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CHEMICAL CHANGES OF THE PAPAYA PLANT DURING DEVELOPMENT, WITH SPECIAL REFERENCE TO ITS PROTEOLYTIC ACTIVITY

by

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INTRODUCTION

The papaya plant (*Carica papaya* L.) attains full maturity in approximately thirteen months of growth, when it bears fruit. During this period of development, several chemical changes take place in the plant. The present investigation was undertaken principally to determine the general trend of variation in the proteolytic activity of the various parts of the plant throughout this period of development. However, other data were collected at the same time, including that referring to the variation in moisture content, hydrogen ion concentration, and total nitrogen. As no data of this nature have previously been recorded, we have thought the same may be of use to those engaged in the study of the papaya plant. For this reason, these data have been included in the present paper, together with those finding related to the distribution of proteolytic activity throughout the plant.

MATERIALS AND METHODS

In the present investigation, the following material and methods were used.

Plant material. The native variety of papaya was utilized. Seeds were sterilized and planted in a greenhouse. The seedlings were then transplanted into pots and, on attaining an age of approximately three months, were in turn placed out in the open. Samples for analysis were taken as recorded in Table I.

TABLE I

Schedule Followed In Collecting Samples for Analysis

Month and year	Age in days after planting seed	Sample of	Amount of samples collected
Sept. 1940	0	Seeds	50 gm.
" 1940	3	"	50 "
" 1940	6	"	50 "
" 1940	13	Whole seedling	50 seedlings
Oct. 1940	43	Whole plant	25 plants
Nov. 1940	73	" "	5 "
Dec. 1940	103	" "	2 "
Jan. 1941	133	" "	1 "
Feb. 1941	163	" "	1 "
Mar. 1941	193	" "	1 "
Apr. 1941	223	" "	1 "
May 1941	253	" "	1 "
June 1941	283	" "	2 "
July 1941	313	" "	1 "
Aug. 1941	343	" "	2 "
Sept. 1941	373	" "	1 "
Oct. 1941	403	" "	1 "

All of the samples were collected early in the morning and immediately sent to the laboratory, where they were placed in cold storage at a temperature of about 8°C. Samples were always utilized within the following twenty-four hours.

Total moisture. Total moisture was determined in the seed, root, stem, leaf, and fruit by placing samples of these various parts in an oven at a temperature of 110°C, until constant weight was attained. The loss in weight was taken as an index of the moisture content of each sample.

Expression of juice. The tissues were thoroughly ground with a small amount of washed quarz in a porcelain mortar and the paste, thus formed, squeezed in a piece of cheesecloth until all the juice was expressed. The juice obtained was used immediately.

Hydrogen ion concentration. The hydrogen ion concentration of the juice was determined with a Leeds and Northrup glass electrode meter.

Milk clotting activity. The milk clotting activity of the juice was one of the indexes used to measure proteolytic activity and the method followed in this determination, was that of Balls and Hoover ([2]). Results obtained in these milk tests were expressed in milk clotting units equivalent to the amount of enzyme required to clot 5 cc. of the standard milk preparation in one minute at 40°C.

Hydrolysis of gelatin. Another index used to measure proteolytic activity was the ability shown by the juice to hydrolyze gelatin; the method followed was the formol titration. A two per cent gelatin solution pH 5 was used as substrate. To 5 cc. of this solution 1 cc. of juice was added. One cc. of this mixture was taken for the initial titration. One cc. of 40 per cent formaldehyde was added to each volume of mixture to be titrated. The indicator used was a solution of 0.2 per cent phenolthalein in 50 per cent alcohol. The incubation period lasted 20 hours at a temperature of 40°C. The strength of the sodium hydroxide solution used for titration was of 0.01N.

Nitrogen content of the tissues examined. All nitrogen determinations were performed in accordance with the Kjeldahl-Gunning-Arnold method ([1]).

Experimental Results

The seed. No measurable amount of proteolytic activity was detected in the seeds before or during the germination period. The nitrogen content of the seed, as planted, was 41.5 mg. per gram of dry seed; samples taken during the thirteen days before germination averaged 44.4 mg. of nitrogen per gram of dry seed. For other determinations performed on the seed, see Table II.

The leaf. Proteolytic activity appeared first in the leaf and then in other parts of the plant as can be seen in Tables III, IV, and V. Samples collected forty-three days after planting the seed showed, on dry basis, a milk clotting activity of 1.80 units per gram and a formol titration of 15 cc. of 0.01N NaOH. The peak of proteolytic activity in the leaf, as indicated both by the milk clotting and formol titration, was reached about the 133rd day after planting of the seed. These values were 11.16 milk units and 56.16 cc. 0.01N NaOH, respectively, on dry basis per gram. From this date on, proteolytic activity of the leaf consistently decreased. The last test performed on the 403rd day gave 4.68 units and 13.61 cc.

0.01N NaOH per gram of dry leaf for the milk clotting test and formol titration, respectively. Nitrogen content of the leaf averaged 42.6 mg. per gram of dry leaf. The highest content was observed on the 253rd day, 49.9 mg., while the lowest, 35.6 mg., was recorded on the 193rd day. For other determinations performed on the leaf, see Tables II, III, and VI.

The stem. Milk clotting activity did not appear in the stem until the 73rd day. This initial activity, as well as that exhibited all throughout the experimental period, was much lower than in the leaf. Average clotting per gram of dry tissue was 2.48 milk units, while the formol titration was 8.18 cc. of 0.01N NaOH. The peak in milk clotting activity, 5.09 units, was reached on the 133rd day. On other hand, formol titration did not show a maximum until the 283rd day when a titration of 13.21 cc. of 0.01N NaOH per gram of dry stem was recorded. Average nitrogen content was 13.7 mg. per gram of dry tissue. A maximum of 20.8 mg. was recorded on the 253rd day and a minimum of 6.6 mg. on the 403rd day. For other data related to the stem, see Tables II, IV, and VI.

The root. Of the various parts of the papaya plant, the root showed the lowest milk clotting activity, although its gelatin hydrolyzing power was the same as that of the stem. Its average milk clotting activity and formol titration per gram of dry tissue were 1.39 milk units and 8.12 cc. of 0.01N NaOH, respectively. The peak in milk clotting activity, 3.43 units, was reached on the 283rd day; the peak in formol titration, on the same day with a value of 17.50 cc. of 0.01N NaOH. Average nitrogen content of the root was 14.3 mg. per gram of dry tissue; its maximum content was recorded on the 223rd day with 19.8 mg. and the lowest on the 373rd day, with 8.5 mg. per gram of dry tissue. For other data on this part of the plant, see Tables II, V, and VI.

The fruit. For the purpose of this study the fruit was divided into two parts: the rind and the pulp. It is well known that the rind contains the largest amount of the latex present in the fruit, while the pulp contains practically none. The fruit was collected at the same time from two different trees, both about thirteen months old. That fruit belonging to the younger inflorescence at the top was small and undeveloped; that from the older inflorescences at the bottom was well developed and medium ripe. Tables VII, VIII, IX, and X, record the inflorescences from the seventh to the first; the seventh being the younger at the top and the first being the older at the bottom of the tree.

(a) *The rind.* The average proteolytic activity of the rind per gram of dry tissue was 36.2 milk units and 26.4 cc. of 0.01N NaOH. Maximum

activity was observed in the fruit belonging to the third inflorescence with values of 94.1 milk units and 36.1 cc. of 0.01N NaOH, respectively, for milk clotting and formol titration. The fruit from this inflo- can be seen from Table VIII. The average nitrogen content of the rind was of 29.9 mg. per gram of dry tissue. For other data, see Tables VII, VIII and X.

(b) *The pulp.* The pulp of the fruit showed a much lower proteolytic activity than the rind, both when such activity was measured by the milk clotting test as well as by formol titration. Milk clotting activity of the pulp was nearly one-tenth that of the rind (36.2 units against 3.3 units per gram of dry tissue). Formol titration of the pulp was about a one-third that of the rind (26.4 cc. against 9.9 cc. 0.01N NaOH per gram of dry tissue). The milk clotting power of the pulp from the second and first inflorescences was zero. On the other hand, its gelatin hydrolytic activity remained quite uniform throughout, showing an average of 9.9 cc. of 0.01N NaOH per gram of dry tissue. The average nitrogen content of the pulp was 14.9 mg. per gram of dry tissue, that is, about half the average nitrogen content of the rind. As in the rind, this nitrogen content also had a tendency to decrease with the age of the fruit. For other data on the pulp, see Tables VII, IX, and X.

Discussión

Outside of the observations made by Balls, Thompson and Jones [3] to the effect that the entire papaya plant, aside from the roots, contained considerable enzyme activity, no other investigation, as far as we know, has been conducted along these lines.

The results obtained by us confirmed the observations of the above mentioned investigators and showed, furthermore, that milk clotting activity made its appearance first, in the leaf of the young plant and later, in the stem and root. The two parts of the plant which at all times showed a high proteolytic activity were the green leaves and the green fruit rind. Whether the formation of papain is closely connected with the presence of chlorophyl we are not in a position to say; however, it is interesting to note the close association that exists between these two substances in the different parts of the papaya plant.

In the leaf, at least, there was a definite variation in proteolytic activity during the first thirteen months of growth. Such activity reached its maximum value on the 133rd day after planting of the seed, but decreased consistently thereafter until the end of the experiment. The same variation was noticed, to a lesser extent, in the stem and in the root.

It must be remembered that these results are subject to considerable variation due to the use of single tree samples for each set of determinations. Lack of planting space made it necessary to take single trees for analysis in such a long time experiment. We feel, however, that in spite of individual variations, these results show definite trends in the distribution and activity of the substances measured.

Summary

1. All parts of the papaya plant, with the exception of the seed, showed varying degrees of proteolytic activity.

2. Milk clotting activity appeared first in the leaf and later, in the stem and root.

3. The largest amount of proteolytic activity was concentrated in the green fruit rind and, in decreasing quantities, in the leaf, fruit pulp, stem, and root.

4. In the leaf and, to a lesser extent, in the stem and root, a definite variation took place in proteolytic activity during the thirteen initial months of growth. Maximum activity was reached between the fourth and ninth months.

5. Data have been collected regarding the moisture, hydrogen ion concentration, and nitrogen content of the various parts of the papaya plant during its initial thirteen months of growth.

SUMARIO

1—Todas las partes de la planta de la papaya, con excepción de la semilla, demostraron poseer distintos grados de actividad proteolítica.

2—La actividad proteolítica apareció primero en la hoja y más tarde en el tallo y las raíces.

3—La actividad proteolítica estaba concentrada en mayor grado en la corteza de la fruta verde y, en cantidades menores, en las hojas, la pulpa de la fruta, el tallo y las raíces.

4—En las hojas y, en menor grado, en el tallo y las raíces, se produjo una variación definida en la actividad proteolítica durante los 13 meses iniciales de crecimiento. La actividad máxima se alcanzó entre los 4 a 9 meses.

5—Se han tomado datos en cuanto al contenido de humedad, variación en el pH y el contenido de nitrógeno de las distintas partes de la planta durante los 13 primeros meses de su desarrollo.

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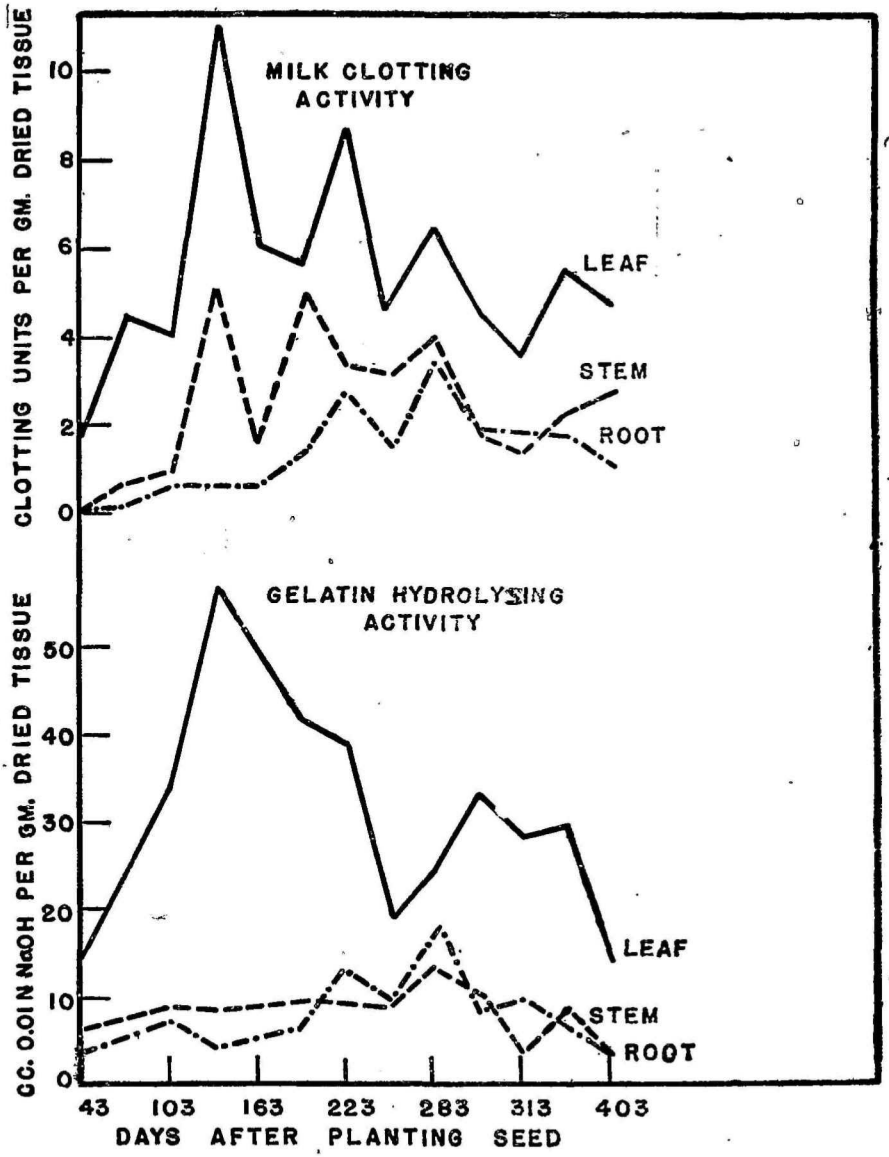

Milk clotting and gelatin hydrolyzing activity of the leaf, stem and root of the papaya plant during the first 403 days of growth.

Table II. *Total moisture and total solids in the root, stem, and leaf of the papaya plant.*

Age in days	Total moisture			Total solids		
	Root %	Stem %	Leaf %	Root %	Stem %	Leaf %
43	86.5	92.8	87.8	13.5	7.2	12.2
73	86.5	88.3	82.5	13.5	11.7	17.5
103	88.5	90.0	84.4	11.5	10.0	15.6
133	89.0	95.5	92.3	11.0	4.5	7.7
163	89.9	86.5	66.0	10.1	13.5	34.0
193	89.7	93.5	75.0	10.3	6.5	25.0
223	90.0	88.0	75.7	10.0	12.0	24.3
253	90.5	87.2	78.8	9.5	12.6	21.2
283	91.2	87.6	79.1	8.8	12.4	20.9
313	87.0	84.9	70.2	13.0	15.1	29.8
343	88.5	80.7	71.2	11.5	19.3	28.1
373	89.9	87.5	78.8	10.1	12.5	21.2
403	87.0	86.5	78.0	13.0	13.5	22.0
Average	89.0	89.0	78.5	11.0	11.0	21.5

Table III. *Milk clotting and formol titration per cc. of leaf juice, per gram of dry leaf and per gram of fresh leaf.*

Age in days	pH of leaf juice	Milk clotting units			Formol titration cc. 0.01N NaOH		
		Per cc. of juice	Per gm. of dry leaf	Per gm. of fresh leaf	Per cc. of juice	Per gm. of dry leaf	Per gm. of fresh leaf
43	6.10	0.25	1.80	0.22	2.08	15.00	1.83
73	6.35	0.97	4.56	0.80			
103	5.77	0.76	4.10	0.64	6.18	33.37	5.22
133	5.70	0.93	11.16	0.86	4.68	56.16	4.32
163	6.58	3.17	6.15	0.21			
193	6.38	1.89	5.67	1.42	13.88	41.64	10.41
223	6.09	2.79	8.64	2.11	12.43	38.53	9.41
253	5.78	1.28	4.75	1.01	5.00	18.60	3.94
283	6.06	1.69	6.42	1.34	6.29	24.28	5.05
313	6.20	1.90	4.56	1.33	13.90	33.36	9.76
343	5.95	1.44	3.60	1.03	11.20	28.00	7.90
373	5.90	1.48	5.48	1.17	7.90	29.23	6.23
403	5.98	1.30	4.68	1.01	3.78	13.61	2.95
Average	6.06	1.53	5.50	1.01	7.95	30.15	6.09

Table IV. *Milk clotting and formol titration per cc. of stem juice, per gram of dry stem and per gram of fresh stem.*

Age in days	pH of stem juice	Milk clotting units			Formol titration cc. 0.0IN NaOH		
		Per cc. of juice	Per gm. of dry stem	Per gm. of fresh stem	Per cc. of juice	Per gm. of dry stem	Per gm. of fresh stem
43	5.75	0.00	0.00	0.00	0.47	6.06	0.44
73	5.80	0.09	0.68	0.08			
103	5.40	0.11	0.99	0.10	1.00	9.00	0.90
133	4.30	0.24	5.09	0.23	0.40	8.48	0.38
163	5.50	0.27	1.73	0.23			
193	5.88	0.35	5.04	0.33	0.68	9.79	0.67
223	5.46	0.46	3.36	0.41	1.25	9.13	1.10
253	5.31	0.42	3.17	0.37	1.30	8.97	1.14
283	5.58	0.49	3.96	0.43	1.86	13.21	1.63
313	5.60	0.34	1.90	0.29	1.88	10.53	1.60
343	5.62	0.32	1.35	0.26	0.77	3.23	0.62
373	5.55	0.33	2.31	0.29	1.20	8.40	1.05
403	5.55	0.43	2.75	0.37	0.50	3.20	0.43
Average	5.48	0.30	2.48	0.26	1.03	8.18	0.91

Table V. *Milk clotting and formol titration per cc. of root juice, per gram of dry root and per gram of fresh root.*

Age in days	pH of root juice	Milk clotting units			Formol titration cc. 0.0IN NaOH		
		Per cc. of juice	Per gm. of dry root	Per gm. of fresh root	Per cc. of juice	Per gm. of dry root	Per gm. of fresh root
43	5.90	0.00	0.00	0.00	0.55	3.52	0.48
73	5.84	0.03	0.19	0.03			
103	5.93	0.09	0.69	0.08	0.68	5.24	0.60
133	5.00	0.09	0.73	0.08	0.55	4.45	0.49
163	5.47	0.07	0.62	0.06			
193	5.62	0.16	1.39	0.14	0.73	6.35	0.66
223	5.50	0.31	2.79	0.28	1.43	12.90	1.24
253	5.55	0.17	1.61	0.15	1.00	9.50	0.91
283	5.55	0.33	3.43	0.30	1.68	17.50	1.53
313	5.50	0.29	1.94	0.25	1.33	8.90	1.16
343	5.55	0.25	1.92	0.22	1.28	9.84	1.14
373	5.55	0.20	1.78	0.18			
403	5.28	0.16	1.07	0.14	0.50	3.34	0.44
Average	5.56	0.17	1.39	0.15	0.97	8.12	0.87

Table VI. *Milligrams of nitrogen per gram of fresh and dry leaf, stem, and root.*

Age in days	Milligrams of N per gram of fresh tissue			Milligrams of N per gram of dry tissue		
	Leaf	Stem	Root	Leaf	Stem	Root
43	5.6	1.3	2.0	47.7	18.4	14.5
73	7.9	1.5	2.5	45.0	12.4	18.4
103	6.0	1.8		38.5	18.2	
133	2.9	0.7	2.1	38.0	15.2	18.7
163	15.2	2.5	1.5	44.8	18.5	14.4
193	8.9	0.8	1.3	35.6	13.0	13.3
223	10.8	1.6	2.0	44.3	13.4	19.8
253	10.6	2.6	1.5	49.9	20.8	15.8
283	9.2	1.6	1.5	44.2	13.0	16.7
313	12.7	1.4	1.3	42.5	9.0	10.0
343	13.0	1.8	1.1	46.5	9.4	9.4
373	8.4	1.2	0.9	39.8	9.9	8.5
403	8.7	0.9	1.6	39.3	6.6	12.6
Average	9.2	1.5	1.6	42.6	13.7	14.3

Table VII. *Total moisture and total solids in the rind and pulp of papayas of different inflorescences.*

Inflorescence (From top to bottom)	Rind		Pulp	
	Total moisture %	Total solids %	Total moisture %	Total solids %
7 younger	92.4	7.6	90.9	9.1
6	89.1	10.9	92.4	7.6
5	88.8	11.2	91.5	8.5
4	87.9	12.1	91.5	8.5
3	90.0	10.0	92.3	7.7
2	88.6	11.4	93.5	6.5
1 older	85.6	14.4	90.4	9.6
Average	88.9	11.1	91.8	8.2

Table VIII. *Milk clotting and formol titration per cc. of fruit rind juice, per gram of dry fruit rind and per gram of fresh fruit rind.*

Inflorescence (From top to bottom)	pH of juice	Milk clotting units			Formol titration cc. 0.01N NaOH		
		Per cc. fruit rind juice	Per gm. dry rind	Per gm. fresh rind	Per cc. fruit rind juice	Per gm. dry rind	Per gm. fresh rind
7 younger	5.38	1.83	22.3	1.69			
6	5.33	1.60	13.2	1.43	2.94	24.2	2.62
5	5.28	2.28	18.0	2.03	3.41	26.9	3.03
4	5.28	4.00	29.2	3.52	3.39	24.8	2.98
3	5.15	10.45	94.1	9.41	4.01	36.1	3.61
2	5.28	5.93	46.3	5.25	3.52	27.5	3.12
1 older	5.08	5.13	30.3	4.39	3.22	19.0	2.76
Average	5.25	4.46	36.2	3.96	3.42	26.4	3.02

Table IX. *Milk clotting and formol titration per cc. of fruit pulp juice, per gram of dry fruit pulp and per gram of fresh fruit pulp.*

Inflorescence (From top to bottom)	pH of juice	Milk clotting units			Formol titration cc. 0.0IN NaOH		
		Per cc. fruit pulp juice	Per gm. of dry pulp	Per gm. fresh pulp	Per cc. fruit pulp juice	Per gm. of dry pulp	Per gm. fresh pulp
7 younger	5.53	0.30	3.0	0.27			
6	5.53	0.56	6.8	0.52	1.03	12.5	0.95
5	5.65	0.28	3.0	0.26	0.73	7.9	0.68
4	5.49	0.25	2.7	0.23	0.67	7.2	0.61
3	5.43	0.65	7.7	0.60	1.15	13.7	0.11
2	5.70	0.00	0.0	0.00	0.82	11.7	0.77
1 older	5.45	0.00	0.0	0.00	1.52	14.3	1.37
Average	5.53	0.29	3.3	0.27	0.85	9.9	0.75

Table X. *Milligrams of nitrogen per gram of fresh and dry fruit rind and pulp.*

Inflorescence (From top to bottom)	Milligrams of N per gram of fresh tissue		Milligrams of N per gram of dry tissue	
	Rind	Pulp	Rind	Pulp
7 younger	2.5	2.1	38.2	22.9
6	2.3	1.5	25.4	16.5
5	2.7	1.4	30.3	15.5
4	2.8	1.3	31.7	13.9
3	3.1	1.2	34.2	13.1
2	2.7	1.0	30.5	10.2
1 older	1.6	1.1	18.1	11.9
Average	2.7	1.4	29.9	14.9

Table XI. *Distribution of Proteolytic Activity and Nitrogen in the Papaya Plant.*

Part of the plant	Proteolitic activity per gram of dry tissue		Milligrams of nitrogen per gram of dry tissue
	Milk clotting units	cc. 0.1N NaOH	
Seed	0.00	———	41.5
Root (Average 13 months)	1.39	8.12	14.3
Stem (Average 13 months)	2.48	8.18	13.7
Fruit pulp (Average 1st to 7th inflorescence)	3.30	9.90	14.9
Leaf (Average 13 months)	5.50	30.15	42.6
Fruit rind (Average 1st to 7th inflorescence)	36.20	26.40	29.9