Influence of gibberellic acid on seedlessness in jamun (*Syzygium cumini* L. Skeels)

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An experiment was conducted using jamun to assess the physiology of seedless fruit formation. Seedless jamuns have higher content of gibberellic acid (GA_3) (0.876 mg g^{-1}) than seeded fruits (0.461 mg g^{-1}) produced in the seedless genotype. Consequently, GA_3 at 100 ppm was sprayed over the jamun trees that resulted in 99.5% seedless fruit production, which was higher than the unsprayed control (72.6%). Also, the application of GA_3 at 300 ppm resulted in 36.4% seedless fruit formation in the seeded genotype. In corroboration with embryo abortion by GA_3, the fruits sprayed with the latter at 100 ppm were analysed for their GA_3 content. The results indicated that GA_3 content was highest (5.609 mg g^{-1}) in these fruits when compared to control (0.683 mg g^{-1}).

Keywords: Fruit production, growth hormone, gibberellic acid, jamun, seedlessness.

JAMUN (*Syzygium cumini* L. Skeels) is an important evergreen tropical fruit tree grown in India, Bangladesh, Burma, Nepal, Pakistan, Sri Lanka and Indonesia. The violet or white fruits of these trees are rich in minerals and vitamins, particularly iron, and vitamins A and C (ref. 1). This anti-diabetic plant is considered an integral part of various alternative systems of medicine. Various extracts of jamun possess a range of pharmacological properties, such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-ulcerogenic, cardio-protective, anti-allergic, anticancerous, radio-protective, anti-oxidant, hepato-protective, anti-diarrhoeal, hypoglycaemic and anti-diabetic effects[2]. Additionally, this plant is rich in compounds containing anthocyanins, glucosides, ellagic acid, isoquercetin, kaemferol and myrecetin[3]. The seeds are claimed to contain the alkaloid jambosine and the glycosides jambolin or antimellin, which halt the conversion of starch into sugar[4].

Large-scale variations with respect to fruit morphology, fruit quality, maturity and productivity have been reported owing to its cross-pollination and seed propagation. Usually, the improved cultivars were superior in all the parameters compared with the other indigenous cultivars, as they were found to be of substandard fruit quality because of the largest seed[5]. Seedless types were also reported in the natural population. Variations were found in J-22 selection, which was propagated through seeds, and for being the most dissimilar genotype from other accessions, it was found to be unique in being seedless[6]. The genotypes possessing thin seeds with negligible seed weight (0.12–0.31 g) might be considered as seedless jamun[7].

Seed setting and fruit development were found to be intimately connected and controlled by the phytohormones[8,9]. Fruit growth can be uncoupled from fertilization and seed development[10] as indicated in the seedless fruit plants. A plant is considered to be seedless when its fruits lack seeds or contain a much reduced number of seeds or aborted seeds[11]. Generally, as seedlessness can contribute to enhancement in fruit quality in terms of taste and nutritional value, it is appreciated by the consumers for raw as well as processing purposes. Jamun is a fruit crop that has few seedless genotypes which can be explored for commercial cultivation. Therefore, the present study was conducted to identify the physiological phenomenon involved in seedless fruits and the reason for the induction of seedlessness through hormonal regulations.

An experiment was conducted to determine the physiological differences between the seeded and seedless fruits of two different jamun genotypes at the Vegetable Research Station, Tamil Nadu Agricultural University, Palur, Cuddalore district, Tamil Nadu, India (11°45′N lat. and 75°40′E long.) from 2014 to 2017. Medium-sized fruits from the plants of seedless (SC1) and seeded (SC2) genotypes were collected within 30 and 50 days after anthesis (DAA) respectively. The gibberellic acid (GA_3) content in them at 254 nm was determined using high-performance liquid chromatography (HPLC)[12]. The mature fruits of seedless plants of genotype SC1 were graded according to their weights as small (<2 g), medium (2–5 g) and large (>5 g), in which the large and medium-sized fruits had tiny seeds weighing 0.5–0.8 g, whereas the small fruits had absolutely no seeds (Figure 1 *a*). The seeds of plants with genotype SC2 weighed about 1.2 g (Figure 1 *b*).

Another experiment was conducted to assess the effect of GA_3 application on the production of seedless fruits. GA_3 was sprayed over the marked branches of jamun plants of SC1 (seedless) and SC2 (seeded) genotypes at different concentrations, viz. T_1 – control, T_2 – GA_3 spray at 100 ppm, T_3 – GA_3 spray at 200 ppm and T_4 – GA_3 spray at 300 ppm. The growth hormone was sprayed twice, i.e. first at the bud-breaking stage and second at the flowering stage over the plants. Then, the total number of buds, flowers, fruits and the percentage of fruit set were recorded for both genotypes. The fruits from SC1 sprayed with the phytohormone at 100 ppm were analysed for their GA_3 content.

The other fruit quality traits like total soluble solids (TSS), pH, acidity, vitamin C, fibre, total sugar and reducing sugar contents were analysed. TSS was estimated using a digital

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bench refractometer[13], whereas moisture content (%) of the fruits was determined using the hot-air oven method. pH of the fruits was measured using a pH meter. Other components, viz. fruit titrable acidity[14], vitamin C[15], total fibre content[16] and sugars (total sugar and reducing sugar)[17] were estimated using standard methods. The overall data were collected and analysed at 5% probability level[18].

Figure 1. Seedless and seeded fruits of jamun. ***a***, C/s of SC1 fruit (bigger fruit with seed and smaller fruit without seed); ***b***, C/s of SC2 fruit (seeded).

Figure 2. Gibberellic acid content in seedless (SC1) and seeded (SC2) jamun fruits.

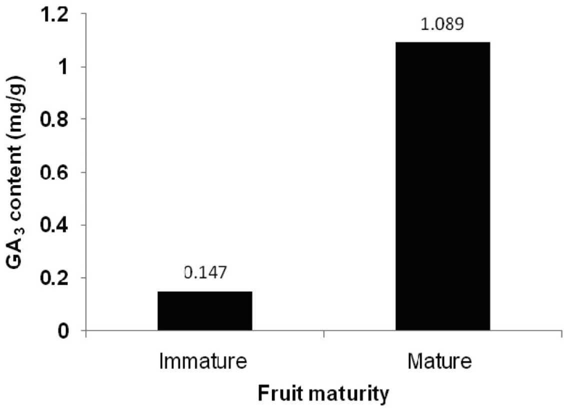

Figure 3. Gibberellic acid content in immature and mature fruits of seedless jamun (SC1).

The results of the analysis showed that the seedless genotype (SC1) contained a higher concentration of GA_3 (0.713 mg g^{-1}) than the seeded genotype (SC2, 0.053 mg g^{-1}) (Figure 2). The increase in GA_3 content with fruit growth and development was determined by collecting immature and mature fruits. The collected immature fruits showed minimum GA_3 concentration (0.147 mg g^{-1}) at 30 DAA, whereas the mature fruits showed a higher concentration (1.089 mg g^{-1}) of this phytohormone (Figure 3). In addition, the large-sized fruits of the seedless genotype had the lowest amount of GA_3 (0.461 mg g^{-1}) followed by the medium and small-sized fruits having GA_3 concentration of 0.713 and 0.876 mg g^{-1} respectively (Figure 4). Generally, the small-sized fruits of the SC1 genotype were devoid of seeds, while the large- and medium-sized fruits had smaller seeds weighing 0.6–0.8 g. Therefore, the fruits of these plants with seedless genotypes possess higher GA_3

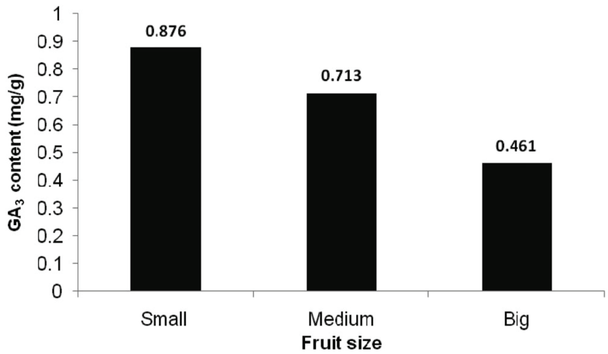

Figure 4. Gibberellic acid content in different fruit sizes of seedless jamun (SC1).

Figure 5. C/s of SC1 fruits (bigger and smaller fruits without seeds after GA_3 application).

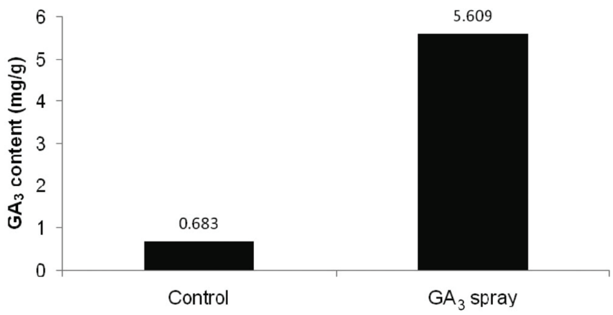

Figure 6. Effect of external GA_3 application on gibberellic acid content produced in seedless jamun (SC1) fruits.

Table 1. Effect of GA_3 application on fruit traits in seedless (SC1) and seeded (SC2) jamun genotypes

	Total no. of flowers per inflorescence		Total no. of fruits per bunch		No. of seedless fruits per bunch		Seedless fruits (%)	
Treatment	SC1	SC2	SC1	SC2	SC1	SC2	SC1	SC2
T_0 – control	216.6	59.3	56.0	19.3	39.7	0	72.6	0
T_1 – GA_3 spray @ 100 ppm	194.3	30.7	71.0	7.0	70.7	0	99.5	0
T_2 – GA_3 spray @ 200 ppm	177.0	57.0	70.0	10.0	70.0	0	100.0	0
T_3 – GA_3 spray @ 300 ppm	151.7	140.0	50.7	30.7	50.7	11.0	100.0	36.4
Mean	184.9	71.8	61.9	16.8	57.8	2.8	93.0	9.1
	Genotype	Treatment	Genotype	Treatment	Genotype	Treatment	Genotype	Treatment
SEd	12.5	17.6	3.8	5.1	2.0	2.8	1.4	2.0
CD (P = 0.05)	26.7	NS	7.6	10.3	4.1	5.9	3.0	4.1

Table 2. Effect of GA_3 application on the biochemical constituents of seedless jamun (SC1)

Treatment	Moisture (%)	Total soluble solids (^{0}Brix)	Acidity (%)	pH	Vitamin C (mg 100 g^{-1})	Fibre (g)	Total sugar (%)	Reducing sugar (%)
Control	87.9	11.0	1.15	3.8	38.8	0.22	10.5	4.7
GA_3 spray @ 100 ppm	87.5	8.7	1.12	4.2	13.0	0.22	7.3	2.2

concentration than the seeded genotypes. The parthenocarpic fruit contains higher levels of GA_3-like substances than the seeded fruits[19], which clearly indicates the presence of high GA_3 concentration at the early stage of fruit development, leading to embryo abortion and resulting in seedless fruit formation. Usually, three fertilization-induced hormones, viz. auxin, gibberellin and cytokinin play a major role in the regulation of fruit set[20], where the parthenocarpic fruit production is induced by auxin that is partially mediated through GA_3. This is because co-treatment with paclobutrazol and auxin greatly reduces parthenocarpy, which could be reversed by the co-application of auxin with GA_3 (ref. 21). The bioactive GA_3 level was elevated in the auxin-induced parthenocarpic fruits due to increased expression of GA_3 biosynthetic genes and reduced expression of GA_3 catabolic genes[22]. In addition, the elevated GA_3 or auxin signalling could induce parthenocarpy or seedless fruit production[23]. Similar findings were also recorded in jamun plants, where higher level of GA_3 produced seedless fruits.

In order to increase the hormonal level in fruits and induce seedless fruit production, GA_3 was sprayed, which resulted in induced production of seedless fruits in both the plants with seedless and seeded genotypes. Generally, plants of the SC1 genotype had a greater number of flowers (216.6) and fruits (56.0) in a bunch than those of the SC2 genotype and the unsprayed control (59.3 and 19.3 respectively) (Table 1). However, the number of flowers and fruits was reduced in plants of genotype SC1 after the application of GA_3. On the contrary, the number of flowers and fruits was increased in the SC2 plants after external application of GA_3 at 200 and 300 ppm. It is evident from the study that the increased GA_3 level in the reproductive system might have caused abscission of flowers and fruits in the seedless genotype, in which it already contained a higher concentration of GA_3 than the seeded fruits. In addition, the number of seedless fruits was higher (39.7%) than the unsprayed control plants of the SC1 genotype (72.6%), which was then increased to 99.5% when treated externally with GA_3 at 100 ppm (Figure 5). Moreover, cent per cent seedlessness was observed in the SC1 plants after treatment with 200 and 300 ppm of GA_3, whereas 36.4% seedlessness was observed in the SC2 plants after the application of GA_3 at 300 ppm. In evidence of seedless fruit production by GA_3 application, the seedless fruits of SC1 genotype showed that the unsprayed control had lower GA_3 (0.683 mg g^{-1}) compared to the fruits sprayed with GA_3 (5.609 mg g^{-1}) (Figure 6). This might be due to embryo abortion by higher concentration of externally applied GA_3. Similar findings related to GA_3 application for seedless fruit production were recorded in grapes[24,25], mandarin[26], rambutan[27], tomato[10,23,28] and pumpkin[29].

GA_3 was effective in grapes, when it was applied between anthesis and petal-fall stages[30]. GA_3 application at 100 mg l^{-1} successfully induced the production of seedless grape berries with enhanced berry size and accelerated fruit development, resulting in earlier ripening of the seedless berries[25]. Single application of GA_3 at 2.5 g ha^{-1} during 80–100% blooming was found to be responsible for berry-thinning, which significantly reduced berry set and increased berry weight rather than multiple applications[31]. Protein synthesis is a major biological process that differentiates between fruit set and fruit abortion, where different protein expression patterns are involved in chemically induced parthenocarpy and natural parthenocarpy[32].

In addition, some of the fruit quality traits like concentration of TSS, vitamin C, total sugar and reducing sugar were reduced in the fruits treated with GA_3 than the control (Table 2). Similarly, the total concentration of phenolics and flavonoids as well as antioxidant activity were greatly reduced in berry skins and pulp after GA_3 application in grapes[25]. Also, GA_3 and 4-CPA significantly increased the concentration of malate in mature fruits, but reduced the concentration of proline compared to unsprayed control[24].

Hence, it can be concluded that GA_3 causes seedlessness of jamun. Application of GA_3 at 100 ppm during bud-breaking and flowering stages enhanced seedless fruit production, which was higher (99.5%) in the seedless genotype than the control (72.6%). Application of GA_3 at 300 ppm induced seedlessness up to 36.4% in the seeded genotype.

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