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Post-infectional biochemical changes associated with soft rot of jackfruit

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Biochemical changes in the jackfruit as a result of infection by a soft rot fungus, *Rhizopus artocarpi* (= *R. stolonifer*) were investigated in respect of sugar and ascorbic acid levels. The total sugar pool in both the host varieties declined gradually with the advancement of infection and the same trend was maintained but more rapidly in 'Ghula' variety than 'Kujja'. The reducing sugar profile, on the contrary, appeared to increase steadily and more evidently in 'Ghula' than the other one. The non-reducing part exhibited significant decrease in both the varieties as the disease proceeded progressively. Ascorbic acid level was found to decline gradually in both the host varieties to a minimum indicating its effective utilisation by the pathogen during the course of its invasion and establishment.

Key words : Soft rot, *Rhizopus artocarpi*, jackfruit (*Artocarpus heterophyllus*), total carbohydrate, reducing sugar, non-reducing sugar, ascorbic acid

INTRODUCTION

Biomacromolecular changes in the host tissues as a result of infection by soft rot and decay fungi altering the nutritional status particularly of the fruit tissues have been studied (Mitra and Samajpati, 1985; Singh *et al.*, 1993; Tiyagi and Kumar, 1994; Ram and Vir, 1995). Numerous diversified reports are available on the changes in total carbohydrate and their reducing and non-reducing fractions in fruits and other plant tissues during pathogensis. An increase in total sugar content following infection has been reported (Inman, 1962; 1965; Lodha *et al.*, 1993). Accumulation of soluble sugars in the infected tissues has also been reported (Vidyasekaran, 1988) while its depletion is more pronounced in diseased fruits than in healthy ones (Prasad and Prasad, 1997; Ram and Vir, 1996). Like saccharides ascorbic acid being the important component of jackfruit functions as one of the biological oxidation-reduction substrate. Post infectional changes in the level of ascorbic acid have been reported by a number of workers (Prasad and Prasad, 1997; Ram and Vir, 1996; Tiyagi and Kumar, 1994; Reddy *et al.*, 1980).

The present work has been designed to study the changes in the levels of carbohydrates together with their reducing and non-reducing fractions and ascorbic acid in jackfruit tissues as a result of infection by *Rhizopus artocarpi*.

MATERIALS AND METHODS

Total carbohydrate content was estimated quantitatively following the method described by Viles and Silverman (1949) and Mc Cready *et al.* (1950) after suitable modifications. One g of fresh host tissues of each healthy and infected jackfruit was homogenised and extracted with hot 80% ethanol maintaining the volume at 10 ml. The extracted ethanolic solution was made colourless with activated charcoal and filtered. The residual plant tissues were treated with 10 ml of 70% perchloric acid to obtain the extract of starch. Finally, the ethanolic extract and the acid extract were made upto the volume of 100 ml separately with the addition distilled water.

One ml of aliquot was taken separately from these two extracts and to each of these aliquots, 4ml of 0.1% anthrone reagent (100 mg anthrone in 100 ml of conc. H_2SO_4) 4 h to 9 days old, was rapidly added, mixed thoroughly with a glass rod and allowed to cool. The tube was placed in a bath of boiling water for 10 min by placing marbel on the top of the test tubes to prevent the loss of water evaporation followed at 620 nm against a reagent blank in a spectrophotometer. The blank set contained 1 ml of distilled water instead of 1 ml of aliquot.

Reducing sugar content was estimated following Dinitrosalicylic acid method (Miller, 1972). For this purpose, alcoholic extracts of both the healthy and infected jackfruit tissues were prepared separately. Three ml of alcoholic extract was taken in a test tube and 3 ml of DNS reagent was added to it. The mixture was then heated for 5 min in a boiling water bath. After the colour has developed, 1 ml of 40% Rochelle salt (40% Sodium potassium tartrate) was added, cooled the tube under a running tap water. The intensity of the colour developed was measured at 575 nm against a reagent blank.

Non-reducing sugar content was calculated from the difference between the total sugar and reducing sugar contents i.e., by deducting the reducing sugar content from the total sugar content and expressed in terms of mg/100 mg of dry tissues (Mahadevan and Sridhar, 1986).

Ascorbic acid was estimated by the method described by Oser (1979). Aliquots of 100 mg fresh tissues of both healthy and infected fruit were homogenised separately with 5 ml of 60% Trichloro acetic acid (TCA). After centrifugation at 5000 rpm for 5 min, the supernatant was collected and a pinch of activated charcoal (Norit) was added to it and filtered. The volume of the filtrate was made up to 100 ml with distilled water. An aliquot of 4 ml was taken in the test tube and 2 ml of 2% 2, 4-dinitrophenyl hydrazine [2 g of 2,4-dinitrophenyl hydrazine in 100 ml of 9(N) H_2SO_4) and one drop of 10% thiourea solution (in 70% alcohol) was added to it. The mixture was then boiled for 15 min in a water bath followed by cooling at room temperature. Then 5 ml of 80% H_2SO_4 was added to the mixture at 0°C and absorbance was taken at 530 nm in a spectrophotometer after 30 min. A standard curve was prepared with analar grade ascorbic acid and the ascorbic acid content in the fruit tissues was calculated and expressed in the terms of mg/100 mg of dry tissue.

Table 1. Changes in carbohydrate level in the tissues of two varieties of jackfruit infected by *R. artocarpi* after different incubation periods

Host variety		Sugar content (mg/100mg of dry tissue)*								
		Healthy tissue	Infected fruits – Incubation period (days)							
			0	2	4	6	8	10	12	14
Kujja	Total sugar	16.4 ±0.05	16.4 ±0.06	15.5 ±0.02	14.5 ±0.05	13.75 ±0.006	13.4 ±0.006	13.16 ±0.005	13.0 ±0.05	12.85 ±0.02
	Reducing sugar	7.9 ±0.011	7.91 ±0.05	8.5 ±0.005	9.49 ±0.005	10.62 ±0.012	10.87 ±0.014	11.18 ±0.014	11.75 ±0.017	11.99 ±0.012
	Non-reducing sugar	8.5 ±0.01	8.49 ±0.05	7.0 ±0.05	5.01 ±0.006	3.49 ±0.051	2.53 ±0.017	1.68 ±0.01	1.25 ±0.02	0.86 ±0.007
Ghula	Total sugar	15.3 ±0.005	15.3 ±0.004	14.2 ±0.011	13.25 ±0.02	12.6 ±0.05	12.0 ±0.06	11.5 ±0.12	11.39 ±0.005	11.37 ±0.006
	Reducing sugar	7.35 ±0.005	7.37 ±0.001	8.45 ±0.02	9.43 ±0.006	10.09 ±0.23	10.72 ±0.001	11.19 ±0.05	11.32 ±0.007	11.36 ±0.01
	Non-reducing sugar	7.95 ±0.006	7.93 ±0.017	5.75 ±0.028	3.82 ±0.011	2.51 ±0.005	1.28 ±0.012	0.31 ±0.004	0.07 ±0.01	0.0 ±0.005

	CD at 5%	
	Sugar types	days
'Kujja'	13.26	7.29
'Ghula'	13.45	7.39

* Mean values of five replicates.

RESULTS

It is evident from the result (Table 1) that the total sugar content varied in the two varieties being maximum in 'Kujja'. With the progress of infection the total sugar declined rapidly up to 6th day of infection in 'Kujja' variety but declined steadily up to 10th day in case of 'Ghula', thereafter the content in both the cases was recorded to be more or less gradually declined. With the advancement of infection for the successful establishment of the pathogen, the reducing fraction in both the cases was noticed to increase significantly to a steady state up to 10 day of infection. On the other hand, the non-reducing fraction declined significantly in both the cases more so in 'Ghula' variety than the other.

It is also clear from the result (Table 2) that the level of ascorbic acid in both the varieties decreased significantly with the advancement of infection more so in 'Ghula' variety.

DISCUSSION

It is apparent from the result that the rot of jackfruit caused by *R. artocarpi* is associated with a gradual decline in total content of sugar in both the host varieties with the advancement of infection. The result is in full agreement with the similar observations in other cases made by Chattopadhyay and Nandi (1978), Ram and Vir (1997) and Prasad and Prasad (1997). The gradual decline in sugar in jackfruit tissues, as a result of progressive establishment of the pathogen may be attributed to the gradual utilisation of sugars by the rot pathogen. Utilisation of the host cell constituents by the pathogenic fungi during host-pathogen interaction has been reported in several cases (Ram and Vir, 1996; Prasad and Prasad, 1997). The loss in sugar, starch and reducing sugar in response to infection in *Lathyrus sativus* by *Peronospora pisi* has been reported by Prasad and Prasad (1997) whereas reducing sugar found to increase in the present experiment may be interpreted as either due to their disturbed photosynthetic activity or utilisation of organic matter already synthesized there. Increase in reducing sugar as a result of infection has been reported by Padmanabhan and Alexandar (1998) and Ram and Vir (1996) which is in full agreement with the present observation. Such an increase on reducing sugar in infected host tissue may be due to the hydrolysis of sucrose into its component sugars, glucose and fructose through the hydrolytic action of the enzyme produced by the pathogen (Ram and Vir, 1996).

Table 2. Changes in the level of ascorbic acid in the infected tissues of two varieties of jackfruit infected by *R.artocarpi* after defferent incubation periods

Host variety	Ascorbic acid content (mg/100mg of dry tissue)*								
	Healthy tissue	Infected fruits							
		Incubation period (days)							
		0	2	4	6	8	10	12	14
'Kujja'	0.01113 ±0.0002	0.01113 ±0.0002	0.0104 ±0.0004	0.0094 ±0.0001	0.0085 ±0.0003	0.0076 ±0.0003	0.0068 ±0.0004	0.0164 ±0.0003	0.006 ±0.0003
'Ghula'	0.0110 ±0.0005	0.011 ±0.0005	0.0101 ±0.0001	0.0085 ±0.0002	0.0068 ±0.0001	0.0052 ±0.0003	0.0038 ±0.0004	0.0024 ±0.0001	0.0018 ±0.0002

CD at 5%	
Host variety	5.66
Days	1.23

* Mean values of five replicates.

Of the two fruit varities, 'Kujja' having higher amount of total sugar along with its reducing fraction i.e. glucose is more easily acceptable to the pathogen causing fruit rot. It is interesting to note that although higher amount of available carbohydrate in host often result in increased susceptibility, the 'Kujja' variety, having higher available carbohydrates in healthy tissues than the 'Ghula' one exhibit less decrease which probably suggests that the former is having higher resistance to infection than the latter.

The decrease in ascorbic acid content in the host tissues in response to infection was reported in several other cases (Sharma and Kaul, 1994; Ram and Vir, 1996) which is in full agreement with the present findings. The loss or decline in ascorbic acid content in the host tissues following infection might be due to the production of ascorbic acid oxidase that take part in the degradation of ascorbic acid present in the host tissues (Tiyagi and Kumar, 1994).

REFERENCES

Chattopadhyay, N.C. and Nandi, B. (1978). Changes in total contents of saccharides, protein and chlorophyll in malformed mango inflorescence induced by *Fusarium moniliforme* var. *subglutinans*. *Biolo Planta* **20**(5) : 468-471.

Inman, R.E. (1962). Disease development, disease intensity and carbohydrate levels in rusted bean plants. *Phytopath*. **52** : 1207-1211.

Inman, R.E. (1965). Quantitative sugar changes in barley infected with a facultative parasite. *Phytopath* **55** : 341-345.

Lodha, S., Mali, P.C. and Barman, N.K. (1993). Development of bacterial blight and changes in biochemical components in the resistance and susceptible genotypes of clusterbean. *Ind Phytopathol*. **46**(2) : 454-459.

Mahadevan, A. and Sridhar, R. (1986). In : *Methods in Physiological Plant Pathology*. 3rd Ed. Sivakam Pub. Madras. pp. 1-328.

Mc Cready, R.M., Guggolz, J., Silviera, V. and Owens, H.S. (1950). In : *Modern methods of Plant Analysis* (Eds. Paech K. and Tracey, M.V.), **2**, pp. 159. Springer-Verlag Berlin, 1995.

Miller, G.L. (1972). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*. **31** : 426-428.

Mitra, S. and Samajpati, N. (1985). Nucleic acid content in leaf blight disease of *Brassica oleracea* var. *botrytis* caused by *Alternaria brassicicola*. *Phytopath. Z*. **114** : 69-72.

Nema, A.G. (1989). Sugar and phenol contents of betevine leaves after inoculation with leaf spot bacteria. *Ind Phytopath*., **42** : 31-37.

Oser, b.L. (1979). In *Hawk's Physiological Chemistry*, Mc Graw Hill Publ. N.Y. pp. 702-705.

Padmanabhan, P. and Alexandar, K.C. (1998). Seedling root rot of sugarcane. *Extension Publication*. No. 5 Sugarcane Institute, Coimbatore.

Prasad, M.M. and Prasad, T. (1997). Changes in L-ascorbic acid contents of Banana fruits under pathogenesis. *Ind. Phytopath*. **30** : 284-285.

Ram, V. and Vir, D. (1996). Post infectional biochemical changes in Banana fruits due to fungal pathogens, *Plant Dis. Res*. **10**(2) : 175-177.

Reddy, S.M., Kumar, B.P. and Reddy, S.R. (1980). Vitamin C changes in infected fruits of tomato. *Ind. Phytopath*. **33** : 511-512.

Sharma, R.L. and Kaul, J.L. (1994). Role of pectolytic trans-eliminases in the rotting of apple by *Monilinia spp*. *Ind. J. Mycol. Pl. Pathol*., **24**(1) : 11-14.

Singh, U.P., Prithiviral, B. and Dubey, R.S. (1993). *Pernospora pisi* spore load on different leaves and its effect on chlorophyll, nucleic acid, protein and phenol contents of pea leaves. *Ind. Phytopath*. **46** : 374-378.

Tiyagi, A, Satraj and Kumar, Rishi, (1994). Changes in ascorbic acid content in tomato fruit due to infection by *Meloidogyne incognita and Rotylenchulus reniformis*. *Ind. Phytopathol*., **47**(2) : 179-180.

Vidyasekaran, P. (1988). *Physiology of Disease Resistance in Plants*. CRC Press. Inc. Bo.Ca raton, Florida. **1** : P. 149.

Viles, F.J. and Silverman, L. (1949). In : *Modern methods of Plant Analysis* (Eds. Paech, K. and Tracey, M.V.), **2** : 162-163, Springer-Verlag, Berlin, 1955.

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