



Selection of Soybean Lines Resistant to *Cercospora sojina* Using CAPS Molecular Markers

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ARTICLE INFO		Abstract
Article Type	: Research Article	Frogeye Leaf Spot (FLS) is one of soybean's most important disease agents. A practical and effective disease control method is using soybean genotypes with disease-resistance genes. In other words, determining soya varieties that are resistant to the disease will be important in the fight against the disease. In this study, 12 soya lines and four different varieties were used. One CAPS molecular marker was used to differentiate resistant and susceptible varieties. According to the results, only one of the 12 lines was found to be sensitive, and the others were resistant. Out of the remaining four varieties, two of them were sensitive, and two of them were resistant.
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1. Introduction

Soybean, a member of the Fabaceae family, is one of the most important food sources worldwide (Khanam *et al.*, 2016). The world population is estimated to increase to 8.5 billion in 2030 and 9.7 billion in 2050 (Gu *et al.*, 2021). Therefore, the importance of sustainable food resources is becoming increasingly critical due to population growth. Soybeans are among the world's top ten most-produced agricultural products regarding food rankings (FAOSTAT, 2024). Besides, soybeans are not only rich in vitamins, minerals, carbohydrates, and fats but also an excellent source of protein. They are utilized extensively for both human nutrition and livestock feed. Additionally, soybeans play a significant role in producing vegetable oil and biodiesel. Although there is an increase in soybean production, fluctuations occur from year to year. The reason for these fluctuations is stress factors. Soybeans are exposed to various abiotic and biotic stress factors that affect their growth and development. Viral, bacterial, and fungal diseases are biotic stress factors common in soybean production. Fungal diseases cause various physiological damages as well as yield loss in soybeans. *Cercospora sojina* is one of the fungi causing disease in soybean agriculture.

Cercospora sojina causes frogeye leaf spots (FLS) in soybean (*Glycine max*) (Mengistu *et al.*, 2011). FLS is a disease that causes significant yield loss in soybeans in countries with hot and humid climates (Mian *et al.*, 2008). The disease is characterized by circular reddish-brown lesions that grow as the infection progresses. FLS mainly affects soybean leaves. However, seeds, pods, and stems can also be affected (Mengistu *et al.*, 2018). If soybean seeds are infected by the fungus *Cercospora sojina*, the fungus spreads to new seedlings the following year (McAllister *et al.*, 2021). It has been stated that yield losses caused by outbreaks of this plant pathogen are due to reduced photosynthetic leaf area, premature leaf fall and reduced seed weight. It is emphasized that the mentioned fungus can survive for a long time in the infected plants and cause new outbreaks (Barro *et al.*, 2023). Fungicides are used to stop this infection caused by *Cercospora sojina* fungus. However, the use of fungicides causes resistance after a while, leading to a decrease in the effect (Zhang *et al.*, 2012). For example fungicides containing quinone outside inhibitor active ingredients, which are frequently used against *C. sojina*, are among the main control methods. However, in the last 10

years, *C. soja* isolates resistant to this fungicide have been isolated in many countries (Piñeros-Guerrero et al., 2023).

It is crucial to develop soybean varieties that are resistant to *Cercospora soja* fungus or to find resistant varieties. Molecular markers are one of the most basic methods to develop resistant varieties to *Cercospora soja* fungus and to find resistant soybean varieties. Molecular markers are nucleotide sequences and can be analyzed by polymorphism between the nucleotide sequences of different individuals (Nadeem et al., 2018). In other words, by looking at molecular markers, resistant or sensitive soybean varieties can be identified quickly and cost-effectively. There are various types of molecular markers. One of these types of markers is the SCAR marker. The SCAR marker is a DNA fragment amplified by PCR using specific 18-26 bp primers (Liu et al., 2020). The SCAR marker, a molecular marker derived from RAPD, was first developed by Paran and Michelmore for downy mildew resistance genes in lettuce (Dahlberg et al., 2002). SCARs have additional specificity and reproducibility advantages over RAPD markers. The separation efficiency of SCAR markers is superior to RAPD markers, depending on the number of reactions required per sample. Furthermore, SCAR marker gels are cleaner and much easier to score. This helps computer automation (Kiran et al., 2010).

Considering this information in this study, a molecular marker was developed for the selection of soybean lines resistant to *Cercospora soja*.

2. Materials and methods

2.1. Soybean Soybeans Seedlings Production

In this study, 16 different soybean genotypes were used. These genotypes were planted in pots containing soil mixed with 50% peat. Soybeans were grown under 16 hours of light and 8 hours of dark photoperiod. Soybeans were watered with Hoagland's solution once a week. On the 30th day, samples were taken for DNA isolation. Samples were stored at -80 °C.

2.2. DNA isolation and SCAR marker

Total DNA isolations were performed using ZYMO Quick-DNA Plant/Seed Kit. 100 mg plant sample was crushed in a mortar and pestle with liquid nitrogen until powdered. The crushed plant samples were taken into a 2 mL microcentrifuge tube cooled with liquid nitrogen and continued according to the kit protocol. The concentration of DNAs obtained at the end of the process was measured by NanoDrop™ 2000c spectrophotometer. The DNA quality was checked by running on 0.8 % agarose gel. Isolated DNA was stored at -20°C.

PCR primers were obtained from (Martins Filho et al., 2002) (Table 1). PCR conditions were optimized using NEB Taq 2X Master Mix. 50 ng/μL DNA was used for the reaction. A total volume of 25 μL was prepared using 1 μL genomic DNA (50 ng/ μL), 0.5 μL forward primer, 0.5 μL reverse primer, 12.5 μL master mix and 10.5 μL sterile nuclease-free ddH₂O. The reaction conditions were pre-denaturation at 95 °C for 3 minutes, denaturation at 95 °C for 30 seconds, and primer binding temperature at 50 °C for 30 seconds. The elongation phase was at 68 °C for 1 minute, the final elongation phase was at 68 °C for 5 minutes, and 30 cycles were repeated. The primer sequences used in PCR studies are given in Table 1.

Table 1. Selected primer sequences and their properties

Primer Name	Primer Sequence
SCARBG5F	5'-GCCGTGAGAAAGGCGAAG-3'
SCARBG5R	5'-AGCCGTGAATTATCCGAT-3'

At the end of PCR amplification, PCR products were separated by running on 1% (W/V) agarose gel at 90 volts for 45 minutes. To visualize the DNA bands, 0.5 μg/ml ethidium bromide was added and visualized in the Biorad Gel Doc XR+ Gel imaging device.

3. Results

In this study, 16 soybean genotypes were subjected to PCR to detect resistant varieties using SCAR markers. PCR conditions were applied as initial denaturation at 95 °C for 3 minutes, 95 °C for 30 seconds, 50 °C for 30 seconds, 68 °C for 1 minute 35 cycles, and 68 °C for 5 minutes in the last step. PCR fragments were separated by 1% agarose gel electrophoresis using 1X TAE buffer and ethidium bromide dye. Gel images were visualized in Biorad Gel Doc XR+ Gel imaging device under UV light. According to the results, resistant and susceptible varieties to *Cercospora soja* fungus were determined. The gel image of the results is shown in Figure 1.

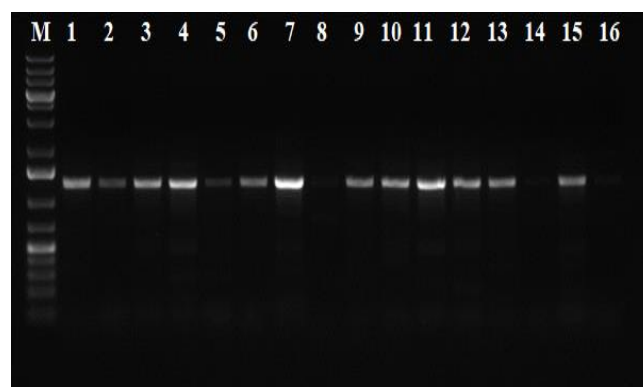


Figure 1. M; 100bp marker, 1-7; Resistant soybean lines 8; Sensitive soybean line, 9-12; Resistant soybean lines, 13; Resistant PG375, 14; Sensitive Emsoy, 15; Resistant Flint, 16; Sensitive Umut

In the present study, 12 soybean lines and four varieties were conducted with SCAR markers. According to the study results, lines 1-7 and 9-12 were found to be resistant to *Cercospora sojina* fungus, while line 8 was found to be susceptible. PG375 and Flint varieties were found to be resistant to *Cercospora sojina* fungus, while Emsoy and Umut varieties were found to be susceptible.

4. Discussion

Today, the effects of biotic and abiotic stress factors on plants are increasing. Fungi, one of the biotic stress factors, causes significant damage to agricultural products that are important for humans. As a result, economic losses are experienced, and people's access to food becomes difficult. Today, the effects of fungi are minimized by combating them with pesticides. However, the adverse effects of pesticides on human health limit their use in agricultural areas (Nicolopoulou-Stamati *et al.*, 2016). Instead, the importance of growing and breeding fungus-resistant plants is increasing.

Molecular markers are used as an auxiliary tool to shorten the breeding period and transfer the desired characteristics to plants. The polymorphism can be determined using a variety of molecular marker techniques. There are advantages and disadvantages to each marker technique. One of the most widely used techniques is SCAR. This marker was developed from RAPD marker (Yang *et al.*, 2013). It is a reliable marker because of its reproducible and codominant (Kumla *et al.*, 2012).

In this study, we tried to determine the resistant soybean lines to *Cercospora sojina* fungus by using SCAR markers. By using these markers, we aimed to shorten the breeding period. According to the results, resistant and sensitive lines were determined. The SCAR markers enable us to develop soybean varieties resistant to *Cercospora sojina* fungus in a shorter period.

Authorship Contribution Statement

All authors contribute equally to the article.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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