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A. R. CHOWDHURY.
H. C. CHATURVEDI.

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ABSCISIC ACID PRODUCTION BY *DIPLOCARPON ROSAE* AND ITS ROLE IN THE DISEASE PRODUCTION

SYNTHESIS of growth regulating substances by plant pathogenic microorganisms has been reported by several workers. A list compiled by Gruen[1] shows that about 80 fungal species including plant pathogens and non-pathogens produce compounds with auxin activity. The synthesis of gibberellin has been extensively studied in *Gibberella fujikuroi* and its imperfect form *Fusarium moniliformae*. The presence of gibberellins in *Agrobacterium tumefaciens* culture has been reported[2]. Ethylene production by microorganisms has been demonstrated with cultures of *Penicillium digitatum*[4], *Ceratocystis fimbriata*[5], and *Pseudomonas solanacearum*[6]. The role of cytokinins in the fasciation disease caused by *Corynebacterium fascians* in *Chrysanthemum*, sweet peas and many other dicotyledon seedlings has been elaborated[7]. A preliminary study to find the cause of severe premature defoliation in rose plants infected by *Diplocarpon rosae* was undertaken.

The culture of *D. rosae* was isolated from the affected leaves of a rose plant collected from the rosery of the University of Agricultural Sciences, Bangalore. The surface sterilized leaf bits were put on the potato dextrose agar medium in the plates and then the culture was purified and its pathogenicity was proved by reinfecting the healthy plants. For isolating the abscisic acid and similar compounds, the fungus was grown in a dialysed sterile 100 ml potato dextrose broth in 250 ml Erlenmeyer flask under stationary conditions. The flasks were incubated for 21 days at 25° ± 2° C. Five flasks were removed at 4, 6, 10, 12, 16 and 21 day intervals and processed. The culture broth along with the mycelial mat was homogenized in a Sorvall Omni-mixer and passed through double layered cheese cloth. The filtrate was centrifuged at 12,000 r.p.m. for 20 min in a refrigerated centrifuge. The supernatant was reduced to 1/4th of its original volume by flash evaporation at 35° C. The concentrated culture filtrate was treated for ABA extraction by following the method of Milborrow[3]. The pH of the filtrate was adjusted to 2·8 (by 1N HCl) and extracted thrice with petroleum ether and these fractions were pooled together and extracted with small volumes of 5% aqueous solution of sodium bicarbonate. The aqueous fraction was adjusted to pH 2·8 with 1 N HCl and extracted again with ether. The petroleum ether fraction was passed through the bed of anhydrous sodium sulphate and dried with compressed air. The samples along with the standard abscisic acid (ABA) were dissolved in petroleum ether and spotted on silica gel TLC plates and the plates were developed in a solvent mixture of benzene : ethyl acetate : acetic acid (50 : 5 : 2, V/V). The developed plates were dried and exposed to UV lamp in a dark room. Similarly, rose plant leaves, healthy and infected by *D. rosae*, were extracted separately by following the same procedure used for *D. rosae* culture and processed accordingly for detecting the presence of ABA.

The growth inhibitory activity of the compound extracted from the culture filtrate was tested by standard wheat coleoptile bioassay method. The coleoptiles were removed individually from the germinated seeds and cut to 5 mm length. Twenty such coleoptiles were placed in small petriplates containing two-fold serial dilutions starting from 500 ppm to 1 ppm of the test compound. The coleoptile lengths were noted after incubation of 12 h.

The dried crude compound obtained from the extract of culture filtrate of *D. rosae* was insoluble in water and white needle-like crystals in appearance. Bright fluorescent spots were observed on TLC plates exposed to short wave UV lamp. Only one fluorescent spot exactly coinciding with that of standard ABA was observed from the samples taken at 4, 5 and 10 day intervals. Whereas the extracts of cultures older than 12 days had three fluorescent spots, only one of the spots coincided with that of standard ABA. The other two had Rf values of 0·54 and 0·91 (Table I). These results indicated that *D. rosae* produced a compound

which was tentatively identified as ABA. The presence of two other compounds in addition to ABA after a 12 day incubation period indicated that the origin of these compounds might be either *D. rosae* or the degradation product of ABA.

TABLE I

Presence of compounds like ABA and other fluoroscent compounds in D. rosae *culture*

Incubation period (days)	Compound corresponding to		
	ABA	0·54 Rf	0·91 Rf
4	+	−	−
6	+	−	−
10	+	−	−
12	+	+	+
16	+	+	+
21	+	+	+

+ Present. − Not present.

TABLE II

Length of wheat coleoptile as affected by different concentrations of the test compound

Test compound concentration (ppm)	Length of wheat coleoptile (mm)*
500	6·37
250	7·62
125	8·22
62·5	8·50
31·25	8·60
15·62	8·65
7·80	8·70
3·90	8·73
1·95	8·75
0·97	9·25
Control	9·25

* Average of 20 observations.

The confirmative results for the growth inhibitory activity of the extracted compound were obtained from the wheat coleoptile bioassay method. The results revealed that the test compound had an inhibitory effect on the growth of the wheat coleoptile. The length of the wheat coleoptile was considerably inhibited (6·37 mm) when put in 500 ppm test solution, whereas, in the control the coleoptile length was 9·25 mm. The test compound was found to be effective in inhibiting the coleoptile growth up to a concentration of 2 ppm. The coleoptile growth was the same as that of the control at a test dose of 1 ppm (Table II).

Abscisic acid is a well-known growth inhibitor. The results of the wheat coleoptile bioassay method and the confirmative TLC method clearly indicated that *D. rosae* synthesised a compound in broth which was tentatively identified as ABA.

A compound with the same Rf value as the standard ABA was obtained only from the ether extract of the diseased leaves but not from the healthy rose leaves. This indicated the possible involvement of ABA (produced by *D. rosae*) in the causation of disease symptoms, particularly of premature defoliation in rose plants.

Department of Microbiology,
University of Agricultural Sciences,
G.K.V.K., Bangalore 560065,
August 3, 1979.

SUHAS P. WANI,*
P. VITTAL RAI,
R. B. PATIL,**

* International Crops Research Institute, for the Semi-Arid Tropics, Patancheru P.O., Andhra Pradesh 502 324, India.

** Agricultural College, Dharwad.

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A NEW SPECIES OF *DICHOMERA* (COELOMYCETES) FROM INDIA

DURING a mycological collection tour to Kerala forests of South India in 1975, an interesting sphaeropsidaceous fungus of the form-genus *Dichomera* Cooke[1] was collected on dead twigs of *Nerium odorum* Soland, which on detailed study proved to be an undescribed taxon. A perusal of literature[2–4] for its identity showed no report of any species of *Dichomera* on *Nerium* or its related hosts of the family Apocynaceae. Further, on comparison, it also differed greatly from *Dichomera capparidis* Munj. and Kapoor[5] (described on *Capparis aphyla* Ridge) in possessing bigger stroma as well as