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ORIGINAL ARTICLE **OPEN ACCESS**

Molecular Characterization of Eggplant *(Solanum melongena* L.) Parental lines by using ISSR marker

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ABSTRACT
Eggplant is the main crop of the tropical and sub-tropical regions of Asia. In southern Asia, it is commonly knownas brinjal. Although, it is called an eggplant due to the fruit shape of certain genotypes, which are white, green, and violet in color and bear a resemblance to eggs in shape. The maintenance of the genetic purity of the Eggplant is a matter of major problem for breeders. The advances in molecular markers allow precise and rapid variety identification. The study was attempted with the objective to check the purity of commonly used farmer's seed stock. The genetic constitution of a variation is its optimum identity, achieving and maintaining genetic purity incrops is essential both from a cultivating as well as breeding point of view. The present investigation was carriedout to identify polymorphic primers where it can distinguish the different parental lines. In this study, 7 ISSR primers were used, out of 7 ISSR primers, only 1 ISSR primer (UBC 815) was scoreable and show a polymorphicband on the agarose gel. UBC 815 was used for the identification of the Eggplant parental lines AB101, AB102, and AB103 line and show 900 base pair (bp), 500bp, and 1200 bp polymorphic bands respectively. These highlyexplanatory primers easily characterize the parent genotype. Genetic diversity helps the plant breeds select lines that improve food security.
KEYWORDS: Eggplant, molecular characterization, genetic purity, inter-simple sequence repeat (ISSR)markers.

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INTRODUCTION

Eggplant (*Solanum melongena L.),* one of the important vegetable crops, belongs to the family Solanaceae and is referred to by various names in different parts of the country as Baingan in Hindi, Badanekai in Kannada, Vangi in Marathi, Katharikai in Tamil, Vankai in Telegu, etc. Internationally, it is known as Eggplant in (England) or Aubergine in (France). Further, in several other countries, it is referred to as Berenjena in (Spain) and Alberenjina in (Arab Countries). India is considered the initial center of origin/diversity of eggplant (9). Confirmation for this fact was made by (4) on the bases of isozyme and morphological variation noticed in large germplasm collections from India. Eggplant is a major vegetable crop in India and is grown all over the year. However, it is widely cultivated in both temperate and tropical regions of the globe mainly for its immature fruits as vegetables (10), but in the temperate regions it is cultivated mainly during warm India is the major producer of Eggplant in the world followed by China, Turkey, Japan, Egypt, Italy, Indonesia, Iraq. Syria, Spain, and the Philippines. Eggplant ranks fair in nutritive value (carbohydrates, proteins, and fiber). It is also known for the presence of alkaloid solanine in roots and leaves. Some medicinal utilization of brinjal tissues and extract involves the treatment of diabetes, asthma, cholera, and bronchitis, and its fruits and leaves are reported to lower blood cholesterol levels (13). It substantially increases dietary intake of vitamins including thiamine, niacin, pantothenic acid, and folacin as well as vital minerals like calcium (Ca), iron (Fe), potash (K), zinc (Zn), copper (Cu), and manganese (Mn). The pickle-making and dehydration sectors, also contain excellent raw materials (1).

MATERIAL AND METHODS

The experimental material comprised of four germplasm of Solanum melongena: AB101(Jhumki), AB102(Pahuja), and AB103(Chandrika) were collected from Adithya Biotech Lab & Research Pvt. Ltd., Raipur.Chhattisgarh). Parent seeds were sown in coco peat trays under the greenhouse to create climates that speed up seed germination.

Figure 1: Eggplant's different parental lines plant samples in the nursery of Adithya Biotech Lab &Research Pvt. Ltd.

Table 1 : **List of Eggplant genotypes used for the present investigations.**

S.NO.	GERMPLASM	LOCAL NAME
1.	AB 101	JHUMKI
2.	AB 102	PAHUJA
3.	AB 103	CHANDRIKA

DNA isolation

The DNA from young and fresh leaves of Eggplant parental lines was successfully extracted through the CTAB extraction method (8).1% TAE agarose gel was efficiently used for the purity and quantification of the extracted DNA.

PCR amplification & ISSR Analysis

ISSR primers synthesized from *Eurofins Genomics India Pvt Ltd* (Bangalore) were used. PCR amplification was carried out in a total volume of 20µl reaction mixture consisting of Standard 10x PCR buffer (2µl), 25mM $MgCl_2$ (1.5µl), 5mM dNTPs (1µl), primer (2µl), 10mg BSA (1µl), Taq polymerase (0.2µl), M.Q. (11.3µl), DNA (1µl); with an initial denaturation step lasting 4 min at 94ºC, 35 cycles of amplification (each cycle consisting of 3 steps: denaturation at 94ºC for 20sec, annealing at 60ºC for 30sec and elongation for 1.30 min at 72ºC) followed by a final extension at 72ºC for 5 minutes. DNA fragments were amplified using horizontal agarose gel electrophoresis (2% w/v) in 10x TBE buffer and visualization by staining with ethidium bromide.

Table No.2 : ISSR (Inter Simple Sequence Repeat) primers name, its sequences & temperature

Primer name	Sequence(5' -3')	Tm(ºC)
UBC 807	AGAGAGAGAGAGAGAGT	50.4ºC
UBC 811	GAGAGAGAGAGAGAGAC	53.0 ºC
UBC 815	CTCTCTCTCTCTCTCTG	47.0 ºC
UBC 820	GTGTGTGTGTGTGTGTGTC	47.0 ºC
UBC 822	TCTCTCTCTCTCTCTCA	45.0 ºC
UBC 824	TCTCTCTCTCTCTCTCG	47.0 ºC
UBC 827	ACACACACACACACACG	47.0 ºC

RESULT

For genetic diversity and polymorphism, 3 Eggplant parental lines (A101, AB102, AB103) were analyzed using different ISSR markers. Out of 7 ISSR primers, only 1 ISSR primer (UBC 815) was scoreable and show a polymorphic band on the agarose gel.

UBC 815 was scored for the identification of Eggplant parental lines(AB101, AB102, AB103) which showed thedifferent polymorphic bands.

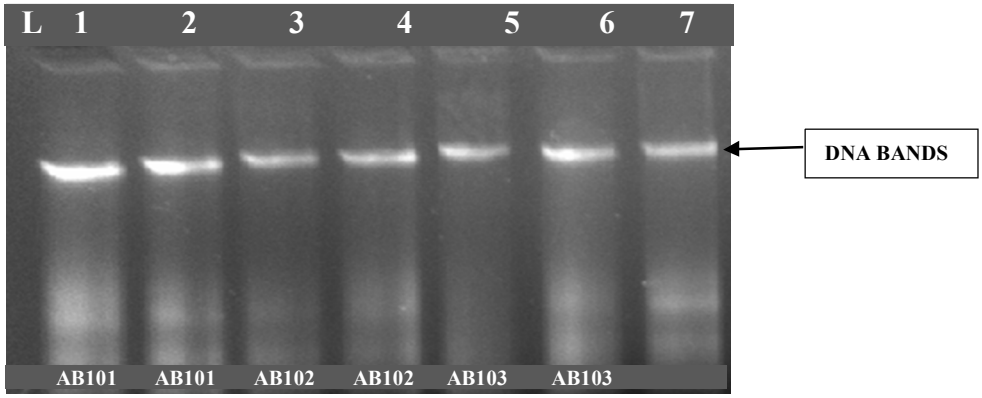

Figure: 2 Isolated genomic DNA of different Eggplant parental lines plant

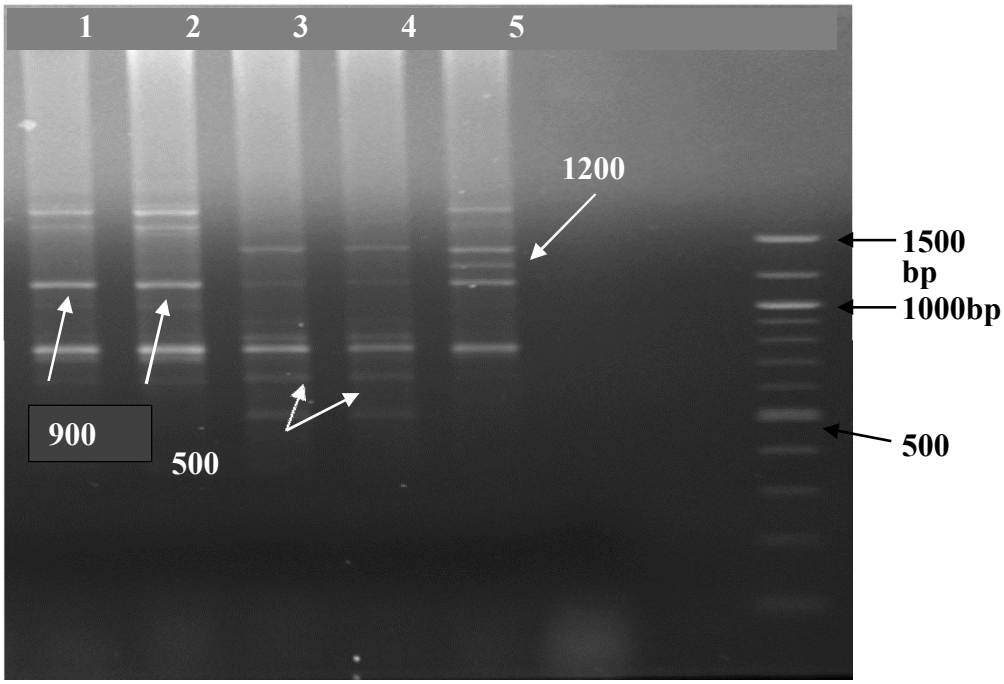

Figure: 3 PCR amplification product of UBC 815.
(Lane L- Ladder of 100 to 1500 bp)

Lane1-2(AB101) has a specific polymorphic band of size 900 bp which was absent in other lanes, Lane (3-4) (AB102)has a specific polymorphic band of size 500 bp which was absent in other lanes., Lane(5) (AB103)has a specific polymorphic band of size 1200 bp which was absent in other lanes.

Table:3 Band size of studied genotype

Genotype	Primer code	Band size	Sequence(5'-3')
AB101	UBC815	900 bp	CTCTCTCTCTCTCTCTG
AB102	UBC815	500 bp	CTCTCTCTCTCTCTCTG
AB103	UBC815	1200 bp	CTCTCTCTCTCTCTCTG

DISCUSSION

Diversity analysis of germplasm collections of several crop species has revealed considerable variability for awide range of traits (15). The wide range of diversity in phenotypic traits has proved a useful tool in the classification of plants and the information obtained could be of high interest to plant breeders in the development of plant species with desirable agronomic and nutritional qualities (11). Genetic diversity assessment of the improved and indigenous eggplant genotypes is an essential component in germplasm characterization and conservation to identify potential parents. Microsatellites are among the most widely used DNA marker for many purposes such as diversity, genome mapping, and varietal identification (2).

Assessment of Genetic diversity helps plant breeders to utilize genetically diverse parents in a breeding program to improve the productivity of varieties of agriculture and horticulture crops, (12). Classical breeding affects genetic diversity by selecting a combination of outcomes from diverse allele frequencies and leads to favorable effects and loss of diversity (14).

The ISSR molecular marker is employed in the current study to determine the genetic diversity of the three different parental lines of eggplant. For the identification of true line breeds in the past, morphological features were studied using tests like the GOT test and biochemical tests. This technique was complicated and demands more accuracy, labor, time, and money as well, and the molecular method brings in accuracy, and less time consumption. The introduction of the molecular marker has been tested in various fruits, flowers, tomatoes, potatoes, and Eggplant. The ISSR marker UBC 815 was employed to amplify the DNA of the eggplant in order to acquire the desired results for the identification of Eggplant parental lines (AB101, AB102, AB103) which showed the different polymorphic bands (900bp,500bp,1200bp) respectively found scorable for the purity assessment of Eggplant. The molecular markers can be a reliable source and can be used to check the hybrid and parental genetic purity for the particular Eggplant line, which will be a boon for the breeder's rights. This technique reduces the cost and the time associated with the selection of suitable plants for hybrid production and can be effectively adapted by the breeders.

CONCLUSION

The results of the current analysis led us to draw the conclusion that the Eggplant sample's genetic purity had been verified and was found to be pure. There is genetic purity among the Eggplant accessions examined using ISSR markers, and this knowledge is crucial for selecting the right parental line for breeding operations. Additionally, the time- and labor-intensive conventional technique of the seed purity test has been eliminated, which makes these seed purity test methods more advantageous. The farmers and breeders will use the seed which is launched in the market by the industrialist.

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ETHICS APPROVAL

The present research study does not involve any human participants, their data, or biological material.

CONSENT TO PARTICIPATE

Not applicable.

CONSENT OF INTEREST

The authors declare no competing interests.

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