

## IN VIVO EFFECT OF SOME METABOLIC INHIBITORS ON GLUTAMATE DEHYDROGENASE AND GLUTAMATE SYNTHASE ACTIVITIES IN EXCISED MAIZE TISSUES

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GLUTAMATE dehydrogenase (NADH-GDH; EC 1.4.1.2) and glutamate synthase (NADH-GOGAT; EC 1.4.1.14) are enzymes involved in the synthesis of glutamate via two different pathways viz GDH and GS/GOGAT pathways and considered to be important in the primary amination reactions in plants<sup>1</sup>. Some amino acid analogs (i.e. methionine sulphoximine, albizzine and azaserine) have been used as marker substances in the determination of potentials of two pathways<sup>2</sup>. However, long term effects of these substances and some other important metabolic inhibitors on these enzymes have not been studied. Moreover, their effects may be different for enzymes from different plant tissues. The present study was undertaken to find out the effect of some important metabolic inhibitors on two enzymes under the same environmental conditions in a nonchlorophyllous (roots) and chlorophyllous (leaves) tissues of young maize seedlings.

Seeds of *Zea mays* L cv GS-2 procured from National Seeds Corporation, New Delhi were grown either for 4 days (for roots) or for 9 days for (leaves) as described earlier<sup>3</sup>. Small pieces (~5 mm) of the

excised roots and secondary leaves were incubated for 24 hr with the desired inhibitor included in the modified half strength Hoagland's solution containing 5 mM  $\text{NH}_4\text{NO}_3$  under the same environmental conditions as used for growth. GDH<sup>5</sup> and GOGAT<sup>6</sup> were extracted and assayed as described earlier. Protein was estimated by the method of Lowry *et al*<sup>7</sup>.

The data shown in table 1 indicate that glutamine analogs; albizzine and azaserine inhibited GOGAT activity in either tissue examined but had no effect on GDH activity during *in vivo* supply to the excised tissues. Leaf soluble protein content, however, was inhibited by 13% with albizzine supply. Methionine sulphoximine, a potent inhibitor of GS catalyzed reaction<sup>2,11</sup>, also inhibited GOGAT activity in both the tissues and to some extent the GDH activity. MSO inhibited leaf protein also but it increased root protein content (table 1). When supplied *in vitro* to the reaction mixture during enzyme assay, the MSO has been reported to inhibit GOGAT activity to some extent along with GS and it causes ammonia accumulation in the tissue<sup>11</sup>. Our experiments reveal that *in vivo* supply of MSO or some metabolic product(s) of MSO may effect the GDH in addition to GS and GOGAT and therefore, entry of ammonia via either pathway. It also appears that these three compounds are not only the markers for the ammonia-assimilating enzymes but they may also affect the process physiologically.

The dicarboxylic acid glutarate and the trimeric dihydrate, glyoxal, whose binding sites are similar to 2-oxoglutarate, inhibit the two enzymes, but the

Table 1 Effect of some amino acid analogs and other metabolic inhibitors on glutamate dehydrogenase and glutamate synthase activities in root and leaf tissues of maize seedlings. Data  $\pm$  SE (n=6). Values in parentheses are percentage relative to control

Inhibitor mM	Root			Leaf		
	Enzyme activity, n mol NADH oxidized (mg protein) <sup>-1</sup> min <sup>-1</sup>		mg protein (g F.Wt.) <sup>-1</sup>	Enzyme activity, n mol, NADH oxidized (mg protein) <sup>-1</sup> min <sup>-1</sup>		mg protein (g F.Wt.) <sup>-1</sup>
	GDH	GOGAT		GDH	GOGAT	
At 0 hr	198 $\pm$ 4	8.03 $\pm$ 0.13	4.98 $\pm$ 0.24	91 $\pm$ 4	8.55 $\pm$ 0.42	10.48 $\pm$ 0.64
After 24 hr incubation						
None (control)	210 $\pm$ 4 (100)	8.25 $\pm$ 0.21 (100)	6.00 $\pm$ 0.05 (100)	94 $\pm$ 4 (100)	9.25 $\pm$ 0.24 (100)	12.20 $\pm$ 0.40 (100)
Azaserine, 0.05	195 $\pm$ 6 (93)	1.52 $\pm$ 0.04 (18)	6.21 $\pm$ 0.12 (103)	98 $\pm$ 6 (103)	0.64 $\pm$ 0.03 (7)	10.61 $\pm$ 0.29 (87)
Albizzine, 1.0	209 $\pm$ 5 (100)	3.34 $\pm$ 0.01 (40)	6.26 $\pm$ 0.16 (104)	110 $\pm$ 5 (117)	5.52 $\pm$ 0.08 (59)	12.93 $\pm$ 0.48 (106)
MSO, 1.0	166 $\pm$ 8 (79)	2.60 $\pm$ 0.08 (32)	6.91 $\pm$ 0.24 (115)	73 $\pm$ 4 (78)	5.83 $\pm$ 0.11 (41)	10.49 $\pm$ 0.21 (86)
Glutarate, 5.0	51 $\pm$ 2 (24)	6.12 $\pm$ 0.26 (74)	6.20 $\pm$ 0.15 (103)	46 $\pm$ 2 (49)	7.67 $\pm$ 0.23 (82)	11.95 $\pm$ 0.28 (98)
Glyoxal, 5.0	134 $\pm$ 4 (64)	2.21 $\pm$ 0.06 (27)	6.10 $\pm$ 0.09 (102)	73 $\pm$ 3 (77)	5.81 $\pm$ 0.12 (62)	11.83 $\pm$ 0.20 (97)
Malonate, 5.0	164 $\pm$ 8 (78)	6.09 $\pm$ 0.12 (74)	7.20 $\pm$ 0.26 (120)	84 $\pm$ 7 (89)	7.95 $\pm$ 0.29 (84)	13.17 $\pm$ 0.20 (108)
DCMU, 0.02	213 $\pm$ 10 (102)	7.83 $\pm$ 0.19 (95)	6.02 $\pm$ 0.08 (100)	77 $\pm$ 4 (81)	6.70 $\pm$ 0.22 (71)	12.16 $\pm$ 0.16 (100)

effect is specific to some extent. For example the effect of glutarate is more pronounced on GDH and that of glyoxal on GOGAT activity (table 1). Malonate, a TCA cycle inhibitor inhibited both the enzymes but the inhibition is more pronounced in the root tissue. The root protein increased by 20% by supply of malonate and consequently it reduced the specific activity of the enzymes. Malonate possibly restricts the supply of carbon skeleton and/or metabolic energy to the process of glutamate synthesis. DCMU[3-(3, 4-dichlorophenyl)-1, 1-dimethylurea] inhibits the activities of GDH and GOGAT only in leaves by 19 and 29% respectively possibly by restricting photosynthetic production of NAD(P)H.

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## AECIDIUM PAINAVUENSIS SP. NOV FROM IDUKKI, KERALA, INDIA

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DURING a survey and study of rust fungi in the forests of Idukki Hydroelectric Project area, the plant *Meliosma pinnata* (Roxb) Walp ssp *arnottiana* (Wight) Beus, was seen to be infected with a fungus. Infection was restricted to the growing tender shoots and a few leaves below the growing tip. Infected shoots were brick-red coloured and showed a typical symptom of witches' broom. The leaves immediately below the infected shoots showed hypertrophied lesions. Infection was not observed on old leaves. A little disturbance to the infected shoots released a cloud of spores into air and such infected plants could easily be detected by their appearance even from a distance. Microscopic study of this fungus revealed that it belongs to the form genus *Aecidium* Pers.

*Aecidium painavuensis* Hosagoudar, sp nov (figures 1-4)

Contagio restringo mollis surculus, efficio witch-e's broom. Pycnia folicola, caulicola, amphigena, subcuticularia, brunnea vel nigra, applanta vel conoidea, hymenium planus, paraphyses terminalibus pallidus vel brunneus, 86-120 × 34-100 μm. Pycniosporae rotundae vel ovalae, minutae, hyalinae vel pallidae. Aecia folicola, caulicola, amphigena, subepidermalia, cupulata, innata, erumpenta ad maturitatus, 243-342 × 135-306 μm;



Figure 1. Infected young tender shoot showing the symptom of witches' broom.