AMINO ACIDS IN ROOT EXUDATES OF HEALTHY AND *HELMINTHOSPORIUM TURCICUM* INFECTED SORGHUM PLANTS

It is well known that micro-organisms in the rhizosphere receive nutrition from the 'root exudates' which are mainly low molecular substances[1]. These substances readily available to micro-organisms are of great importance for the population of plant root surfaces and are, from all aspects, the determining factors of rhizosphere effect[2].

Studies on changes in rhizosphere microflora due to pathogenic condition in plants is receiving increased attention. This is understandable because any diseased condition in plants results in changed physiology and such changes cause alterations in the chemical composition of root exudates, resulting in altered rhizosphere effect[3]. In the present investigation attempt was made to study the influence of pathogenicity on the quantitative-qualitative occurrence of amino acids in root exudates of sorghum.

Seedlings of CO. 18 sorghum strain (*Sorghum subglabrascens*) were raised in a root exudation apparatus adopting the method and apparatus developed by Balasubramanian and Rangaswami[4]. Plants were inoculated with spore suspension (10,000 spores/ml) of *Helminthosporium turcicum* Pass, causing leaf blight disease of sorghum, on the 15th day and after a fortnight the root exudates from the inoculated plants were pooled and analysed for amino acids. Exudates from healthy plants were also analysed simultaneously for comparison. The exudates so collected after desalting were analysed for amino acids by passing through ion-exchange resin columns (Dowex-1 and Dowex-50 resins) following a modified procedure of Husain and McKeen[5]. Pyridine (0·1 M) was used to elute amino acids from Dowex-50, *n*-butanol acetic acid-water (4 : 1 : 1 v/v) and phenol-water (3 : 1 v/v) solvent systems were employed in two-dimensional ascending chromatographic method for separation and identification of amino acids. Ninhydrin positive spots were quantitatively estimated by the method of Demetriades[6].

It is evident from Table I that while the concentration of asparagine, aspartic acid, cysteine/cystine, glutamic acid, leucine(s), methionine, proline, serine and threonine reduced, there was increase in the concentrations of alanine, histidine and phenylalanine in the root exudates of diseased plants as compared to that in the healthy plants. In addition, unlike in healthy plants, the root exudates of infected plants did not contain glycine, lysine and valine. Thus the present results conclusively prove that disease incidence which results in pronounced changes in physiology of host, could bring both quantitative and qualitative changes in the root exudation pattern. The significance of decreased concentration of certain amino acids like asparagine, glutamic acid, glycine and leucine(s) and other sulphur containing amino acids in root exudates of diseased plants deserves some attention as this would greatly affect the rhizosphere microflora.

TABLE I

A comparison of the amino acids present in the root exudates of healthy and diseased sorghum plants
(Concentration expressed in mμg/plant)

Amino acids	*A	B
Asparagine	.. 270	120
Aspartic acid	.. 320	210
Alanine	.. 110	130
Cysteine/Cystine	.. 120	80
Glutamic acid	.. 480	320
Glycine	.. 70	..
Histidine	.. 80	120
Leucine(s)	.. 100	60
Lysine	.. 90	..
Methionine	.. 120	70
Phenylalanine	.. 140	170
Proline	.. 230	130
Serine	.. 260	190
Threonine	.. 280	150
Tryptophan	.. 170	200
Tyrosine	.. T	60
Valine	.. T	..
Unidentified	.. 1	2
Total corcentration	.. 2,840	2,010

* A — Amino acids in the root exudates of healthy plants. B — Amino acids in the root exudates of diseased plants.
— Not detectable.
T Present in trace.

Division of Microbiology, V. VELRAJU.
Faculty of Agriculture, N. N. PRASAD.
Annamalai University,
Annamalainagar, P.O., *January* 30, 1974.

1. Rovira, A. D., *Bot. Rev.*, 1969, 35, 35.
2. Schroth, M. N. and Hildebrand, D. C., *Annu. Rev. Phytopathol.*, 1964, 2, 101.

3. Rangaswami, G., *Bull. Indian Phytopath. Soc.*, 1968, 4, 120.
4. Balasubramanian, A. and Rangaswami, G., *Mysore J. agric. Sci.*, 1967, 1, 233.
5. Husain, S. S. and McKeen, W. E., *Phytopathology*, 1963, 52, 541.
6. Demetriades, S. P., *Nature* (Lond.), 1956, 177, 95.
7. Wallace, R. H. and Lochhead, A. G., *Soil Sci.*, 1950, 28 C, 1.

AFFINITY OF SHEEP POX VIRUS (SP_s STRAIN) FOR HETEROLOGOUS SYSTEMS

ON reviewing the literature on sheep pox it has been observed that workers have mostly attempted to cultivate sheep pox virus in homologous system in the hope to produce vaccine virus for large scale immunization of sheep. Evidently, heterologous system need to be investigated in greater detail to that virulence is lowered; therefore, this study was undertaken to passage sheep pox virus in different heterologous systems.

Sheep pox virus (SPV) was received as infected skin of lambs suspended in buffered glycerine saline from the department of bacteriology of U.P., Vety. College, Mathura. The virus was purified by Arcton according to the method of Epstein (1958).

Tissue Culture

Mouse embryo fibroblast monolayers were prepared, according to the method of Evans and Salaman (1965) except we used inactivated lamb serum.

Chick embryo fibroblast were prepared from 10-day-old white leghorn embryonated eggs. Cell preparation was same as in mouse embryo except tissue was trypsinised for 40 min. and number of cells was adjusted to 300,000/ml.

Inoculation in Unirradiated Animals

The SPV (10,000 SID 50) was inoculated intracranially in 4-day-old mice and rats and intratesticularly in 20, 40, 60-day-old male mice and rats at the rate of 0·03 ml/animal. After 6 days of observation the animals were sacrified and such 6 and 4 blind passages were given respectively.

Rabbits and guinea pigs (6 months old) were given, in each testis, 0·5 ml of SPV (10,000 SID 50). The testis and animals were observed for 6 days. Such four successive passages were given.

The sheep pox virus (10,000 and 100 SID 50) was inoculated in 12-day-old embryonated eggs by chorio-allantoic (CAM) route at the rate of 0·2 ml/egg. After 96 hours at 37° C virus was harvested and such 4 blind passages were given each time the CAM was examined visually.

In Irradiated Mice

X-Ray irradiation, A200 KV-X-Ray apparatus (Siemens) with an irradiation rate of 200 *r*/3 mins. 10 sec., of 252·5 and 505 *r*, was given to mice in two groups of 20 each according to their body weight. Ten mice, in each group (5 gm and 10 gm), were not exposed to serve as control. Mice from each high and low dose irradiated groups and the unirradiated control were then challenged with SPV (10,000 SID 50) intracranially at the rate of 0·03 ml per mouse. Encephalitic symptoms and brains were observed at the end of 10 days for any gross lesions.

In Tissue Culture

The monolayers of chick and mouse embryo fibroblast in 4 oz. bottle were infected with SPV (10,000 SID 50) by inoculating 1 ml per bottle. After virus adsorption at 37° C for 1 hr 2 ml of maintenance medium was added and cultures were reincubated at 37° C for 140 hours. The virus was liberated by freezing and thawing and centrifugated at 3000 rpm. for 10 min. to remove coarse particles. Such four successive passages were given.

The virus from passaged material from animals and tissue cultures were inoculated i/d in lambs to observe any loss in virulence.

In Intact Animals

Macroscopic examination of brains of 4-day-old mice and rats inoculated intracranially in each passage, revealed no lesions.

Hyaluronidase is known to facilitate the spreading of virus, and testis are known to be rich in this enzyme (Monroe *et al.*, 1949; Sen, 1968). Male mice and rats of varying age groups, *i.e.*, 20, 40 and 60 days, were, therefore, inoculated intratesticularly. Macroscopic examination revealed no change.

Passage of SPV in rabbits and guinea pig testis did not bring about any gross change.

The visual examination of the SPV inoculated CAM revealed thicknening of the membrane which increased progressively on successive passages, such thickening was not found in the control membranes.

The passaged materials obtained from mice, rats, rabbits, guinea pigs and embryonated eggs were inoculated in lambs skin intradermally, neither they produce any gross lesion nor any rise of body temperature upto 10th day. On challenge with virulent SPV, no protection of the inoculated lambs was observed, showing that SPV apparently did not multiply in mice and rats brain, mice, rats, rabbits, guinea pig testis as well as on CAM of eggs.

Groups of mice were irradiated with 252·5 *r* and 505 *r* prior to inoculation of the SPV intracranially, observation revealed that irradiation of mice apparently did not have any effect on virus adapta-