:

- 100 ng/μL of DNA template
- 12.5 μL of 2x GoTaq® Green Master Mix (Promega, Madison, WI, USA)
- 1 μL of 20 μM primer
- 1 μL of 10 μM MgCl₂
- Sterile distilled water to a final volume of 25 μL

PCR products were amplified using a thermal cycler, and the resulting fragments were separated by electrophoresis on a 0.7% agarose gel. Ethidium bromide staining allowed visualization of the bands under UV radiation. Fragment sizes were determined by comparison with the BenchTop 100 bp DNA ladder.

Results and discussions

The results of the PCR amplification with SCoT primers revealed distinct banding patterns that provided insights into the genetic purity of the AH1023 sunflower hybrid. Using the SCoT 34 primer, up to 11 bands were generated, 5 of which were polymorphic. This primer demonstrated a high capability for detecting genetic variability, making it highly effective for assessing genetic purity between individual plants within the hybrid population.

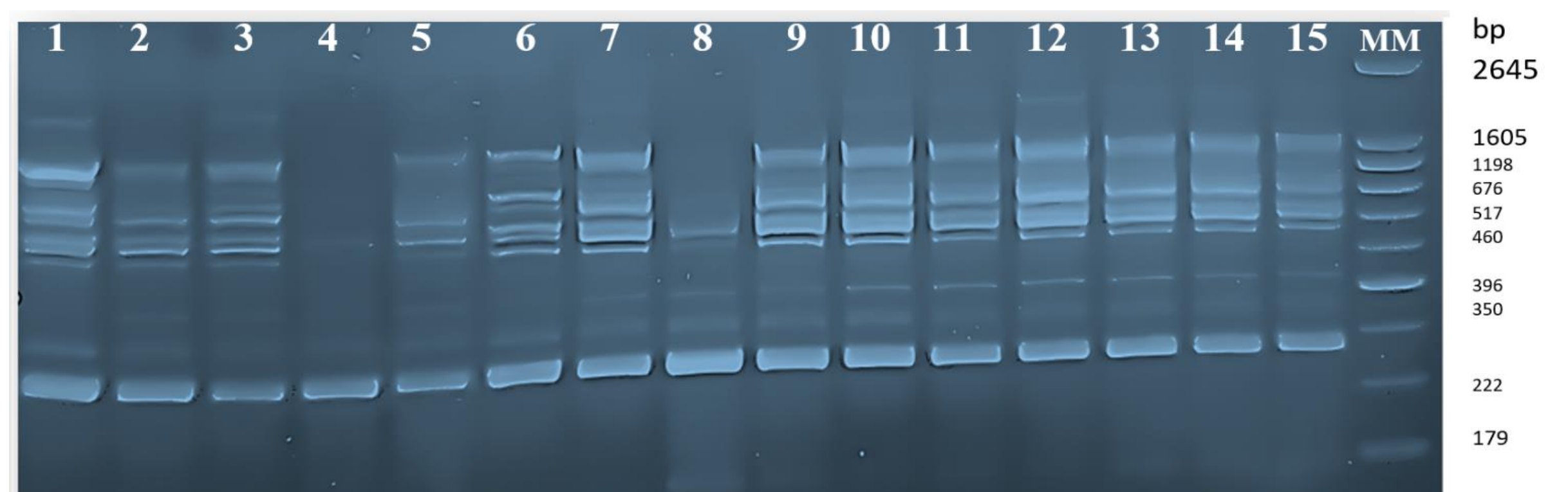


Figure 1. Agarose gel-electrophoresis results SCoT 34

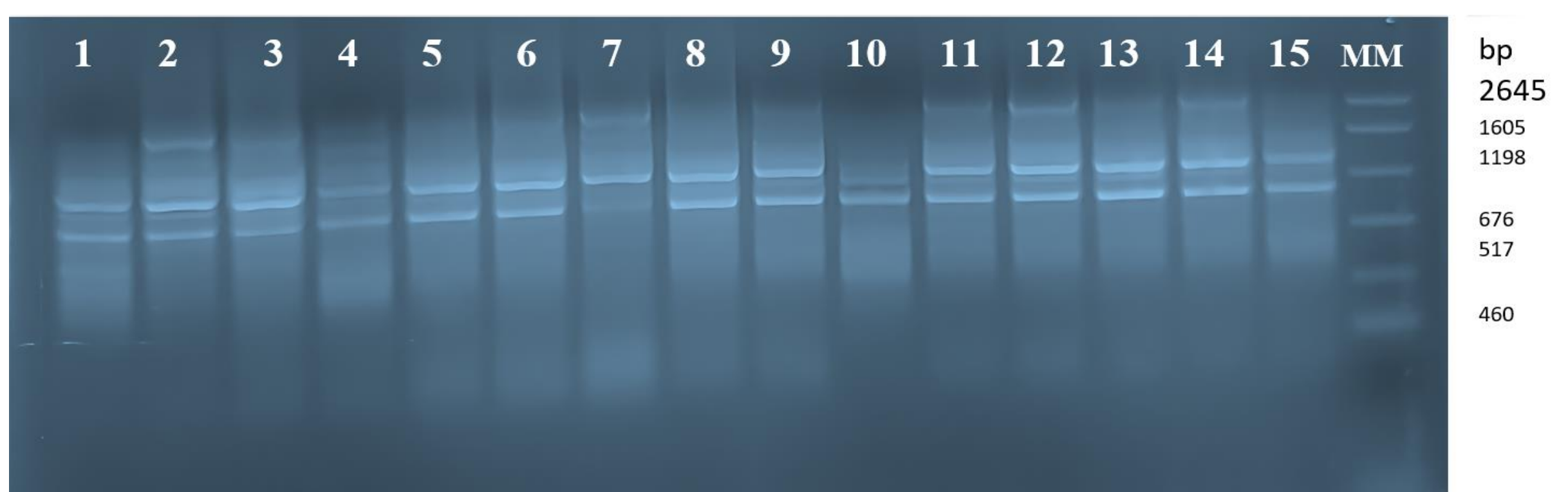


Figure 2. Agarose gel-electrophoresis results for SCoT 7.

In contrast, the SCoT 7 primer generated only 4 bands, suggesting a lower resolution in detecting genetic diversity. Despite this, the bands produced were sufficient to contribute to the overall genetic analysis, highlighting the primer's utility in complementary applications. The findings indicate that primers generating a higher number of bands, such as SCoT 34, facilitate a clearer observation of genetic purity. The increased number of detectable polymorphic bands allows for a more precise differentiation of genetic variability among plants. This supports the use of SCoT primers, particularly those with high amplification efficiency, as robust tools in determining genetic uniformity in sunflower hybrids.

Conclusions

The study demonstrates the effectiveness of SCoT molecular markers in assessing the genetic purity of sunflower hybrids. SCoT 34 proved to be particularly efficient, generating a higher number of polymorphic bands, which facilitated clearer differentiation of genetic variability among plants. These findings underscore the utility of SCoT markers as reliable tools for genetic analysis and hybrid purity determination, contributing significantly to the improvement of breeding programs for sunflower.

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