DOI : 10.5958/0974-0112.2017.00005.6

Indian J. Hort. 74(1), March 2017: 6-10

Efficiency of morphological, physiological and biochemical parameters related to sex expression in papaya

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ABSTRACT

The papaya (*Carica papaya* L.) is a polygamous plant, it has three types of sex forms, *viz.* male, female and hermaphrodite. The efforts were made to identify sex expression based on morphological, physiological and biochemical parameters. The matured plants were uniformly maintained for nutrition and intercultural operations in the nethouse and parameters were recorded accordingly. For the tabulation and data analysis, four genotypes were grouped in male (M), female (F) and hermaphrodite (H) making eight treatments, *i.e.*, Pusa Nanha (F), Pusa Nanha (M), P-7-2 × SA M (F), P-7-2 × SA M (M), Red Lady (F), Red Lady (H), P-9-5 (F) and P-9-5 (H). Results showed that the maximum number of plants expressed their sex type in a range of 46-60° orientation of petiole, higher leaf chlorophyll 'a' content (2.573 mg/g f.wt.), in hermaphrodite sex plants in Red Lady and P-9-5 and lower levels in male plants. The phenolic content was significantly higher in the female plants followed by the hermaphrodite and male counterparts at seedling stage. The lowest total phenols content (29.25 and 39.50 mg/100 g f.wt.) was in hermaphrodite plants of Red Lady and P-9-5, respectively compared to female plants but higher than male plants among the dioecious genotypes at 10-leaf stage. The significant difference in total phenols content among hermaphrodite types was in Red Lady and P-9-5. Leaf gas exchange parameters were also indicative of sex expression at seedling stage. In female plants, the maximum stomatal conductance (0.523 mmol $m^{-2}s^{-1}$) was recorded in Red Lady followed by (0.353 mmol $m^{-2}s^{-1}$ in P-9-5 in comparison to hermaphrodite (0.435 mmol $m^{-2}s^{-1}$) plant in Red Lady followed by (0.280 mmol $m^{-2}s^{-1}$ in P-9-5. However, minimum stomatal conductance was recorded in male plants. The higher stomatal conductance indicated feminism in papaya followed by hermaphrodite plants.

Key words: Chlorophyll, papaya, total phenols, sex expression, stomatal conductance.

INTRODUCTION

The papaya (*Carica papaya* L.) is a native of Central and South America and belongs to family Caricaceae. It is a commercial fruit crop cultivated throughout the tropical and sub-tropical regions of the India and ranks fifth with regard to area and production. Both production and consumption of papaya has increased several folds in the last two decades.

Seed propagation in papaya is commercial method of raising the crop, being a polygamous plant, growers cannot identify the productive and unproductive plants at nursery stage, so as to have desired planting ratio of male and female plants. Identification of the desirable plants at seedling stage would help in raising the orchard with appropriate design. The leaf extracts of a large number of sexually undifferentiated seedlings at nursery stage, with modified Almen's reagent were analysed. Based on this colorimetric reaction, it was found to be 67% true in case of male and 87% in case of female sex form (Singh *et al*., 8). A colorimetric test for total phenols can differentiate females (86%) from males (77%), but was unable to detect the bisexual plants (Jindal and Singh, 4). Paper chromatography also indicated that trans-cinnamic acid to be has expressed in the leaves of hermaphroditic seedlings, but females and males could not be differentiated (Poller, 6). In addition, isozymes have been exploited to identify markers that could co-inherit with sex types in papaya. Using the banding patterns of cationic peroxidase, male plants could be differentiated from females, but female plants could not be distinguished from hermaphrodites (Sriprasertsak *et al*., 9). However, these studies were primarily with dioecious papaya genotypes. Hence, present study was undertaken to distinguish the three types of sex forms through morphological, physiological and biochemical parameters at seedling stage.

MATERIALS AND METHODS

The present investigation was carried out at the Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi during 2014-2015, to study the morphological, physiological and biochemical traits related to sex expression in dioceious and gynodioecious papaya genotypes. The differentiated plants of four genotypes were grouped in male (M), female (F) and hermaphrodite (H) making eight

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treatments, *i.e.*, Pusa Nanha (F), Pusa Nanha (M), P-7-2 × SAM (F), P-7-2 × SAM (M), Red Lady (F), Red Lady (H), P-9-5 (F) and P-9-5 (H). Twenty five seedlings were uniformly maintained under proper seedlings nutrition and intercultural operations in the net house. Only 20 seedlings were subjected for taking observations. For the tabulation and data analysis, in each genotype, data was collected on four seedlings and pooled for drawing a mean of one treatment. Seedlings were selected at random and tagged and transplanted in field adopting randomized block design.

Plant tissue were sampled from seedlings at 10-leaf stage for estimating the chlorophyll contents -'a', 'b' and 'total' and 'a/ b' ratio as per the methods suggested by (Hiscox and Israelstam, 3). The total phenols were determined by Folin-Ciocalteau reagent method. The photosynthesis (*A*, μmol $m^{-2}s^{-1}$), stomatal conductance of water (*gs*, mol $m^{-2}s^{-1}$), PAR incidence on leaf surface (Q leaf, μmol $m^{-2}s^{-1}$) were measured by using IRGA (LCi-SD portable photosynthesis system). The leaf gas exchange parameters were also recorded at 3 leaf stage at 5 intervals from the individual plants across the genotypes and based on their sex expressed, four plants were selected for each replication. The observations were recorded at seedling stages and flowerings were also recorded later in the same plants to establish the relation and efficiency of various parameters in prediction of sex types.

RESULTS AND DISCUSSION

The efforts were made to identify morphological, physiological and biochemical parameters related to sex expression, the data present in Table 1 shows stem colour in papaya seedlings consisting of male, female and hermaphrodite plants. The greater number of productive plants were recorded based on their sex expression where light green stem colour at seedling stage in both Pusa Nanha and Red Lady. The male plant type had express all four colours of stem, *i.e.* Light green, green, purple and light purple in P-7-2 × SAM which might be due to heterozygous nature of genotype where either parent contribute purple or light purple stem colour. The unique purple colour was noticed uniformly in P-9-5 seedlings and higher numbers of the hermaphrodite plants were observed in green purple colour at the base of the stem. Among the dioecious and gynodioecious genotypes, the light purple colour may be the useful morphological marker for the P-9-5 to enhance the prediction of female or hermaphrodite plants within the population. The concepts of botanical traits related to sex expression was described by Storey (10).

Table 1. Stem colour of papaya seedlings in relation to sex expression.

Genotype	Stem colour			
	Light green	Green	Purple	Light purple
Pusa Nanha (F)	7	5	0	0
Pusa Nanha (M)	4	4	0	0
P-7-2 × SAM (F)	5	3	0	1
P-7-2 × SAM (M)	3	7	1	1
Red Lady (F)	4	5	0	0
Red Lady (H)	6	5	0	0
P-9-5 (F)	0	0	4	9
P-9-5 (H)	0	0	4	3

Data presented in Table 2 reveal a wide range of variation among the dioecious and gynodioecious plants. The maximum number of plants expressed their sex type in the range of 46-60° orientation. There was not a uniform trend of sex prediction in a particular range of orientation or in a particular genotype. However, maximum female plants were

Table 2. Orientations of petiole with stem in relation to sex expression at seedling stage in papaya.

Genotype	Orientation (degrees)					
	41-45	46-50	50-54	54-60	60-64	65-70
Pusa Nanha (F)	0	2	3	4	2	1
Pusa Nanha (M)	0	1	3	2	1	1
P-7-2 × SAM (F)	0	1	2	4	2	0
P-7-2 × SAM (M)	1	3	4	2	1	0
Red Lady (F)	1	1	5	2	1	0
Red Lady (H)	1	2	2	4	2	0
P-9-5 (F)	1	3	2	4	2	1
P-9-5 (H)	0	1	2	3	2	0

recorded in Pusa Nanha, P-9-5 and P-7-2 × SAM in (54-60°) for leaf orientation. The findings are in close conformity with report of Kumar (5). However, results are in partial conformity with earlier reports (Bojappa, 2; Kumari, 5).

The data present in Table 3 shows the chlorophyll status in papaya seedlings of male, female and hermaphrodite forms. Chlorophyll 'a' content in leaf was recorded to be maximum (2.573 and 2.480 mg/g f.wt.) in hermaphrodite plants of Red Lady and P-9-5, respectively followed by female plants of P-9-5 (2.303 mg/g f.wt.), Red Lady (2.293 mg/g f.wt.), Pusa Nanha (1.938 mg/g f.wt.) and P-7-2 × SAM (1.475 mg/g f.wt.), whereas the lowest was observed in male plants P-7-2 × SAM (0.520 mg/g f.wt.) followed by Pusa Nanha (0.328 mg/g f.wt.). However, there were significant differences for chlorophyll 'a' observed in Pusa Nanha, while in rest of the genotypes it was non-significant.

Among eight papaya plant types, chlorophyll 'b' content was recorded to be maximum 2.623 (mg/g f.wt.) in P-7-2 × SAM (F) followed by 2.418 mg/g f.wt.in Red Lady (F) and 1.108 mg/g f.wt. in Red Lady (H). There were no significant difference for chlorophyll 'b' content observed in Pusa Nanha and P-9-5, though minor differences among the sex types within the genotypes was noted.

Total chlorophyll content of papaya leaf was observed to be maximum in female plants of Red Lady (3.438 mg/g f.wt.) followed by P-7-2 × SAM (3.165 mg/g f.wt.), P-9-5 (1.853 mg/g f.wt.) and Pusa Nanha (1.645 mg/g f.wt.), whereas in hermaphrodite plants of Red Lady (2.285 mg/g f.wt.) and P-9-5 (2.155 mg/g f.wt.), while lowest content in male plants of P-7-2 × SAM (0.608 mg/g f.wt.) followed by Pusa Nanha (0.470 mg/g f.wt.). There was significant difference for total chlorophyll in genotype P-7-2 × SAM, though the rest of the genotypes showed non-significant differences. The results are in partial conformity with earlier reports (Jindal and Singh, 4; Bojappa, 2; Kumari, 5).

There was a clear trend of increasing levels of total phenols with the increase in plant age from 6- to 10-leaf stages across the genotype and sex type (Table 4). The phenolic level was significantly higher in the female plants followed by the hermaphrodite and male plants at different stages of the sexually undifferentiated papaya seedlings. The maximum leaf phenolic content (27.950 mg/100 g f.wt.) was observed in plant of the P-9-5 at 6-leaf stage followed by 24.575 mg/100 g f.wt. in Red Lady, 23.475 mg/100 g f.wt. in P-7-2 × SAM and 24.025 mg/100 g f.wt. in Pusa Nanha among their female plants. However, there was non-significant difference recorded in male and female and female and hermaphrodite plants within a genotype at 6-leaf stage. The maximum phenolic content (31.050 mg/100 g f.wt.) in leaf was observed in plants of the P-9-5 followed by Red

Table 4. Total phenols leaf in relation to sex expression at different in stages of papaya seedlings.

Genotype	Total phenols (mg/100 g f.wt.)		
	6-leaf stage	8-leaf stage	10-leaf stage
Pusa Nanha (F)	24.025	25.625	28.700
Pusa Nanha (M)	19.400	21.100	22.975
P-7-2 × SAM (F)	23.475	26.500	28.600
P-7-2 × SAM (M)	23.438	25.120	28.080
Red Lady (F)	24.575	25.825	30.675
Red Lady (H)	25.250	26.350	29.250
P-9-5 (F)	27.950	31.050	41.250
P-9-5 (H)	27.650	29.600	39.500
$LSD_{(p<0.05)}$	4.638	4.590	6.172

Table 3. Chlorophyll status in the leaf of papaya seedlings in relation to sex expression.

Genotype	Chlorophyll 'a' (mg/g f.wt.)	Chlorophyll 'b' (mg/g f.wt.)	Total chlorophyll (mg/gf.wt.)	Chlorophyll a:b ratio
Pusa Nanha (F)	1.938	0.753	1.645	2.865
Pusa Nanha (M)	0.328	0.333	0.470	0.673
P-7-2 × SAM (F)	1.475	2.623	3.165	0.630
P-7-2 × SAM (M)	0.520	0.378	0.608	1.383
Red Lady (F)	2.293	2.418	3.438	1.933
Red Lady (H)	2.573	1.108	2.285	3.743
P-9-5 (F)	2.303	0.780	1.853	2.950
P-9-5 (H)	2.480	1.015	2.155	2.595
$LSD_{(p<0.05)}$	1.499	1.211	1.692	NS

Table 5. Leaf gas exchange parameters among papaya genotypes in relation to sex expression at seedling stage.

Genotype	Leaf internal CO_2 conc. (μmol mol^{-1} $m^{-2}s^{-1}$)	Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)	Stomatal conductance (mmol m^{-2} s^{-1})	Photosynthesis rate (μmol CO_2 $m^{-2}s^{-1}$)
Pusa Nanha (F)	286.250	6.665	0.325	11.825
Pusa Nanha (M)	242.250	7.770	0.283	11.505
P-7-2 × SAM(F)	321.750	9.025	0.315	9.555
P-7-2 × SAM(M)	340.750	8.433	0.268	8.425
Red Lady (F)	286.750	9.543	0.523	16.848
Red Lady (H)	310.500	9.400	0.435	14.190
P-9-5 (F)	321.250	8.943	0.353	14.648
P-9-5 (H)	357.750	8.593	0.280	12.830
$LSD_{(p<0.05)}$	NS	NS	NS	3.550

Lady (26.350 mg/100 g f.wt.), P-7-2 × SAM (26.50 mg/100 g f.wt.) and Pusa Nanha (25.625 mg/100 g f.wt.) for the female plants at 8-leaf seedling stage. The range of phenolic content (28.70 to 41.25 mg/100 g f.wt.) was 24 among the plant at 10-leaf stage of the sexually undifferentiated seedlings. The maximum total phenolic content (41.25 mg/100 g f.wt.) was recorded in P-9-5 followed by in Red Lady (30.675 mg/100 g f.wt.), in P-7-2 × SAM (28.6 mg/100 g f.wt.) and in Pusa Nanha (28.70 mg/100 g f.wt.) among female plants, whereas, it was minimum (22.975 mg/100 g f.wt.) in Pusa Nanha followed by in P-7-2 × SAM (28.08 mg/100 g f.wt.) among the papaya male seedlings. However, phenol content (29.25 and 39.50 mg/100 g f.wt.) was estimated in hermaphrodite plants of Red Lady and P-9-5, respectively compared to female plants but it was higher than male plants among the genotypes at 10-leaf stage. The data (Table 3) further revealed that there was a significant difference among hermaphrodite plants in gynodioecious genotypes (Red Lady and P-9-5) and non-significant differences were recorded in male plants of dioecious genotypes (Pusa Nanha and P-7-2 × SAM) of the study. The findings are partially in accordance with the report of (Jindal and Singh, 4; Begum *et al.*, 1).

Wide variation for stomatal conductance (0.28 to 0.523 mmol $m^{-2}s^{-1}$) among the investigated genotypes but was non-significant (Table 5). Among the female plants, the maximum stomatal conductance (0.523 mmol $m^{-2}s^{-1}$) was recorded in Red Lady followed by P-9-5 (0.353 mmol $m^{-2}s^{-1}$) and Pusa Nanha (0.325 mmol $m^{-2}s^{-1}$) and in P-7-2 × SAM (0.315 mmol m^{-2} s^{-1}). In case of hermaphrodite plants, the maximum stomatal conductance (0.435 mmol $m^{-2}s^{-1}$) was recorded in Red Lady followed by in P-9-5 (0.280 mmol $m^{-2}s^{-1}$), whereas, minimum (0.268 mmol $m^{-2}s^{-1}$) was recorded in male plants of P-7-2 × SAM followed in Pusa Nanha by (0.283 mmol m^{-2} s^{-1}). The analysis of the data suggests that higher stomatal conductance predicted feminism in papaya followed by hermaphroditism and masculinity in a population of a genotype.

There was a significant difference among the genotypes and sex forms genotype on photosynthesis rate of the papaya seedling with a wide range of variation (8.425 to 16.848 μmol CO_2 m^{-2} s^{-1}). The higher photosynthetic rate (16.848 μmol CO_2 $m^{-2}s^{-1}$) was recorded in Red Lady followed by in P-9-5 (14.648 μmol CO_2 $m^{-2}s^{-1}$), Pusa Nanha (11.825 μmol CO_2 $m^{-2}s^{-1}$) and in P-7-2 × SAM (9.555 μmol CO_2 $m^{-2}s^{-1}$) among the female plants studied. The analysis of data suggests that photosynthetic rate was lower in case of hermaphrodite and male plants within a genotype. Similar findings were reported (Begum *et al.*, 1) for sex differentiation in papaya (Sánchez-Vilas and Retuerto, 7). They reported that sex-specific variations in eco-physiological traits in response to water availability determine the performance of each sex in different habitats

From the above discussion, it is concluded that the purple stem colour in the seedling predicted female sex form in P-9-5 papaya line. Among physiological parameters, higher leaf chlorophyll 'a' content (2.573 mg/g f.wt.) hermaphrodite type sex plants in Red Lady and P-9-5 and lower level for male plants. The phenolic level in the leaf was significantly higher in the female plants followed by the hermaphrodite and male plants at seedling stage. The higher stomatal conductance indicated female sex form in papaya followed by hermaphrodite plants.

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Received : October, 2015; Revised : September, 2016; Accepted : December, 2016