



Using Molecular Markers as a Powerful Tool for Accelerating Hemp Breeding Programs

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Abstract

Industrial hemp (*Cannabis sativa* L.) is a multipurpose crop prized for its diverse applications, from textiles to pharmaceuticals. However, traditional hemp breeding programs are often constrained by the laborious and time-consuming nature of phenotypic screening for key traits such as plant gender and cannabinoid profiles. Traditional methods are generally inefficient and delay selection until later developmental stages. This study investigated the application of a PCR Plant Screening by SCAR markers (SCAR119 and MADC2) and commercial youPCR assays (Gender Assay, CBDA Markers, and THCA Markers) for rapid and accurate early-stage screening of ten industrial Turkish hemp landraces. The research focused on achieving early and precise identification of sex and cannabinoid chemotypes to accelerate breeding cycles. By employing these molecular markers, we were able to confirm the gender and predict the cannabinoid chemotypes of all accessions within one week of germination. These molecular identifications showed high agreement with later-stage visual observations, thus validating the efficacy of the applied method. The ability to rapidly identify male plants and cannabinoid chemotypes significantly reduces the time, labor, and resources required for trait selection in traditional style breeding. The study highlights the breeding potential of incorporating genetic tools into hemp breeding strategies. These findings demonstrate that the implementation of molecular markers can substantially improve the efficiency and effectiveness of hemp breeding programs. The demonstrated benefits are not limited to a faster selection process, but also facilitate targeted hemp cultivation and advancements in both agricultural and industrial applications. The current method supports the faster development of improved cultivars tailored to specific industrial and medicinal applications.

Keyword: Industrial hemp breeding, Molecular markers, PCR, Gender, Cannabinoid chemotype

Özet

Endüstriyel kenevir (*Cannabis sativa* L.), tekstilden ilaçlara kadar çeşitli uygulamaları nedeniyle değer verilen çok amaçlı bir üründür. Ancak, geleneksel kenevir yetiştirme programlarında genellikle bitki cinsiyeti ve kannabinoid profilleri gibi temel özellikler için fenotipik taraması zahmetli ve zaman alıcı işlemler yüzünden kısıtlanmaktadır. Geleneksel yöntemler genellikle etkisizdir ve seçimi daha sonraki gelişim aşamalarına kadar geciktirmektedirler. Bu çalışmada, on adet endüstriyel Türk kenevir yerel çeşidinde erken aşamada hızlı ve doğru bir şekilde taranması için SCAR belirteçleri (SCAR119 ve MADC2) ile PCR Bitki Tarama ve ticari youPCR analizleri (Cinsiyet Analizi, CBDA Belirteçleri ve THCA Belirteçleri) yaparak bir araştırma yapılmıştır. Bu Araştırmada, üreme döngülerini hızlandırmak için cinsiyet ve kannabinoid kimotiplerinin erken ve kesin bir şekilde tanımlanmasına odaklanmıştır. Bu moleküler belirteçleri kullanarak, çimlenmeden sonraki bir hafta içinde tüm örneklerin cinsiyetini doğrulayabilmiş olup, kannabinoid kimotiplerini tahmin edilebilmiştir. Bu moleküler tanımlamalar, daha sonraki aşama görsel gözlemlerle yüksek düzeyde uyum göstererek uygulanan yöntemin etkinliğini doğrulamıştır. Erkek bitkileri ve kanabinoid kimotiplerini hızla tanımlama yeteneği, geleneksel tarzdaki yetiştirmede özellik seçimi için gereken zamanı, emeği ve kaynakları önemli ölçüde azalmıştır. Çalışma, genetik araçları kenevir yetiştirme stratejilerine dahil etmenin yetiştirme potansiyelini vurgulamaktadır. Bu bulgular, moleküler belirteçlerin uygulanmasının kenevir yetiştirme programlarının verimliliğini ve etkinliğini önemli ölçüde artırabileceğini göstermektedir. Gösterilen faydalar daha hızlı bir seleksiyon süreciyle sınırlı değildir, aynı zamanda hedeflenen kenevir yetiştiriciliğini ve hem tarımsal hem de endüstriyel uygulamalardaki ilerlemeleri kolaylaştırmaktadır. Mevcut yöntem, belirli endüstriyel ve tıbbi uygulamalara yönelik geliştirilmiş çeşitlerin daha hızlı gelişimlerini desteklemektedir.

Anahtar Kelime: Endüstriyel kenevir ıslahı, Moleküler markörler, PCR, Cinsiyet, Kannabinoid emotipi

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1. INTRODUCTION

Industrial hemp (*Cannabis sativa* L.) is a highly versatile crop with a wide range of applications, including textiles, biofuels, food, and pharmaceuticals, making it a cornerstone of sustainable agriculture (Visković et al., 2023; Yazici et al., 2019). Its ability to thrive in diverse climates and its low environmental footprint further enhance its appeal as a sustainable crop (Atmf et al., 2022). However, the cultivation of hemp for cannabinoid production presents unique challenges, particularly the need for precise control over plant gender and chemotype, as these traits critically influence yield, product quality, and compliance with legal regulations (Ingvarlsen et al., 2023). Male plants, for instance, are undesirable in cannabinoid production due to their inability to produce cannabinoid-rich flowers, while the ratio of cannabinoids such as tetrahydrocannabinolic acid (THCA) to cannabidiolic acid (CBDA) determines the plant's suitability for specific industrial or medicinal applications (Weiblen et al., 2015; Kamle et al., 2024). Traditional breeding methods for hemp rely heavily on labor-intensive and time-consuming phenotypic screening, often requiring weeks or months to identify key traits such as gender and chemotype (Barcaccia et al., 2020). This delay in the selection process not only increases costs but also limits the efficiency of breeding programs, particularly in the context of rapidly evolving market demands (Krishna et al., 2023). Furthermore, the phenotypic expression of these traits can be influenced by environmental factors, leading to inconsistencies in selection accuracy (Alsaleh and Yılmaz, 2025). Recent advancements in genetic analysis have revolutionized precision breeding, offering molecular tools to overcome these limitations (Gudi et al., 2022). Molecular markers, such as sequence-characterized amplified region (SCAR) markers and quantitative PCR (qPCR)-based assays, enable early and accurate screening of plants at the seedling stage, allowing breeders to eliminate undesirable genotypes before significant resources are invested (Barcaccia et al., 2020). These tools have been particularly effective in identifying gender-specific markers and cannabinoid synthase genes, which are critical for optimizing cannabinoid profiles and ensuring compliance with regulatory standards (Atmf et al., 2022). The integration of molecular tools into hemp breeding programs has the potential to significantly accelerate the development of high-quality cultivars tailored to specific industrial needs (Alsaleh and Yılmaz, 2025). For example, the use of PCR-based platforms for rapid screening of gender and chemotype can reduce the time and cost associated with traditional breeding methods, while also improving the accuracy of trait selection (Krishna et al., 2023). This is particularly important for the development of hemp cultivars with optimized cannabinoid profiles, which are increasingly in demand for pharmaceutical and nutraceutical applications (Taşkesen and Tüfekci, 2025). This study investigates the application of a PCR-based plant screening platform to rapidly identify gender and cannabinoid synthase genes in ten industrial hemp Turkish landraces. By demonstrating the efficacy of molecular tools in streamlining trait selection, this research highlights their potential to revolutionize hemp breeding programs, improve efficiency, and support the development of cultivars optimized for diverse industrial applications. The findings of this study are expected to contribute to the growing body of knowledge on precision breeding in hemp, providing valuable insights for breeders and researchers working to meet the demands of a rapidly expanding industry.

2. MATERIALS AND METHOD

2.1. Plant Material

Ten industrial hemp Turkish landraces were obtained from the Hemp Research Institute at Yozgat Bozok University (Table 1). Seeds from each landrace were germinated under controlled conditions in 2024. One-week post-germination, tissue samples from randomly selected seedlings, pre-labelled for subsequent sex confirmation, were collected for molecular genetic analysis. This analysis was performed at the Hemp Research Institute at Yozgat Bozok University. To validate the accuracy of the molecular identification method, the seedlings were grown to maturity under controlled greenhouse conditions. At the flowering stage, the gender of each plant was visually confirmed based on morphological characteristics, such as the presence of male flowers (staminate) or female flowers (pistillate). These visual observations were then compared with the molecular identification results to assess the agreement between the two methods.

2.2. Molecular Marker Analysis

DNA extraction was performed according to (Alsaleh and Yilmaz, 2025), the Cetyl trimethyl ammonium bromide (CTAB) protocol was employed. Following isolation, the DNA was diluted to a concentration of 25 ng μl^{-1} and used for molecular analysis. Molecular marker analysis was performed using two approaches. First, SCAR markers, including SCAR119 (Borin et al., 2021) and MADC2 (Toth et al., 2020), were utilized for gender determination. The primer sequences for SCAR119 were 5'-GCT CTA CAA TCC AAT CCC TC-3' (Forward) and 5'-GCT CTA CAA TCC AAT CCC TC-3' (Reverse). The primer sequences for MADC2 were 5'-GAA TTC TGC TTC AAG TCA TCC-3' (Forward) and 5'-GAA TTC TGC TTC AAG TCA TCC-3' (Reverse). For SCAR119 amplification, PCR was performed according to the protocol outlined by Borin et al. (2021) with slight modifications. The reaction consisted of an initial denaturation at 94°C for 2 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute. A final extension step was performed at 72°C for 4 minutes. For MADC2 amplification was based on (Toth et al., 2020), the PCR protocol was identical except for the annealing temperature, which was increased to 51.5°C. Second, commercial youPCR® assays were employed, including the Gender Assay, which detects the presence of the Y chromosome for male plant identification, the CBDA Marker, which detects active CBDA synthase alleles, and the THCA Marker, which detects active THCA synthase alleles. The youPCR® assays were conducted as recommended by the manufacturer. Positive and negative controls were included in each run to ensure assay accuracy.

The SCAR-PCR products were then run on 2.5% Agarose gel submerged in 1X of Tris, Borate, and Ethylenediaminetetraacetic acid (TBE) buffer using an electrophoresis chamber alongside a standard size marker (100 bp DNA Ladder) to estimate the molecular size. The gels were stained with ethidium bromide solution (at a final concentration of 0.5 $\mu\text{g}/\text{ml}$) for 20 min, and the PCR fragments were visualized on an ultraviolet (UV) transilluminator (Alsaleh and Yilmaz, 2025). The results from SCAR markers and youPCR gender assay were scored and compared to visual observations to assess accuracy. Gender was confirmed through visual observation during the flowering stage.

Table 1. List of hemp strains used in the study, including their origin, genotype type, and sex type

Strain number	Strain Name	Origin	Sex Type
1	Eminönü-3	Turkish Landraces	Dioecious
2	Maltepe-2	Turkish Landraces	Dioecious
3	Maltepe-4	Turkish Landraces	Dioecious
4	Eminönü-3	Turkish Landraces	Dioecious
5	Kavacık	Turkish Landraces	Dioecious
6	Mısır çarşısı	Turkish Landraces	Dioecious
7	Eminönü-2	Turkish Landraces	Dioecious
8	Maltepe-3	Turkish Landraces	Dioecious
9	Maltepe-1	Turkish Landraces	Dioecious
10	Van-1	Turkish Landraces	Dioecious

3. RESULTS AND DISCUSSION

The results presented in Figure 1 and Table 2 highlighted the breeding potential of PCR-based screening in hemp breeding programs, particularly for early gender and cannabinoid chemotype identification. Clear bands of primers (SCAR119) (a) at 119 bp, 390 bp in MADC2 (b), while the pink colour in youPCR Gender Assay were associated only with the male phenotype (not shown). Both SCAR markers and the youPCR Gender Assay successfully identified male and female plants in all ten landraces within one week of germination, demonstrating remarkable efficiency. Male plants were identified in 20% of the strains (2 out of 10), and these results were 100% consistent with visual observations during flowering. This early detection allows for the timely removal of male plants, which are undesirable in cannabinoid production, thereby conserving weeks of resources that would otherwise be expended on their cultivation. Furthermore, the PCR assays targeting CBDA and THCA synthase genes revealed two distinct chemotypes among the accessions: CBDA-dominant (70%, 7 strains), and mixed chemotypes (30%, 3 strains).

These findings provide breeders with invaluable early insights into the genetic potential of each strain, enabling the prioritization of plants with desirable cannabinoid profiles for further breeding. This approach not only streamlines the selection process but also enhances the precision and efficiency of hemp breeding, paving the way for the development of cultivars tailored to specific industrial or medicinal applications.

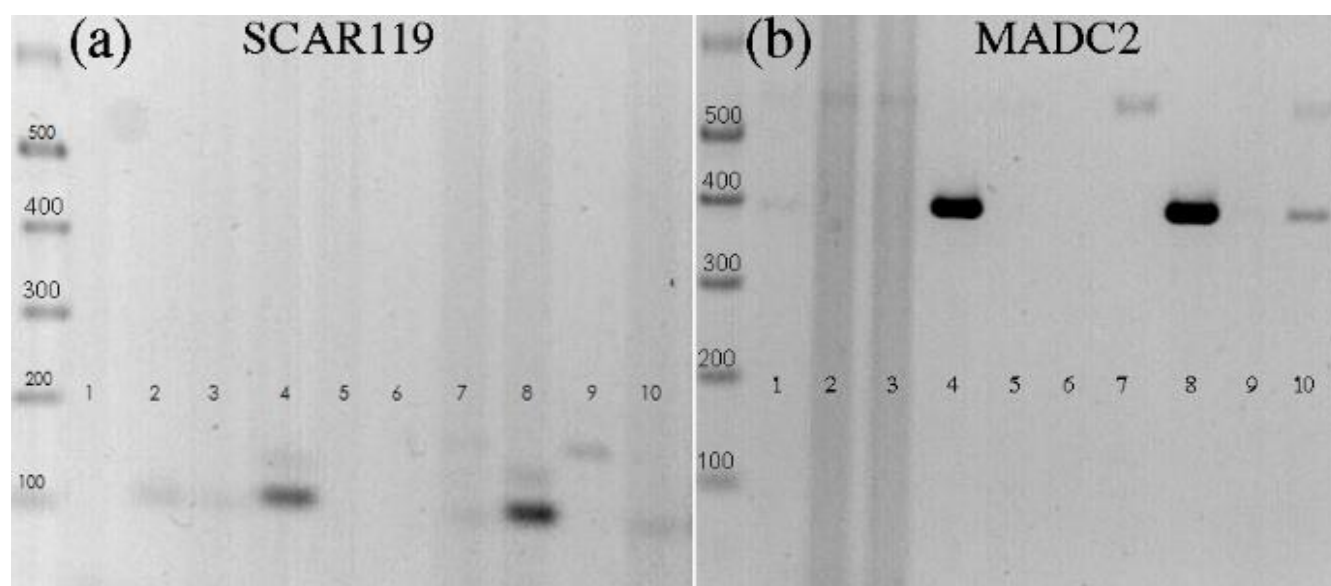


Figure 1. PCR analysis of SCAR119 (a) and MADC2 (b) markers for gender identification in industrial hemp seedlings revealed a distinct band of approximately 119 bp and 390 bp, respectively, which was consistently observed in male plants, but was absent in female plants. The reference legend is based on the 100 bp size marker.

The integration of genetic analysis into hemp breeding represents a significant advancement over traditional phenotypic methods. By enabling the identification of male plants and cannabinoid chemotypes at the seedling stage, breeders can optimize resource allocation and significantly accelerate the selection process (Barcaccia et al., 2020; Toth et al., 2020). This study highlights the PCR Platform as a powerful tool for early trait screening, particularly in large-scale breeding programs where time and resource efficiency are of paramount significance. The ability to predict cannabinoid chemotypes early in the plant lifecycle is particularly impactful. While environmental factors can modulate cannabinoid concentrations, the relative ratios of THCA to CBDA are genetically determined by Laverty et al., (2019) and Grassa et al., (2021). By screening for active synthase alleles, breeders can accurately predict chemotypes, facilitating targeted crossbreeding to develop strains with specific cannabinoid profiles (Toth et al., 2020). The consistency between molecular marker results and visual observations underscores the reliability of these tools. However, it is important to acknowledge

that genetic analysis alone cannot predict absolute cannabinoid concentrations, as these are influenced by environmental and agronomic factors (Trancoso et al., 2022).

Table 2. Characterization of Hemp Accessions: Strain Number, Strain Name, Sex Type, and Cannabinoid Chemotype

Strain number	Strain Name	Sex Type by SCAR119	Sex Type by MADC2	Sex Type by Gender Assay	Chemotype
1	Eminönü-3	♀	♀	♀	CBDA dominant (Type III)
2	Maltepe-2	♀	♀	♀	CBDA dominant (Type III)
3	Maltepe-4	♀	♀	♀	CBDA dominant (Type III)
4	Eminönü-3	♂	♂	♂	CBDA dominant (Type III)
5	Kavacık	♀	♀	♀	CBDA dominant (Type III)
6	Mısır çarşısı	♀	♀	♀	CBDA dominant (Type III)
7	Eminönü-2	♀	♀	♀	CBDA dominant (Type III)
8	Maltepe-3	♂	♂	♂	CBGA dominant (Type IV)
9	Maltepe-1	♀	♀	♀	CBGA dominant (Type IV)
10	Van-1	♀	♀	♀	CBGA dominant (Type IV)
11	Positive control			♀	THCA dominant (Type I)
12	Negative control			♂	CBDA dominant (Type III)

The findings of this study are consistent with previous research on the application of molecular markers in hemp and other dioecious plants. For instance, Moliterni et al. (2004) successfully used SCAR markers to identify male-specific regions in hemp, while Borna et al. (2017) demonstrated the utility of qPCR-based assays in chemotype determination. Our results further validate the efficacy of these molecular tools in the context of Turkish hemp landraces, highlighting their potential for regional breeding programs. The future research should focus on integrating genetic data with phenotypic and environmental variables to refine breeding strategies further. Such an integrated approach will enhance the robustness of hemp breeding programs, ensuring the development of high-performing cultivars that meet the diverse needs of the agricultural and industrial sectors.

4. CONCLUSION

The use of molecular markers, including SCAR markers and commercial PCR assays, offers significant advantages over traditional phenotypic screening. By identifying gender and cannabinoid chemotypes at the seedling stage, breeders can save time, reduce costs, and focus resources on plants with desirable traits. This study demonstrates that molecular tools provide rapid, accurate, and cost-effective designing of hemp breeding programs. This will pave the way for sustainable and efficient hemp cultivation as the first application of such genetic tools in Türkiye, ultimately supporting the development of improved cultivars tailored to specific industrial and medicinal applications.

AUTHORS' CONTRIBUTION

All authors contributed to the study's conception and design. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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