

Thanks are due to Birbal Sahni Institute of Palaeobotany, Lucknow for permission to conduct the SEM studies.

28 April 1986; Revised 5 September 1986

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ALPHA-AMYLASE AND ISOPEROXIDASES IN DIFFERENTIATING CALLUS CULTURES OF *DATURA INNOXIA*

R. K. JAIN, N. MAHERCHANDANI and V. K. CHOWDHURY

Department of Genetics, Haryana Agricultural University, Hisar 125 004, India.

REGULATION of growth and differentiation has remained a mysterious problem in plant tissue culture. Until now, only in a few species, it is possible to make the cultured tissue differentiate at will because the nutritional and hormonal requirements of different cells are not well understood. We have been studying the biochemical changes pro-

duced in plant cell cultures during differentiation with a view to identify some cellular changes, which would serve as an early indication of differentiation. This communication reports the changes produced in the enzyme activity and isoenzyme pattern of α -amylase and peroxidase during differentiation in anther derived callus cultures of *Datura innoxia*.

The calli were initiated from explants of anther derived haploid plantlets¹ on Murashige and Skoog's² (MS) medium supplemented with 2 mg/l of 2,4-D (2,4-dichlorophenoxyacetic acid). This medium is henceforth designated as MS₂ medium. These cultures were transferred to fresh MS₂, B₃ and MSR (MS medium + 0.1 mg/l of indoleacetic acid) media for callus growth, shoot regeneration and root differentiation respectively. All the cultures were kept in dark at 27 ± 1°C except for shoot regeneration, where they were incubated under constant illumination of 4000 lux. Alpha-amylase activity was assayed by the method of Naylor⁴. The method of Seevers *et al*⁵ was followed for the enzyme assay of peroxidase. Protein was assayed by the folin-phenol method of Lowry *et al*⁶. Polyacrylamide gel electrophoresis (anionic system)⁷ was performed to detect the isoenzyme pattern of α -amylase⁸ and peroxidase⁵.

The effect of different media on the enzyme activity of α -amylase and peroxidase is shown in table 1. The specific activity of both the enzymes increased considerably under differentiating conditions. Further, activity of these enzymes was greater in cultures incubated in MSR medium as compared to that in B₃ medium promoting shoot regeneration.

Table 1 Alpha-amylase and peroxidase specific activities* in cultured cells of *Datura innoxia* after 20 days of incubation in different media

Medium	Growth hormones	Response	α -amylase	Peroxidase
MS ₂	2, 4-D (2.0 mg/l)	Callus formation	3.3 ± 0.8	48 ± 11
MSR	IAA (0.1 mg/l)	Root differentiation	20.2 ± 4.5	1842 ± 155
B ₃	Kinetin (2.56 mg/l) and IAA (4.0 mg/l)	Shoot regeneration	13.6 ± 1.7	1143 ± 210

Enzyme unit = 0.1 change in optical density,

Specific activity = enzyme units × minute⁻¹ × mg⁻¹ protein

Alpha-amylase isoenzyme pattern of callus cultures remained unchanged, when cultured onto MSR or B₃ medium. Only one band with a R_f values of 0.158 was observed in each case. However, distinctive changes in the isoperoxidase pattern were observed during root or shoot differentiation in callus cultures. Peroxidase isoenzymes could be classified into two groups (i) those with intermediate electrophoretic mobilities (R_f—0.25 and 0.27) and (ii) fast moving isoperoxidase (R_f—0.40 and 0.50) (figure 1). The most significant change was observed in the fast migrating group, which developed in cultures incubated in MSR and B₃ media. While in cultures incubated in MSR medium, only one band with a R_f value of 0.40 was observed, two bands with R_f values 0.40 and 0.50 developed in cultures incubated in B₃ medium.

Comparison of α -amylase and peroxidase activities in callus promoting and root and shoot forming conditions revealed that there was more pronounced activity of the two enzymes prior to shoot and root formation. Similar increases in α -amylase and peroxidase activities have been demonstrated prior to root or shoot differentiation⁹⁻¹¹. Increased α -amylase which precede differentiation may be necessary for increased mobilization of carbohydrate reserves concomitant with high synthetic activities which occur during organogenesis⁹. Differences in isoperoxidase pattern associated with shoot and root dif-

ferentiation have also been elegantly demonstrated^{9,11}. Fast moving anodic bands have been shown to be associated with lignification¹². The changes in band patterns in this study can be interpreted as creating situations conducive to shoot or root formation. Such isoperoxidases thus provide useful biochemical signals for morphogenetic events that follow.

6 May 1986

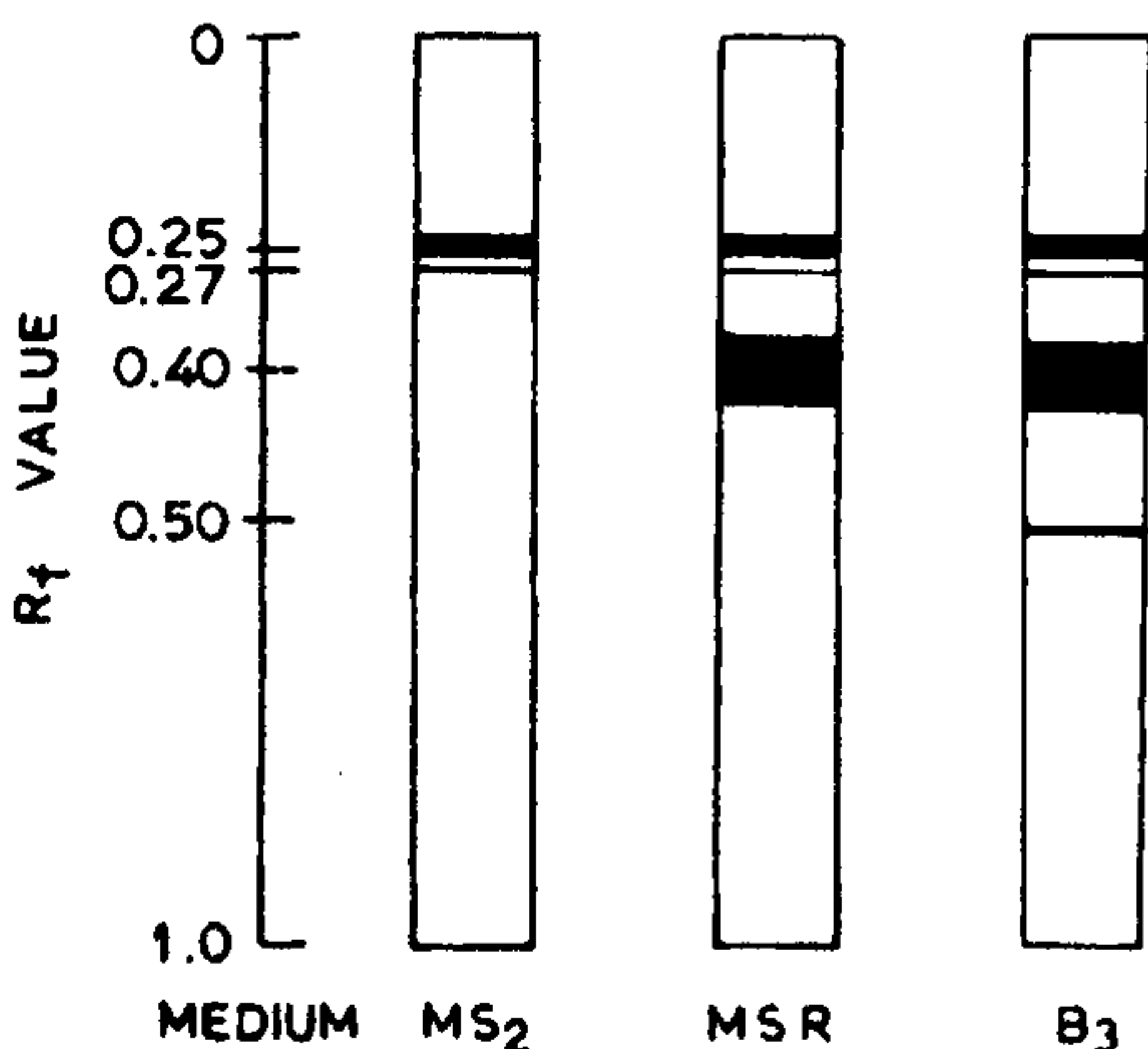


Figure 1. Peroxidase isoenzyme pattern in cultured cells of *Datura innoxia* after 20 days of incubation in different media.

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RELATIONSHIP OF RICE GRASSY STUNT VIRUS WITH ITS PLANTHOPPER VECTOR

S. K. MATHEW* and A. N. BASU

Mycology and Plant Pathology Division, Indian Agricultural Research Institute, New Delhi 110 012, India

*Present address: College of Horticulture, Vellanikkara, Trichur 680 654, India.

GRASSY stunt of rice, a disease caused by grassy stunt virus (GSV), was first observed in the Philip-