CHANGES IN THE PROTEIN-BOUND AMINO-ACIDS OF TOMATO FRUITS UNDER PATHOGENESIS

THE investigations carried out by the authors[1] on the free amino-acids of healthy and diseased fruits of tomato (*Lycopersicum esculentum* Mill.) infected with *Drachslera australiense* (Bugn.) Subram. and Jain indicated that in general, the amount of most of the free amino-acids increased in the infected tissues of the fruits. A further survey was undertaken to determine the changes in protein-bound amino-acids of healthy and diseased tomato fruits infected by the same organism.

Just ripe fruits of the same age were inoculated with *D. australiense* and were incubated at 25° ± 1° C. The extract of healthy/diseased tissues was made with ethanol after 10 days of infection. This ethanol extract was analysed chromatographically to determine the free amino-acid contents. For chromatographic assay of protein-bound amino-acids, the alcohol extracted residue, containing the protein nitrogen and the colloidal protein (left after centrifugation of soluble protein) were hydrolysed by 6 N HCl in the autoclave at 15 lb. pressure for 20 minutes. A pinch of stannous chloride was added to avoid humin formation and destruction of amino-acids in the presence of carbohydrates. The hydrolysed residue was filtered and the volume of hydrolyzate so obtained was adjusted to g./ml. fresh weight in 20% ethanol. The method followed for two-dimensional ascending chromatography was similar to that reported in earlier publication.[1] Proline, which gave a weak reaction with ninhydrin as a yellow spot, was confirmed by spraying with Isatin reagent of Saifer and Orekes.[2] The method for colorimetric estimation of the separated amino-acids and amides suggested by Thompson *et al.*[3] and also previously described by the authors[1] was followed. The results are summarized in Table I.

It is evident from Table I that leucine/isoleucine, α-alanine, glutamic acid, threonine, aspartic acid, serine/glycine, histidine/lysine were present both in healthy and diseased fruits, while analysis of the protein of the infected tomato fruits revealed the additional presence of valine, proline, arginine and glutamine. The concentration of all the bound amino-acids increased in the diseased tissues, and in this respect the results were similar to those reported by the authors for free amino-acids.[1] The conspicuous increase of the protein-bound amino-acids appears to be due to the association of fungal mycelium with the host tissue which is responsible for higher quantities of protein-bound amino-acids. An attempt was also made to determine if the organism could synthesize the various amino-acids and for this purpose the fungus was grown on modified Asthana and Hawker's liquid medium "A" in which glucose was substituted by 12 different sources of carbon. It was observed that the pathogen synthesized from 3 to 13 non-protein and 8 to 13 protein-bound amino-acids and amides. The synthesis of amino-acids and amides varied with the source of carbon.[4,5]

TABLE I

Showing protein-bound amino-acids and amides in healthy and infected tomato fruit (μg./gm. fresh weight)

Amino-acids	Protein-bound	
	Healthy	Infected
Leucine/isoleucine ..	60·0	80·0
Valine ..	—	60·0
Proline ..	—	260·0
α-alanine ..	60·0	410·0
Glutamic acid ..	60·0	240·0
Threonine ..	120·0	180·0
Arginine ..	—	360·0
Aspartic acid ..	140·0	360·0
Serine/glycine ..	260·0	480·0
Glutamine ..	—	60·0
Histidine/lysine ..	180·0	1160·0
Total ..	880·0	3680·0

—, indicates the absence of amino compound.

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