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Original Article

Utilizing Marker-Assisted Selection (MAS) for Morphological Traits in the F3 Generation of Tomato (Solanum lycopersicum).

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Abstract

The use of molecular markers has become a pivotal component of molecular breeding programs in tomato. In the third generation of this research, two lines derived from the crossbreeding of commercial hybrids were subjected to detailed analysis using molecular markers by ten RAPD-based primers. Among these primers, six (OPA01, OPA05, OPA07, OPA08, OPA09, and OPB10) successfully amplified targeted DNA regions. Significantly, primer OPA07 uniquely linked specific traits in the first line to distinct bands of 280 and 180 base pairs. In the second line, all six primers achieved amplification, with four primers (OPA07, OPA08, OPA09, and OPB10) associating selected traits with distinct bands of 450, 500, 350, and 450 base pairs, respectively. These bands are likely to serve as marker-assisted selection (MAS) tools, facilitating the selection of plants with desired phenotypic traits in tomato breeding. These markers are expected to greatly improve marker-assisted selection techniques and potentially reveal novel genetic resources, thus enriching the genetic diversity within tomato breeding programs for future advancements.

Keywords: Marker-assisted, MAS, Molecular breeding, RAPD, Solanum lycopersicum and Tomato hybrid.

Introduction

Tomato (*Solanum lycopersicum* L.) is a critically valuable vegetable crop cultivated worldwide, valued for fresh and processed products. It serves as an extensive source of essential nutrients, vitamins as well as a variety of antioxidants (Tiwari *et al.*, 2023). In 2022, global tomato production reached 189.13 million tonnes, cultured across an area of 5.16 million hectares, lead to an average yield of 36.60 tonnes per hectare (Tiwari *et al.*, 2023). Traditional breeding methods face challenges such as prolonged breeding cycles, limited genetic diversity, and unpredictable outcomes. These issues delay the efficient development of new crop varieties with improved yield (Khan *et al.*, 2024). Traditional breeding methods typically require multiple generations to achieve the desired traits, whereas molecular approaches can accelerate this process by directly manipulating the genes associated with those traits (Khan *et al.*, 2024). The efficient and effective use of molecular markers in crop

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improvement programs increases the effectiveness of selection accuracy and speeds up the breeding cycle to create new cultivars with desirable traits (Mapari and Mehandi, 2024). Marker-Assisted Selection (MAS) represents an indirect method of selection, wherein a target trait is chosen by leveraging an associated marker. The marker employed in this selection process is closely linked to the gene or locus of the desired trait due to Genetic Linkage (Rosyara, 2006) .

Marker-assisted selection (MAS) is a molecular breeding approach that employs molecular markers, including specific DNA sequences, to identify and select desirable traits in crops. MAS operate on the principles of linkage and association mapping, which entail detecting correlations between target traits and genetic markers (Elsharawy and Refat, 2023). Utilizing molecular marker genotyping streamlines the process by reducing the number of generations breeders need to be assessed to confirm the incorporation of the desired gene(s) combination into the preferred genotype (Oladokun and Mugisa, 2019). Marker-assisted selection (MAS) has been studied as a method to improve the precision and efficiency of trait selection in tomato breeding programs (Osei *et al.*, 2018). In tomato, the use of molecular marker techniques has been developed in the last 10 years and marker-assisted selection (MAS) is a quite common method that has been used in plant breeding programs by researchers (Aktaş and Aydin, 2022). Marker-assisted breeding has been employed to develop tomato lines with resistance to multiple diseases, followed by their evaluation for horticultural (Kumar *et al.*, 2022), and to target complex traits such as tomato fruit quality (Lecomte *et al.,* 2004).

The objective of this study is to identify molecular markers within the third generation of tomato hybrids using the RAPD technique. These markers are intended to be employed in the selection processes targeting diverse desirable traits in the pursuit of advancing tomato breeding practices.

Materials and Methods

A total of thirteen distinct tomato F3 segregation lines were cultivated within the nursery, with each individual line comprising twenty-five tomato plants in the experimental farm, Agricultural Research Center, University of Sadat city, Egypt. Uniform treatments encompassing fertilization, irrigation, and pesticide application were administered across all lines. Following a span of four months, a discerning process led to the selection of only two F3 lines. Specifically, number 9 emerged as a result of a cross between the hybrid Giza 80 as the male parent and Super Marmande as the female parent. Likewise, number 13 originated from a cross between Giza 80 ♂ and Cal-Ace ♀ hybrids. The selection criteria encompassed multiple traits, including the count of branches, flowers, plant length, early maturation, fruit quantity, fruit morphology (shape, length, width, thickness), fruit weight, and the total fruit count.

In accordance with these criteria, ten plants were meticulously chosen. This subset

comprised five plants that exhibited the utmost expression of the desired trait and an additional set of five plants that demonstrated the minimum expression of the desired trait. To enable a comprehensive comparison, the parental plants used in the initial crosses were also cultivated alongside the third-generation plants. This allowed for an in-depth evaluation of trait differences between generations. Statistical analysis of the data was conducted using a t-test to determine the significance of observed differences in traits between parental and third-generation plants.

A collection of twenty-four fresh leaf samples were assembled for analysis, comprising the following breakdown: ten samples sourced from the third generation F3 of the initial line 9, encompassing five samples exemplifying the highest trait expression and an additional five samples with the lowest trait expression. An analogous setup was established for the second line 13, wherein ten samples from its third generation (F3) were gathered, comprising five specimens showcasing the utmost trait expression and an equivalent number representing the lowest trait expression. To round off the sample set, four additional samples were acquired, denoting the parental lines, i.e., number 9 and 13.

For each leaf sample, a quantity of one gram was meticulously sectioned into small fragments. Subsequently, the fragments were pulverized in the presence of liquid nitrogen, while concurrently introducing the extraction solution as stipulated by the adjusted CTAB protocol (Gawel and Jarret, 1991). RAPD 10 mer random primer from Operon inc, were used in the PCR amplification process Table 1.

Table 1: Sequences, melting temperature (Tm), and annealing temperature (Ta) of ten RAPD primers utilized for DNA amplification of tomato F3 segregation lines.

No.	Primer	Primer sequences (5′–3′)	GC%	Melting °C (Tm)	Annealing °C (Ta)
1	OPA01	5′ - AGG GGT CTT G– 3′	60	32	29
2	OPA05	5′ - CAG GCC CTT C– 3′	70	32	29
3	OPA07	5′ – GAA ACG GGT G– 3′	60	28	25
4	OPA08	5′ – GTG ACG TAG G– 3′	60	30	27
5	OPA09	5′ – GGG TAA CGC C– 3′	70	36	33
6	OPB-10	5′- CTG CTG GGA C- 3′	70	32	29
7	OPB-11	5′- GTA GAC CCG T- 3′	60	30	27
8	OPC- 01	5′- TTC GAG CCA G- 3′	60	34	31
9	OPC- 05	5′- GAT GAC CGC C- 3′	70	36	33
10	OPC- 06	5′- GAA CGG ACT C- 3′	60	30	27

Amplification was conducted utilizing a Thermal cycler (Biometra, Germany T-Gradient Thermoblock), employing a total reaction volume of 20 µl. This reaction mixture included

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1 µl (30 ng) of total genomic DNA, 0.2 µl (10mM) of dNTPs, 1.7 µl (25) of MgCl2, 2 µl of a 10X buffer solution (composed of 10 mM Tris, pH 8.0, 50mM KCl, and 50 mM ammonium sulphate), 0.1 µl of Taq polymerase 10U / µl produced by (iNtRON BIOTECHNOLOGY), 4 µl of primer (15 pmol/µl), and 11 µl of nuclease-free water. The amplification process followed a sequential pattern: initial denaturation at 94 °C for 5 minutes, involving 35 cycles of denaturation at 94 °C for 60 seconds, primer annealing at the specific temperature for 90 seconds, and extension at 72 °C for 2 minutes. A final extension was performed at 72 °C for 7 minutes. Subsequently, the amplified DNA fragments, combined with loading dye, were loaded onto a 1.5% agarose gel, and the gel was subjected to electrophoresis at 30 V for a duration of 180 minutes in 1X TAE buffer (30 mM). Post-electrophoresis. The gels were treated with ethidium bromide stain and visualized under UV light, typically by placing the gel on top of a UV transilluminator.

Results and discussion

The results from the t-test statistical analysis performed on the third-generation plants reveal significant differences in two traits in line 9, whereas they reveal significant differences in four traits in line 13 (Table 2).

Table 2. The utmost and lowest phenotypic trait data of the third-generation plants (line 9 and 13).

	line 9		line 13	
Trait	**Min**	**Mix**	**Min**	**Mix**
Branches Number	18.0	21.8	14.0[a]	19.0[b]
Flowers number	7.6[a]	15.6[b]	6.4[a]	11.2[b]
Plant length/cm	54.0[a]	73.0[b]	49.6[a]	65.2[b]
Early maturity/day	82.6	74.4	82.6	87.6
fruits number	3.6	3.6	2.4	4.0
fruit length/cm	6.2	6.0	3.6	3.3
Fruit width/cm	4.0	4.0	3.0	2.9
fruit thickness/cm	3.2	3.1	2.6	2.3
Fruit weight/gm	173.8	190.2	90[a]	161.0[b]

In the context of line 9, the analysis demonstrated noteworthy variations in the number of flowers among the five designated plants, with the trait's range spanning from 7.6 to 15.6 flowers per plant. Similarly, the parameter of plant length exhibited a range of 73 cm to 54 cm, by comparing these significant distinctions in traits with identifiable DNA markers, the potential arises to establish a correlation between these traits and the resultant DNA bands, thus presenting these markers as valuable tools. This approach holds the promise of streamlining the genetic selection process for this trait, ultimately conserving both

time and effort. Uçar and Şensoy, (2022) also use molecular markers-assisted selection in breeding programs aimed at enhancing resistance in tomato against pathogens. The molecular analysis of the F3 of line 9 employed ten RAPD primers, out of which only six demonstrated successful amplification. These successful primers included OPA01, OPA05, OPA07, OPA08, OPA09, and OPB10. In this subset of six RAPD primers, only one primer, OPA07, successfully demonstrated an association between the identified traits and two distinct DNA bands, with molecular weights of 280 base pairs and 180 base pairs, as shown in Figure 1. In contrast, although primer OPA9 achieved successful amplification, it did not generate any distinguishable bands, as depicted in Figure 2**.**

In a related study, Pereira da Costa *et al.,* (2016) utilized the correlation between phenotypic and molecular variations in the second generation F2 of tomato to identify supplementary markers for certain fruit traits. The primer OPA07 exhibited four bands characterized by varying molecular weights. Among these, two bands were observed to be monomorphic, measuring 340 base pairs and 240 base pairs, respectively. Consequently, these two bands lacked discriminatory potential in identifying plants with the sought-after traits. Conversely, the band weighing 280 base pairs, depicted in Figure 1, is categorized as a negative band due to its exclusive presence in the five plants exhibiting the lower limit of the trait range, and its absence in plants with the upper limit. This specific band can be traced back to the mother plant, where its presence was evident, unlike in the father's genetic profile where it was absent. The band characterized by a molecular weight of 180 base pairs is identified as a favourable trait-specific band, given its exclusive presence in the plants featuring the upper limit, while it remains absent in those with the lower limit. This band's origin traces back to the father's plant, as it is also observed in the father's profile and not in the mother's genetic makeup. In a similar vein, Saleh *et al.*, (2015) observed positive and negative bands using the RAPD marker in grape plants.

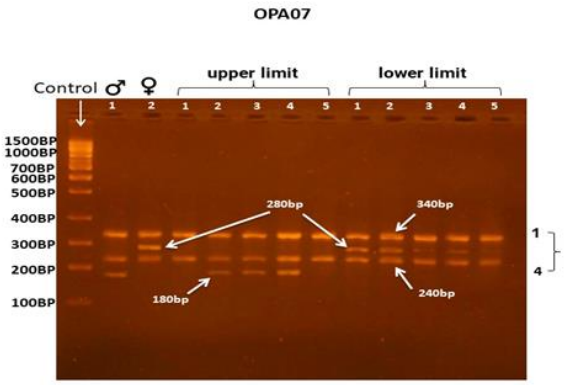

Figure 1: The molecular analysis of OPA07 (RAPD) primer-generated polymorphism in banding patterns of F3 tomato line 9 within Parents.

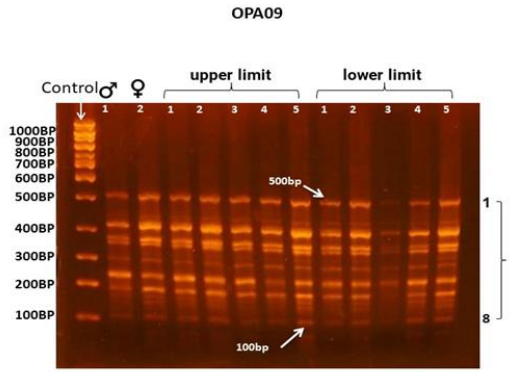

Figure 2: The molecular analysis of OPA09 (RAPD) primer-generated monomorphism in banding patterns of F3 tomato line 9 within Parents.

The outcomes derived from line thirteen unveiled notable variations across the five designated plants in terms of branch number, flower count, plant length, and fruit weight. The upper limit for these traits stood at 19 branches, 11.2 flowers, 65.2 cm plant length, and 161 grams in fruit weight, while the corresponding lower limits were 14 branches, 6.4 flowers, 49.6 cm plant length, and 88 grams in fruit weight. By scrutinizing the significant discrepancies in these traits with the aid of indicative DNA markers, a potential linkage emerges between these three characteristics and the resultant DNA bands. Consequently, these bands could be regarded as auxiliary markers, contributing to a more efficient trait assessment process.

The F3 of line 13 underwent evaluation using ten RAPD primers, of which six successfully achieved amplification (OPA01, OPA05, OPA07, OPA08, OPA09, and OPB10). Among these six primers, the linking of selected traits was not achieved by two primers, namely OPA01 and OPA05. However, the remaining four primers (OPA07, OPA08, OPA09, and OPB10) exhibited success in generating bands that can serve as assisting markers (MAS). A study by Wang *et al.*, (2007) corroborated that employing molecular markers and comparing them alongside morphological and physiological data led to parallel conclusions, thereby establishing a correlation with phenotypic traits. This finding underscores the practical value of molecular analysis. In a similar vein, Chamikara *et al.*, (2015) integrated molecular markers into pepper breeding programs in Sri Lanka, unveiling the influence of fifteen alleles on fruit size traits out of the forty-four alleles, while twenty-three alleles were linked to fruit shape. This insight serves as validation markers and can be pivotal in the strategic planning of chili pepper breeding and enhancement initiatives.

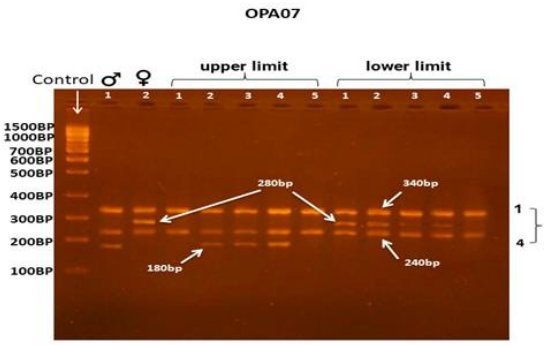

Figure 3. The molecular analysis of OPA07 (RAPD) primer-generated polymorphism in banding patterns of F3 tomato line 13 within Parents.

The F3 of line 13 with OPA07 primer yielded a set of three bands characterized by diverse molecular weights, among which only one band stood as monomorphic, featuring a molecular weight of 350 base pairs. Consequently, this specific band lacked the discriminatory capacity

required for identifying plants with sought-after traits. On the other hand, the band weighing 550 base pairs, as portrayed in Figure 3, is designated as a negative trait-associated band. Notably, this band's lineage can be traced back to the mother plant. In contrast, a distinctive positive band with a molecular weight of 450 base pairs was also identified. Figure 3 provides further clarity, showcasing that this band's origin is attributed to the father's plant. This deduction arises from the absence of this band in the mother's genetic profile while being present in the father's profile. The OPA08 primer with the F3 of line 13 revealed four distinct bands characterized by varying molecular weight. Among these, three bands were monomorphic, specifically measuring 250, 300, and 380 base pairs in molecular weight. Consequently, these bands lack the discriminatory potential necessary for distinguishing between plants. Conversely, the band weighing 500 base pairs, depicted in Figure 4, serves as a negative characteristic band, stemming from maternal inheritance.

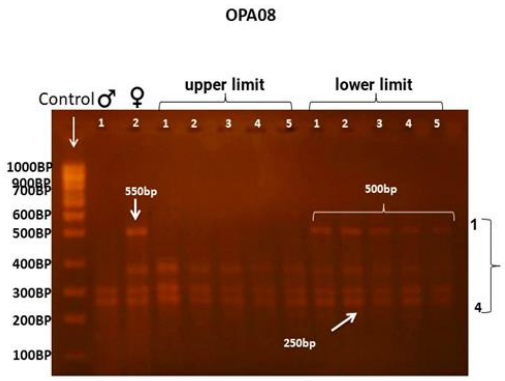

Figure 4: The molecular analysis of OPA08 (RAPD) primer-generated polymorphism in banding patterns of F3 tomato line 13 within Parents.

Employing Marker-Assisted Selection (MAS) within crop enhancement endeavors not only holds the promise of curtailing the expenses involved in developing novel tomato varieties but also enhances the precision and efficiency of selection within the breeding program. This approach concurrently reduces the timeline required for the production of a new crop variety (Osei *et al.*, 2018).

The OPA09 primer with the F3 of line 13 yielded a set of four bands distinguished by their diverse molecular weights. Among these, three bands exhibited monomorphic attributes, specifically measuring 600, 450, and 280 base pairs in molecular weight. Consequently, these bands lack the capacity to differentiate between plants. Conversely, the band weighing 350 base pairs stands as a negative characteristic band, stemming from maternal inheritance Figure(5).

In the case of the OPB10 primer Figure (6), three bands with distinct molecular weights were identified, of which two were monomorphic, measuring 400 and 200 base pairs, rendering them unsuitable for discerning between plants. The band weighing 450 base pairs, as depicted in the figure, serves as a positive characteristic band, originating from the father's plant. Genetic markers constitute biological traits capable of transmission across generations. They serve as experimental probes or identifiers, facilitating the tracking of individuals, tissues, cells, nuclei, chromosomes, or genes (Osei *et al.*, 2018).

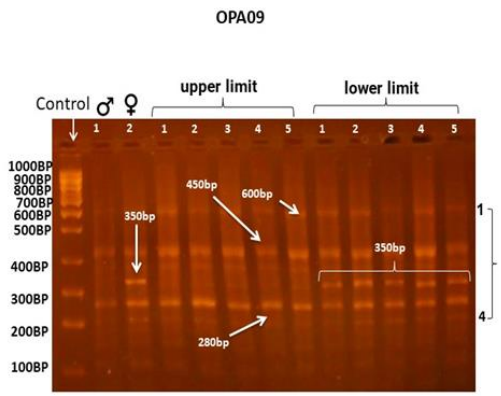

Figure 5: The molecular analysis of OPA09 (RAPD) primer-generated polymorphism in banding patterns of F3 tomato line 13 within Parents.

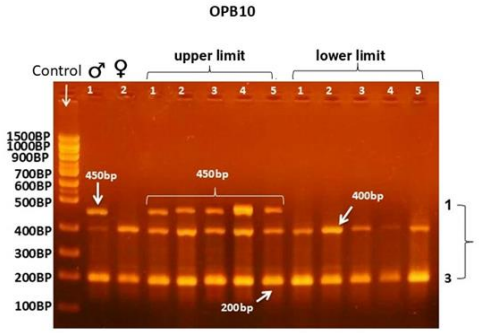

Figure 6: The molecular analysis of OPB10 (RAPD) primer-generated polymorphism in banding patterns of F3 tomato line 13 within Parents.

Conclusion

The implementation of the RAPD technique has validated the presence of seven distinct loci, serving as markers that aid in the selection of phenotypic traits within tomato. Among these markers, four negative ones measuring 280, 550, 500, and 350 base pairs can be traced back to the maternal parent. Conversely, three positive markers 180, 450, and 450 base pairs originate from the male parent. These findings collectively contribute to a streamlined breeding process for the development of novel tomato varieties, significantly reducing both time requirements and the level of effort compared to conventional breeding methodologies.

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استخدام تقنية الاختيار بمساعدة العلامات الجزيئية (MAS) للصفات الظاهرية في الجيل الثالث من هجن الطماطم

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المستخلص

يعد استخدام العلامات الجزيئية محوراً مركزياً في برامج التربية الجزيئية للطماطم، وفي هذا البحث تم تحليل الجيل الثالث لسلالتين مشتقتين من هجن تجارية باستخدام الواسمات الجزيئية المعتمدة على تقنية RAPD ، حيث تم استخدام عشر بادئات، نجحت ستة بادئات هي: OPA01، OPA05، OPA07، OPA08، OPA09 و OPB10 في تضخيم قطع الحمض النووي، وقد تميزت من بينها البادئةOPA07 في ربط الصفات المحددة في السلالة الأولى بحزم محددة ذات أوزان جزيئية قدرها 280 و 180 زوج قاعدي، أما بالنسبة للسلالة الثانية فقد حققت جميع البادئات الست التضخيم بشكل فعال، ومن بين هذه البادئات كانت أربع بادئات هي: OPA07، OPA08، OPA09 و OPB10 قادرة على ربط الصفات المختارة بحزم ذات أطوال مختلفة (450 ، 500 ، 350 و 450 زوج قاعدي). ومن المتوقع أن تكون هذه الحزم بمثابة علامات مساعدة (MAS) في زراعة الطماطم لاختيار الأفراد بناءً على صفات ظاهرية محددة. هذه الحزم المرتبطة بالصفات الظاهرية تساعد بشكل كبير في تعزيز ممارسات الاختيار المدعوم بالعلامات، وقد تكشف عن موارد جينية جديدة يمكن دمجها في برامج تربية الطماطم، مما يسهم في إثراء التنوع الجيني ويطور برامج تربية وتحسين محصول الطماطم.

الكلمات الدالة: العلامات المساعدة، MAS، التربية الجزيئية، هجين الطماطم،RAPD، Solanum Lycopersicon.

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