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INFLUENCE OF BIOCHEMICAL CONSTITUENTS ON THE SEX EXPRESSION OF PAPAYA

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ABSTRACT

Studies were made on the biochemical substances of papaya, in two varieties (CO 5 and SRS) at three stages of crop growth in eight different seed groups. It was found that the leaf protein content was higher in the black and bold seeds; and also in the males followed by hermaphrodites and low in females. Phenol content was lower in females than males and hermaphrodites. The reverse was true with RNA and DNA contents. IAA oxidase activity was higher in the leaves of male papaya followed by the hermaphrodites and the lower in females. The females had more cytokinin and lower GA than the males and hermaphrodites.

KEY WORDS : Papaya, seed groups, sex expression, biochemical constituents

Sex in papaya seems to be highly influenced by the biochemical substances present in the plants before flowering. Many workers have tried sex forecast in papaya on the basis of colour reaction of dried tissue through chemical tests. However, 100 per cent forecast has not been possible. Jindal and Singh (1976) observed specific patterns of protein in the male and female flowers and plants of papaya, and also changes in the patterns, specific to each sex, as the flowers developed. Dutta and Mazumdar (1989) reported that the leaf protein content was higher in the female trees of papaya over male trees. Jindal and Singh (1975) reported that the amounts of free - acid and alkali hydrolysable phenolic compounds were considerably higher in male plants. A comparison was attempted between the typically dioecious male and female trees of papaya by Nandi and Mazumdar (1990), who reported a higher level of endogenous RNA and histone protein content in the female plants. Hasdiseve *et al.*, (1989) reported that male papaya had the highest levels of auxin - and gibberellin - like substances while females had the lowest. Khryanin (1989) reported that in hemp and spinach cytokinin activity was greater in female plants than males ; GA activity was more in males than in females.

MATERIALS AND METHODS

The study was conducted at the Agricultural College and Research Institute, Madurai during 1991-92. Two varieties of papaya were taken up for study namely, SRS (V_1) and CO 5 (V_2). The seeds were grouped into eight classes based on their size as extra large (S_1), large (S_2), medium (S_3), and small (S_4) using BSS wire mesh sieves, and based on their colour as black (C_1) and brown (C_2). The biochemical constituents of papaya leaves such as

Table 1. Effect of seed grouping on leaf protein (mg/g)

Treatments / Stages		CoSo	C_1				C_2				SED	CD 0.05
			S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4		
Stage 1	V_1	95.3	132.0	87.0	158.0	95.2	113.2	128.7	121.3	106.0	3.7	7.5
	V_2	111.7	92.0	116.0	110.0	107.0	106.0	115.0	100.0	90.0		
Stage 2	V_1	105.7	145.3	177.0	160.3	96.9	127.1	137.1	130.0	115.2	4.7	9.6
	V_2	84.4	100.8	131.9	116.9	120.7	131.2	134.2	105.9	91.4		
Stage 3												
Sex - 1	V_1	114.6	109.8	100.6	78.0	131.3	108.3	85.4	94.1	95.6	5.6	11.4
	V_2	103.3	95.0	102.1	97.5	91.4	109.0	101.4	100.0	88.0		
Sex - 2	V_1	110.8	108.1	96.3	77.7	121.0	128.4	127.5	104.6	97.5	9.1	18.5
	V_2	126.7	111.3	103.0	102.5	95.7	112.5	107.5	101.9	92.7		

Stage 1 - Seedling stage
Stage 2 - Vegetative stage
Stage 3 - Reproductive stage
Sex - 1 - Females
Sex - 2 - Hermaphrodites in SRS (V_1) and Males in CO 5 (V_2)

CoSo - Control Bulk
C_1 - Black seeds
C_2 - Brown seeds
S_1 - Extra large seeds
S_2 - Large seeds
S_3 - Medium seeds
S_4 - Small seeds

protein, phenol, nucleic acids and the enzyme IAA oxidase were estimated at three stages of crop growth, *viz*., seedling, vegetative and reproductive stages by normal methods. The growth regulating substances such as gibberellic acid and cytokinin were also estimated.

RESULTS AND DISCUSSION

Protein

The varietal and size difference were marginal as also the colour variation (Table 1). Regarding the protein content over stages, vegetative stage recorded significantly higher protein content than seedling stage. Also, the protein content was higher in the males followed by the hermaphrodites and the least in the females. No significant correlation was observed between the protein content at any stage of the crop and sex ratio. The differences among sex can be explained in the light of larger metabolic sink of females which need greater amount of metabolites for the developing flowers and fruits. However, Dutta and Mazumdar (1989) and, Nandi and Mazumdar (1990) reported that females had higher protein content than the other sexes.

Phenol

The difference in phenolic content among various size groups was negligible (Table 2). As

Table 2. Effect of seed grouping on leaf phenol (mg/g)

Treatments / Stages		CoSo	C_1				C_2				SED	CD 0.05
			S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4		
Stage 1	V_1	0.450	0.527	0.570	0.618	0.605	0.574	0.600	0.644	0.628	0.002	0.004
	V_2	0.421	0.513	0.526	0.480	0.571	0.513	0.592	0.627	0.635		
Stage 2	V_1	0.480	0.419	0.529	0.435	0.476	0.410	0.439	0.484	0.517	0.016	0.032
	V_2	0.529	0.455	0.505	0.441	0.559	0.499	0.563	0.606	0.621		
Stage 3												
Sex - 1	V_1	0.318	0.341	0.350	0.365	0.370	0.330	0.276	0.349	0.339	0.009	0.081
	V_2	0.376	0.366	0.321	0.302	0.345	0.392	0.361	0.421	0.401		
Sex - 2	V_1	0.401	0.351	0.361	0.363	0.372	0.332	0.346	0.351	0.335	0.017	0.034
	V_2	0.442	0.355	0.342	0.345	0.323	0.448	0.443	0.481	0.461		

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Table 3. Effect of seed grouping on leaf DNA (mg/g)

Treatments / Stages		CoSo	C_1 S_1	C_1 S_2	C_1 S_3	C_1 S_4	C_2 S_1	C_2 S_2	C_2 S_3	C_2 S_4	SED	CD 0.05
Stage 1	V_1	2.152	2.116	3.096	1.948	2.927	2.124	2.972	1.208	1.004	0.059	0.120
	V_2	1.028	1.030	0.238	0.143	2.100	1.272	0.466	0.179	1.578		
Stage 2	V_1	3.288	4.006	4.419	2.837	4.128	3.459	5.062	1.371	1.081	0.483	0.981
	V_2	1.244	1.524	0.446	0.280	2.723	2.059	0.798	0.277	2.315		
Stage 3												
Sex - 1	V_1	0.224	0.471	0.303	0.431	0.246	0.472	0.136	0.100	0.290	0.006	0.011
	V_2	0.274	0.492	0.163	0.252	0.092	0.196	0.353	0.384	0.305		
Sex - 2	V_1	0.180	0.250	0.195	0.225	0.104	0.392	0.125	0.097	0.125	0.021	0.042
	V_2	0.260	0.343	0.138	0.226	0.090	0.135	0.259	0.295	0.201		

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the age of crop advanced, the leaf phenol content decreased. A highly significant negative correlation was observed between the phenol content at seedling stage and the female sex ratio (r = -0.705**). At the vegatative stage also, a significant negative association was observed (r = -0.475*) and, a significant positive association with that of males and hermaphrodites (r = 0.475*). The females had significantly lesser amounts of phenols than the hermaphrodites ; males had higher amounts. There was no significant correlation found to exist between the phenol content at flowering stage and sex ratio. The results of the present study are in confirmity with the earlier repors of Bhattacharya and Rao (1982). Many of the polyphenolic substances such as caffeic acid and quercetin, identified in the papaya leaves have been found to synergise the action of IAA (Tomeszewski, 1964). This is not necessary for males, and hence the phenolic content of males was high. In the case of females, since the auxins play an imporant role in feminization the role of synergetic phenols of IAA is more, whereas. the role of inhibitory phenols of IAA is reduced. possibly for the conversion to female flowers.

Nucleic acids

DNA : The maximum leaf DNA content was recorded at the vegetative stage, followed by the seedling stage, and the least at flowering stage.

Table 4. Effect of seed grouping on leaf RNA (mg/g)

Treatments / Stages		CoSo	C_1 S_1	C_1 S_2	C_1 S_3	C_1 S_4	C_2 S_1	C_2 S_2	C_2 S_3	C_2 S_4	SED	CD 0.05
Stage 1	V_1	0.234	0.183	0.206	0.152	0.132	0.167	0.219	0.109	0.263	0.009	0.018
	V_2	0.273	0.429	0.271	0.163	0.321	0.039	0.123	0.092	0.073		
Stage 2	V_1	0.161	0.010	0.050	0.403	0.129	0.136	0.531	0.065	0.020	0.022	0.045
	V_2	0.037	0.039	0.064	0.813	0.312	0.079	0.252	0.591	0.021		
Stage 3												
Sex - 1	V_1	0.604	0.728	0.659	0.557	0.535	0.542	0.761	0.512	0.636	0.022	0.045
	V_2	0.753	0.765	0.899	0.555	0.869	1.030	0.812	0.728	0.525		
Sex - 2	V_1	0.605	0.715	0.600	0.540	0.525	0.499	0.682	.454	0.640	0.017	0.034
	V_2	0.550	0.750	0.510	0.450	0.670	0.465	0.443	0.306	0.337		

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S_4 - Small seeds

Table 5. Effect of seed grouping on leaf IAA Oxidase activity (ug of IAA oxidized/2 hours/g)

Treatments / Stages		CoSo	C1				C2				SED	CD 0.05
			S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4		
Stage 1	V_1	0.73	0.81	0.80	0.91	0.76	0.81	0.71	0.86	0.90	0.02	0.04
	V_2	0.79	0.81	1.30	0.76	0.96	0.75	0.42	0.33	0.55		
Stage 2	V_1	8.58	8.62	8.10	9.37	7.90	8.20	7.53	8.70	9.30	0.21	0.43
	V_2	7.53	8.27	11.13	7.53	9.57	7.37	4.30	6.50	5.50		
Stage 3												
Sex - 1	V_1	2.47	3.74	3.11	3.89	2.66	2.98	2.81	3.80	4.00	0.14	0.28
	V_2	3.83	3.20	4.40	2.83	3.17	3.77	4.12	3.52	3.10		
Sex - 2	V_1	3.57	3.37	4.13	2.84	3.13	2.91	3.91	4.13	0.13	0.26	
	V_2	4.11	3.37	4.60	2.97	3.21	3.90	4.33	3.79	3.43		

Stage 1 - Seedling stage
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Among the various sex groups, maximum DNA content was observed in females followed by males and then the hermaphrodites (Table 3). There was no significant correlation observed between the DNA content at any stage and sex ratio of the crop. Though the protein content was lower in the leaves of female papayas, the DNA content was higher. The DNA may regulate the synthesis of specific proteins of females. It may be assumed that a specific protein of females and a specific cytokinin formed a hormone - acceptor complex in the cells, which regulated the activity of genes responsible for the female sex expression.

RNA : Significant increase in leaf RNA content was noted with increasing age of the crop (Table 4). The leaf RNA content of males and hermaphrodites was found to have a significant negative correlation with the sex ratio ($r = -0.486^*$). Also, at the seedling stage, a significant positive correlation was found to exist between the RNA content and the female sex ratio ($r = 0.474^*$). Similarly, a significant but negative correlation was noticed between the RNA content at seedling stage and the sex ratio of males and hermaphrodites ($r = -0.474^*$). Females had significantly more leaf RNA content followed by the hermaphrodites and males. Thus, the present study confirms the relatively

Table 6. Effect of seed grouping on leaf hormonal content (ug/g)

Treatments / Stages		CoSo	C1				C2				SED	CD 0.05
			S_1	S_2	S_3	S_4	S_1	S_2	S_3	S_4		
Gibberellic acid												
Sex - 1	V_1	131.4	105.5	34.5	91.5	288.1	107.2	70.4	88.8	114.7	3.1	6.4
	V_2	67.5	42.1	19.1	23.2	39.7	98.3	83.6	120.8	127.5		
Sex - 2	V_1	282.5	307.3	368.3	355.9	316.3	275.9	323.0	308.0	159.2	7.4	15.0
	V_2	233.3	274.3	445.4	318.1	303.4	106.7	208.6	112.5	160.4		
Cytokinin												
Sex - 1	V_1	96.4	88.6	96.1	92.8	115.3	85.4	90.8	95.4	98.4	2.0	4.0
	V_2	87.3	87.3	91.2	96.7	101.3	73.4	77.3	81.0	85.7		
Sex - 2	V_1	72.5	84.4	80.7	89.9	108.7	69.0	65.3	74.3	79.3	2.1	4.2
	V_2	84.1	84.3	80.2	90.0	92.4	70.0	75.1	65.4	78.4		

Sex - 1 - Females
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higher metabolic status of females over males and hermaphrodites.

IAA oxidase : The activity of IAA oxidase in terms of amount of IAA oxidase was estimated. In the different colours and sizes of seed groups, the enzyme activity was significantly more in those which were in favour of maleness, the brown colour (C_1) and the smaller seed groups (S_3 and S_4). Thus, the IAA oxidase activity can be considered as one of the aids of sex differentiation. However, a direct correlation between the enzyme activity and the sex ratio could not be seen. Higher amount of IAA was oxidised in the males followed by the hermaphrodites and the lower in the females.

The enzyme activity was low in the females, since IAA is a feminising hormone which activates by inducing ethylene evolution (Khryanin, 1989). Also, the IAA content of males was very low compared to females (Galun *et al.*, 1965). This was proved in the present study also.

Growth regulating substances

Gibberellic Acid (GA) : The small seed size recorded the maximum GA content, and also the brown colour (Table 6). The hermaphrodites had significantly higher amount of GA followed by the males and then the females. There was no significant correlation observed between the GA content and sex ratio.

Cytokinin : The plants of black seeds had more cytokinin content whereas, the smaller seeds also recorded more. The males and hermaphrodites had lower cytokinin content than the females. No significant association was found to exist between the cytokinin content and the sex ratio. However, a significant positive correlation was observed between the GA content of males and hermaphrodites and the cytokinin content of females ($r = 0.523^*$). Thus, the results clearly indicate that the endogenous hormonal levels are the deciding factors of the sex ratio.

Heslop-Harrison (1963) proposed a hypothesis stating that the sexual differentiation was controlled by the endogenous level of auxins in regions neighbouring the flowering primordia ; during flowering, the formation of pistillate organs may be favoured by high auxin levels in the vicinity of differentiating primorida, and of staminate organs by low auxin levels. Thus analysis of hormone content at the tip of the plant is necessary. Rudich *et al.*, (1972) reported that the increased levels of auxins and inhibitors with a decrease in gibberellin content resulted in the female flower production. The present study also support this view.

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