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ISSR PCR ANALYSIS OF SELECTED SOMACLONAL MUTANTS INDUCED BY MICROWAVE IRRADIATION IN *SOLANUM MELONGENA*. L VAR PPL

Deepika Guduru[1], T. Christopher Reuben[2*].

[1,2] Department of Biotechnology, Chaitanya Deemed to be University, Himayathnagar, Moinabad, Hyderabad, Telangana – 500 075

**Corresponding Author: Dr. T. Christopher Reuben.*

ABSTRACT:

Solanum melongena L a member of Solanaceae is generally cultivated as fruit vegetable in sub-tropical and tropical regions of the world. After being treated with microwave irradiation for duration as 0.0,1.0,2.0,3.0 & 5.0 mins and the source of microwave was 630 w (Panasonic). *Solanum melongena* L seeds were planted for two generations, M1 and M2. The treated plants were identified as the selected mutants that produced desirable characteristics like plant height, number of fruits, fruit size, and early fruiting, among other things. To filter out the genetic variants at the molecular level, seeds from these mutants were gathered and subjected to M3 generation. According to the results of the current investigation, we found that every line that was screened displayed a different banding pattern when compared to the control plant, which is equal to a genetic variation frequency of 36.06%. The fragments that each primer produced, which varied in size from 100 to 1,500 bp, were amplified using PCR. With an average of six bands per primer, the three tested primers produced 35 scorable bands altogether. The bands were repeatable. Each primer has anywhere from five to nine bands. When comparing the ISSR profiles of the mutants to the control, new bands or the absence of bands were found. ISSR test using primer 2 (ISSR-2) yielded 52 bands total, 5 of which were novel bands. were noted, and there were no 06 bands in the lanes. A total of 25 bands were obtained using the ISSRassay with primer 3 (ISSR-3), of which 9 were new bands and 5 were not visible. The ISSR test using primer 9 (ISSR-9) yielded 57 bands in total, of which 5 were new and 5 were not seen, according to theresults.35 of the 134 bands that were assessed, with an average polymorphism percentage of 26.11, were polymorphic. The primer ISSR-9 provided the lowest polymorphism (17.54%), while ISSR-3 displayed the highest polymorphism (56.00%) out of the three. Compared to control plants, the seven mutant lines showed notable differences in the banding pattern.

Key Words - *Solanum melongena* L, EMS, ISSR-PCR, Dendrogram**.**

INTRODUCTION

Eggplant belongs to the very large genus Solanum, as well as its largest sub genus, *Leptostemonum*, which includes many wild relatives, as well as other cultivated species, such as the Gboma eggplant (*Solanum macrocarpon* L.) and the scarlet eggplant (*Solanum aethiopicum* L.) grown mostly in Africa for their fruits and leaves. We will focus here on *S. melongena* (Daunay *et.al*., 2001). Eggplant (*Solanum melongena* L) is a widely adaptive and highly productive vegetable crop of tropical and subtropical regions world, which suffers from various abiotic and biotic stresses particularly insect-pests (Singh *et.al.*, 2000; Kaur *et.al.,* 2013). Tissue extracts of brinjal can be used in the treatment of asthma, bronchitis, cholera and dysuria. Fruits and leaves of brinjal can be used for lowering blood cholesterol and can be given to diabetics and obese patients as it is low in calories and high in potassium (Kashyap

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et.al., 2003; Singh and Kumar 2006; Rajam and Kumar 2007). Plant tissue culture offers an efficient method for pathogen free materials and germplasm preservation. Tissue culture techniques are widely used for the improvement of various crops. In vitro shoot induction from callus culture can induce genetic and epigenic changes in the regenerated plants. These genetic changes have been coined "Somaclonal variation" (Larkin *et al.*, 1981). These somaclonal variations are mainly caused by newly generated mutations arising from tissue culture process (Sato *et.al.,* 2011). In recent years, there has been an acceleration of biotechnological interventions in eggplant breeding, particularly in genetics and genomics, aimed at developing improved varieties of the crop (Sharma *et.al.,*2020).

MATERIALS AND METHODS

Plant material
The seeds of *Solanum melongena* L. var. PPL were collected from the Telangana State Seeds Development Corporation, Hyderabad (TSSDC).

Explants
Mature zygotic embryos, hypocotyl and cotyledon were used as explants.

Preparation of MS medium
Murashige and Skoog medium (1962) was used in the present experiment.

Surface sterilization:

Mature zygotic embryos
The seeds of S. melongena L. var. PPL were thoroughly washed in running tap water and then surface sterilized with 0.1 % Mercuric chloride (HgCl2) for 2 mins., followed by 2-3 times rinsing in sterile distilled water to remove traces of (HgCl2). The surface sterilized seeds were excised using a sterile blade (10 No.) and intact mature zygotic embryos were inoculated on MS+NAA (1.0 – 10.0 mg/l

Hypocotyl and cotyledon explants
Fresh seeds of *S. melongena* L. var. PPL were inoculated on MS basal medium to raise axenic cultures. Hypocotyl (1.0 cm in length) and cotyledon (1.0 cm c.a.) were inoculated on MS medium supplemented with various concentrations and combinations of IAA, NAA & IBA (1.0-10.0 mg/l) and BAP & Kn (1.0-10.0 mg/l).

Incubation
Culture tubes were incubated in culture room for 4 weeks under 16/8 hrs photoperiod at 25 ± 2^0C and relative humidity of 75%.

Acclimatization
Complete plantlets were transferred to earthen pots for 4 weeks until acclimatization in green house.

Morphological characteristics
Morphological characters like plant height(cm), number of branches, number of fruits per plant, days to flowers, number of seeds per fruit, weight of 100 seeds (g) and length of fruits (cm) of M1 plantlets derived from mutation breeding experiments was recorded.

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Table 1: Individual mutants and the changes observed in M2 generation of *Solanum melongena* L

Plant	Morphological changes
MW7	Tall, early maturing, prolific with more number of branches & leaves and six petal flower, more number of fruits. Early ripening, long pods.
	smaller in size, large fruit sizes. More number of fruits per plant.
MW39	Tall, early maturing with more number of leaves and branches, sterile flower buds.
MW46	Dwarf, fertile, more number of leaves, 7 petals.
MW2	Early maturing, more number of leaves, different shape of leaves, 7 petals.
MW36	Tall, Sterile (petaloid structures of flower buds), more number of leaves, originally stem was divided into two branches of equal size each was acting as main stem.
MW26	More number of leaves, more number of branches, bushy habit, prolific with 7 petals in flowers.
MW86	Dwarf, more number of leaves, originally stem was divided into two branches of equal size each was acting as main stem.

MW indicates the "Microwave irradiation" at 630w.

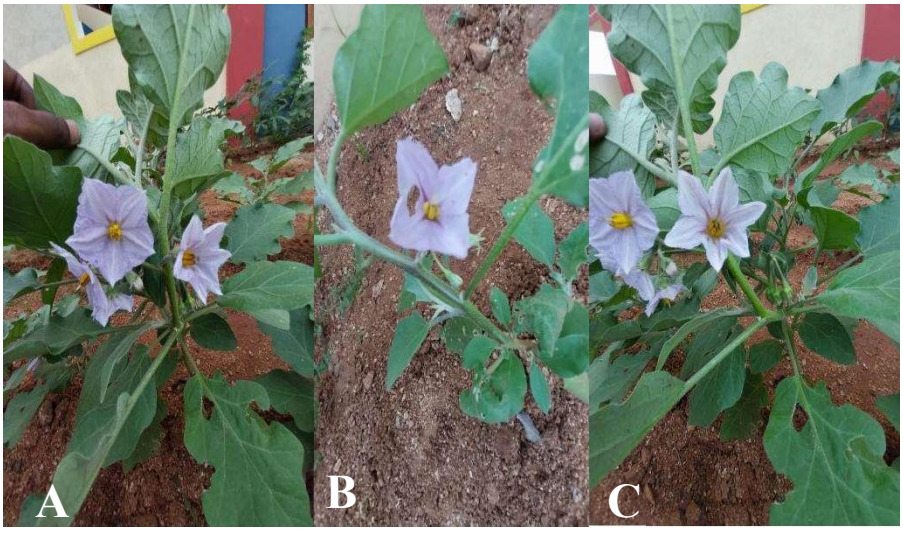

Figure 1: Flower Mutants in Brinjal **A)** Control plant with five petals, **B)** Mutant flower with Four petals, **C)** Mutant flower with Six Petals.

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Image 2: A) &B) Mutants showing Early Flowering **C)** plant with more number of branches **D)** Mutant with colored fruit. **E)** Healthy fruiting in selected mutant

Mutagenesis:

Microwave irradiation:
Soaked (24 hrs) seeds of Solanum melongena L. var PPL were irradiated using a microwave oven (Domestic). Each experimental group consisted of Twenty (20) seeds were taken in a beaker with sterile distilled water and exposed to microwaves for duration as 0.0,1.0,2.0,3.0 & 5.0 mins. The source of microwave was 630 w (Panasonic). Before each experiment, it was confirmed that the microwave irradiation and chemical mutagens like EMS was spread out uniformly over the entire target area (Rajender Vadluri et.al.,). An equal number of seeds was used as un-irradiated control. Some of these seeds were excised and zygotic embryo was inoculated on MS+NAA medium. 4 weeks old seeds were used for hypocotyl and cotyledon explants, the studies were carried up to R1 generations

Survival percentage:
Microwave irradiated seeds (100 seeds) were germinated in the nursery. The seedling survival percentage was recorded after 4 weeks of germination in M_3 generations. Mutation breeding studies were carried up to R_2 generations.

Planting technique:
One month old seedlings along with control were transplanted in the research field in completely Randomized Block Design (RBD) in four replications with inter row distance of 90cm.During

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harvest time, seeds were collected plant wise from different doses. This constitutes M_1. Mutation breeding studies were carried up to M_2 generation.

Morphological parameters:
During the course of investigation, data was scored for seven quantitative characters. viz.

Statistical analysis:
Data collected on these seven characters was statistically analysed, which includes mean (X), Standard Errors (S.E) and Analysis of Variance (ANOVA).

ISSR-PCR:

A total of 20 arbitrary primers were used for initial screening in control plants. For screening the mutants, seven primers were selected for PCR amplification. Each amplification reaction mixture volume 20 µl contains 1X PCR buffer, Mg2+ 1.5 mM, 200 µM of each of dATP, dGTP, dCTP and dTTP, 0.55 µM primer, 20 ng template DNA and 0.1 U/µl of Taq polymerase.

Reaction mixtures were processed to the following incubation steps: 94^0C for 3 min, followed by 40 cycles of 1min for 94^0C, 40s for 45^0C, 2min 30s for 72^0C, and a final 5 min extension at 72^0C. Amplification products were visualized with DNA marker on 1.2% agarose gels under UV light and were photographed. The genetic variation frequency is calculated as the number of lines with variationin banding pattern divided by the total number of lines for ISSR analysis.

Agarose gel electrophoresis (AGE):

The ISSR-PCR products were electrophoresed on 1.2% of agarose gel, in 1X TBE buffer at 60 V for 3 h. The size of the amplicons was estimated using 100bp DNA ladder as standard (Fermentos, USA). DNA bands were visualized using Gel Documentation System (Bio-Rad, USA) and photographed. Theexperiment was repeated thrice to establish reproducibility of DNA banding pattern.

RESULTS & DISCUSSION:

Microwave irradiation:
The number of green spots and somatic embryoids decreased over control at 1-5 minutes exposure time(Table 2).

Molecular level:

Comparison of quantitative characters in M_3 generation:

Morphological variations:
The objective of the present study was to statistically analyse 7 quantitative characters, namely (i) Plantheight, (ii) Number of branches, (iii) Number of days to flowers, (iv) Number of fruits per plant, (v) Fruit length, (vi) Number of Seeds per fruit and (vii) Weight of 100 seeds in 92 M_3 generation obtainedfrom NAA, NAA+2,4-D+BAP treatments.

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We observed that variability in these morphological characters was significant in somaclonal variantsobtained from NAA treatment (Table 3).

ISSR analysis:
DNA 260/280 ratio averaged 1.5-2.3, indicating that the DNA samples can promote for ISSR analysis and as well long-term storage. Among 20 ISSR primers used in the primary screening, 7 were choosenfor their reproducibility, amplification and scorability of banding patterns. These seven ISSR primers were employed for all the genomic DNA mutant lines. Among these seven ISSR primers, 3 primers (ISSR-2, ISSR-3 and ISSR-9) were amplified and remaining primers are not shown any banding patterns (The results are represented in table 4 and 5).

Table:2. Induction of somatic embryoids and plantlet conversion in hypocotyl, Zygotic embryo and cotyledon in microwave irradiation explants of *S.melongena* L var. PPL.

Microwave irradiated seeds No of Somatic Embryoids			
Mints	Zygotic Embryo	Hypocotyl	Cotyledon
0 (Control)	22.09±0.34	20.56±0.64	19.46±0.62
1	20.21±0.37	19.49±0.24	17.82±0.34
2	19.54±0.12	17.08±0.53	16.15±0.45
3	18.04±0.43	15.17±0.52	14.09±0.32
5	21.07±0.50	18.21±0.35	16.03±0.52

Table:3. Comparisons of 07 quantitative characters in microwave irradiated M1 plantlets of *S.melongena* L var. PPL

Characters	Control	1 Min	2 Min	3 Min	5 Min
Weight of 100 seeds(g)	55.53±0.63	52.31±0.38	51.48±0.40	50.31±0.38	50.18±0.22
Length of fruits(cm)	08.63±0.21	08.46±0.37	08.35±0.54	07.46±0.37	06.35±0.54
Plant Height (cm)	18.24±0.27	18.14±0.48	17.32±0.39	16.34±0.48	15.32±0.39
Number of branches	46.26±0.59	47.17±0.46	44.35±0.45	43.37±0.46	42.35±0.45
Number of fruits per plant	57.45±0.34	57.34±0.42	57.37±0.54	56.34±0.42	65.37±0.54
Days to flower	0.27±0.40	0.26±0.39	0.26±0.43	0.23±0.39	0.22±0.43
Number of seeds per fruit	3.46±0.49	3.39±0.52	3.28±0.39	3.14±0.52	3.23±0.39

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Table 4: Results of ISSR Amplified products in selected mutant DNA bands compared with Control plant

Primer code	Mutant Lines								Total No of Amplicons
	Control	1 (MW 7)	2 (MW3 9)	3 (MW 46)	4 (MW 2)	5 (MW3 6)	6 (MW 26)	7 (MW86)	
ISSR-2	-	2	-	-	1	1	-	1	5
ISSR-3	-	1	2	1	1	1	1	2	**9**
ISSR-9	-	1	-	1	1	-	1	1	5

Table 5: Results of ISSR Amplified products of DNA bands absent, in selected mutants compared with Control plant

Primer code	Mutant Lines								Total No of bands absent
	Cont rol	1 (MW7)	2 (MW39)	3 (MW46)	4 (MW2)	5 (MW36)	6 (MW26)	7 (MW86)	
ISSR-2	-	1	2	2	1	-	-	-	6
ISSR-3	-	2	1	1	-	1	-	-	5
ISSR-9	-	-	1	3	-	-	-	1	5

Table 6: Analysis of ISSR banding pattern for Brinjal (*Solanum melongena* L.)

S. No	Primer Code	Total number of bands	Polymorphic Bands	Monomorphic bands	% of polymorphism
1	ISSR-2	52	11	41	21.15
2	ISSR-3	25	14	11	**56.00**
3	ISSR-9	57	10	47	17.54
	Total	134	35	99	94.69
	Average	44.66	11.66	33.00	26.11

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From the above studies, we observed that all the lines screened were showed variation in banding pattern compared with control plant, equivalent to a genetic variation frequency of 36.06%. PCR amplified fragments generated by each primer, ranged in size from 100 to 1,500 bp. The three tested primers showed reproducible bands, yielding a total of 35 scorable bands, with an average of 6 bands per primer. The number of bands for each primer varied from 5 to 9.

Figure 3: ISSR Primer-2 amplified products in selected mutant lanes
(*Lane- 123- 6hr; 4,5- 12 hr; 6,7- 24 hr)*

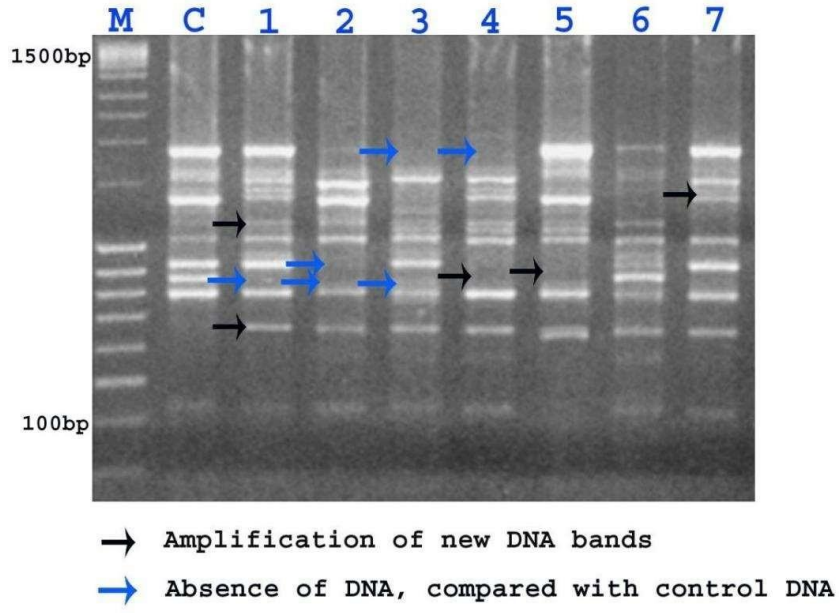
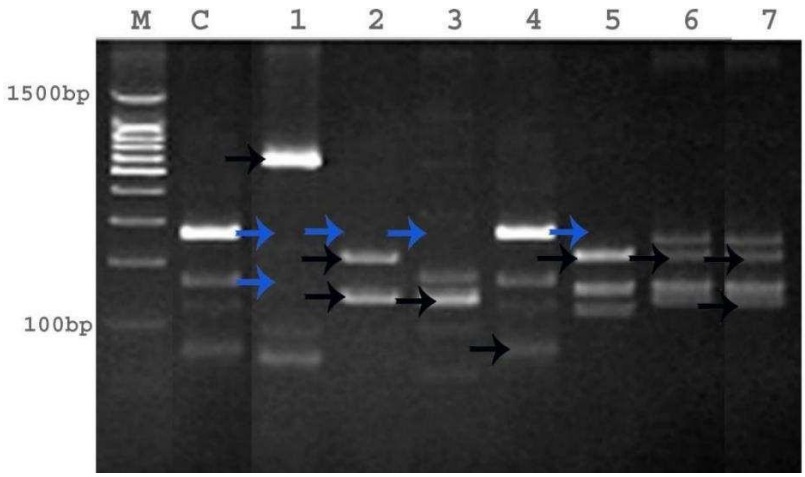


Figure 4: ISSR Primer-3 amplified products in selected mutant lanes
(Lane- 123- 6hr; 4,5- 12 hr; 6,7- 24 hr)



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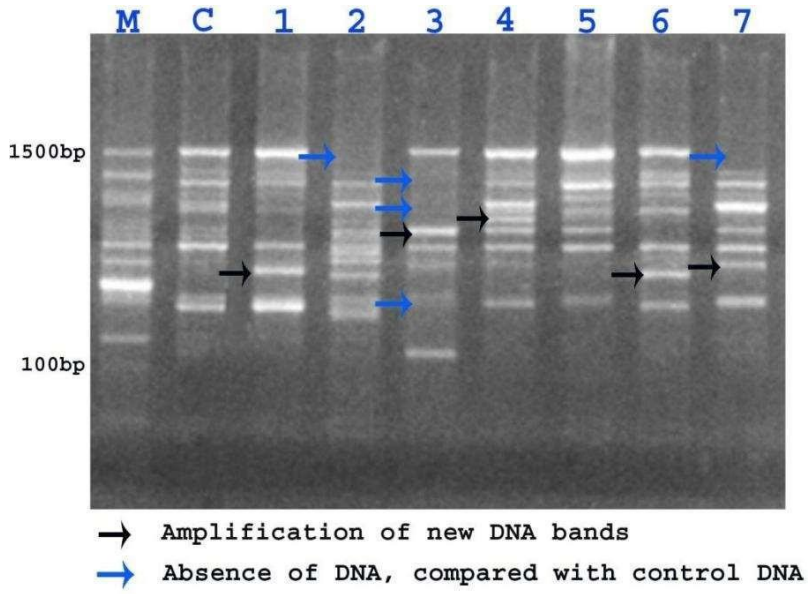
From the ISSR profiles, new bands or the absence of bands were observed among mutants incomparison with the control one. Figure 3 shows an ISSR assay with primer 2 (ISSR-2), which produced a total number of bands 52 in which 5 new bands were noticed and 06 bands were not observed in the lanes.

Figure 4 shows an ISSR assay with primer 3(ISSR-3) produced a total of 25 bands in which 9 new bands were noticed and 5 bands were not observed. Figure 5 shows an ISSR assay with primer 9 (ISSR-9) produced a total of 57 bands in which 5 new bands were noticed and 5 bands were not observed.

Figure 5: ISRR Primer-9 amplified products in selected mutant lane



Lane -123-6hrs; 4,5-12 hrs; 6,7-24 hrs

Table 7: Selected ISSR primers for amplification of mutant lines

S	ISSR Primer Code	Primer sequence	No. of bp	Amplified/ not
1	ISSR-1	AGAGAGAGAGAGAGAGC	17	NO
2	ISSR-2	AGAGAGAGAGAGAGAGG	17	**YES**
3	ISSR-3	GAGAGAGAGAGAGAGAT	17	**YES**
4	ISSR-5	GAGAGAGAGAGAGAGAC	17	NO
5	ISSR-9	TCTCTCTCTCTCTCTCC	17	**YES**
6	ISSR-16	TCTCTCTCTCTCTCTCG	17	NO
7	ISSR-27	AGAGAGAGAGAGAGAGYT	18	NO

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A total of 134 bands scored of which 35bands were polymorphic with an average of 26.11 percentage polymorphism. Out of three, ISSR-3 showed highest polymorphism (56.00%) and the primer ISSR-9 gave the lowest polymorphism (17.54%) (Table 6) Significant variations in the banding pattern were noticed in the seven mutant lines as compared to control plants.

Table 8: Purity of DNA 260/280 ratio per 1 gram of plant material collected form M3 mutants

S.No	Sample	260/280	ratio	DNA conc. (ng)
1	MW7	22.01	2.0	49
2	MW39	**23.89**	**2.3**	**72**
3	MW 46	21.01	2.1	61
4	MW 2	19.45	1.8	36
5	MW 36	18.22	1.5	41
6	MW 26	18.30	1.6	40
7	MW 86	15.56	0.5	25
8	Control	16.13	0.8	31

CONCLUSION

Based on the results of this study, it can be concluded that using Microwave irradiation at varying intervals during treatment has the ability to cause mutations. The desired mutation in economic features can then be chosen and tested to improve *Solanum melongena* L. In molecular tests, we found that all of the screened lines had different banding patterns from the control plant, which translates to a 36.06% genetic variation frequency. The fragments that each primer produced, which varied in size from 100 to 1,500 bp, were amplified using PCR. With an average of six bands per primer, the three tested primers produced 35 scorable bands altogether. The bands were repeatable. Each primer has anywhere from five to nine bands. When comparing the ISSR profiles of the mutants to the control, new bands or the absence of bands were found. Primer 2 (ISSR-2) was used in the ISSR assay, yielding 52 bands total, of which 5 werenovel and 06 were not seen in the lanes. A total of 25 bands were obtained using the ISSR assay withprimer 3 (ISSR-3), of which 9 were new bands and 5 were not visible. The ISSR test using primer 9 (ISSR-9) yielded 57 bands in total, of which 5 were new and 5 were not seen, according to the results.35 of the 134 bands that were assessed, with an average polymorphism percentage of 26.11, were polymorphic. The primer ISSR-9 provided the lowest polymorphism (17.54%), while ISSR-3 displayed the highest polymorphism (56.00%) out of the three. Compared to control plants, the sevenmutant lines showed notable differences in the banding pattern.

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