GROWTH AND PHENOLIC PRODUCTION IN CALLUS CULTURES OF *CROTALARIA*

PHENOLIC compounds are produced by *Crotalaria* seedings[1] and tissues derived from it[2]. Qualitative analysis of callus tissues revealed nine different phenolic acids[3] such as chlorogenic, coumaric, *p*-hydroxybenzoic, protocatechuic, gallic and methoxybenzoic acid derivatives. Methods of inducing increased concentrations of these compounds would be very useful. L-Phenylalanine and L-tyrosine are well established intermediates in the pathway leading to the synthesis of phenolic compounds. Further, a few reports[1-5] suggested that the accumulation of phenolic compounds was favoured by the growth of the cells. The present investigation was aimed to study the effect of L-phenylalanine and L-tyrosine on growth and production of phenolic compounds in *Crotalaria juncea* callus cultures.

The callus cultures derived from the seedlings of *C. juncea* and established on Murashige and Skoog's (MS) medium[6] for over 3 years were subjected to different concentrations and combinations of L-phenylalanine and L-tyrosine. Tissue masses weighing 300 ± 30 mg fresh weight were inoculated separately on 30 ml agar medium. The culture flasks were incubated under uniform conditions of light (1000 lux) and temperature (26 ± 2° C) and a fixed number of replicates was havested at the end of culture period (4 weeks) for the estimation of total phenolics and for the growth determination. Growth measurements were followed by the increase in fresh and dry weights of the tissue. Phenolic compounds were extracted with ice-cold 80% (v/v) ethanol and estimated by the Folin method of Swain and Hillis[7] using chlorogenic acid as standard.

TABLE I

Influence of L-phenylalanine and L-tyrosine on growth and phenolic production in seedling cultures of Crotalaria*

Inoculum : 300 ± 30 mg fresh tissue on 30 ml MS medium supplemented with 2·0% sucrose, 2·0 mg/l 2, 4-D and amino acids as given below.

Incubation : 4 weeks in light at 26 ± 2° C.

Treatment	Conc. of amino acid (%)	Fresh wt. (mg)	Dry wt. (mg)	Phenolic compounds	
				µg/culture	µg/100 mg dry wt.
Control	0·0	6772	229	1892	826
L-Phenylalanine	0·05	856	29	319	1100
	0·1	898	32	480	1500
	0·2	575	20	250	1250
L-Tyrosine	0·05	961	34	663	1950
	0 1	3440	113	4068	3600
	0·2	1621	53	1669	3150
L-Phenylalanine + L-Tyrosine	0·05 + 0·05	1251	41	645	1575
L-Phenylalanine + L-Tyrosine	0·1	3552	103	2292	2225

* Data represent average of six replicates.

The data presented in Table I clearly showed that the addition of L-phenylalanine did not enhance the production of phenolic compounds per culture when compared with the control. At all the levels of L-phenylalanine tested, there was pronounced inhibition of growth and phenolic production. However, on percentage basis, L-phenylalanine supported more phenolic materials than the control. With tyrosine, on the other hand, suppression of growth was less than the phenylalanine and the production of phenolic compounds was more than even the control. Highest (3·6%) accumulation of phenolic compounds was registered in tissues grown on 0·1% tyrosine containing medium. Further, a sort of antagonism was observed when phenylalanine and tyrosine were added

together in the medium. Tyrosine appreciably relieved the pronounced reduction in growth and phenolic production caused by phenylalanine but its own promoting effect when used alone was found much less in combination with phenylalanine.

Thus, *Crotalaria* cells failed to grow satisfactorily when phenylalanine or tyrosine or both were incorporated into the medium containing nitrate. Among the hypotheses which were considered to account for the amino acid effects was the idea that they inhibit growth by inhibiting the biosynthesis of other amino acids. The pathway of nitrate assimilation would seem a likely place, as also suggested by Filner[8], for one amino acid to prevent the synthesis of others. Phenylalanine seems to be more inhibitory than tyrosine as more reduction in growth resulted with phenylalanine medium.

Both the amino acids, phenylalanine and tyrosine, are direct precursors of phenolic synthesis.. In the light of this, increased production of phenolic materials per cell is obvious when they are incorporated into the medium. However, addition of tyrosine has enhanced the production of phenolic compounds substantially. Tyrosine is nearly four times less inhibitory to growth than phenylalanine. The effect of these amino acids on the growth may be due to their inihibition of nitrate assimilation pathway, particularly the glutamate generating system[8]. The synthesis of phenylalanine and tyrosine is dependent on the transamination reactions involving glutamate. This means, the endogenous synthesis of phenylalanine may be nearly four times less than tyrosine as evident from their effect on growth. The overall result is that tyrosine supported maximum whereas phenylalanine enhanced very little phenolic production. However, detailed investigation is demanded before concluding with confidence as the control mechanism of the pathway includes many complex enzyme systems[2].

One of us (RRS) is grateful to the University Grants Commission for financial support.

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August 26, 1977.

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MECHANICAL TRANSMISSION OF WHITE FLY-BORNE YELLOW MOSAIC VIRUS OF *LABLAB NIGER* MEDIKUS (*DOLICHOS LABLAB* L.)

THE yellow mosaic disease is known to infect many leguminous crop plants and its successful transmission only by the white fly *Bemisia tabaci* Genn has been reported[1,4,5], while attempts to transmit the disease by mechanical inoculation were unsuccessful. However, recently a similar white fly-borne golden mosaic disease of bean was transmitted successfully by sap-inoculation[6]. The present study was therefore taken up to find out the possibility of transmitting a yellow mosaic disease of *Lablab niger* Medikus and the results are reported in this communication.

Lablab cultivar Co. 8 plants that were inoculated by the viruliferous *Bemisia tabaci* were used as virus sources in the present study. An enamel tray was filled to three-fourth of its capacity with tap water. A pair of pestle and mortar was kept in the tray which was then placed in a freezer till the water was frozen. Phosphate buffer 0·1 M at pH 6·6, 6·8, 7·0, 7·2, 7·4, 7·6, 7·8 and 8·0 was prepared and cooled in the freezer. Young leaves showing clear symptoms of yellow mosaic disease were macerated in the mortar kept in the ice tray with the phosphate buffer added at the rate of 3 ml/g of leaf material. The extracted sap was rubbed with the pestle on the cotyledonary leaves of 5 days old test plants that had been dusted with 600-mesh silicon carbide gently by having a thin cardboard pad below the leaves. Plants rubbed with the buffer alone served as control. The excess inoculum was washed away with tap water using a wash bottle. The plants after inoculation were kept in the glasshouse (temperature variation 21°–35°). The results are given in Table I.

The present white fly-borne yellow mosaic disease could successfully be transmitted by sap inoculation as per the method followed in the present study. The virus appears to remain infective in the extra cellular environment provided during this study. It may be seen from Table I that the