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GENETIC ANALYSIS OF DRAGON FRUIT *SELENICEREUS* SPECIES

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ABSTRACT

Dragon fruit is considered an important economic fruit species worldwide due to its rich nutrient contents and strong to lerance to drought stress. In order investigate Genetic Analysis of Dragon fruits in Telangana 6 Germplasms were subjected to RAPD. The size of RAPD bands measured and score. The RAPD bands were scored as 1 if present and 0 if absent of bands of same molecular weight Five primers (Df series) amplified a total length of 39 loci with a size of 150-1400 bp. Amplified from the genomic DNA of Germplasm using 25 Decamer primers of random sequence were initially screened on DNA samples of 6 dragon fruit germplasm to evaluate their sustainability for amplifying DNA sequences, which could be accurately scored. A final subset of 5 primers. Dendrogram was generated by using NTSYSpc software that differentiated all the six dragon fruit samples. Respectively indicating Jaccard similarity coefficient. The similarity indices (SI) Vary from 0.11 to 0.88. The highest SI value (0.88) was found between Df-5, and Df-1 Germplasm whereas lowest observed between Df-6 and Df-3.

KEYWORDS: RAPD MARKER, Monomorphism, Genetic Distance, Gene Analysis, *Selenicereus* species.

INTRODUCTION

Dragon fruit also known as pitaya or *Selenicereus*, a climbing cactus, is a tropical fruit that is native to Central America but is now cultivated in various regions of the world. The Dragon fruit plant has long, vining, and succulent stems that can grow up to several meters in length

(Balendres and Bengoa, 2019). These stem have aerial roots and require support, such as a trellis or a study structure, to climb and grow (Pushpakumara et al. 2005).

Dragon fruit is low in calories and rich in vitamins, minerals, and antioxidants. It is good source of vitamin C, iron and magnesium (Hossainet al. 2021). The plant climbing cactus that needs support to grow, such as a trellis or a sturdy structure (Thokchom et al. 2019). There are several varieties of Dragon fruit available with variations in fruit colour, shape and size. Some popular species include the (*Selenicereusundatus*, *Selenicereuscostarcens is* and *Selenicereuspolyrhizus*, and) white, red and pink fleshed fruit, (Hao et al. 2022).

MATERIAL and METHODS

Collection and preservation of samples:

From each representative plant of the parental lines, younger plant tissue of about two to three grams of portion of young tissue was collected and stored at -20°C.

Genomic DNA extraction

DNA was extracted from each individual sample from tender stem tissue following CTAB method (Xin and Chen, 2012) with some modifications.

The lyophilized tissue was cut into small pieces and was grounded into fine powder in motor and pestles using liquid nitrogen.

Immediately after grinding the powdered tissue was transferred to 1.5 ml micro centrifuge tubes, homogenized and digested with extraction buffer (2% cetyltrimethylammonium bromide, 1% polyvinyl pyrrolidone, 100 mMTris-HCl, 1.4 M NaCl, 20 mM EDTA, 3% β-mercaptoethanol) and the tubes were placed in a water bath at 65 °Cfor 1 hr.

The tubes were removed from the water bath and centrifuged at 12000 rpm for 15 minsat 4° C. The supernatant was transferred into 1.5 ml fresh micro centrifuge tubes and to the tissue lysate Chloroform: Isoamyl alcohol (24:1, v/v) was added and centrifuged. (Repeat the Chloroform: Isoamyl alcohol step for two times).

The supernatant was then transferred to fresh centrifuge tube, add chilled isopropanol to precipitate the DNA.

The precipitated DNA was pelleted down by centrifugation step (12000 rpm for 15 mins at 4°C).

The DNA pellet was washed by adding 100µl of 70 % Ethanol and centrifuged.

The tube was centrifuged at 12000 rpm for 10 mins and 70% ethanol was discarded and the pellet was kept for air drying.

Finally, after air drying, the DNA pellet was dissolved in 100 µl of 1X TE buffer and stored at 4°C.

The purity and amount of isolated genomic DNA were estimated qualitatively and quantitatively following the below steps.

Qualitative analysis using 0.8% Agarose gel

The quality of isolated genomic DNA was checked on 0.8% agarose gel. 1 µl of genomic DNA sample was mixed with 2 µl of loading dye (1X). The solution was mixed thoroughly and loaded into the gel and subjected to 80 V for 45 mins on electrophoresis unit. The DNA bands were visualized using gel documentation system. The high molecular weight DNA appeared as a thick intense band, fig1.

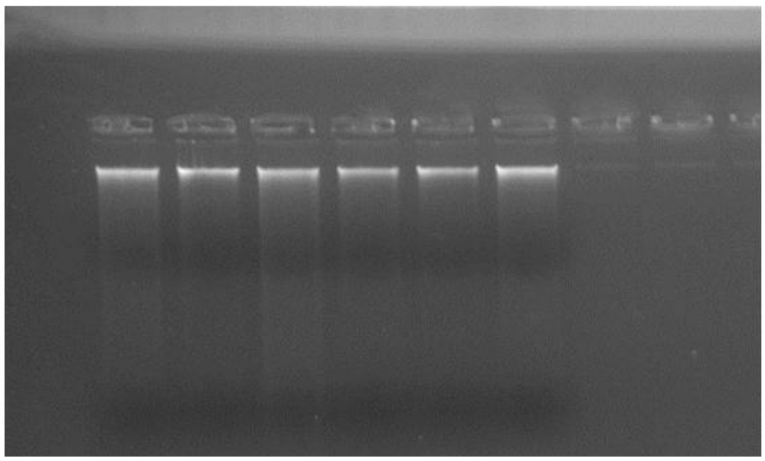


Fig. 1: Genomic DNA samples testing on 1 % Agarose gel.

Quantification of genomic DNA using Nanodrop

The genomic DNA was quantified by using spectrophotometer (Nano-Drop ND-1000, Thermo Scientific).

The computer and the spectrophotometer were interconnected with each other. Initially both were switched on, Nucleic acid option was selected after double clicking the Nano drop icon.

After adding 2 µl of sterile water with the help of a micropipette on to the lower pedestal and closing of the sampling arm it initiates the spectrophotometer. After the initialization step, option DNA - 50 module was selected
The blank was set by adding 2 µl of TE buffer.

2 µl of genomic DNA was loaded on the lower pedestal and the absorbance was measured; Based the absorbance instrument determines the concentration of DNA in nano-grams.

The ratio of absorbance at 260 / 280 nm is used to access the purity of DNA. A ratio of ~1.8 is generally accepted as "Pure" for DNA. The concentration of DNA in the solution was calculated by using the formula.

DNA Concentration (µgml−1) = (OD@260x50 µg /mlxdilutionfactor) /1000

RAPD Marker selection

A total of 25 decamer primers of random sequence were initially screened on DNA samples of 7 dragon fruit germplasm to evaluate their sustainability for amplifying DNA sequences, which could be accurately scored. A final subset of five primers (Table1: Primers details) was selected on the basis of intensity or resolution of bands, their reproducibility, consistency within the individuals and potentiality for discrimination of genotypes. In order to ensure reproducibility of RAPD bands further, amplification of DNA samples of 2 dragon fruit germplasms using the selected 5 primers was repeated twice. Similar banding pattern was observed in each case.

PCR analysis

The selected RAPD primers were used to amplify whole set of genomic DNA from dragon fruit germplasms. PCR reactions were carried out in 20 µl reaction mix containing 2 µl (100 ng) of genomic DNA, 1 µl of 10x Taq buffer, 1 µl of dNTPs (250 µM each) (Takara, Japan), 2 µl (10 µM) of primer (Invitrogen), 0.2 (1 unit) of Taq DNA polymerase (Takara, Japan) and required amount of sterile water.

PCR was performed in a Applied biosystems as follows: initial denaturation at 94ºC for 3 min; 40 cycles of denaturation at 94° C for 1 min, annealing at 36° C for 1 min and extension

at 72° C for 2 min. A final extension step at 72° C for 7 min was employed to ensure complete amplification of all DNA fragments followed by holding at -4° C.

Agarose Gel Electrophoresis

PCR amplified products were separated on 2.0% agarose gel. Molecular weight markers (1kb and 100 bp DNA markers Invitrogen, UK) were also run alongside the gel. Gels were stained with ethidium bromide (10 mg/ml), visualized and photographed using a gel documentation system (Alpha imager Mini)

RESULTS

Data Analysis

The size of RAPD bands was measured and scored. The RAPD bands were scored as '1' if present and '0' if absent of bands of same molecular weight.

Five primers (Df series) amplified a total length of 39 loci with a size range of 150 –1400 bp (Figure2 a, b, c, d, e: Gel Pictures) from genomic DNA of six dragon fruit germplasms collected from different geographical locations. Three primer (DF_2, 5 and 6) generated highest number of bands (9) whereas primer DF_10 produced least number of bands (4). The details of the primers were mention in the table2.

Dendrogram was generated by using NTSYSpc software that differentiated all the six dragon fruit samples into different clusters and sub-clusters by jaccard's similarity coefficient value of 0.40 on the basis of geographical locations of them (Figure: Dendrogram). Cluster analysis or dendrogram based on the Jaccard similarity coefficient segregated six dragon fruit genotypes into two major clusters (Table1: Jaccard Similarity coefficient). Cluster 1 consisted of Df-2, Df-4 and Df-3, whereas cluster 2 has Df-1, Df-5 and Df-6.

The similarity indices (SI) vary from 0.11 to 0.88. the highest SI value (0.88) was found between Df-5 and Df-1 germplasm whereas lowest were observed between Df- 6 and Df-3, The similarity with s3 and s2 0.77 and s4 and s3 0.77.

Gel Figures.

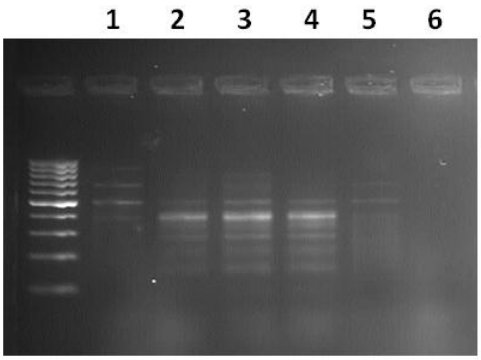


Fig. 2a: (df1- Primer)

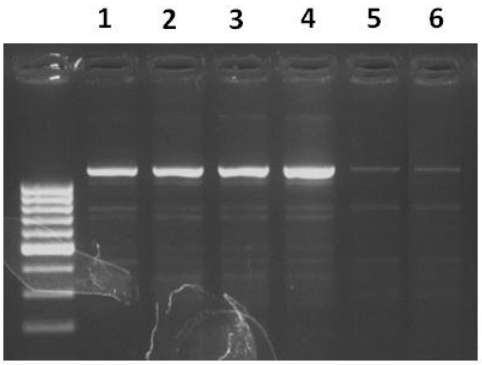


Fig.2b: (DF2-Primer)

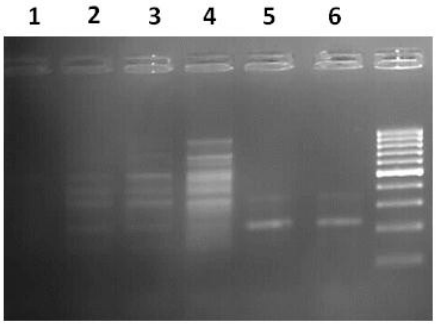


Fig. 2c: (df5-Primer)

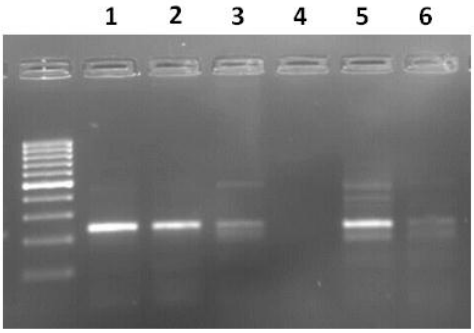
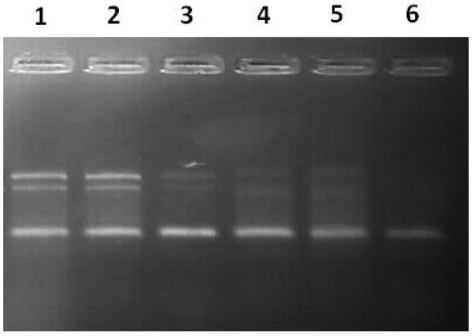


Fig. 2d: (DF6-Primer)



(DF-10-Primer).

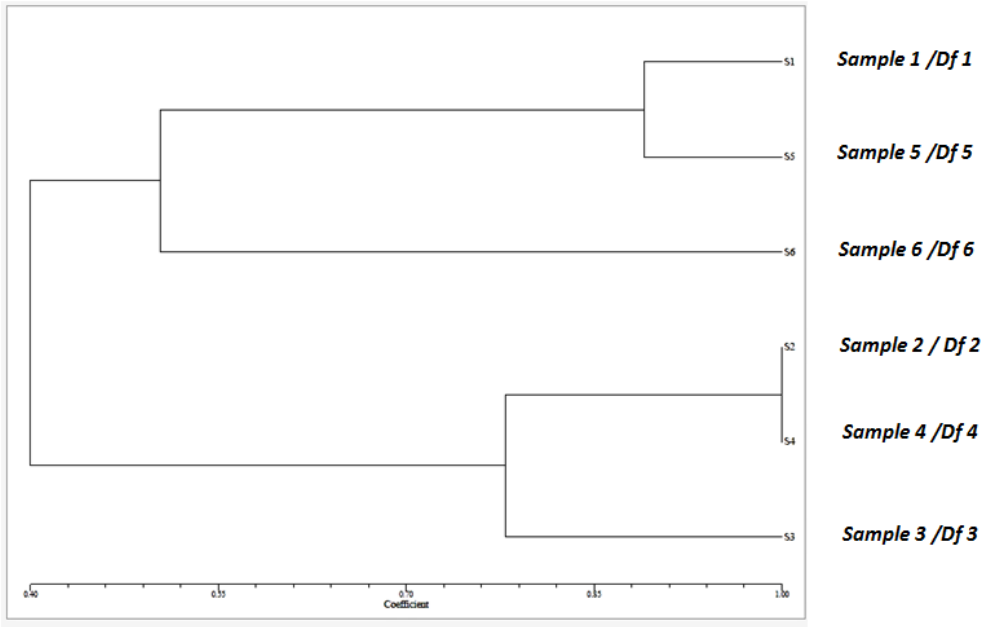
The gelfigures developed in six samples each with a specific primer

Table1: RAPD primers were used to amplify whole set of genomic DNA from dragon fruit germplasms.

S. No	Primer	Seq (5' - 3')	GC%	Tm	No of bands	Size range (bp)
1	DF_1	ACGGATCCTG	60	32	8	150-1000
2	DF_2	GAGGATCCCT	60	32	9	200-1325
3	DF_5	CCGAATTCCC	60	32	9	150-900
4	DF_6	GGGAATTCGG	60	32	9	100-1100
5	DF_10	GGAAGCTTGG	60	32	4	200-950

Table 2: Jaccard Similarity Coefficient.

S. No	S1	S2	S3	S4	S5	S6
S1	1					
S2	0.33	1				
S3	0.55	0.77	1			
S4	0.33	1	0.77	1		
S5	0.88	0.44	0.66	0.44	1	
S6	0.55	0.33	0.11	0.33	0.44	1



***Selenicereus undatus* 5, 6 white fleshed fruit. *Selenicereus costarcensis* 1 redfleshed fruit and *Selenicereus polyrhizus* 2, 3, 4, pink fleshed fruit.**

Fig. 3: Dendrogram based on Jaccard similarity coefficient, summarizing data on genetic variation between dragon fruit (Df) germplasm according to RAPD analysis.

DISCUSSION AND CONCLUSION

Detection of genetic differences and elucidation of genetic relationships among genotypes of any plant are of high importance for both proprietary rights protection, conservation, utilization and improvement of its genetic resources (Liu et al., 2011). The performed a systematic baseline analysis of genetic variation in 06 dragon fruit germplasms using RAPD markers and related this molecular based variation with their some important characteristics.

Five primers (Df series) amplified a total length of 39 loci with a size range of 150 –1400 bp (Figure2a,b,c,d,e: Gel Pictures) from genomic DNA of six dragon fruit germplasms collected from different geographical locations. Three primer (DF_2,5 and 6) generated highest

number of bands (9) whereas primer DF_10 produced least number of bands (4). The details of the primers were mention in the table1.

Dendrogram was generated by using NTSYSpc software that differentiated all the six dragon fruit samples into different clusters and sub-clusters by jacquard's similarity coefficient value of 0.40 on the basis of geographical locations of them (Figure3: Dendrogram). Cluster analysis or dendrogram based on the Jaccard similarity coefficient segregated six dragon fruit genotypes into two major clusters (Table2: Jaccard Similarity coefficient). Cluster 1 consisted of Df-2, and Df-4 and Df-3, whereas cluster 2 has Df-1 and Df-5 and Df-6.

The similarity indices (SI) vary from 0.11 to 0.88. the highest SI value (0.88) was found between Df-5 and Df-1 germplasm whereas lowest were observed between Df- 6 and Df-3.

In the present study, RAPD primers generated good number of bands was clearly distributed. Good quality genetic profiling was obtained with using a total number of 25 decamer primers of random sequence were initially screened on DNA Samples of 6 dragon fruit germplasm to evaluate their sustainability for amplifying DNA sequence, which could be accurately scored. A final subset of five primers was selected on the basis of intensity or resolution of bands, their reproducibility, consistency within the individuals and potentiality for discrimination of genotypes.

Similarly results are reported by other Author Demeke, T., Adams, R. P and Chibbar, R. 1992. Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in Brassica, Theor. Appl. Genet. 84: 990-994.

Cluster analysis

similarity matrix was prepared on the basis of all amplified products of six accessions with twenty The Genetic five primers, with the help of dendrogram which was generated cluster analysis of Jacquard" similarity coefficient. It showed two clusters with a wide range of variability among six accessions. Cluster A consist of three accessions (sample – 2, 4 and 3*Selenicereus polyrhizus* 2,3,4) and Cluster B consist of 3 accession(Sample – 1 *Selenicereuscostarcensis*1, 5 and 6*Selenicereus undatus*5, 6) Similar results reported, in *Curcuma caesia* by Ranemma and karnakar, 2017.

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