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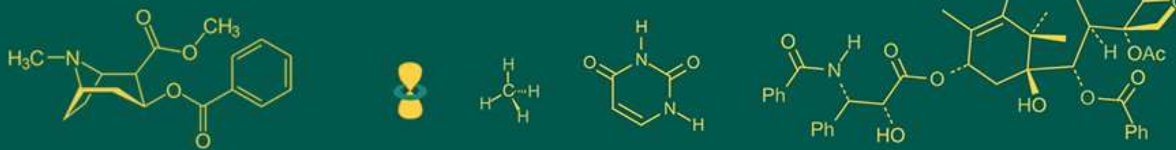

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Genetic exploration of dwarf traits in red banana mutants: Integrating molecular markers for sustainable cultivation

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Abstract
This study set out to investigate red banana mutants in-depth and used a careful molecular analysis to determine the genetic cause of dwarfism. The study, which used 3,200 red banana plants grown *In vitro* and exposed to various gamma irradiation dosages, determined that an LD_{50} dose of 20 Gy was necessary for the best possible growth and survival. The use of Sequence Characterized Amplified Regions markers in DNA analysis revealed the presence of the dwarf gene in select samples, with Sample No. 1 leading to notable findings and subsequent identification of specific dwarf plants Samples 14, 18, and 52 at both group and individual plant levels. This research is important because it emphasizes the integration of morphological observations with molecular investigation, going beyond genetic discoveries. The study's strength lies in the convergence of molecular markers with morphological and biochemical analyses, enhancing the overall validity of its findings. Considering these findings, the thorough molecular screening serves to illuminate the wider implications for breeding programs as well as the genetic characteristics associated with dwarfism in red banana mutants. In essence, this research bridges the gap between genetic insights and practical applications, paving the way for sustainable crop development and enhancing the adaptability of red banana cultivation in diverse environmental conditions.

Keywords: Banana, red banana, dwarf plant, molecular markers, SCAR marker

Introduction
Bananas, a cornerstone of global fruit crops, have evolved into a fundamental food source, propelling economies in tropical and subtropical regions (Aurore *et al.,* 2009) [1]. Belonging to the *Musa* genus, these beloved fruits come in various species and edible varieties, renowned for their delightful taste, nutritional richness, and digestive ease (Keskar *et al.,* 2019) [9]. With a nutrient profile of carbohydrates (22.8 gm), proteins (1.1 gm), sugar (12.2 gm), fiber (2.6 gm), and essential minerals like calcium, magnesium, potassium, and phosphorus in a 100-gram serving, bananas stand out as a nutritious powerhouse. Beyond their appeal as a fresh snack, bananas play a versatile role in processed products such as chips, puree, jam, jelly, juice, wine, and halwa. Even the fragile pseudo-stems of harvested banana trees are useful as vegetables and in the production of fiber silk.
In the global context, bananas are a key food crop cultivated and consumed in over 100 countries in the tropics and subtropics. India stands out as the world's leading producer, contributing 30.8% to the total global banana production, followed by China, Indonesia, Brazil, and other nations. Among Indian states, Andhra Pradesh leads in banana production with a 16.27% share, followed by Gujarat, Maharashtra, Tamil Nadu, Uttar Pradesh, and Karnataka. Maharashtra, a significant contributor to the national production, showcased a noteworthy output of 3,924.1 thousand metric tons, with the red banana variety accounting for 10% of this total. India, a major player in banana production, contributed approximately 34.9 million metric tonnes, with red bananas comprising around 5% of the total output, according to the Ministry of Agriculture and Farmers Welfare. Maharashtra, along with states like Tamil Nadu, Karnataka, Gujarat, and Andhra Pradesh, plays a pivotal role in banana cultivation, boasting remarkable productivity that surpasses the national average,

with Maharashtra leading at 65.7 metric tons per hectare.
The red banana, distinguished by its red skin and yellow flesh, holds a special place in Maharashtra, India. Its popularity among farmers and consumers is attributed to its high yield, exceptional quality, and distinct flavor. The cultivation of dwarf bananas is favored in agriculture due to its advantageous traits, including a decreased risk of lodging, improved harvesting efficiency, and heightened planting density. Despite the benefits, the genetic foundation of dwarfness in banana plants remains poorly comprehended. The identification of dwarf plants emerges as a pivotal development with promising potential for the future of red banana cultivation, particularly benefiting farmers in hilly regions like the Kokan area where red banana plants thrive. Red banana plants are often cultivated in hilly areas with well-draining soils. The specific variety of red bananas and local environmental conditions can influence the success of cultivation in each area. The dwarf stature of these plants proves to be a practical advantage in such terrains, providing resilience against adverse weather conditions, notably heavy winds, and ensuring a more robust and stable crop yield. Molecular markers serve as crucial tools in plant breeding, enabling the identification and selection of genes linked to specific traits. These markers operate by pinpointing variations in DNA sequences associated with distinct phenotypic characteristics (Miri *et al.,* 2009) [10].

Material and Methods

Experimental Setup

All experiments were conducted at the laboratory of the Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. Field-grown plants were chosen for the current study, focusing on the detection of dwarf plants through PCR. Leaf samples were collected from red banana plants grown in the fields of the Plant Biotechnology Centre, Dapoli, and Agricultural Research Station, Shirgaon, Dist. Ratnagiri.

Sample Source

Red banana explants, with an 80% establishment rate, underwent shoot multiplication and gamma irradiation at Bhabha Atomic Research Centre. Ten dosage levels (0-100 Gys) were applied, revealing the LD_{50} dose for *In vitro* shoots (Sawardekar *et al.,* 2022) [13]. Notably, 20 Gy played a crucial role in determining optimal outcomes. Red banana *in vitro* culture exposed to 20 Gy of radiation was specifically collected. Leaf samples from field-grown and *In vitro*-raised irradiated banana plants were sourced from the Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli.

Plant Selection and DNA Isolation

The extraction of genomic DNA followed the procedure described by Edwards *et al.,* 1991 [8], with minor modifications to the buffer composition and concentration. A total of 3,200 plants were chosen for the present experimental investigation. At the hardening stage, the plantlet's DNA was isolated. Before field planting, 32 pooled DNA samples, each containing the DNA of 100 plants, were examined by PCR.

Isolation of DNA and Analysis

Total genomic DNA was extracted from young leaves of both Control and irradiated red banana plants utilizing a modified DNA method adapted from Edwards *et al.,* 1991 [8]. To evaluate the quantity and quality of DNA, comparisons were made by assessing band intensities on an agarose gel. Dwarf-specific primers, derived from Ramage *et al.,* 2004 [11], and Suprasanna *et al.,* 2008 [15], designed as follows: A1 (5' CCG AAC ACG GGA CTT ATA CA 3'), A2 (5' CCG AAC ACG GGC TAA CCT AG 3'), B1 (5' CTG TGG TTG CAT TCT CAT AC 3'), B2 (5' GTG AAT CAT ACT CGC GAA CC 3'), and Dw1 (5´ CTGTGGTTGCATTCTCATAC3´), and Dw2 (5 ´ C T G A A T C A T A C T C GCGAACC3´) respectively. The PCR mixture of 10 µl included the primers (1 µl each, 250 nM), Taq DNA polymerase (1 µl, 3 U/µl), 200µM of each dNTP (0.5 µl), $MgCl_2$ 25 mM 0.25 µl, Molecular Grade Water 5.75 µl, 10X PCR Taq buffer 1.25 µl (10 mM Tris-HCl, 4.5 mM MgCl2, 50 mM KCl, pH 8.3), and 20-25 ng of genomic DNA as the template. The thermocycler was programmed at 94 °C for an initial denaturation of 3 min, followed by 35 amplification cycles, each comprising steps at 94 °C for 10 Sec, (55 °C for B1 B2 & Dw1 Dw2, 50.2 °C for A1 A2) for 10 sec, and 72 °C for 60 Sec, with a final extension for 5 min. The resulting amplified products were analyzed using agarose gel electrophoresis (1.4%), and gel visualization and photography were performed on a UV transilluminator after staining with ethidium bromide following the protocol by Sambrook *et al.,* (1989) [12]. The amplicon size of bands was measured by using UviTec software.

Results and Discussion

The Bhabha Atomic Research Centre embarked on a significant study involving the inoculation of Red Banana explants, which exhibited an 80% survival rate following exposure to gamma radiation. This investigation aligns with Sawardekar *et al.,* 2022 [13] findings, highlighting an LD_{50} of 20 Gy in the red banana variety. The consistent LD_{50} values emphasize the robustness of sensitivity to gamma radiation, underscoring potential applications in induced mutation techniques for red banana varieties. The resonance between the studies reinforces the reliability of observed effects, shedding light on dose-dependent responses and their implications for the development of desirable mutants.

In this study, analysis was conducted on 3200 carefully selected red banana plants, isolating their DNA for subsequent screening. Employing a bulk analysis approach, these plant samples were organized into 32 pooled samples, as detailed in Table 1. Further dilution in ratios of 1:20 and 1:50 was performed on these pooled samples, each containing DNA from 100 plants, forming the basis for screening for dwarfness. Utilizing Polymerase Chain Reaction (PCR) with Sequence Characterized Amplified Region (SCAR) Primers A1/A2, B1/B2 (Ramage *et al.,* 2004) [11], and Dw1/Dw2 (Suprasanna P *et al.,* 2008) [15], each pooled sample underwent scrutiny.

The screening outcomes are presented in Table 2, elucidating the results of Dwarf Gene detection in the *In vitro* raised irradiated red banana plants using SCAR Markers. Notably, among the 32 samples, only Sample No. 1 exhibited a positive result ("+"), signifying the presence of the dwarf gene, while the remaining samples tested negative ("-"). The PCR process involved three primers: A1/A2, B1/B2, and Dw1/Dw2. Positive results were distinctly observed with B1/B2 and Dw1/Dw2 primers, whereas A1/A2 primers yielded results that were challenging to interpret accurately, leading to their categorization as

negative. This observation aligns with the study by Ramage *et al.,* (2004) [11], where B1/B2 SCAR primers demonstrated notable results in *In vitro* dwarf and wild-type banana leaf tissue, establishing consistency with the current findings.

Furthermore, the outcomes of this study, particularly those involving Dw1/Dw2 primers, harmonize with Suprasanna P *et al.,* 2008 [15] research on the characterization of radiation-induced and tissue culture-derived dwarf types in bananas using a Sequence Characterized Amplified Region (SCAR) marker. Summarily, the positive outcome in Sample No. 1 indicates the presence of the dwarf gene, contrasting with the negative results in other samples indicative of tall plant traits. The utilization of specific SCAR markers, including B1/B2 and Dw1/Dw2, has proven effective in identifying dwarf traits, aligning with similar studies in the field.

Dwarf and giant varieties of red bananas are commonly found in the wild, with dwarf varieties showing a tendency to resist returning to their usual forms (Smith and Drew, 1990) [14]. Distinguishing characteristics of dwarf variants include notably shorter pseudo stems compared to their normal counterparts (Suprasanna P *et al.,* 2008) [15]. Moreover, the reduced height of dwarf plants can function as a protective measure against lodging during adverse weather conditions such as heavy winds or rain (Suprasanna P *et al.,* 2008) [15]. Optimal selection conditions are attained when plants are cultivated under uniform and vigorous growth conditions (Suprasanna P *et al.,* 2008) [15].

Further examination of Sample No. 1, comprising DNA from 100 plants (Table 2), a subsequent division into ten groups, each containing DNA from 10 plants (Table 3), was conducted. The outcomes detailed in (Table 3) unveil that among the ten sample groups, Sample No. 2, and Sample No. 6 displayed positive results, indicating the presence of specific genetic markers, while the remaining eight groups tested negative. Following positive results in Sample Groups 2 and 6, a more in-depth analysis was undertaken at the individual plant level, as elaborated in Table 4. This phase involved the extraction of genomic DNA from 10 plants in each of these groups, yielding 20 distinct samples from Sample Groups 2 and 6 red banana plant samples. The primer, namely A1 A2 and B1 B2, was initially developed by (Damasco *et al.,* 1996) [7] and previously applied by Ramage *et al.,* 2004 [11], for the early detection of dwarf off-types in micropropagated Cavendish bananas (*Musa spp*. AAA), was employed. The analysis outcomes revealed positive results for Sample No. 14, Sample No. 18, and Sample No. 52, signifying the presence of specific genetic markers associated with dwarfism. Conversely, the remaining eighteen samples tested negative, implying the absence of these markers. In summary, it is deduced that Sample No. 14, Sample No. 18, and Sample No. 52 can be categorized as dwarf plants, with this characterization specifically applicable to plants in the *In vitro* conditions (Table 4).

Suprasanna *et al.,* (2008) [15] undertook a study focused on characterizing radiation-induced and tissue culture-derived dwarf types in bananas using a Sequence Characterized Amplified Region (SCAR) marker. In the analysis of the control group, a distinct amplified fragment of 811 bp was observed, as depicted in the gel photograph (Fig. 1). This characteristic band at 811 base pairs was conspicuously absent in the irradiated plants, specifically in Sample No. 14, Sample No. 18, and Sample No. 52. The expected banding pattern aligns with the descriptions provided by Damasco *et al*. (1996) [7] and Ramage *et al*. (2004) [11]. To validate that the absence of this band in irradiated plants indicated the dwarf condition rather than PCR failure, a distinct 85 bp band was observed in Fig 1 (Ramage *et al.,* 2004 & Suprasanna *et al.,* 2008) [11, 15]. This technique, while offering a reliable and reproducible means for detecting dwarf off-types, is sensitive to the type of material chosen for analysis, as highlighted by Ramage *et al*. (2004) [11]. Optimal results hinge on the careful selection of young, actively growing tissue, as older tissue may lead to PCR failure (Ramage *et al.,* 2004) [11]. The presence of an 85 bp band in Fig 1 serves as a reliable marker for PCR success, ensuring that normal plants are not misclassified as off-types and enabling micro propagators to maximize the production of normal plantlets (Ramage *et al.,* 2004) [11]. Regarding plant height, the control plant measured 337 cm, while Plant No. 14 measured 287 cm, Plant No. 18 stood at 296 cm, and Plant No. 52 reached 282 cm. These height measurements were meticulously recorded after the plants had fully matured and produced fruits in the field following a comprehensive hardening process. The integration of morphological observations, such as plant height, with biochemical data significantly strengthens the overall validity of the results obtained in this study. Biochemical analysis at an early developmental stage suggests that meaningful results can be obtained early in the growth process, eliminating the need to await full maturity before relying solely on morphological observations and analysis.

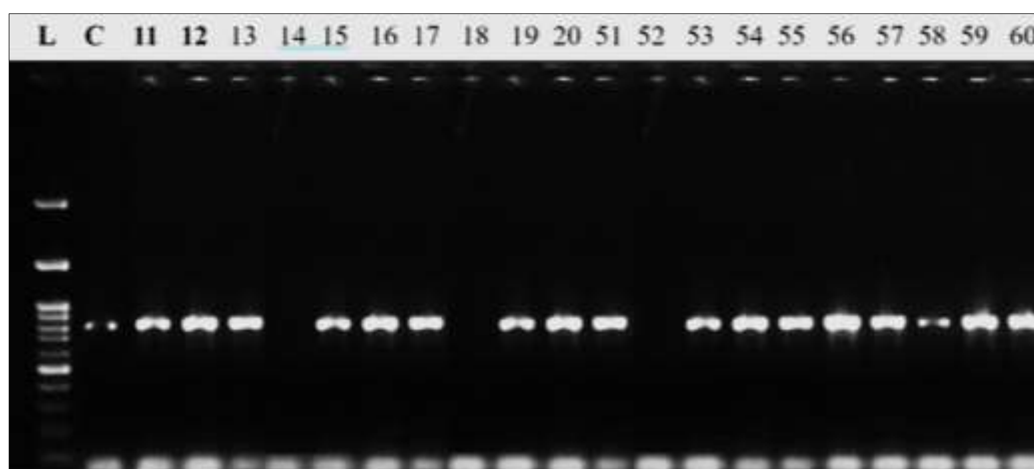

Fig 1: Characterization of radiation-induced dwarf types in Red Banana by using SCAR (Dw1 & Dw2) marker (811 bp). Lanes L- Ladder, C—Control; 14, 18 and 52 —Dwarf, 11 – 13, 15-17, 51, and 53-60 — Tall

Table 1: DNA Extraction from *In vitro* raised irradiated material of Red Banana

Sr. No.	No. of Pooled samples of DNA	Sample No.	Sr. No.	No. of Pooled samples of DNA	Sample No.
1.	1 – 100	1	17.	1601 – 1700	17
2.	101 – 200	2	18.	1701 – 1800	18
3.	201 – 300	3	19.	1801 – 1900	19
4.	301 – 400	4	20.	1901 – 2000	20
5.	401 – 500	5	21.	2001 – 2100	21
6.	501 – 600	6	22.	2101 – 2200	22
7.	601 – 700	7	23.	2201 – 2300	23
8.	701 – 800	8	24.	2301 – 2400	24
9.	801 – 900	9	25.	2401 – 2500	25
10.	901 – 1000	10	26.	2501 – 2600	26
11.	1001 – 1100	11	27.	2601 – 2700	27
12.	1101 – 1200	12	28.	2701 – 2800	28
13.	1201 – 1300	13	29.	2801 – 2900	29
14.	1301 – 1400	14	30.	2901 – 3000	30
15.	1401 – 1500	15	31.	3001 – 3100	31
16.	1501 – 1600	16	32.	3101 – 3200	32

Table 2: Screening of *In vitro* raised irradiated material of red banana through PCR for Dwarf Gene using SCAR Markers

Sr. No	Sample No	No. of Pooled samples of DNA	SCAR Marker		
			A1 & A2	B1 & B2	Dw1 & Dw2
1.	1	1 – 100	-	+	+
2.	2	101 – 200	-	-	-
3.	3	201 – 300	-	-	-
4.	4	301 – 400	-	-	-
5.	5	401 – 500	-	-	-
6.	6	501 – 600	-	-	-
7.	7	601 – 700	-	-	-
8.	8	701 – 800	-	-	-
9.	9	801 – 900	-	-	-
10.	10	901 – 1000	-	-	-
11.	11	1001 – 1100	-	-	-
12.	12	1101 – 1200	-	-	-
13.	13	1201 – 1300	-	-	-
14.	14	1301 – 1400	-	-	-
15.	15	1401 – 1500	-	-	-
16.	16	1501 – 1600	-	-	-
17.	17	1601 – 1700	-	-	-
18.	18	1701 – 1800	-	-	-
19.	19	1801 – 1900	-	-	-
20.	20	1901 – 2000	-	-	-
21.	21	2001 – 2100	-	-	-
22.	22	2101 – 2200	-	-	-
23.	23	2201 – 2300	-	-	-
24.	24	2301 – 2400	-	-	-
25.	25	2401 – 2500	-	-	-
26.	26	2501 – 2600	-	-	-
27.	27	2601 – 2700	-	-	-
28.	28	2701 – 2800	-	-	-
29.	29	2801 – 2900	-	-	-
30.	30	2901 – 3000	-	-	-
31.	31	3001 – 3100	-	-	-
32.	32	3101 – 3200	-	-	-

Note: "+" Indicates the Presence of a dwarf gene.
"-" Indicates the Absence of a dwarf gene.

Table 3: Sample No. 1 and Results: A Positive Outcome and 100 DNA Samples Organized into 10 Groups

Sr. no.	Sample No.	Sample group	Result
1.	1	1-10	Negative
2.	2	11-20	Positive
3.	3	21-30	Negative
4.	4	31-40	Negative
5.	5	41-50	Negative
6.	6	51-60	Positive
7.	7	61-70	Negative
8.	8	71-80	Negative
9.	9	81-90	Negative
10.	10	91-100	Negative

Table 4: Sample Group 2, 6 shows positive Results and contains 20 DNA Samples

Sr. no.	Sample No.	Result	Sr. no.	Sample No.	Result
1.	11	Negative	11.	51	Negative
2.	12	Negative	12.	52	**Positive**
3.	13	Negative	13.	53	Negative
4.	14	Positive	14.	54	Negative
5.	15	Negative	15.	55	Negative
6.	16	Negative	16.	56	Negative
7.	17	Negative	17.	57	Negative
8.	18	Positive	18.	58	Negative
9.	19	Negative	19.	59	Negative
10.	20	Negative	20.	60	Negative

Table 5: Distinctive Features of Control Red Banana and Irradiated Red Banana

Sr no	Plant No	Days for Flowering	Plant Height (Cm)	Pseudostem Circumference (Cm)	Pseudostem Diameter (Cm)	Hands/ Bunch	Total Fingers/ Bunch
1	C-158	273	337	92	29.29	6	109
2	I-18	234	296	83	26.43	5	77
3	I-14	239	287	78	24.84	5	66
4	I-52	278	282	75	23.88	5	68

Conclusion

In conclusion, the study has shed light on the genetic markers associated with dwarfism in red bananas, unveiling vital information about their genetic structure. A dwarf gene was found to be present in the distinctly different Sample No. 1, which merits further exploration at the group and individual plant levels. With a more in-depth investigation, Sample Nos. 2 and 6 were revealed to have dwarf traits and specific dwarf plants that stood out from the rest were identified as Samples No. 14, 18, and 52. Using molecular markers such as SCAR Primer (Dw1 & Dw2 primers has provided an extensive genetic profile of these red banana anomalies. The positive findings in Samples No. 14, 18, and 52 that exhibit specific genetic markers signify promising candidates for future cultivation. By integrating molecular markers with morphological observations and biochemical analyses, we have reinforced the credibility of our results, underlining the importance of an interdisciplinary approach. Identifying dwarf plants in red bananas upholds significant implications for cultivation enhancement, sustainability, and crop improvement strategies. By understanding the genetic foundations of desirable traits, the future of crop enhancement lies in our hands.

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