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Biochemical changes in pigeonpea (*Cajanus cajan* (*L.*) Millsp.) leaves in relation to resistance against sterility mosaic disease*

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Abstract. Changes in different biochemical parameters like total phenolic content, protein pattern, polyphenol oxidase, peroxidase and isozymes of peroxidase were compared in sterility mosaic resistant (Hy3C) and susceptible (Type-21) pigeonpea varieties at different growth stages both under inoculated and uninoculated conditions. Resistant variety was characterized by the presence of specific isoperoxidase and proteins but only little difference was recorded between resistant and susceptible variety with respect to preformed or induced total phenolics and peroxidase activity. The activity of polyphenol oxidase increased substantially in susceptible variety following infection. Role of these changes is discussed in relation to disease resistance.

Keywords. Pigeonpea; pigeonpea sterility mosaic virus; phenolics; peroxidase; polyphenol oxidase.

Introduction

Sterility mosaic of pigeonpea, considered to be viral in etiology (Nene, 1980), is one of the most serious diseases of the crop. The pigeonpea sterility mosaic virus (PSMV) is transmitted and perpetuated through a mite, *Aceria cajani* (Nene and Rathi, 1972).

Certain resistant germplasm lines have been made available to the pulse breeders in the recent past (Nene and Reddy, 1976) and no information is available on the biochemistry of resistance mechanism in these germplasms. A role for phenolics and phenol oxidizing enzymes like polyphenol oxidases (PPO) and peroxidases in plant resistance against viral diseases have been implicated by several workers, but also contradicated by others (Loebenstein, 1972; Fric, 1976). Therefore, the present investigation was undertaken to find out the levels of total phenolics and their oxidizing enzymes in the leaves of two lines of pigeonpea in relation to their resistance against PSMV.

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Abbreviations used: PSMV, Pigeonpea sterility mosaic virus; PPO, polyphenol oxidase; PO. peroxidase.

Materials and methods

Plant material

Two varieties of pigeonpea *(Cajanus cajan (L.)* Millsp.), one highly susceptible (Type-21) and other resistant (Hy3C) to PSMV, were obtained from International Crop Research Institute for Semi-Arid and Tropics (ICRISAT). Patancheru (Hyderabad). They were raised in glass house in earthen pots filled with a mixture of soil and farm yard manure (10:1). Fifteen surface sterilized seeds were planted in each pot (30 cm) and after emergence, 10 seedlings per pot were maintained.

Inoculation

The inoculum of PSMV was obtained from naturally infected plants of pigeonpea cv. Sharda. The diseased leaflets infested with the mites served as inoculum. The seedlings were inoculated at the two leaf stage *(i. e.* 12 day old) by following the 'leaf pinning technique'. Two leaflets from diseased plant having sufficient number (20-50 mites per leaflet) of eriophyid mites were pinned with the help of Entomological pin No 20 (F. F. Taylor and Co., Birmingham, UK) to each primary leaf of seedling in such a manner that the healthy leaf was sandwitched between the lower surfaces of the two infected leaflets. Control plants were pinned in the same manner with healthy leaflets. In order to obtain better infection, inoculated plants were kept in shade for 12 h to facilitate the migration of more mites to healthy leaves. After 5 days of inoculation, the leaflets pinned to the healthy leaf were removed and the entire plant was sprayed with 0·1% metasystox.

Sampling

The primary leaves and first trifoliate of same size and position were harvested after washing them with distilled water from both inoculated and control plants after 0, 7, 14 and 28 days of inoculation. The harvested leaves were immediately transferred to ice bags and pooled replication wise. Three replications were taken for each interval. A part of each lot was used for the electrophoretic separation of soluble proteins and peroxidase (PO) isozymes. Half of the rest was weighed and stored at - 20°C to determine the PO and PPO activities. The remaining half of the each lot was oven dried at 60°C for 48 h, powdered with the help of mortar and pestle and stored in desiccator for the quantitative estimation of phenolic compounds and soluble proteins.

Determination of total phenolic content

Phenolic compounds were extracted from leaves using the method as described by Kuc *et al.* (1956). AOAC colorimetric method (AOAC, 1965) was followed for the quantitative estimation of total phenolic content. Tannic acid was used as standard.

Determination of PO activity

Peroxidase was extracted and assayed by the method of Retig (1974). Guaiacol was used as substrate. One unit is defined as the amount of enzyme that caused an increase in A470 of 0·01 in 1 min at 30°C.

Determination of PPO activity

PPO was extracted and assayed by the method of Tripathi *et al.* (1975) using pyrocatechol as a substrate. One unit of enzyme is defined as the amount of enzyme that caused an increase in A410 of 0·01 in 1 min at 30°C.

Electrophoretic separation of soluble proteins and PO isozymes

The first leaves were crushed in chilled mortar and pestle with 0·2 M cold phosphate buffer (pH 7·0). The homogenate was centrifuged at 14000 *g* for 40 min at 4°C. The soluble proteins and PO isozymes were separated on 7·5% Polyacrylamide gel and subsequently stained by following the method of Davis (1964) except that ammonium persulphate was used as a polymerizing agent in the spacer gel instead of riboflavin. About 0·1 mg protein was loaded in each gel. Proteins were stained in Coomassie blue while peroxidase isozymes were detected by staining with benzidine and H_2O_2.

Results

Total phenols

As shown in table 1, total phenolic content of healthy leaves of resistant variety was significantly higher than that of the susceptible one at all the stages of sampling except at 21 and 28 days where differences were non-significant. Phenolic content of the leaves increased progressively with increasing plant age upto 21 days after inoculation, thereafter, it declined in both the varieties.

Upon inoculation, phenolic content increased in both resistant and susceptible varieties. However, the magnitude of increase was higher in the resistant variety than in the susceptible one particularly during initial intervals *i.e.* 7 and 14 days after inoculation (table 1).

Protein pattern

A total of 34 different protein bands were observed at different stages of sampling but none of the two varieties showed all 34 bands at any stage (figure 1). Proteins appearing at band positions: 1, 2 , 6 , 8 , 10. 13, 14, 18, 19, 21, 23 and 31 were found in both the varieties at one stage or other. A group of 6 proteins was Hy3C specific, appearing at band positions: 5, 12, 16, 24, 27 and 33. Four proteins, band positions: 11, 26, 30 and 32, were specific to Type-21. Certain protein bands (1 at 14 day and 19 and 21 at 21 day) were stage specific (figure 1).

Peroxidase

Irrespective of the stage of sampling higher PO activity was recorded in healthy leaves of resistant variety than in the susceptible one. However, differences were significant only at 14 and 21 day stages. The activity of enzyme was observed to increase with age of the leaves in both the varieties (table 2) but the increase was slight and non-significant between any two consecutive intervals.

Upon inoculation with PSMV, initially there was a slight increase in the enzyme activity in the leaves of both susceptible and resistant varieties, but after 14 days, it

Table 1. Total phenolic content in the leaves of two pigeon pea varieties inoculated with pigeon pea sterility mosaic virus.

Days after inoculation	Type-21			Hy3C		
	Uninoculated	Inoculated	Increase (+) or decrease (−) over uninoculated (%)	Uninoculated	Inoculated	Increase (+) or decrease (−) over uninoculated (%)
0	9·40*	9·00	−4·25	10·50	10·35	−1·61
7	10·05	11·05	+9·95	10·86	12·90	+18·78
14	10·55	11·45	+8·53	11·41	12·65	+10·86
21	11·95	12·10	+1·25	11·81	12·20	+3·30
28	11·00	10·35	+5·90	11·66	11·85	+1·62

* Phenolic compounds (mg per gram of dry leaf).
l.s.d. ($P = 0·05$) = 0·99.

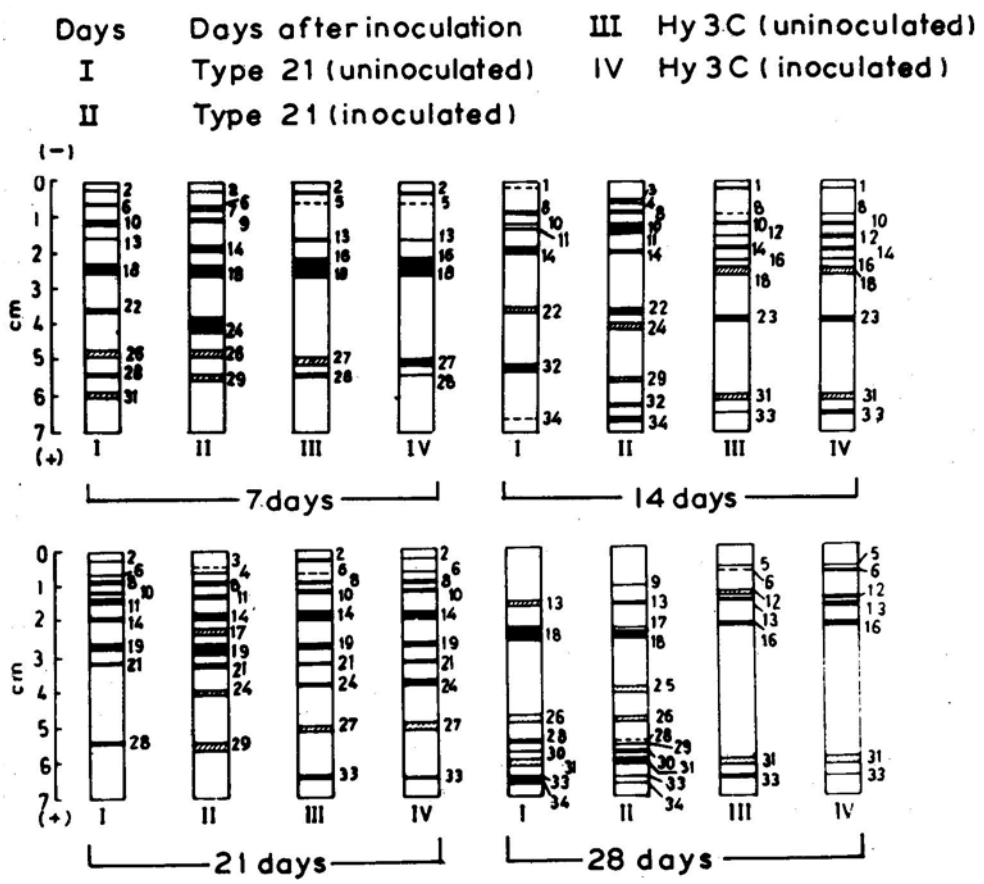

Figure 1. Protein pattern of pigeonpea leaves of susceptible (Type 21) and resistant (Hy3C) varieties at different intervals after inoculation with PSMV.

recorded significant decline in the susceptible variety falling below the control level at the following stages *i. e.* at 21 and 28 days after inoculation. In resistant variety, however,activity continued to increase (statistically not significant) till 21 days after inoculation, followed by a decline thereafter (table 2).

Isozymes of peroxidase

In the healthy leaves of resistant variety 7 (a, b, c, d, e, f and h) and in the susceptible, 6 (a, b, d, e, f and h) isozymes of PO were detected at 7, 14 and 21 day stages. One additional isozyme 'g' was added in susceptible variety at 28 day stage. Bands c and g were specific to resistant and susceptible varieties, respectively. Upon inoculation of PSMV, no quantitative deviation from the control was observed in the isozymes of PO of resistant variety while many such changes were recorded in susceptible one (figure 2). Intensity of all the bands diminished after infection except band 'a' whose width and intensity remained unaltered.

Table 2. Peroxidase activity in the leaves of two varieties of pigeon pea inoculated with pigeon pea sterility mosaic virus

Days after inoculation	Type-21			Hy3C		
	Uninoculated	Inoculated	Increase (+) or decrease (−) over uninoculated (%)	Uninoculated	Inoculated	Increase (+) or decrease (−) over uninoculated (%)
0	3869*	3843	−0·7	3993	3994	+1·7
7	3923	4081	+4·0	4091	4166	+4·1
14	4004	4121	+2·9	4198	4324	+3·0
21	4039	3746	−7·2	4293	4404	+2·6
28	4209	3493	−17·0	4274	4349	+0·7

* Peroxidase activity (enzyme units per gram of fresh tissue) in leaves. One unit indicates amount of enzyme required to increase in A470 by 0·01 per min at 30°C.
l.s.d. (P = 0·05) = 145.

Table 3. Polyphenoloxidase activity in the leaves of two varieties of pigeon pea inoculated with pigeon pea sterility mosaic virus.

Days after inoculation	Type-21			Hy3C		
	Uninoculated	Inoculated	Increase (+) or decrease (−) over uninoculated (%)	Uninoculated	Inoculated	Increase (+) or decrease (−) over uninoculated (%)
0	104*	98	−5·7	111	110	−0·9
7	118	149	+26·0	131	139	+6·2
14	129	203	+56·5	144	148	+3·0
21	115	201	+74·5	124	127	+2·5
28	134	218	+62·2	138	143	+3·9

* Polyphenoloxidase activity (enzyme units per gram of fresh tissue) in leaves. One unit indicates amount of enzyme required to increase in A410 by 0·01 per min at 30°C.
l.s.d. (P = 0·05) = 13.

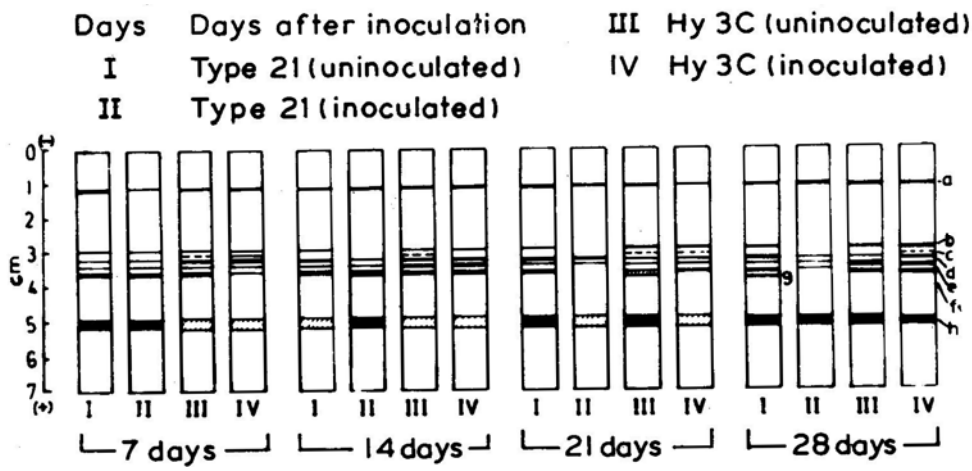

Figure 2. Isozyme pattern of PO in pigeonpea of susceptible (Type 21) and resistant (Hy3C) varieties at different intervals after inoculation with PSMV.

Polyphenol oxidase

Healthy leaves of resistant variety Hy3C, exhibited slightly higher PPO activity than the corresponding leaves of susceptible variety, Type-21, at all the stages of sampling (table 3), but the differences were statistically not significant for most of the intervals except day 14.

Inoculation resulted in significantly increased PPO activity in susceptible variety but not in the resistant one. Unlike healthy leaves, inoculated leaves of susceptible variety exhibited significantly higher PPO activity in comparison with the corresponding leaves of resistant variety at all the stages of sampling except at day 0 (table 3).

Discussion

Constitutive phenolics and their oxidizing enzymes like PO and PPO have been implicated in resistance in certain viral diseases (Fric, 1976; Kosuge, 1969; Loebenstein, 1972; Tripathi *et al.,* 1975). However, difference between resistant and susceptible varieties of pigeonpea with respect to these parameters was too little to be of any significance in providing resistance against PSMV. Similar observations have been recorded in certain other viral diseases (Barbara and Wood, 1972; Cabanne *et al.,* 1971; Fric, 1976).

Upon inoculation with PSMV, the only appreciable change recorded was the activity of PPO in susceptible variety where it increased considerably after 7 days of inoculation and continued to increase till 28 days after inoculation. In the resistant variety its activity was practically unaffected following inoculation. This indicates the possible involvement of PPO in symptom expression as also indicated by few other investigations (Barbara and Wood, 1972; Suseno and Hampton, 1966).

Constitutive isoperoxidases appearing at position 'c' and 'g' were unique for the resistant and susceptible plants, respectively. Altered zymogram pattern of isoperoxidases of susceptible variety, following infection with PSMV, suggested inactivation of existing isoperoxidases, activation of inactive form and/or synthesis of new isoperoxidases. No such alterations were recorded in resistant variety.

The difference in protein pattern of the two genotypes might be due to their genetic differences. Some of the specific proteins may also be associated with susceptibility or resistance to PSMV. Resistance associated proteins are reported in several virus-host systems (Sela, 1981).

The present findings indicate non-involvement of total phenols. PO and PPO in imparting resistance to pigeonpea against sterility mosaic disease. PPO may be involved in or associated with symptom expression. Proteins specific to resistant variety were observed. However, the question whether these proteins are varietal specific or are involved in imparting resistance could not be ascertained.

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References

A O A C (1965) *Official Methods of Analysis* 10th edition (Washington: Association of Oficial Agricultural Chemists) p. 657.

Barbara, D. J. and Wood, R. K. S. (1972) *Physiol. Plant Pathol.,* **2,** 167.

Cabanne, F., Scalla. R. and Martin. C. (1971) *J. Gen. Virol.,* **11,** 119.

Davis. B. J. (1964) *Ann. N.Y. Acad. Sci.,* **121,** 404.

Fric. F. (1976) In *Physiological Plant Pathology* (eds R. Heitefuss and P. H. Williams) (Berlin: Springer-Verlag) Vol. 4. p. 617.

Kosuge, T. (1969) *Annu. Rev. Phytopathol.,* **7,** 195.

Kuc, J., Henze. R. R., Ulistrup. A. J. and Quanchenbush. F. W. (1956) *J. Am. them. Soc.,* **78,** 3123.

Loebenstein. G. (1972) *Annu. Rev. Phylopathol.,* **10,** 177.

Nene, Y. L. (1980) *A world list of pigeonpea (Cajanus cajan (L.)* Millsp.) *and chickpea(Cicer arietinum* L.) *Pathogens.,* Pulse Pathology Progress Report-8. ICRISAT, Patancheru.

Nene, Y. L. and Rathi, Y. P. S. (1972) *A survey of Pulse Diseases in Uttar Pradesh* (ed. Y. L. Nene). (Pantnagar: G. B. Pant University of Agriculture and Technology) pp. 157-159.

Nene, Y. L. and Reddy, M. V. (1976) *Plant Dis. Rep.,* **60,** 1034.

Retig, N. (1974) *Physiol. Plant Pathol.,* **4,** 145.

Sela, I. (1981) *Adv. Virus Res.,* **26,** 201.

Suseno, H. and Hampton, R. E. (1966) *Phytochemistry.* **5,** 819.

Tripathi, R. K., Vohra, K. and Beniwal. S. P. S. (1975) *Indian J. Exp. Biol.,* **13,** 513.