Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2022; 11(1): 101-103
Received: 01-11-2021
Accepted: 03-12-2021

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Identification of nucellar and zygotic seedlings of Jamun (*Syzygium cumini* Skeels.) using RAPD marker

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Abstract
Jamun (*Syzygium cumini* Skeels.) is an important minor indigenous fruit of India. It belongs to the *Myrtaceae* family. Though native to India and Myanmar, it has naturalized throughout the South East Asia and Pacific regions. Jamun is an important fruit crop with a special character called polyembryony. This trait could be effectively utilized for propagation in nursery or hybridization work. The proper identification of zygotic and nucellar seedlings may lead to proper management of the elite germplasms in mass scale propagation. Conventional techniques of 'Off type' rouging and other morphological identification techniques are not full proof. Molecular marker may be used for discrimination of different seedlings. Cheap and cost effective RAPD technique is used in seedlings of a single seed of jamun with 20 non-specific primers. Primer OPP-04, OPT-06 and OPT-07 gave optimum polymorphism as compared to other primers. Total 11 amplicons were identified with these 3 primers that are effective in zygotic and nucellar discrimination. Sequencing of the amplicons could generate nucleotide sequence associated with the trait. Polyembryony character is regulated by several environmental factors, hence specific marker selection for specific Eco-region is crucial. The universal primers identified here are important for detection of polyembryony trait of jamun of this subcontinent.

Keywords: Jamun, polyembryony, RAPD, zygotic, nucellar seedlings, molecular markers

Introduction
Jamun, botanically known as *Syzygium cuminii* or *Eugenia jambolana* belongs to the *Myrtaceae* family. It is also commonly known as java plum, black plum, jambul, and Indian blackberry (Misra and Bajpai,1971) [15]. Jamun is used for the treatment of various diseases such as Chronic diabetes, chronic diarrhoea and other enteric disorders (Migliato *et al*., 2011). However, little work has been done on the systemic breeding and propagation in this crop which might be due to the specific character, Polyembryony.

Polyembryony is a condition in which multiple embryos arise within the embryo sac by budding or by cleavage of the zygotic pro-embryo or from the synergids and antipodal cells (Maheswari and Sacher, 1963) [13]. Adventitious embryos are initiated directly from the maternal nucellar tissue, which surround the embryo sac containing a developing zygotic embryo (Aleza *et al*., 2010) [2]. Therefore, in polyembryonic crops, the identification of the zygotic embryo has great importance (Villegas and Andrade, 2008) [17]. Polyembryony is a common phenomenon in Jamun with four embryos was reported by Sivasubramaniam and Selvarani, (2012) [16].

Different morphological and biochemical markers have been used to distinguish nucellar from zygotic seedlings, but none is as efficient as molecular markers (Andrade-Rodríguez *et al*., 2004; Rao *et al*., 2008) [3, 14]. Out of all molecular markers, RAPD is more simple, cost effective and reliable. RAPDs have been extensively used in assessing relationship amongst various Citrus accessions (Das *et al*., 2004) [4], genotype identification (Deng *et al*., 1995), estimation of relationship (Machado *et al*., 1996) [12] and zygotic and nucellar detection (Lima *et al*., 2000) [11]. In citrus and mango, different workers have reported some molecular markers for distinguishing zygotic from nucellar seedling. However, in jamun (*Syzygium cuminii*), no work has been reported on the identification of zygotic and nucellar seedling using molecular markers. Keeping this in view, a preliminary study was carried out to distinguish zygotic and nucellar seedling of jamun through RAPD markers.

Materials and Methods

Plant Material

Single plant, from Hessaraghatta (Karnataka, India), was marked as mother plant on the basis of various quality *viz* infection-free plant, high productivity, regular bearing and fruit quality. Mature fruits were collected from mother plant and brought to the laboratory. 100 seeds were selected, washed, disinfected and sown in tray containing cocopeat in the nursery.

Isolation of Genomic DNA

Young, fully expanded leaves from the mother plant (from which the fruits were collected) and seedlings grown in tray in the nursery were collected for genomic DNA isolation. Total genomic DNA was extracted according to the method described by Doyle and Doyle (1990) [6]. DNA concentration was primarily checked by using 0.8% agarose gel electrophoresis. In addition, DNA concentration and purity were quantified by Nanodrop 1000 spectrophotometer (Invitrogen, Waltham, Massachusetts, USA) at 260/280 nm absorbance.

PCR Analysis

20 RAPD primers were used for PCR analysis of genomic DNA from mother and progeny plants. The process of PCR amplification was carried out by using thermocycler (Eppendrof Master Cycler Gradient) with final volume of 25 µl reactions containing 25 ng of template DNA, 0.1 mM total dNTPs, 0.3 µM primer, 2.5 µl of 1X PCR buffer with 15 mM of magnesium chloride and 0.5 unit of Taq DNA polymerase. The PCR cycling profile was: initial denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 1 min., 36 °C for 1 min., 72 °C for 2 min and a final extension at 72 °C for 7 min followed by cooling at 4 °C. About 10 µl of PCR-amplified product (with 2 µl of 6X loading buffer) was analyzed on a 2% agarose gel in 1X TAE buffer stained with 10 mg/ml of ethidium bromide for visualization of bands for 3 h at 80 V and examined under UV transilluminator using the UVitech gel documentation system (Bangalore genei, Bangalore, India).

Results and Discussion

20 RAPD primers listed in table 1 were used to analyse gDNA of Jamun seedlings and mother plant, among them Five RAPD primers (*viz*., OPP-04, OPT-06, OPT-07, OPH-07 and OPH-
20) were chosen for the individual analysis of the seedlings. 27 Seedlings, polyembryony as well as moloembryony, were chosen for the analysis. Among the selected primers OPP-04, OPT-06 and OPT-07 showed clear banding patterns in both parents and the seedlings whereas, OPH-07 and OPH-20 did not show clear banding patterns in parents as well as in certain seedlings (Fig 1 and 2). The primer OPP-04 showed a polymorphic band between 600 and 800bp and this particular band is seen in the seedlings 6, 7, 9, 15 and 21. Even the primer OPT-06 showed a polymorphic band around 1000bp, this polymorphic band is seen in 2, 6, 7, 9, 12, 16, 17, 18, 19 and 20 seedlings. The seedlings mentioned above could be nucellar seedlings based on the preliminary observations.

Polyembryony has ecological significance as it increases the probability of survival under varied conditions. Nucellar polyembryony is the only practical approach to raise virus-free clones of polyembryonatic citrus species in nature. Disease-free plants can also be obtained through nucellar embryo culture (Kishore 2017) [10]. In crop science, aassessment of genetic diversity of cultivated crop plants is very crucial to select proper genotypes for any hybridization programme. Molecular marker can be used as an important tool of crop improvement programme and can also be helpful in protecting the biodiversity of various agro-economically important varieties of crops. It is also routinely being used in ecological, evolutionary, taxonomical, phylogenic and genetic studies of plant sciences (Escribano *et al*., 2004; Iqbal *et al*., 1997) [7, 8]. Ahmad *et al*. (2012) [1] studied the molecular character of wild jamun from Andaman and Nicobar Islands. They have co-related the wild varieties from island and variety from mainland India by using different molecular markers of RAPD and ISSR. These findings are also in accordance with Shakya *et al*. (2010) [15] in *Syzygium cuminii*, and Kingdom *et al*. (2007) [9] in *Annona spp*.

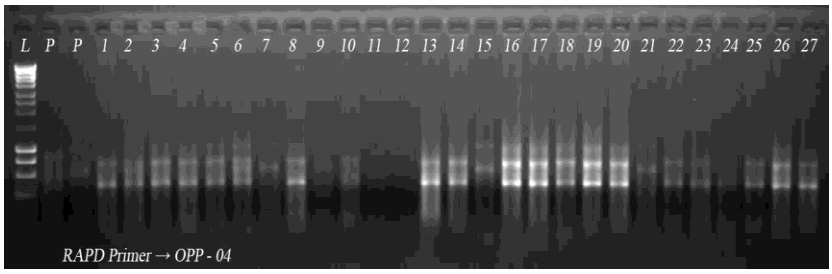

Fig 1

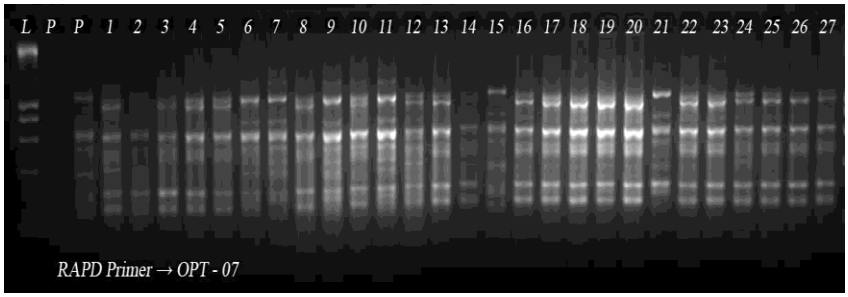

Fig 2

Fig 1, 2: Represents the Agarose gel pictures showing the amplifications by RAPD Primers *viz*., OPP-04 and OPT-07 in Jamun mother plant and seedlings gDNA.

Symbols L- Hyper Ladder- 1 (200, 400, 600, 800, 1000, 1500, 2000bp and so on.) P- Mother Plant; Numbers –Designated Seedlings

Table 1: List of RAPD primers and % polymorphism

S. No.	Primer	Sequence 5'-3'	% of GC content	Polymorphism (%)
1	OPA-02	TGCCGAGCTG	70	57.1
2	OPA-04	AATCGGGCTG	60	63.9
3	OPA-05	AGGGGTCTTG	60	60.0
4	OPA-06	GGTCCCTGAC	70	59.9
5	OPA-07	GAAACGGGTG	60	61.0
6	OPA-10	GTGATCGCAG	60	44.8
7	OPF-02	GAGGATCCCT	60	63.1
8	OPF-03	CCTGATCACC	60	50.0
9	OPF-04	GGTGATCAGG	60	42.9
10	OPF-07	CCGATATCCC	60	63.6
11	OPH-07	CTGCATCGTG	60	72.6
12	OPH-20	GGGAGACATC	60	70.0
13	OPP-04	GTGTCTCAGG	60	75.4
14	OPP-05	CCCCGGTAAC	70	33.3
15	OPQ-01	GGGACGATGG	70	47.1
16	OPQ-02	TCTGTCGGTC	60	49.5
17	OPQ-03	GGTCACCTCA	60	62.5
18	OPQ-04	AGTGCGCTGA	60	50
19	OPT-06	CAAGGGCAGA	60	69.9
20	OPT-07	GGCAGGCTGT	70	75.0

Conclusion

To conclude the study, the polyembryony is very important characteristic not only in jamun but also in various other horticultural crops. However, the genetic study in this crop is not advanced as compared to other crops. RAPD primers are often not reproducible but it can be used for primary screening and further studies may help in identifying the loci which is capable of differentiating the nucellar seedlings from zygotic seedlings in Jamun, which helps in recognizing true to type seedlings. Moreover, Seedlings classified as zygotic have a different RAPD profile from that of the mother plant or nucellar seedlings.

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