

*ptium* and *D. aristatum* mentioned in the literature have been presently examined by observations on living and herbarium specimens. A total of 105 populations of these species were studied for the spike length and the prolongation of the rachis. It is seen that the spike length varies from 2–4.5 cm in *D. aegyptium* and from 1 to 4.5 cm in *D. aristatum* without any discontinuity. This character, therefore, cannot be used in separating *D. aristatum* from *D. aegyptium*. The prolongation of rachis, on the other hand, was observed to be 1–2.5 mm in *D. aegyptium* and 2.5–5 mm in *D. aristatum*. A number of other morphological characters such as the growth habit, plant length, internode length, number of racemes per spike, sheath length, lamina length, lamina breadth, lamina hairiness and characters of glumes and lemma were then studied in order to assess their taxonomic utility. Of all these characters, the character of lamina hairiness was helpful in separating populations of *D. aristatum* from those of *D. aegyptium*. As a result of these studies, Bor's key for the separation of *D. aristatum* from *D. aegyptium* is modified as under:

1. Plants stoloniferous; erect, prostrate to decumbent; tip of the rachis shortly produced, upto 2.5 mm long; leaf lamina sparsely hairy . . . *D. aegyptium*.
2. Plants not stoloniferous; erect to prostrate; tip of rachis upto 5 mm long (always more than 2.5 mm); leaf lamina profusely hairy . . . *D. aristatum*.

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## PRODUCTION OF CELLULOLYTIC ENZYME BY *RHIZOCTONIA SOLANI* KÜHN

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*RHIZOCTONIA SOLANI* Kühn the casual organism of sheath blight disease of rice, is a soil-borne facultative saprophyte, known for its secretion of heat-stable metabolites as well as the cell wall degrading enzymes<sup>1</sup>. The disease incited by *R. solani* is one of the major diseases of rice in tropical Asia. The aim of the present investigation is to know about the quantitative changes in the production of cellulolytic enzymes by differentially aggressive isolates of *R. solani*.

Fungal mat from 6-day old cultures of less aggressive ( $R_1$ ) and aggressive ( $R_5$ ) isolates of *R. solani* grown on potato dextrose agar (PDA), washed thoroughly with sterile-distilled water, was used as inoculum. Czapek's broth, which supported the maximum growth of the fungus was used by substituting carboxy methyl cellulose (CMC, 0.25%) for sucrose. The cell-free samples were collected on alternate days after centrifugation at 3,000 rpm. The enzyme cellulase activity was determined by the viscosimetric method<sup>2</sup> and the reaction components were temperature equilibrated and mixed in the following proportions: 2 ml of the culture filtrate, 8 ml of 1% CMC in 0.05 M acetate buffer at pH 4.5. Viscosity of the reaction mixture was determined by a viscosimeter at 30°C after an incubation period of 10 min. Boiled culture filtrate served as control. The activity was converted to viscosimetric units by calculating the reciprocal of the time required for 1 ml of the enzyme solution to reduce the viscosity of the substrate by 50%. The enzymatic activity was also determined colorimetrically by estimating the reducing sugars<sup>3</sup>. The reactive mixture was the same as that used for the viscometric method. The developed

**Table 1** Cellulase production by the less aggressive ( $R_1$ ) and aggressive ( $R_5$ ) isolates of *R. solani*

Days of incubation	Enzyme activity (units)		Reducing sugars ( $\mu$ g of glucose)		Growth (mg dry wt)	
	( $R_1$ )	( $R_5$ )	( $R_1$ )	( $R_5$ )	( $R_1$ )	( $R_5$ )
2	40	80	50	55	4	5
4	85	180	120	250	7	8
6	55	120	80	115	10	12
8	50	110	50	95	16	18
10	45	100	40	55	22	25

blue colour was read at 720 nm. The standard curves of glucose were prepared to estimate the cellulase activity.

A close correlation was obtained between the enzyme production and the aggressiveness of the isolates (table 1) as it has been previously reported in *R. solani*<sup>4</sup> and in *Verticillium*<sup>5</sup>. The aggressive isolate R<sub>5</sub> has interestingly recorded a maximum activity on day 4 which followed a sudden decrease in the latter periods of incubation. Whereas the less aggressive isolate R<sub>1</sub> showed a slight increase in activity, only after prolonged period of incubation. That the increased production of cellulase only by the aggressive isolate further substantiates the earlier work of Weinhold and Motta<sup>6</sup> in cotton, where it has been showed that the cell wall degrading enzymes are responsible for the host damage, even before the symptoms are apparent. A thorough investigation of isoenzymes present both in the culture filtrates and in host tissues infected with aggressive and less aggressive isolates will throw more light on the nature of enzymes involved during early pathogenesis.

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## CYTOKININ GLUCOSIDE ACTIVITY IN GUAVA (*PSIDIUM GUAJAVA* L.) SEEDS

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CYTOKININ glucosides have been suggested to be the storage forms and serve to regulate the levels of free forms of cytokinins in the plants<sup>1</sup>. The occurrence of zeatin glucoside in coconut milk<sup>2</sup> and glucosylated

forms of zeatin riboside and the dihydrozeatin riboside has been reported as a metabolite of 8 [<sup>14</sup>C]zeatin in the seedlings of *Phaseolus vulgaris*<sup>3</sup>. In an earlier paper<sup>4</sup> five different cytokinins were detected from immature seeds of guava. While three of them were identified as zeatin, zeatin riboside and zeatin nucleotide, a suggestion was put forward<sup>4</sup> that either of the rest one may be zeatin glucoside. The present paper reports the tentative identification of zeatin glucoside and zeatin riboside glucoside from the immature seeds of guava *Psidium guajava* L.

From about 2000 immature guava fruits (cv. Allahabad safeda) seeds were separated and homogenised twice with chilled 80% methanol. The combined methanolic extract was evaporated to aqueous *in vacuo* below 40°C and from it the fractions A and B were separated as described earlier<sup>4</sup>. Descending paper chromatography of fraction B (ammonia eluate of Zeo-karb column) was performed on Whatman No. 1 paper strip (45 × 10 cm) at 25–26°C using *n*-butanol:acetic acid:water (12:3:5 v/v) solvent

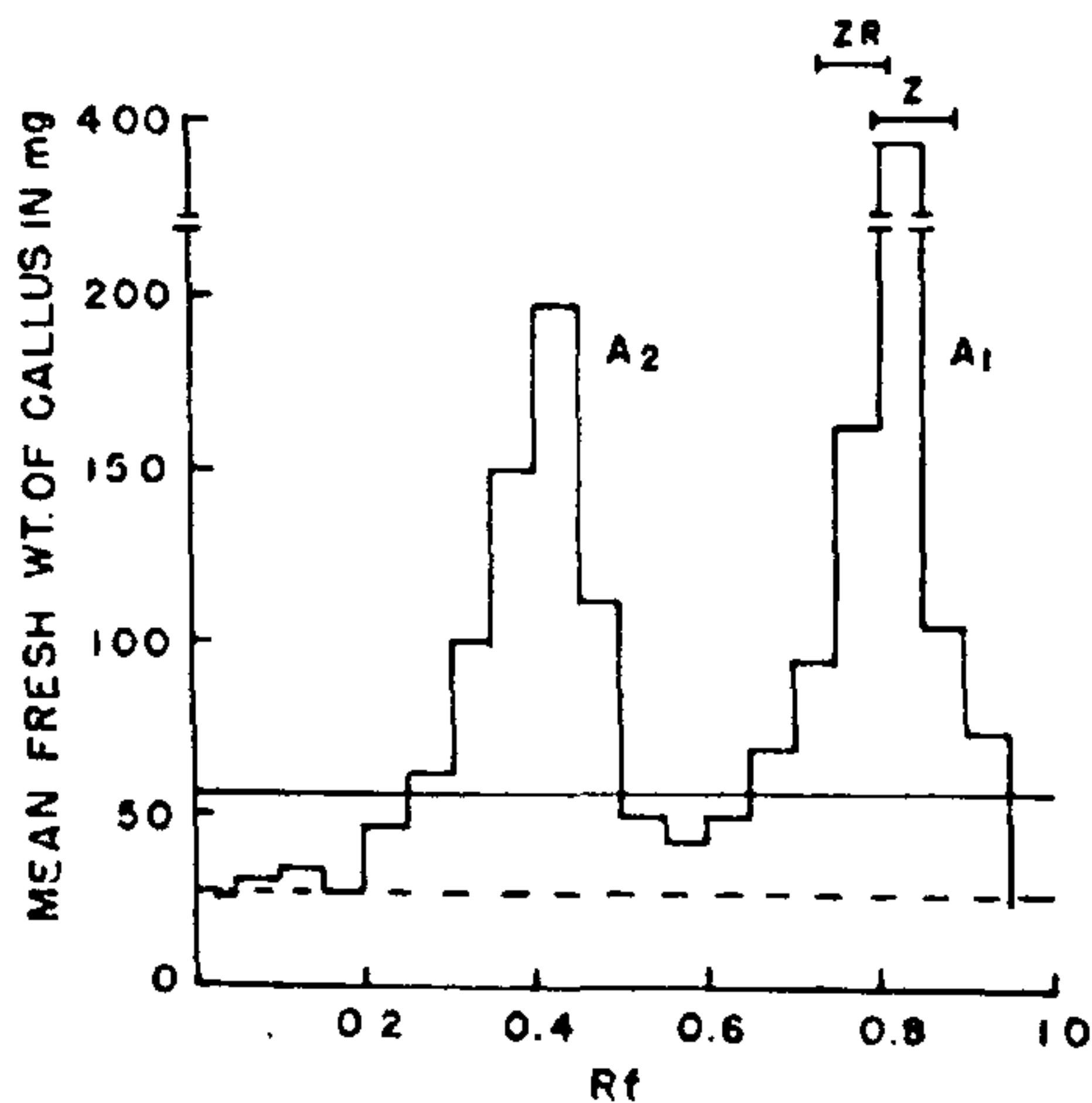


Figure 1. Cytokinin activity as measured by soybean callus test after paper chromatography in *n*-butanol:acetic acid:water solvent system of ammonia fraction obtained by Zeo-karb 225 column chromatography of guava seed extract. Quantity equivalent to 20 g fresh weight of tissue was chromatographed. The broken and solid horizontal lines represent control and least significant differences at 5% level respectively.