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Identification of carrying alien DNA fragments in *Solanum melongena* x *Solanum incanum* interspecific progeny by using COSII marker

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Abstract: Cultivated eggplant (*Solanum melongena* L.) is produced in many countries with temperate and tropical climates and has great economic importance. In recent years, resistance to biotic and abiotic stress conditions in addition to increasing yield and quality has gained importance in plant breeding including eggplant breeding. Therefore, the wild relative *S. incanum* is an important parent in breeding studies as it provides resistance to some important biotic and abiotic stresses for eggplant. It is possible to obtain a fully fertile hybrid between the two species, as well as to establish F2 lines. However, it is a mystery whether there is gene introgression from wild ancestor in F2 individuals. The phenotypic distinguishing and elimination in selection of the nonhybrid plants by classical breeding methods requires a long time and intensive labour. For this reason, there is a need for solutions in breeding studies in which gene transfers from wild relatives can be detected in the early development stages of plants. In this study, it was aimed to determine whether the F2 individuals obtained from interspecific cross of an inbred line P45 belonging to *S. melongena* x *S. incanum* crosses, when they were at the seedling stage, were *S. melongena*, *S. incanum* or carry genes from both ancestors by using T1480 COSII marker. Among a total of 94 F2 eggplant individuals and two parent eggplant species, it was determined that 51 individuals carried DNA fragments from both parental eggplant species, while 22 individuals belonging to the *S. incanum* species and 23 individuals belonging to the *S. melongena* species were identified. The resulting segregation ratio was 1:2:1, which was in accordance with Mendelian genetics. The results clearly showed that T1480 could be a useful marker for distinguishing whether seedling stage F2 plants carry genes from *S. melongena*, *S. incanum*, or both parents. As a conclusion, T1480 COSII marker can be used in eggplant breeding studies and will enable the plant improvement to progress more reliably than classical approaches.

Key words: Eggplant, interspecific hybridization, PCR, MAS breeding

1. Introduction

Eggplant ranks 4th among vegetables produced in the world, following tomatoes, peppers, and cucumbers, with approximately 55 million tons of eggplant produced worldwide in 2021 (FAO, 2021). China produces 35.5 million tons of eggplant and has a share of 64.5% in the world eggplant production. China is followed by India with 12.7 million tons of production, Egypt with 1.2 million tons, and Türkiye with 835 thousand tons of production.

The most important eggplant species consumed is *Solanum melongena* L., and it is located in the subgenus Leptostemonum (Dunal) of the genus *Solanum* L. from the family Solanaceae (Eggplantaceae). It is reported that there are many species in the family Solaneceae (Geboloğlu and Ellialtıoğlu, 2022). Eggplant is significant due to its vitamin and mineral content and holds considerable economic value in many countries, which is why it ranks third in production within the family Solanaceae, after potato and tomato (Doganlar et al., 2002a).

Eggplant is cultivated both in the greenhouse and in the open field (Shimira and Taşkın, 2022). In eggplant cultivation, cultural practices such as irrigation and fertilization are crucial for increasing both yield and quality (Ali et al., 2021), so is breeding. The use of hybrid varieties in eggplant greenhouse cultivation is very common. Breeding studies in eggplant has been carried out to increase yield and quality, and to resist biotic and abiotic stress conditions. First known commercial hybrid eggplant variety, "Millionaire", was developed in 1961 in Japan. The main countries conducting eggplant breeding programs are Japan, Spain, China, Italia, India, Türkiye, France, Spain, and the Netherlands (Boyacı, 2021).

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Eggplant cultivars, like other *Solanaceae* vegetables, are susceptible to many biotic and abiotic stress conditions (Rotino et al., 2014; Boyaci and Ellialtioglu, 2018). Although natural diversity in cultivated eggplant is rich across various agroclimatic zones, it is not sufficient for sustainable production. However, wild relatives of eggplant may serve as an important genetic source to adapt to adverse conditions (Toppino et al., 2022). In recent years, the great efforts have been made to improve the genome of the eggplant by using wild relatives (Boyaci, 2020). In particular, *S. incanum* is one of the most popular genetic resources having useful genes in current eggplant breeding programs. Great progress has been achieved in transferring the gene(s) of resistance to Fusarium, drought, and high temperatures to cultivated forms ,as well as in developing breeding lines at different levels and introgression lines (ILs) through backcrossing methods (Boyaci et al., 2010; Hurtado et al., 2015; Gramazio et al., 2017; Boyaci, 2020; Mangino et al., 2021; Cebeci et al., 2023). MAGIC populations, a powerful tool for eggplant genetics and breeding studies in eggplant, were created by using interspecific cross between *S. incanum* and *S. melongena* (Mangino et. al., 2022). However, conventional breeding methods could be time-consuming, laborious, and expensive. Molecular markers have already played a major role in the genetic characterization (Boyacı et al., 2015; Keçe and Kamberoğlu, 2016). Conventional and molecular breeding techniques are commonly used together to obtain new varieties in recent years (Frary et al., 2007; Polat, 2018; Nadeem et al., 2018; Hasan et al., 2021).

DNA markers improved the productivity and accuracy of conventional breeding by means of MAS-marker-assisted selection (MAS). Molecular MAS has considerably shortened the time for developing new crop varieties (Hasan et al., 2021). Single-copy orthologs (COSII) marker, from Conserved Ortholog Set (COS) genes, designed to amplify intronic and/or exonic regions, provides greater flexibility in the identification of polymorphisms (Wu et al., 2006; Enciso-Rodríguez et al., 2010; Wang et al., 2010). Highly promising results were obtained in one of the previously conducted study by using molecular markers to map the characteristics of the interspecies F2 population produced from the crosses between *S. melongena* L. and its wild relative *S. linnaeanum* (Doganlar et al., 2002b). In a study conducted by Villarroya (2009), F2 genotypes obtained from *S. incanum* x *S. melongena* crosses were effectively distinguished using the COSII marker which was developed with the support of a mapping study by Wu et al. (2009).

The parent selection is a critical step to carry out a successful breeding program. *S. incanum* is a wild eggplant species, and although it is not suitable for consumption, it constitutes a source of resistance/tolerance to many biotic and abiotic stresses. For this reason, it is extensively used in breeding studies with *S. melongena* (Prohens et al., 2013; Rosa-Martínez et al., 2022).

Therefore, the objective of this study was to distinguish the genotypes obtained from *S. incanum* x *S. melongena* crosses at the F2 stage (a ratio of 1:2:1) by using the COSII molecular marker. Since the molecular tests can be conducted at the seedling stage of the plants using the COSII marker, it is assumed that time, cultivation space, and labour, and costs would be saved in eggplant breeding studies.

2. Materials and methods

The inbred line P45 genotype (Figure 1a), belonging to *S. melongena* L., was developed in a breeding program carried out by Bati Akdeniz Agricultural Research Institute (BATEM) Antalya, Türkiye. The wild parent *S. incanum* L. group C (MM684) (Figure 1b) was obtained from INRA (Unit of Genetics and Breeding of Fruits and Vegetable, Avignon, France). All of F2 progenies were produced by self-pollination of their hybrid.

A total of 96 genotypes were used in the present study: two parents (*S. incanum* and *S. melongena*) and 94 individual F2 plants obtained from the cross of *S. incanum* x *S. melongena*.

Total genomic DNA was extracted from young leaves, as described by Doyle and Doyle (1990). Molecular-marker-assisted selections to genotypes were performed according to Villarroya (2009) with some modification.

Cleaved amplified polymorphic sequences (CAPS) marker, named T1480 primer (F: ACC ACC TTG GAT GAA TAC CG and R: TGC AAC AGC TTT TCC CTC TC), was used as the primer (Villarroya, 2009). All PCR amplifications were performed in a 25-µL reaction volume containing 15 µL of mastermix (Fermentas K0171), 2 µL (0.3 µM each primer) of forward and reverse CAPS primer, 6 µL of ddH2O, and 2 µL of eggplant DNA (25 ng) in a gradient thermal cycler (Biorad DNA-Engine Gradient 84 Cycler, Hercules, CA, USA). The PCR reaction condition was as follows: an initial denaturation step of 2 min at 94 °C followed by 35 cycles of 2 min at 94 °C, 1 min at 52 °C, and 2 min at 72 °C; the program ended with a 10-min elongation step at 72 °C. All of the PCR products were separated on 2% high-resolution agarose gel (Amresco SFR, OH, USA) in 1X TAE (Tris-Acetate-EDTA) buffer at 120 V for 2 h, and photographed under UV light (ENDURO GDS Gel Documentation System) in dye (EZ-ONE N472-KIT, Ambresco). PCR products were digested with HindIII restriction enzyme at 37 °C for 16 h according to the following conditions: 8 µL of the PCR amplification, 2 µL of the HindIII, and 2 µL of buffer in a final volume of 32 µL. Products were separated on a 2% high-resolution

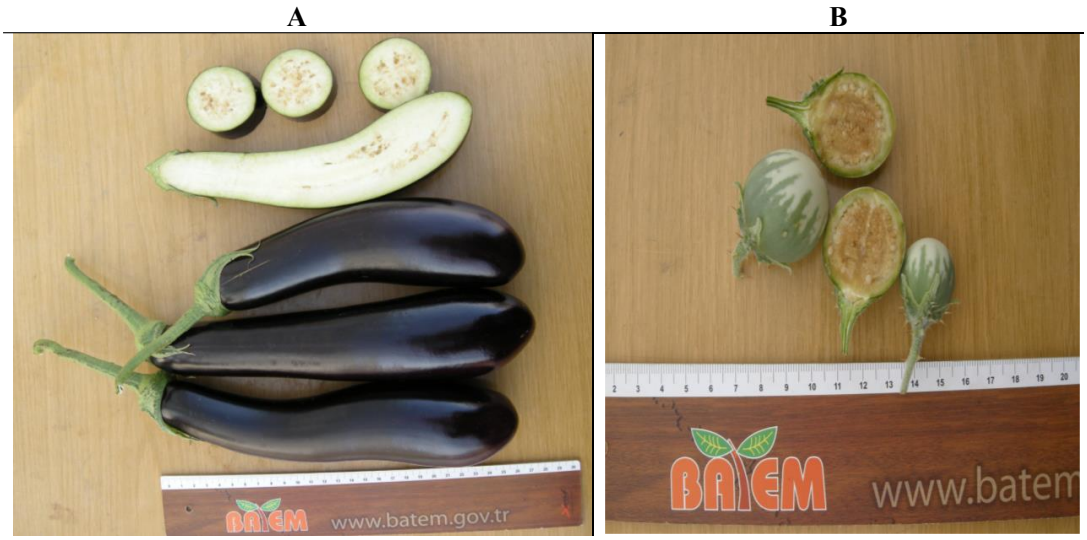

Figure 1. The fruit appearance of the parents: A) the inbred line belonging to *S. melongena* L., B) the wild parent *S. incanum* L. group C (MM684) used in this study.

agarose gel in 1X TAE buffer and photographed under UV light for further analysis. A 100-bp DNA ladder was used as a molecular standard to confirm the marker.

The results were evaluated according to the presence and absence of bands. The software PAST (Paleontological Statistics) (http://folk.uio.no/ohammer/past/) was used for statistical analysis and UPGMA cluster and principal coordinate analysis (PCoA) were constructed based on Dice's coefficient (Dice, 1945).

3. Results and discussion

In this study, two parents (*S. melongena* and *S. incanum*) and 94 F2 individuals from their crosses were tested using the COSII molecular marker T1480 for determination of hybrid genotypes. As a result, 22 genotypes as *S. incanum*, 23 genotypes as *S. melongena*, and 51 genotypes as hybrid individuals were determined. The result of gel image obtained by using Hind-III cutting enzyme for some genotypes was given in Figure 2.

A similarity matrix based on the COSII marker T1480 primer data was calculated using the Dice coefficient (1945). The Dice's similarity was used for the cluster analysis to generate a dendrogram illustrating the genetic diversity between the F2 genotypes and their respective parents (Figure 3). The cophenetic correlation (r) between ultrametric similarities of the tree and the similarity matrix was high ($r = 0.89$, $p < 0.01$), suggesting that the cluster analysis strongly represents the similarity matrix. Correlation coefficient matrix is very good, good, weak, or very weak if $r \geq 0.9$, $0.8 \leq r < 0.9$, $0.7 \leq r < 0.8$ or $r < 0.7$, respectively (Aka-Kacar et al., 2005). In our cluster analysis, hybrid genotypes were indicated as a separate group from parents. Moreover, 22 genotypes of *S. incanum*, 23 genotypes of *S. melongena*, and 51 genotypes were determined as hybrids, which is consistent with 1:2:1 ratio according to Mendelian genetics (Miko, 2008). Similarly, the results of the PCoA analysis indicate the differentiation between hybrid genotypes and those within *S. melongena* and *S. incanum*, as depicted in Figure 4.

Conserved ortholog set (COS) markers are suitable for evolutionary, phylogenetic, taxonomy studies and comparative genomic studies in a wide array of plant species (Fulton et al., 2002).

COS markers are an important functional genomics resource that has greatly improved orthology detection in Asterid species including Solanaceae (Wu et al., 2006; Lindqvist-Kreuze et al., 2013; Ranade and Yadav, 2014). COSII markers are PCR-based markers developed from a set of single-copy conserved orthologous genes (COSII genes) (Wang et al., 2010). Since COSII can be relatively easily detected by transcriptome sequencing, thereby simplifying the procedure to identify suitable molecular markers (Liu et al., 2013; Li et al., 2017). Enciso-Rodríguez et al. (2010) proposed COSII markers as sound tools for molecular studies, conservation, and breeding of *Solanum quitoense* Lam. and *Solanum betaceum* Cav. Sendt. species. On the other hand, the tomato conserved orthologous set was utilized to map populations derived from interspecific hybrids to increase the overall low DNA polymorphism observed in eggplant (Gramazio et al., 2018).

COSII SNP markers developed for cacao would be used for MAS breeding programs (Kuhn et al., 2012). The T1480 COSII marker was used in the eggplant mapping population produced from interspecific hybridization

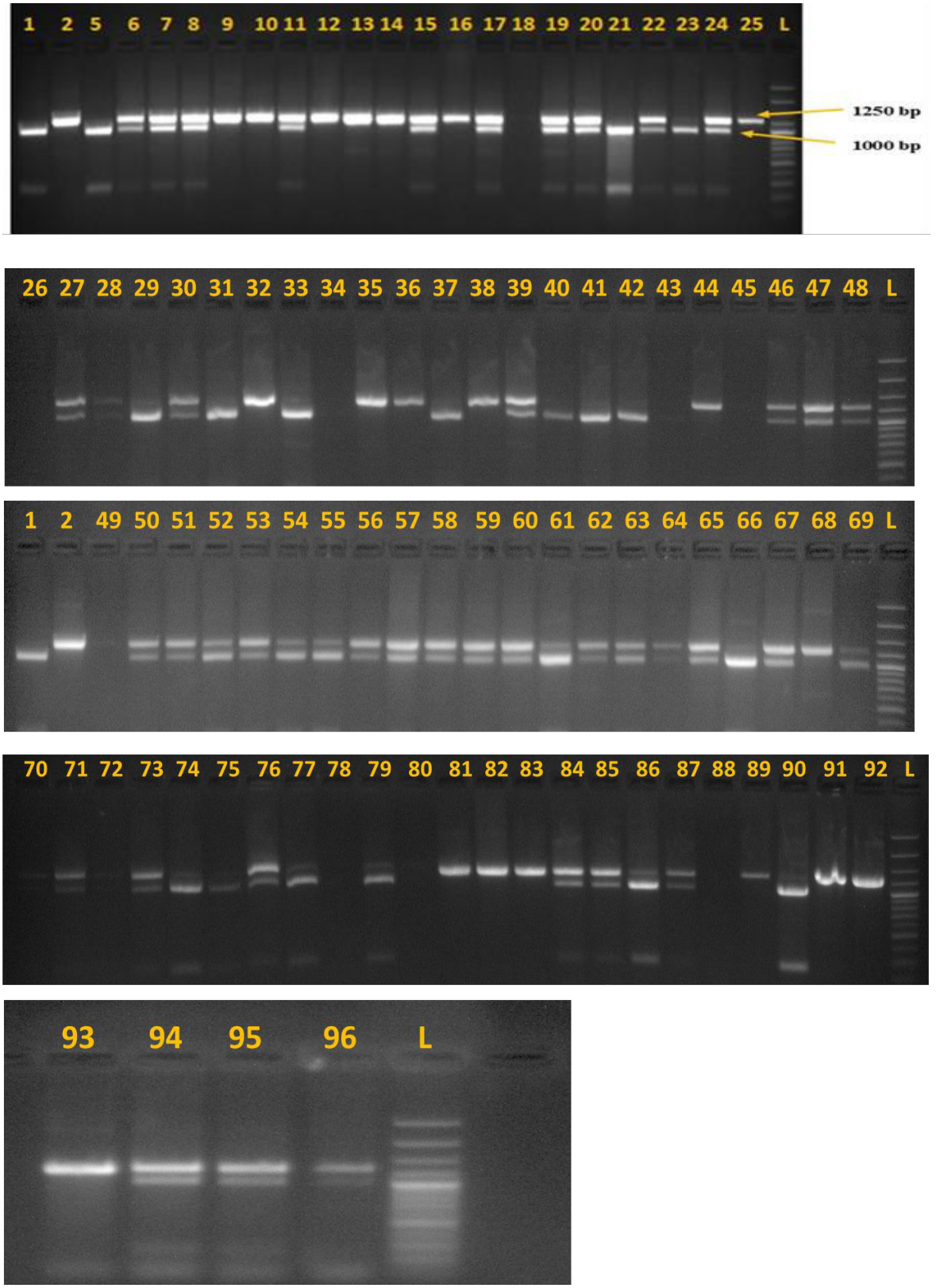

Figure 2. The gel image obtained by using Hind-III cutting enzyme for some genotypes. 1: *S. incanum*, 2: *S. melongena*, 3-96: F2 genotypes, L: 100 bp DNA ladder.

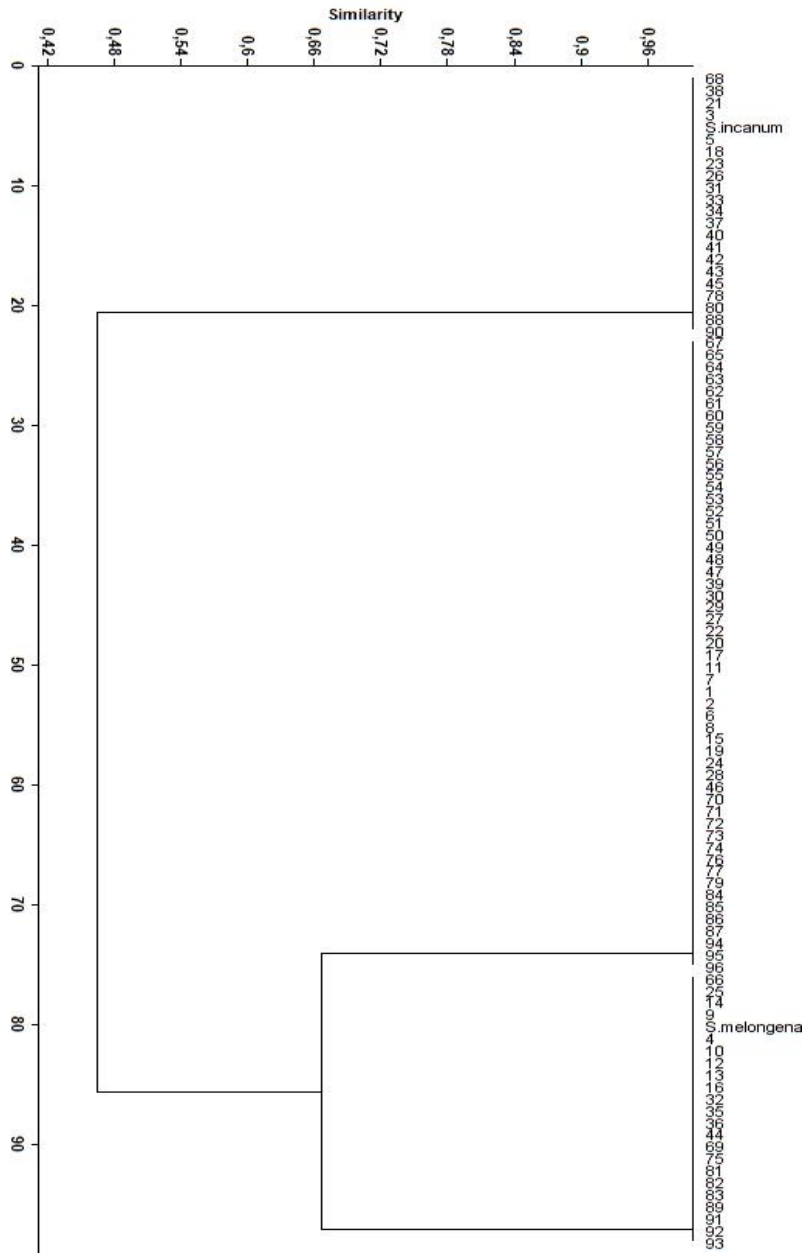

Figure 3. UPGMA dendrogram of the T1480 primer for 96 genotypes

between the *S. melongena* and *S. linneanum* and it was determined that the marker corresponds to E2 (105 cM) of the eggplant linkage groups in the eggplant haploid chromosome set (Wu et al., 2009). COSII T1480 primer was developed as a result of SNP single nucleotide polymorphisms (SNP) analysis (Villarroya, 2009). The present study revealed that the use of stated molecular marker could distinguish hybrid individuals. In recent years, application of SNPs increased our knowledge about genetic diversity among individuals and a better understanding on crop improvement (Huq et al., 2016; Morgil et al., 2020). Especially, SNP analysis enables the selection of desired genotypes in large-scale populations. Therefore, it can be used for the improvement of the crop more economically using new-generation technologies (Wei et al., 2020; Berdugo-Cely et al., 2021). In order to obtain SNP-based markers, studies have been carried out to support breeding studies in eggplant (Villarroya, 2009; Barchi et al., 2011; Wei et al., 2020; Ro et al., 2022) as well as in many important members of the family Solanaceae,

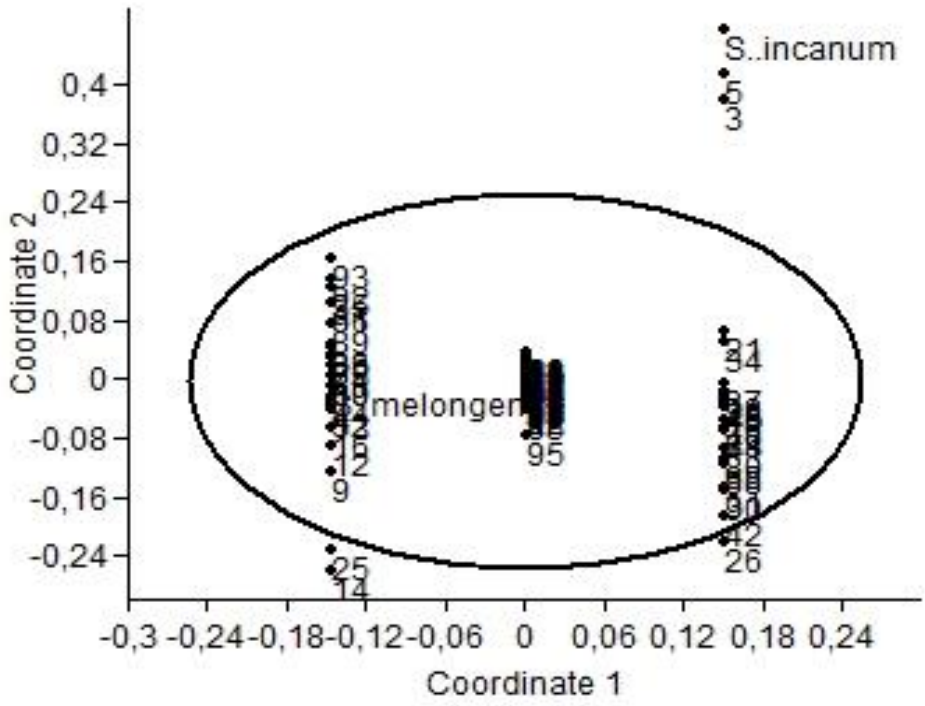

Figure 4. Principal coordinate analyses of T1480 primer for 96 genotypes

such as tomatoes (Víquez-Zamora et al., 2013; Osei et al., 2019; Park et al., 2022) and potatoes (Vos et al., 2015; Berdugo-Cely et al., 2021).

The species of consumed eggplant is *S. melongena*, which is sensitive to many biotic and abiotic stress conditions, as stated earlier (Mutlu et al., 2008; Boyacı et al., 2015; Rosa-Martínez et al., 2022). Although, many resistances and tolerances to biotic and abiotic stresses have been described in eggplant wild relatives, just a few species have been made to transfer them to the cultivated eggplant, and *S. incanum* is the most commonly preferred wild species (Vilorroya, 2009; Rotino et al., 2014; Gramazio et al., 2017; Rosa-Martínez et al., 2022). Therefore, the *S. melongena* x *S. incanum* hybridization combination is very important in eggplant breeding. Considering that the breeding work is time-consuming and challenging, identifying hybrid individuals at the F2 stage when the plants are still very young (2–3 young leaves) would save both money and time in addition to reducing the overall breeding time.

Gramazio et al. (2017) used *S. incanum* to screen the full set of ILs in eggplant with eggplant-specific COSII, SSR, and SNP markers. They stated that no region of the donor parent was lost during the backcross process with culture form, and the entire *S. incanum* genome was inserted into advanced backcrosses (ABs), but some materials would need further backcrossing and self-construction to produce ILs. However, present study showed that *S. incanum* gene entries can be even lost during the producing self-pollination of interspecific hybrids. This could be explained by paracentric inversions and translocations occurring between these two species (Wu et al., 2009).

In conclusion, the *S. melongena*, *S. incanum*, and their 94 F2 individuals have been genetically characterized by using the COSII marker. Within the materials studied, considerable variation has been found for the analysed characters. The molecular marker used in our study is suitable for marker-assisted selection in a wide range of genetic intercrossing. It could be possible to distinguish hybrid genotypes. In this study, SNP-based T1480 CAPS marker was found to be effective in distinguishing hybrid individuals of *S. melongena* x *S. incanum* crossing as the results are also compatible with Mendelian genetics with a ratio of 1:2:1. This marker could also be used successfully in future eggplant breeding programs.

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