PAL-ASE ACTIVITY IN THE ROOTS OF *SORGHUM BICOLOR* (L.) INOCULATED WITH *AZOSPIRILLUM*

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AZOSPIRILLUM, the nitrogen-fixing diaztroph, is widely used as a bio-fertilizer for sorghum and other millets as a supplement to fertilizer nitrogen. Recent observations reveal that the use of *Azospirillum* bio-fertilizer for sorghum both as seed and soil inoculation increased the total phenolic content in young sorghum plants offering resistance against a major pest, the shootfly, *Atherigona soccata* Rond[1]. Khurana and Verma[2] observed that the total phenol content was negatively correlated to sorghum shootfly susceptibility. Studies with phenolic compounds imply that the enzymes involved in polyphenol synthesis may be formed in response to mechanical injury. Among the several enzymes involved in phenolic-biosynthesis in plants, the phenylalanine ammonia lyase (PAL-ase) is the most important enzyme[3]. It has been amply illustrated that post-infectionally synthesized phenolic compound plays an important role in disease resistance[4]. We report in this communication the PAL-ase activity in the roots of sorghum plants inoculated with *Azospirillum* bio-fertilizer.

Assay of PAL-ase activity

Seeds of the sorghum cultivars, CSH 9 and Co 26 respectively susceptible and moderately resistant to shootfly attack were inoculated with *Azospirillum* bio-fertilizer and raised under controlled conditions. When the seedlings were 20-day-old, the roots were collected, washed in tapwater, and blotted dry. A weighed quantity (5 g) of the tissue was cut into small bits and macerated to a fine consistency in a pre-chilled pestle and mortar. To the slurry, 20 ml of acetone was added, stirred well, and filtered through a Buchner funnel. The precipitate left over the filter paper was collected, air-dried and used as the enzyme source[5]. The enzyme assay procedures were essentially the same as detailed by Zucker[6].

The present results reveal a significant increase in the PAL-ase activity in the roots of both the sorghum cultivars (table 1). An increase of 10.2 and 27.2 units of PAL-ase per 100 mg of the enzyme protein over the control was observed after 24 h and 48 h of inoculation in the moderately resistant Co 26 sorghum. The hybrid, CSH 9 also registered an increase of 12.2 and 6.5 units of PAL-ase over the control after 24 h and 48 h of inoculation.

Table 1 *PAL-ase activity in the roots of Azospirillum inoculated sorghum cultivars*

Period after inoculation (h)	Sorghum cultivar		Unit of* PAL-ase	Per cent increase over control
24	Co. 26	Control	31.0	–
	Co. 26	Inoculated	41.2	32 90
	CSH. 9	Control	27.2	–
	CSH 9	Inoculated	29 8	9.56
48	Co. 26	Control	18.85	–
	Co. 26	Inoculated	46.05	144 29
	CSH. 9	Control	18.85	–
	CSH. 9	Inoculated	19.35	2.65

*per 100 mg of enzyme protein; 1 Unit of PAL-ase = 3.3 μmol of cinnamic acid formed

It is interesting to note that *Azospirillum* inoculated Co 26 exhibited a steady increase of PAL-ase activity (from 41.2 units to 46.05 units) even after 24 h as compared to the uninoculated control and in the cultivar, CSH 9, the activity dropped after 24 h of inoculation. Purushothaman[5] found that the activity of PAL-ase was higher in TKM 6 paddy, which is resistant to several pests and diseases than in the susceptible cultivars, Co 13 and IR 8. Probably, the faster rate of increase of PAL-ase activity in Co 26 may be associated with its moderate resistance. Uritani[4] reported that the PAL-ase is not activated from pre-existing inactive forms but rather synthesized 'de novo'. Hence it is probable that from the present investigation *Azospirillum* inoculation activates the enzyme, PAL-ase, implicated in the biosynthesis of phenolics resulting in increased phenolics in plants. This appears to be a plausible reason for resistance towards shootfly incidence.

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LIPIDS IN MUCOSAL EPITHELIUM OF THE INTESTINE OF MICE FED ON ZN-DEFICIENT DIET

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THE loss of appetite and growth retardation associated with sterility, oesophageal parakeratosis, aplocia and impaired wound healing have been frequently reported in mammals under Zn-deficient conditions[1]. The causes of most of the symptoms have been related to depression in the activities of many Zn-dependent enzymes[2]. However growth retardation and anorexia in such animals point toward malfunctions of alimentary canal besides other factors. An attempt has been made to study the absorption rate of lipids under Zn-deficient conditions through perfusion experiments[3].

Lipids have been associated with the inhibitory effect on gastric secretion and stomach emptying process when they are present in mucosal epithelium of the intestine[4,5]. Cytochemical localization of lipids in mucosal epithelial cells, therefore, can provide us the morphological evidence for such a phenomenon. This paper reports the effect of severe Zn-deficiency on the cytochemically detectable lipids in mucosal epithelium of the intestine of mice.

Twenty male mice, *Mus musculus* of Lacca strain weighing 18–20 g, were equally divided into two groups. The animals in the first group (ZD) were fed *ad libitum* on semisynthetic diet containing: EDTA-treated casein: 30% sucrose: 51% corn oil: 8% mineral mixture[6]: 4% vitamin mixture[6]: 5% methionine: 0.8% and agar agar 1.2%. The analysis of this feed by atomic absorption spectroscopy has shown that it contains 0.5–1 ppm of Zn. The animals in the second group (ZS) that served as the control were fed on Zn-supplement diet which was identical to Zn-deficient diet except that $ZnSo_4$ was added to the mineral mixture to raise the Zn content to 100 ppm. Triple distilled deionized water was given to the animals *ad libitum*.

The symptoms of Zn-deficiency characterized by low food intake, spiny hair coat, skin lesions, growth retardation in terms of weight gain, started appearing in mice of ZD diet group after 2 weeks of the treatment and the severity of symptoms increased as the duration of feeding on low Zn diet was prolonged to 3 weeks.

The average food intake during the first 2 weeks was statistically insignificant between the two groups. Thereafter, the ZD group started consuming less food. At the end of the third week, they consumed 28% less ration than ZS control and lost at an average of 23.04 g/kg body weight. The feeding was then suspended for 3 h and 8 h and the animals of both groups were dissected feeding for 3 h and 8 h. The duodenum and jejunum were cut into small pieces and fixed in formalin-calcium and neutral formalin fluids. The cold gelatin sections were cut at 10 μ and stained in Sudan black B (SBB), acid haematin (AH) and Nile blue sulfate (NBS) following Pearse[7].

The cytoplasm of columnar, long and narrow absorptive epithelial cells of duodenum and jejunum that line the luminar surface of the villi stained intensely and homogeneously with SBB and NBS in ZD animals in contrast to a moderate reaction limited to a few granules concentrated more apically than basally in ZS animals sacrificed after 3 h of their meals (figures 1 and 2). The sudanophilia in ZD mice was so intense that the cytoplasm appeared pitch black in SBB preparation. A slight reduction in SBB and NBS reaction was noticed at 8 h stage in ZD mice. However, the reaction was far more intense than ZS animals which practically lacked sudanophilic granules at this stage.

These cytochemical results suggest a massive lipid accumulation, predominantly triglycerides, far more in excess in mucosal epithelial cells of ZD mice than ZS control at 3 h and 8 h stages despite the equal amounts of corn oil in their diets. This envisages a slower rate of lipid transport to lacteal. These results are in conformity with those of Koo and Turk[3] who through electronmicroscopic and chromatographic studies concluded that the exit block to the movement of lipid droplets out of mucosal cells occurs due to the failure of mucosal synthesis of proteins required for the formation of chylomicrons in Zn-deficient rats. Almost similar results have been obtained following treatment of rats with protein synthesis inhibitors[8,9]. The essentiality of Zn in protein synthesis is well-established[10,11].

The persistence of lipids for a longer duration in mucosal epithelial cells in ZD mice than ZS control perhaps is responsible for imposing a prolonged inhibitory effect on stomach emptying process through feedback mechanism which may be one of