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STERILITY MOSAIC INFECTION IN DIFFERENT PIGEONPEA GENOTYPES. II. STUDIES ON DIFFERENT METABOLITES AND SOME OXIDISING ENZYMES OF THE HOST

BY

B. L. SUBBA RAO AND Y. L. NENE

Plant Virology Division, Central Institute of Medicinal and Aromatic Plants (CIMAP-CSIR), Post Bag No, 1, P. O. RSM Nagar, Lucknow-226016, India, and Pulse Pathology, ICRISAT, Pattancheru Post, A. P. 502 324

Pigeonpea cultivars expressing different types of symptoms upon sterility mosaic infection were selected alongwith the two resistant cultivars to study the hostpathogen interaction in terms of different plant metabolites like chlorophyll content and chlorophyllase activity, ascorbic acid levels and its oxidative enzyme, alcohol soluble sugars, total phenol content, peroxidase and polyphenol oxidase. A general reduction in the contents of chlorbphyll, ascorbic acid, alcohol soluble sugars and increase in total phenol content and in the activities of peroxidase, polyphenoloxioxidase, chlorophyllase and ascorbic acid oxidase was noticed in the highly susceptible cultivar (BDN-1) followed by the tolerant cultivar (ICP-2376) due to sterility mosaic infection. The resistant cultivars were relatively unaffected. Pronounced increase in the activity of polyphenoloxidase was noticed in the tolerant cultivar (ICP-2376) in comparison with the other cultivars consequent to sterility mosaic infection.

INTRODUCTION

The sterility mosaic of Pigeonpea is widely prevalent in the Indian subcontinent. This disease is economically important as in some fields 100% incidence was noticed (Nene, 1972). The eriophid mite *(Aceria cajani* channabasavanna) borne disease produces main symptoms as normal typical systemic mosaic mottle, reduction in leaf size and partial or complete sterility of plants (Capoor, 1952 ; Nene, 1972 ; Ramkrishan and Kandaswamy, 1972). Some cultivars (eg. ICP-2376) produce distinct ring spots and no sterility was observed (Reddy and Nene, 1979). Among the many defence mechanisms exhibited by plants against the invasion of the pathogen, there are some which are strictly of metabolic nature (Sempio, 1950). Major shifts in metabolic sequences differ according to the susceptibility of the host. We have therefore attempted to understand the host-pathogen interaction in different pigeonpea cultivars with known disease reaction. The present study deals with changes in some oxidising enzymes, the chlorophyll contents, sugars and phenolic and ascorbic acid contents in different pigeonpea genotypes infected with sterility mosaic.

MATERIALS AND METHODS

Hosts : Four pigeonpea cultivars with known disease reaction against sterlity mosaic, a highly susceptible, BDN-1, a tolerant (or ring spot genotype), ICP-2376 and resistant ICP-7035, 7119 were selected for the present studies.

Maintenance of experimental plants : All pigeonpea cultivars BDN-1, ICP-2376, 7035, 7119 were grown in white enamel painted GI sheet trays measuring 28" L x 12" W x 6" H. These trays were filled with 25 kg of vertisol (black soil) uniformly, taken from one source and was thoroughly mixed. Later the top soil was properly levelled and 50 clean seeds of each cultivar were sown. Water was poured twice every day. 13 day old uniformly grown seedlings were inoculated with sterility mosaic with the help of infected leaflets of BDN-1 having good number of mites following the leaf stapling technique (Nene and Reddy, 1976). The plants with both the treatments of all the cultivars were grown with all the precautions to ensure uniformity in growth and without any mineral toxicity or deficiency.

Determination of chlorophyll contents : Chlorophyll 'a' 'b' and total contents were estimated following the method of Yoshida *et al* (1972) and the amounts were expressed in mg/g fresh weight.

Determination of ascorbic acid content : The ascorbic acid was extracted and estimated following the method of Roe (1954) and the quantities were expressed in mg/100g fresh weight.

Estimation of sugars : To estimate the total sugars present 0.5g of dried defatted leaf powder was refluxed with 100 ml of 80% ethanol for 6 hrs and this volume was reduced to 10 ml. 0.1 ml of this extract was diluted to 10 ml (Loomis and Shull, 1937). One ml of the extract was reacted with 5.5 ml of phenolsulphuric acid reagent and absorbance was read at 490 nm after cooling. The values were converted to percent values using the factor 92.31 to give the total sugar content per gram leaf tissue.

Estimation of individual sugars was done by TLC from the original extract, 50 μl aliquots were spotted along with a mixture of standard sugars, separately on a same TLC plate (20 x 20 cm) coated with 40% keiselgel 13% type 60 to give a thickness about 0.75mm. The spotted TLC plates were chromatographed in a solvent consisting of chloroform : acetic acid and water (120 : 140 : 20). After 3 hrs the dried plates were sprayed with aniline diphenylamine reagent (freshly prepared). The sprayed plates were incubated for 30 min at 100°C For quantitative studies, colours of standard sugars were only developed and the corresponding zones of the samples were scraped, dissolved in 5 ml of deionised distilled water and centrifuged. The clear supernatant was used for the estimation of sugar quantity by the above mentioned phenol-sulphuric acid method. The individual sugars were expressed in percent values (Subba Rao, 1980).

Estimation of tatal phenols : One g. of leaf material was cut in to bits and extracted in boiling ethanol for 5 minutes in hot water bath, and cooked. The material was homogenized thoroughly in a mortar filtered through muslin cloth. The residue was again reextracted with small quantity of 80% ethanol. Both the extracts were pooled and filtered through Whatman No. 41 filter paper and the final volume of the extract was adjusted to 10 ml with the help of vacuum flash evaporator (Mahadevan *et al,* 1965). The total phenols were determined following Folin Dennis method of Swain and Hills (1959).

Estimation of chlorophyllase activity :

Preparation of enzyme : 2 g of leaf material was extracted in 85% aq. acetone thoroughly and filtered through Whatman No. 41 filter paper. The filter paper was washed with several volumes of 85% acetone till no green colour was seen on the filter paper. The acetone was evaporated to optimum level. The resultant bright coloured precipitate was resuspended in a known amount of distilled water, the insoluble enzyme (in the ppt) was recovered by centrifugation (5 min. at 3000g). 500 mg of the precipitate (as enzyme) was made to react with 15 ml of the chlorophyll extract, after the determination of the chlorophyll content in 66% acetone in dark for 24 hours at 22°C with slow constant shaking. After the reaction was over, chlorophyll content was estimated in clarified acetone extracts (Ardao and Vennesland, 1960). The enzyme source was taken both from healthy and sterility mosaic infected plants of each cultivar and was made to react with the healthy chlorophyll extracts of the respective cultivar. A blank was always maintained of each cultivar without enzyme source (Subba Rao, 1978).

Ascorbic acid oxidase : The enzyme was extracted from 1 g leaf samples in 10 ml of 0.1 N sodium phosphate buffer at pH 7.1 and clarified. The activity of the enzyme was assayed from the reaction mixture containing 0.1 ml of extract, 0.1 ml of 0.2 M phosphate buffer at pH 6.2, 0.1 ml 0.001 M ascorbic acid and 1.8 ml of distilled water. The absorbance changes were recorded in a Beckman DU Spectrophotometer at 265 nm exactly after 10 minutes. Values at '0' time were observed. The activity of the enzyme was expressed as changes OD's after 10 minutes. Control cuvetts contained the same concentrations of the reaction mixture as that of the sample except the enzyme source (Oberbacher and Vines 1963).

Peroxidase (PO) and Polyphenoloxidase (PPO) PO and PPO were extracted from one g fresh leaf samples in 10 ml of 0.1 M phosphate buffer (pH 6. 8), clarified and diluted to 25 ml and used for assay (Kar and Mishra, 1976). The assay mixture for peroxidase comprised of 0.1 ml of the extract, 1.0 ml of 0.001 M pyrogallol in phosphate buffer pH 6.8, 0.1 ml of 2% H_2O_2 and 1.8 ml of distilled water (Hampton, 1963). The assay mixture for poly-

phenol oxidase was of same composition except for the absence of H_2O_2. The activity in either cases was estimated by measuring absorbancy at 420 nm after stoping the reaction by adding 0.5 ml of 5% sulphuric acid (Kar and Mishra, 1976).

RESULTS

Chlorophyll content : Progressive increase in chlorophyll content in uninoculated plants of four cultivars with the age was noticed. Decrease in chlorophyll content was noticed in BDN-1 along with increase of the mosaic disease and slight reduction in the chlorophyll content was also noticed in ICP-2376. It appears that chlorophyll 'b' was more affected in BDN-1 compared to chlorophyll 'a'. The same was noticed in the early samplings with infected ICP-2376. No reduction in the chlorophyll content was noticed, with the other two cultivars ICP-7035 and ICP-7119 upon inoculatiod (Table 1).

TABLE 1. *Changes in total chlorophyll, chlorophyll 'a' and chlorophyll 'b' contents in pigeonpea cultivars due to sterility mosaic infection (mg/g fresh wt.)*

Age of the plant in days	Days after inoculation	BDN-1		ICP-2376		ICP-7035		ICP-7119	
		H	I	H	I	H	I	H	I
Total chlorophyll									
18	5	1.81	1.56	1.80	1.44	1.50	1.52	1.49	1.43
27	15	2.10	1.13	1.91	1.60	1.81	1.69	1.81	1.81
42	30	2.07	0.14	2.02	1.94	1.98	1.83	2.05	2.09
Chlorophyll 'a'									
18	5	0.57	0.56	0.54	0.51	0.57	0.57	0.62	0.61
27	15	0.71	0.40	0.59	0.61	0.70	0.63	0.79	0.79
42	30	0.76	0.32	0.64	0.59	0.74	0.74	0.82	0.84
Chlorophyll 'b'									
18	5	1.26	0.99	1.25	0.92	1.02	0.94	0.86	0.81
27	15	1.38	0.72	1.31	0.98	1.10	1.05	1.02	1.02
42	30	1.30	0.08	1.38	1.33	1.23	1.08	1.22	1.25

H-healthy ; I-inoculated

Ascorbic acid content : The ascorbic acid content of all the four cultivars showed increasing trend with the age of the plants. Reduced amounts of the ascorbic acid content was noticed in the mosaic infected BDN-1 in all the

samplings. Slight reduction was noticed in the tolerant cultivar ICP-2376 inoculated plants. But ICP-7035 showed slightly increased ascorbic acid content after inoculation, whereas no clear change was noticed in ICP-7119 (Table 2).

TABLE 2. *Changes in ascorbic acid content of pigeonpea cultivars in response to sterility mosaic infection (mg/100g fresh wt.)*

Age of the plant in days	Days after inoculation	BDN-1		ICP-2376		ICP-7035		IPC-7119	
		H	I	H	I	H	I	H	I
18	5	51.17	43.34	52.03	52.40	60.64	61.55	55.10	55.35
27	15	69.25	59.41	71.34	64.36	77.86	99.13	73.80	72.57
42	30	102.46	79.95	97.91	86.10	104.55	119.71	87.08	86.55

H-Healthy ; I-Inoculated

Total sugar content : Data presented in the Table 3 showed increase in the total sugar content with the age in all the four inoculated cultivars. The total sugar content appears to be progressively decreasing with inoculated BDN-1 with the progress of the infection and is more pronounced than in inoculated ring spot tolerant cultivar ICP-2376. No appreciable changes were noticed in both the resistant cultivars upon sterility mosaic infection.

TABLE 3. *Changes in total sugar content in pigeonpea cultivars in response to sterility mosaic infection (in percent values)*

Age of the plant in days	Days after inoculation	BDN-1		ICP-2376		ICP-7035		ICP-7119	
		H	I	H	I	H	I	H	I
19	5	7.00	6.65	7.00	7.75	6.09	5.90	5.17	6.09
27	15	8.30	7.01	7.38	6 50	7.20	7.38	6.46	6.65
42	30	9.23	7.38	9.60	9.23	9.05	9.23	8.67	9.05

H-Healthy ; I-Inoculated

No qualitative changes were noticed due to sterility mosaic inoculation in all the cultivars. The sugars observed were 'verbascose' stachyose, raffinose, sucrose, glucose, fructose and two unidentified sugars. Sucrose content was more in all the cultivars in both the treatments. All the four cultivars showed low quantities of 'verbascose' with the increase in age of the plants in both the

treatments. However in the final sampling the sugar was not noticed (except in the uninoculated BDN-1 and ICP-7035 as traces). Sterility mosaic infection reduced almost all the sugars in all the three samplings in the highly susceptible BDN-1. No definite trend was noticed in both the tolerant and resistant cultivars (Table 4).

TABLE 4. *Effect of sterility mosaic on sugar contents of pigeonpea cultivars (in percent values)*

Name of the sugar	BDN-1		ICP-2376		ICP-7035		ICP-7119	
	H	I	H	I	H	I	H	I
5 Days after inoculation								
'Verbascose'	5.0	5.0	5.0	4.5	4.8	4.9	4.1	3.7
Stachiose	10.0	10.0	9.0	9.5	9.7	9.5	8.3	8.0
UIS-1	5.0	4.0	4.0	6.0	6.2	6.1	5.4	5.6
Raffinose	11.0	9.0	10.0	9.0	6.0	6.0	5.0	5.5
UIS-2	6.0	6.0	5.0	4.5	5.0	5.1	4.8	5.2
Sucrose	30.0	29.0	33.0	33.0	31.0	30.0	27.6	28.0
Glucose+Fructose	10.0	9.0	9.0	9.5	8.2	9.6	8.6	8.5
15 Days after inoculation								
'Verbascose'	3.0	4.0	2.0	2.0	3.0	4.0	2.5	2.5
Stachyose	10.0	9.2	19.2	10.0	10.0	10.5	9.5	7.2
UIS-1	9.5	4.0	4.0	8.2	8.0	8.5	9.0	6.0
Raffinose	11.0	8.2	8.2	13.0	10.9	10.0	10.5	11.0
UIS-2	12.0	5.1	5.1	9.6	10.2	11.0	10.3	11.0
Sucrose	30.0	26.0	26.0	34.2	34.0	33.0	33.6	35.0
Glucose+Fructose	10.2	8.2	8.9	8.5	9.0	9.6	9.6	10.5
30 Days after inoculation								
'Verbascose'	—				—			
Stachyose	12.5	8.2	11.2	11.5	16.5	17.0	14.5	15.0
UIS-1	4.0	4.0	5.3	4.5	6.1	7.0	8.0	9.0
Raffinose	15.0	7.1	17.4	18.0	16.7	16.5	16.0	16.0
UIS-2	16.0	7.2	16.7	15.0	18.0	17.9	12.0	11.0
Sucrose	32.0	28.0	34.0	34.0	35.2	35.0	34.0	33.7
Glucose+Fructose	12.0	9.0	11.0	12.0	11.0	12.0	11.5	13.0

H-Healthy ; I-Inoculated ; —Traces ; UIS-Un identified sugar.

Total phenol content : The total phenol content increased with the age of the plants in all the cultivars. Sterility mosaic infection increased the phenol content in both BDN-1 and the tolerant ICP-2376, the later showing maximum increase in all the samples compared to the other three cultivars except in the last sample where the susceptible changes were noticed in ICP-7119 (Table 5).

TABLE 5 : *Changes in total phenol content of pigeonpea cultivars due to sterility mosaic infection (mg/g fresh wt.)*

Age of the plant in days	Days after inoculation	BDN-1		ICP-2376		ICP-7035		ICP-7119	
		H	I	H	I	H	I	H	I
18	5	10.6	12.6	12.0	20.6	10.6	10.2	12.4	12.6
27	15	13.2	15.4	14.0	23.2	15.0	12.4	15.4	12.6
42	30	15.6	25.6	15.0	24.08	16.2	15.60	16.8	16.0

H-Healthy ; I-Inoculated

Chlorophyllase activity : Increased chlorophyllase activity was noticed right from five days after inoculation in the highly susceptible BDN-1, the activity being more pronounced in the sample taken 30 days after inoculation. A slight increase in the enzyme activity was noticed in ICP-2375 and ICP-7119 (Table 6).

Ascorbic acid oxidase activity : Sterility mosaic infection induced high activity of ascorbic acid oxidase in the highly susceptible BDN-1, where as only a slight increase in the activity was noticed in the inoculated tolerant ICP-2376 plants. The resistant inoculated cultivars showed activity nearing to their uninoculated counterparts (Table 7).

Peroxidase and polyphenoloxidase activities : Increased peroxidase activity was noticed consequent to sterility mosaic infection in BDN-1, ICP-2376 and ICP-7035. The enzyme activity was more pronounced in the inoculated ring spot tolerant cultivar ICP-2376 in all the samplings. One of the resistant cultivars, ICP-7035 showed decrease in activity as the plant aged whereas no trend was noticed in the other resistant cultivar ICP-7119 (Table 7). However the polyphenoloxidase showed a different trend in its activity in BDN-1, ICP-2376 and ICP-7119. High activity was noticed in the ring spot tolerant ICP-2376 and slight in one of the resistant cultivars ICP-7119 in all the inoculated samplings. The highly susceptible BDN-1 showed less activity and no definite trend was observed in the other resistant cultivar ICP-7035, upon infection.

TABLE 6. *Changes in chlorophyll content of pigeonpea cultivars due to chlorophyllase activity*

Cultivar	Enzyme source	Chlorophyll content in healthy extracts	Changes in chlorophyll content by addition of chlorophyllase	Percent of chlorophyll recovered	Percent decrease	Chlorophyll in healthy extracts	Changes in chlorophyll content by addition of chlorophyllase	Percent of chlorophyll recovered	Percent decrease	Chlorophyll content in healthy extracts	*Changes in chlorophyll content by addition of chlorophyllase	**Percent of chlorophylly recovered	**Percent decrease
		5 days after inoculation				15 days after inoculation				30 days after inoculation			
BDN—1	Uninocultated	1.15	1.10	95.65	4 34	1.59	1.50	94.33	5.66	1.56	1.49	95.50	4.48
	Inoculted		1.04	90.43	9.56		1.18	74.21	25.78		1.01	55.00	35.25
ICP—2376	Uninoculated	1.15	1.10	95.65	4.34	1.56	1.50	96.15	3.84	1.56	1.49	95.50	4.48
	Inoculted		1.07	93.04	6.95		1.45	92.94	7.05		1.42	91.02	8.97
ICP—7035	Uninoculated	1.50	1.10	95.65	4.34	1.62	1.53	94.44	5.55	1.59	1.50	94.33	5.66
	Inoculted		1.10	95.65	4.34		1.50	92.59	7.40		1.47	92.45	7.54
ICP—7119	Uninoculated	1.30	1.10	97.34	2.65	1.62	1.53	94.44	5.55	1.59	1.53	96.22	3.77
	Inocutoted	1.10	1.10	97.34	2.65		1.50	92.59	7.40		1.47	92.45	7.54

* in milli grams

** $\frac{100 \times \text{chlorophyll recovered}}{\text{chlorophyll added}}$

DISCUSSION

The present paper aims at comparing different pigeonpea showing two types of symptom expression that is normal systemic mosaic mottle in highly susceptible cultivar (BDN-1) and chlorotic ring spots on leaves in the tolerant cultivar (ICP-2376) in terms of different plant metabolites like chlorophyll content and chlorophyllase activity, content of ascorbic acid and ascorbic acid oxidase activity, alcohol soluble sugars, total phenol content and its oxidising enzymes like PO and PPO activities upon sterility mosaic infection, in comparison with the resistant cultivars (ICP-7035, ICP-7119). In pigeonpea sterility mosaic disease the commonly expressed symptom is the mosaic mottling of the leaves. Table 1,6 indicates progressive increase in uninoculated plants of all the four cultivars as influenced by age. Sterility mosaic infection has increased the chlorophyllase activity in highly susceptible and tolerant pigeonpea cultivars together with progressive reduction in the total chlorophyll content depending on the susceptibility of the cultivar. Chlorophyll 'b' was generally more affected than chlorophyll 'a' in the infected leaves. The resistant cultivars were far from affected by infection. The loss In the amounts of chlorophyll in the susceptible and the tolerant cultivars may be due to either the synthesis or hydrolysis of the already formed chloroplasts (Diener 1963). The loss may also be due to the break down of already developed chloroplasts by the activation of chlorophyllase as evidenced in the present study. It is known that due to chlorophyllase activity the chlorophyll is removed from the lamellar structure of the chloroplast and the chlorophyllide crystallises in the cell (Goodman *et al* 1967). Reduction in chlorophyll content and a simultaneous increase in chlorophyllase activity was noticed in hoja blanca virus and rice tungro virus diseases of rice (Long and Black, 1970, Subba Rao, 1978), bean yellow mosaic virus infected *D. lablab* leaves (Chinnadurai and Nair, 1971), sterility mosaic infected pigeonpea leaves (Narayanaswamy and Ramakrishnan, 1965), and different viruses infecting tobacco (Peterson and Mckinney, 1938). Our data showing a greater increase in the activity of chlorophyllase together with increased reduction in chlorophyll content in the highly susceptible BDN-1 than in the tolerant variety ICP-2376, is in clear agreement with all the above findings.

Sterility mosaic infection caused a more pronounced decrease in the ascorbic acid content and increased ascorbic acid oxidase activity in the highly susceptible cultivar than in the tolerant cultivar and variable effect was noticed in the other two resistant cultivars (Tables 2, 7). Reduction in ascorbic acid levels was noticed in chillies infected by PVY (Jayaranjan and Ramakrishnan, 1968) and in sterility mosaic infected pigeonpea (Narayanaswamy and Ramakrishnan, 1966) and no characteristic trend in different varieties of cowpea infected with cowpea mosaic virus (Khatri, 1968). Chloroplasts are the seats of ascorbic acid synthesis (Reid, 1942). Hence it is more than possible that

the low levels of ascorbic acid content with infected plants may be attributed to the reduction in chlorophyll content. However the reduction may also be due to its oxidase enzyme the ascorbic acid oxidase (Jayarajan and Ramakrishnan, 1968). In sterillity mosaic infected plants the decrease in ascorbic acid levels may be logically attributed to the combined effect of the reduction in chlorophyll content and increase with activity of the ascorbic acid oxidase as we have noticed in our studies.

No qualitative differences were noticed in the individual sugars due to sterility mosaic infection in all the four cultivars. However considerable decrease of all the sugars was noticed in the highly susceptible BDN-1 upon infection. The total alcohol soluble sugar content which showed an increase in all the four uninoculated cultivars with age (Table 3) showed progressive decrease in the infected susceptible cultivar. The degree of susceptibility of the cultivar appears to be having a bearing on the reduction in the soluble sugar content. This decrease in the infected plant may be due to the inhibition of carbohydrate synthesis and the utilisation of the sugars for the increased respiration which was noticed earlier by Ramakrishnan *et al* (1969).

An increase in the phenolics content was noticed in the tolerant (ICP-2376) cultivar followed by the highly susceptible (BDN-1) cultivar (Table 5). No appreciable changes were noticed in the resistan cultivar. An increase in the activity of the peroxidase was noticed consequent to sterility mosaic infection in the highly susceptible, tolerant, and initially one of the resistant cultivar (ICP-7035). The enzyme activity was more pronounced in the tolerant cultivar. The infected tolerant cultivar showed high activity of polyphenoloxidase followed by the susceptible cultivar and the resistant ICP-7119, (Table 7). The relationship between systemic viral infection in plants and altered phenol metabolism is not fully understood. More work on the role of phenols in the above situation in necessary to elucidate the relationship. Peroxidase activity was found to be relatively higher in dwarf varieties than tall varieties (Kamerbeek, 1956). A similar degree of inverse relationship between peroxidase activity and growth was also found in pigeonpea cultivars as the infection caused stunting. As Loebensfein and Linsey (1961) have suggested greater break down of carbohydrates through the HMP pathway produces precursers of phenolic compounds which can be oxidised by peroxidase. In the present study also we have noticed an increase in the phenolics content in the infected pigeonpea plants (Table 7). However it is more clear in the case of polyphenol oxidase where its enhancement is correlated to ring spot symptoms produced (Forkas and Solymosy, 1965). In the tolerant cultivar ICP-2376 ring spots are produced due to sterility mosaic infection (where high activity of PPO was noticed) which differs from the normal systemic symtoms produced in the susceptible pigeonpea cultivars.

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