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Morphological and biochemical methods for sex determination in papaya (*Carica papaya*) seedlings

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Abstract
A single female papaya plant normally produces as many as 100 fruits in its life cycle and about 250 gm of crude papain a year. Thus, an increase in the number of fruit bearing trees per hectare of land would directly increase in the yield of fruits and papain, making the cultivation more profitable. A field study was carried out at Crop Physiology Unit, Department of Agronomy, Agricultural College & Research Institute, Killikulam, Vallanad, Tuticorin district of Tamil Nadu. Two varieties of papaya differing in their sex behaviour viz., CO 8 a dioecious variety and Red lady, a hermaphrodite variety were selected. The seed physiological characters such as germination percentage, pH and EC of seed leachate were studied. The biochemical constituents of the papaya leaves such as protein, phenol and IAA oxidase activity were estimated. It was found that a highly significant positive correlation was found to exist between 100 seed weight and male sex, but a negative correlation with females and hermaphrodite. The IAA oxidase activity was considered as one of the aids for sex differentiation.

Keywords: Papaya seedling, Papain, Sex differentiation, pH, EC and IAA oxidase

Introduction
Papaya exhibits wide morphological and biological diversity of its types with prominent sex specific characters. The papaya plants can be either dioecious with male and female flowers occurring in separate plants or gynodiocious with male and female parts befalling in the same flower. Monoecy, presence of male and female flowers on the same plant is also found in some related species of papaya. In papaya the change of sex occurs in some trees at high temperature, where short stalked male flowers are produced instead of usual perfect flowers. Male or bisexual plants changing completely too female plants after being beheaded and some "all male" plants occasionally producing small flowers with perfectly pistils leading to abnormally slender fruits are also instances of change of sex in papaya. If the sex of dioecious papaya is identified at seedling stage, prior to their transplantation to the field, cultivation of male and female plants in a desired ratio would be achieved and resources like planting space, fertilizers and water could be devoted to female plants. A single female papaya plant normally produces as many as 100 fruits in its life cycle and about 250 gm of crude papain a year. Thus, an increase in the number of fruit bearing trees per hectare of land would directly increase in the yield of fruits and papain, making the cultivation more profitable. Therefore, it is of immense agricultural importance to identify sex of papaya plants at juvenile stage.
The cultivated papaya varieties grown all over the world are mainly of two types: Dioecious and Hermaphrodite. Papaya production in Hawaii is based on hermaphrodite variety Solo, where as in Africa, Dioecious variety Hortus gold is considered to be the most important (Manasha Ram 2009) [12]. Since papaya fruit is the sole source of commercial papain, cultivation of high papain yielding varieties is becoming very essential to meet the need of domestic and international markets. The dioecious papaya varieties are always preferred for extraction of papain all over the world, because the yields and proteolytic activity of crude papain obtained from female fruits are greater than that of hermaphrodites (Madrigal *et al*., 1980) [9]. Marked variations are reported among different dioecious and hermaphrodite varieties grown in India for yield of papain. Among 70 varieties evaluated by Muthukrishnan and Irulappan (1995) [13], a dioecious variety Coimbatore (CO 2) has recorded highest papain

held of 686.29 g per plant in a period of 6 months as against an average yield of 125 g of papain produced by other varieties. Hence, it is considered as the most important variety in India and Srilanka for commercial papain production. Cultivation of dioecious varieties is therefore recommended for places where hermaphrodite types are excessively susceptible to environmental factors.

Materials and Methods

A field trial is being operated from 2013 at Crop Physiology Unit, Department of Agronomy, Agricultural College & Research Institute, Killikulam, Vallanad, Tuticorin district. Two varieties of papaya differing in their sex behavior viz., CO 8 a dioecious variety and Red lady, a hermaphrodite variety were taken up for the study. Seeds of these two varieties were classified based on their size which saved as treatments. S_0 – Control (Unclassified bulk); S_1 - Extra large (6 mm) ; S_2 - Large (5mm) ; S_3 – Medium (4mm) ; S_4 - Small (3mm). The sowing was taken up in the evening hours at the rate of five seeds per polythene bags. After 45-50 days, the seedlings were planted in the main field in individual pits of 45 x 45 x 45 cm size dug out at a spacing of 1.8 meters either way. The pits were filled with sand and red earth with 25 kg of farmyard manure. Immediately after planting, shade was provided to young seedlings and watering was given with rose can. Pot watering was continued until the seedlings established well. The plants were manured with Urea and phosphate 100g each and Muritate of Potash 200 g at regular intervals of three months. The work was carried out to cover the physiological and biochemical aspects. The seed morphological characters such as 100 seed weight were noted. The seed physiological characters such as germination percentage, pH and EC of seed leachate were studied. The biochemical constituents of the papaya leaves such as protein, phenol and IAA oxidase activity were estimated. Protein content of the leaves was estimated by Lowry's method and expressed as mg/g of fresh tissue. The Phenol content of papaya leaves was estimated as per the procedure suggested by Malik and Singh (1980), and result was expressed as mg/g of fresh tissue. The enzyme activity was calculated and expressed as µg of auxin oxdised / 2 hours / g of fresh tissue (Mahadaeven, 1975) [11]

Result and Discussion

Regarding the 100 seed weight between two varieties, CO 8 had higher 100 seed weight (1.87 g) compared to Red lady (1.43 g). The 100 seed weight in CO 8 ranged from 1.25 to 2.00 g and in Red lady from 1.00 to 1.83 g. The 100 seed weight noted from S_1 (2.00) followed by S_2 (1.93), S_3 (1.67) and S_4 (1.25). The same pattern was observed in Red lady. CO 8 was found to be significantly superior to Red lady with regard to 100 seed weight. The 100 seed weight was more in S_1 than others. A highly significant positive correlation was found to exist between 100 seed weight and male sex, but a negative correlation with females and hermaphrodite. In contrast, Diware *et al.* (1992) [4] working on CO 5 papaya found higher female: male ratio when bold seeds were used. Again, they ascertained that black coloured seeds had produced more females than males.

Table 1: Effect of seed size on seed morphology and Physiological parameters in *papaya* seedlings.

S. No.	Treatment	Sizes						
		S_0	S_1	S_2	S_3	S_4	SE (d)	Cd
1.	100 Seed weight (g) CO 8 Red lady	1.87 1.43	2.00 1.83	1.93 1.71	1.67 1.62	1.25 1.00	0.05	0.11
2.	Germination (%) CO 8 Red lady	58.20 49.67	72.24 69.67	8.11 61.72	59.51 50.00	33.33 29.33	2.61	5.11
3.	Seed leachate pH CO 8 Red lady	7.27 7.99	6.97 7.11	7.27 7.28	7.43 8.10	7.70 8.30	0.19	0.24
4.	EC m mhos/cm	0.270 0.271	0.261 0.257	0.263 0.264	0.269 0.272	0.283 0.283	0.002	0.004

CO 8 had a higher germination percentage of 58.20 for control and Red lady recorded 49.67 per cent. The germination percentage ranging from 33.33 to 72.24, similarly in Red lady the values ranging from 29.33 to 69.67 per cent. Higher germination was recorded by S_1 (72.24) followed by S_2 (68.11), S_3 (59.51) and S_4 (33.33) percent. In Red lady, S_1 had a higher germination of 69.67, followed by S_2 (61.72), S_3 (50.0) and S_4 (29.33 per cent)

pH: The seeds were soaked in water for 24 hrs at room temperature and the pH and EC of seed leachate were measured. The seed-steep water was decanted and referred to as seed leachate. The pH, one of the estimates of nutrient availability, was higher in Red lady (7.99) compared to CO 8 (7.27). In CO 8, S_4 had a higher pH of 7.70 followed by S_3 (7.43), S_2 (7.27) and S_1 (6.97). In case of Red lady, S_4 had a higher pH of seed leachate (8.30) followed by S_3 (8.10), S_2 (7.28) and minimum was noted in S_1 (7.11). No significant correlation between the sex and pH of the seed.

EC: Measurements of Electrical conductivity of the seed leachate showed that both varieties has similar values of 0.270 and 0.271mmhos/cm in CO 8 and Red lady respectively. The EC of S_4 was 0.283 followed by S_3 (0.269), S_2 (0.263) and S_1 (0.261). In the case of Red lady also similar trend was noticed significant negative correlation between EC of seed leachate and 100 seed weight. However no significant correlation was observed between EC of seed leachate and sex. The study of seed leachates indicated that the leachate of small seeds had high pH and EC. As the seed size increases, the pH of the seed leachate decreased. Also, a positive correlation was observed between moisture content and EC of seed leachate (r = 0.896**); in addition, a negative correlation was obtained for 100 seed weight and EC of seed leachate (r = -0.827**). (Bass (1975) also confirmed this earlier with his work on papaya seeds and reported that the seed lots with the highest moisture content on storage, showed the largest germination decline. These results clearly indicated that the quality of smaller seeds was very poor compared to the bigger seeds. This may be because of the rapid conversion of soluble metabolites which could be leached out from the seed resulting in the depletion of nutrients for the germinating seeds. The leaching out of metabolites is possibly due to the permeability of the weaker cell membrane allowing the leachates to diffuse out. Heydecker (1973) [7] attributed weakening of cell membrane as the cause of leaching of metabolites, electrolytes and other soluble components through their membrane into the imbibing medium. Also, the

negative association of seed leachate tests with seed quality was proved beyond doubt by Takayanaki and Murakami (1968) [15]. Dharmalingam *et al*. (1976) [3] also reported that increased leaching of electrolytes were the indicators of loss of viability.

Table 2: Effect of seed size on sex ratio

Treatment	Variety	S_0	S_1	S_2	S_3	S_4	SE (d)	CD
Ratio (%)	Female							
	CO 8	46.7	33.3	46.7	73.3	66.7	6.7	13.7
	Red lady	66.7	40.0	40.0	63.3	30.0		
	Male							
	CO 8	53.3	66.7	53.3	26.7	33.3	6.8	13.7
	Red lady Hermaphrodite	33.3	60.0	60.0	36.7	28.5		

The number of female plant produced in each seed group of two varieties was counted and expressed in percentage. In CO 8, S_3 (medium) recorded the maximum number of females (73.3) followed by S_4 (66.7), S_1 recorded minimum percentage of 33.3 female plants.

Male and Hermaphrodite

The number of male and hermaphrodite produced by each seed group was observed in CO 8 and Red lady respectively. In CO 8, S_1 (Extra large) recorded more male (66.7 per cent), followed by S_2 (53.3) and the minimum by S_3 (26.7 per cent). In Red lady S_1 and S_2 showed the higher number of hermaphrodites (60.0 per cent) followed by S_3 (36.7 percent) and minimum by S_4 (28.5). With regard to size of seeds, in CO 8, the two larger size S_1 and S_2 recorded significantly greater number of males than S_3 and S_4. In Red lady S_4 recorded more hermaphrodite followed by S_2 and S_3.

Between the two varieties CO 8 recorded significantly more number of females than Red lady.

Protein content of papaya leaves was estimated at seedling stage of crop (45 DAS) and expressed as mg per g fresh tissue, In CO 8, S_1 recorded greater amount of protein (158.0) compared to S_2 (132.9), S_3 (95.2) and S_4 (87.0mg). In Red lady again S_1 recorded the maximum leaf protein of 116.2 followed by S_2 (110.1) S_4 (107.5) and S_3 (92.0 mg). Between two varieties Red lady had significantly more protein than CO 8. Among the different size groups S_1 had significantly more protein content. There was no significantly correlation between the protein content at seedling stage and sex ratio.

Table 3: Effect of seed size on Biochemical parameters in *papaya*

S. No.	Treatment	Variety	S_0	S_1	S_2	S_3	S_4	SE (d)	CD
1.	Leaf protein mg/g	CO 8	97.8	158.0	132.9	95.2	87.0	3.8	7.7
		Red lady	125.5	116.2	110.1	92.0	107.5		
2.	Phenol mg /g	CO 8	0.437	0.623	0.605	0.511	0.529	0.002	0.004
		Red lady	0.453	0.671	0.526	0.480	0.513		
3.	IAA oxidase activity (µg / g)	CO 8	0.842	0.919	0.801	0.761	0.815	0.005	0.011
		Red lady	0.771	1.301	0.961	0.764	0.846		

At the seedling stage, in CO 8 S_1 recorded greater phenol content of 0.623 mg, followed by S_2 (0.605), S_3 (0.511) and S_4 (0.529 mg/g). In the case of Red lady S_1 produced leaves with higher phenol content (0.67 mg), compared to S_2 (0.526) S_4 (0.513) and S_3 (0.480). Jindal and Singh (1975) [8] reported a higher amount of free and bound phenolics in male plants compared to females.

Red lady had significantly more amount of phenol than CO 8. Among the various sizes S_1 had significantly more phenol in both the varieties. A highly significantly negative correlation was observed between the phenol content at seedling stage and female sex ratio. Again, among the sexes, the males had significant amounts of leaf phenol than the hermaphrodites and lower in the females. Similar results were also reported by Jindal and Singh (1975) [8] and Bhattacharya and Rao (1982) [2]. The type and amount of phenolic compounds in the plant may regulate the level of metabolically active auxins (Nitsch and Nitsch, 1962) [14]. The role of auxin in sex differentiation, especially feminization had been proved beyond doubt by Galun *et al*. (1965) [5]. Since many of the polyphenolic substances such as caffeic acid and quercetin which were identified in the papaya leaves (Jindal and Singh, 1975) [8] have been found to synergise the action of IAA (Tomaszewski, 1964) [16] which is not necessary for males and hence the phenolic content of males, was high. In the case of females, since the auxins play an important role in feminization, the role of synergetic and inhibitory phenols of IAA is reduced possibly for the conversion of female flowers.

The leaves of papaya were analysed for IAA oxidase activity at seedling stage and the results were expressed as IAA oxidased (µg/g/two hours of fresh tissue). In the seedling stage it was found that greater IAA oxidase activity was shown by S_1 of CO 8(0.919 µg), followed by S_2 (0.815), S_4 (0.801) and S_3 (0.761). In Red lady, S_2 showed the greater activity of the enzyme (1.301 µg) followed by S_1 (0.961), S_2 (0.840), and S_4 (0.764 µg/g) (Above all treatments extra large (S_1) of CO 8 and Red lady S_2 (0.846) recorded maximum IAA oxidase activity. Medium size of CO 8 of large and Red lady recorded minimum amount. The enzyme activity was very high in the leaves of male plants, followed by the hermaphrodites and the least in the females. This is because, the IAA content was higher in the females since it is a feminizing hormone which effects its activity by inducing the Etheral evolution. Also, the IAA content of males was very low compared to the females.In the different colours and sizes of seed groups, the enzyme activity was significantly greater in those which were in favour of maleness (the brown colour (C_2) and the smaller seed groups S_3 & S_4). Thus, the IAA oxidase activity can be considered as one of the aids for sex differentiation.

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