TRITERPENOID FEEDING DETERRENT OF *RAPHIDOPALPA FOVEICOLLIS* L. (RED PUMPKIN BEETLES) FROM *MOMORDICA CHARANTIA* L.

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RAPHIDOPALPA faveicollis L. (red pumpkin beetles) causes severe damage to the cotyledonary and tender leaves of many cucurbitaceous crops[1]. However, cotyledons and leaves of *Momordica charantia* L. (bittergourd), also a member of cucurbitaceae, are not fed upon by red pumpkin beetles. This observation evoked interest for the present investigation in elucidating the chemical basis for this phenomenon as no morphological factor has been assigned any role and the presence of feeding inhibitor has not been reported so far.

Cotyledons of *M. charantia* were collected soon after seedling emergence and leaves were collected from plants at flowering and fruiting stages. The cotyledons and leaves were dried in air at 60° C.

Bioassay: Cotyledons of *Cucurbita maxima* Duch. (pumpkin), which are normally fed upon by red pumpkin beetles, were used as a bioassay material. Test samples, at different stages of extraction (corresponding to 2.5 g of fresh material) dissolved in 80% ethyl alcohol, were applied on both the sides of the excised cotyledons. They were offered to beetles (collected from field grown plants of pumpkin and bottlegourd) in a petri dish and were examined for feeding damge after 24 hr. Cotyledons treated with 80% ethyl alcohol served as the control. Absence of feeding damage was taken as positive evidence of the presence of a feeding inhibitor in the applied extract.

Isolation of feeding deterrent: The materials isolated in different steps were monitored by the bioassay. Powdered leaves/cotyledons (40 g) dry weight were extracted by percolation with 90% methanol at ambient temperature. The percolate was evaporated and the residue was partitioned between petroleum ether (60-80° C) and 80% methanol. The methanol phase was reextracted thrice with petroleum ether. Evaporation of the methanol phase left a residue with high feeding deterrent activity. The residue was partitioned between ethyl acetate and water. Only the ethyl acetate phase had high feeding deterrent activity. The ethylacetate extract was chromatographed on a column of alumina (20 g neutral) and washed with ethyl acetate till the eluent was colourless. The active constituents were then eluted with methanol. Upon evaporation of the methanol fraction, a pale yellow solid (150 g) was obtained. Further purification was achieved by column chromatography on silica gel (15 g, 60–120 mesh) and the active fraction was eluted with ethylacetate. On thin layer chromatography using silica gel G, the feeding deterrent moved to $R_f = 0.36$ in a solvent systems of ethyl acetate:methanol:water (16:1:1). The plate was developed by spraying H_2SO_4 (50%) and heating to 110° C for 10 minutes. The colour changes of the spot were light pink to pink, purple to dark purple. The compound was crystallised from MeOH-H_2O mixture.

The feeding deterrent had a bitter taste and a melting point of 185-195° C. The IR spectra (recorded on a Perkin-Elmer IR spectrophotometer as a KBr pellet) revealed the following peaks at 3400, 2940, 2860, 1600, 1360, 1060 and 770 cm^{-1}. The compound underwent hydrolysis in 1.5 N methanolic H_2SO_4 (at 4° C for 24 hr). The only sugar produced was identified as D-Glucose on paper chromatography (Whatman No. 1) with authentic sample of D-Glucose, using a solvent system of butanol:acetic acid:water (12:3:10 upper phase) and ethyl acetate:acetic acid:water (3:1:3 upper phase). The NMR spectra of the aglycone (recorded in $CDCl_3$ using tetramethylsilane as internal standard on a 270 MHZ spectrometer) exhibited signals of eight methyl groups (0.85 × 2, 0.88, 0.90, 0.91, 1.34, 1.74 and 1.78 ppm) along with olefinic protons at 5.62 and 6.00 ppm.

The glycosidic nature of the feeding inhibitor was indicated by acid hydrolysis followed by identification of sugar (D-Glucose) on paper chromatography. The aglycone gave a positive Salkowski reaction and a pink colour with the Liebermann-Burchard reagent characteristic of a triterpenoid compound. The inhibitor was thus identified as triterpenoid glycoside.

Recently Okabe *et al*[2] isolated bitter principles (Momordicosides) from seeds of *M. charantia* and characterised them as glycosides of cucurbit-5-ene-3, 22, 23, 24, 25 pentaol. The NMR spectra of the aglycone and the IR spectra of the bitter triterpenoid glycoside, isolated from leaves in the present study were similar to those of the cucurbit-5-ene derivatives. The bitter triterpenoid glycoside and momordicosides A and B were compared by thin layer chromatography on silica gel G using solvent system ethylacetate:methanol:water (16:1:1) and chloroform:methanol:water (7:3:1 upper layer). The R_f value of the isolated compound did not agree with those of the standards used and this suggests that the triterpenoid glycoside isolated from the leaf is apparently different from momordicosides A and B.

The bioassay study revealed that a concentration of 2 mg and above of the triterpenoid glycoside inhibited beetle feeding completely and this emphasises the chemical aspect of insect host relationship. While cucurbitacins, the tetracyclic triterpenes widely distributed in cucurbitaceae, are specific feeding attractants of the red pumpkin beetles[3,4], the triterpene derivatives of *M. charantia* act as a feeding inhibitor of the same species. This contrasting feeding response of the above triterpenoids may be due to differences in their chemical structure. Studies on the structure-activity relationship of the various cucurbitane derivatives could throw light on the functional group responsible for inhibition or stimulation of insect feeding.

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EFFECT OF ABSCISIC ACID ON ENDOGENOUS CYTOKININS IN RICE (*ORYZA SATIVA* L.) LEAVES

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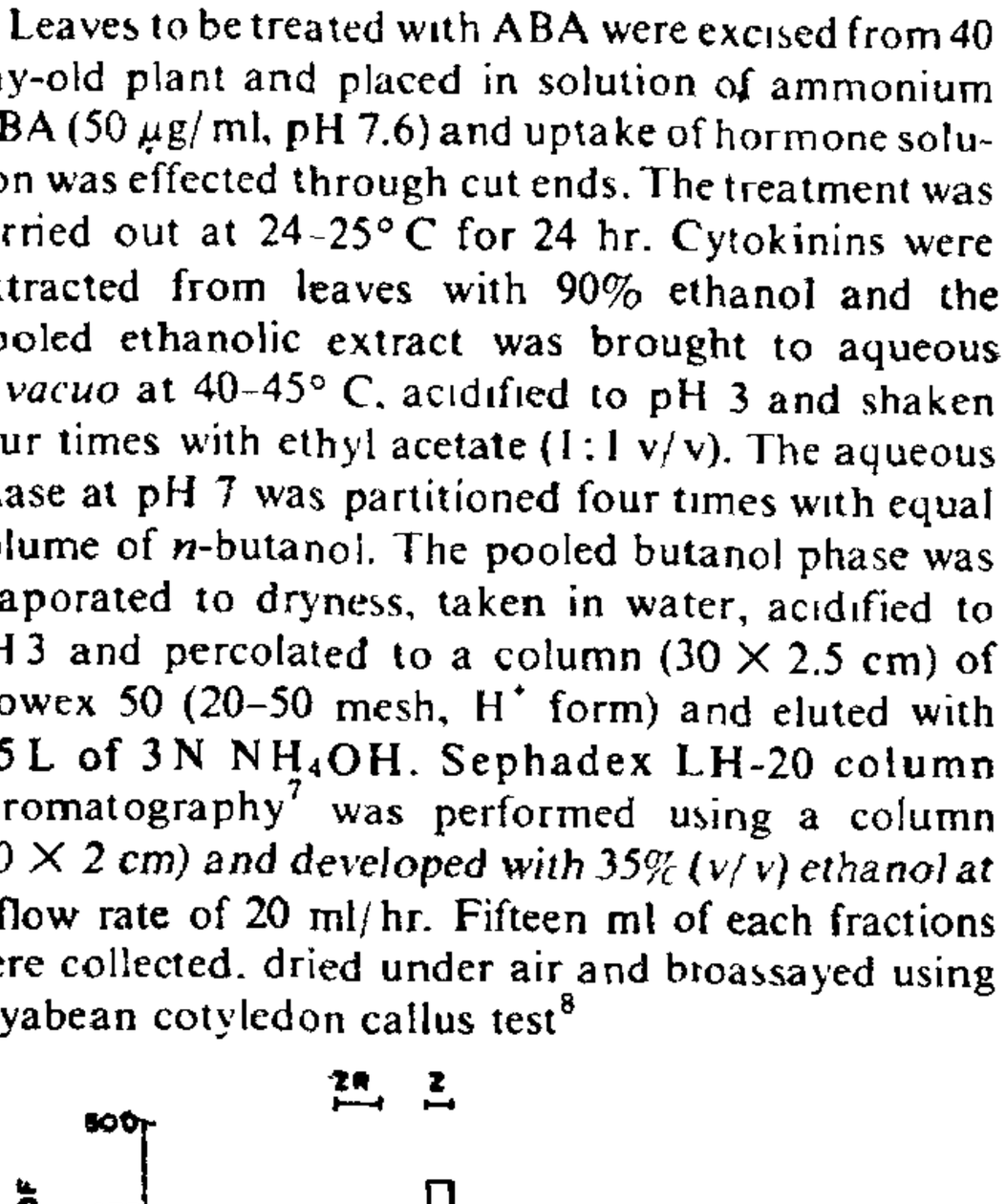
As a natural growth substance, abscisic acid (ABA) has been identified acting as an inhibitor in various biological systems[1,2] and counteracting responses induced by other plant hormones[3,4]. ABA and cytokinin have been reported to counteract the effects of each other in several plant systems and at certain proportions ABA reportedly even promotes growth callus tissues[5,6]. In the present paper the direct influence of abscisic acid on the endogenous cytokinin activity in leaves of rice *Oryza sativa* L. is presented and discussed.

Leaves to be treated with ABA were excised from 40 day-old plant and placed in solution of ammonium ABA (50 μg/ml, pH 7.6) and uptake of hormone solution was effected through cut ends. The treatment was carried out at 24–25° C for 24 hr. Cytokinins were extracted from leaves with 90% ethanol and the pooled ethanolic extract was brought to aqueous *in vacuo* at 40–45° C, acidified to pH 3 and shaken four times with ethyl acetate (1 : 1 v/v). The aqueous phase at pH 7 was partitioned four times with equal volume of *n*-butanol. The pooled butanol phase was evaporated to dryness, taken in water, acidified to pH 3 and percolated to a column (30 × 2.5 cm) of Dowex 50 (20–50 mesh, H^+ form) and eluted with 1.5 L of 3N NH_4OH. Sephadex LH-20 column chromatography[7] was performed using a column (40 × 2 cm) and developed with 35% (v/v) ethanol at a flow rate of 20 ml/hr. Fifteen ml of each fractions were collected, dried under air and bioassayed using soyabean cotyledon callus test[8]



Figures 1&2. Separation of cytokinin activity following Sephadex LH20 column chromatography of Dowex 50 eluate of **1.** untreated leaf material; **2.** leaf material after ABA (50 μg/ml) treatment. Quantity equivalent to 25 g fresh weight of leaf tissue was chromatographed on Sephadex LH20 column. The elution patterns of zeatin (Z) and zeatin riboside (ZR) are shown by horizontal bars over the histogram.