Identifying Cucurbitacin in Cotyledons of Cucurbita pepo L. cv. Black Zucchini

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Abstract. Florisil column chromatography and silica-gel thin-layer chromatography were excellent preparatory steps for separation of cucurbitacins in cotyledons. It was difficult to identify closely related cucurbitacins by thin layer chromatography. Unstable cucurbitacins derivatives limited the use of gas-liquid chromatography for cucurbitacin identification; however, mass spectrometry was an effective method for cucurbitacin B.

Cucurbitacins are ecologically important secondary plant substances. They both attract insects (2, 9) and repel them (2). Initially found in Cucurbitaceae (4), these tetracyclic triterpenes have since been reported in Cruciferae (5), Scrophulariaceae (6), and Begoniaceae (3). At present, 14 cucurbitacins are known and most of them have been chemically characterized (7). Because they are closely related, chemical separation and identification in plant materials are difficult. Rehm et al. (8) reported small amounts of cucurbitacin D with B, and I with E in the genera Cucurbita and Luffa. Analytical techniques to identify the cucurbitacin in fully expanded cotyledons of 'Black Zucchini' are discussed in this study.

We planted seeds in flats containing sand and vermiculite (1:1), and harvested 1 kg of fully expanded cotyledons and immediately froze them. We used a clarified ethanolic extract obtained by a procedure modified from Enslin (4), shaken twice with 250 ml of petroleum ether (bp 60-80°C); separated interfering lipoidal substances in the ether layer; shook the ethanol fraction twice with 300 ml chloroform; washed the chloroform fraction with equal amounts of distilled and deionized water, dried it over sodium sulfate and flash evaporated it, which gave a dark yellowish gum. On silica gel G thin layer plates, we spotted pure cucurbitacins A, B, C, D, E, and I, along with the unknown from the cotyledons. As a preparatory step, we streaked the remainder of the extract on several thin layer chromatography (TLC) plates, and used vanillin and UV light as visualizing agents. The crude cucurbitacin from TLC was concd to 2 ml and then charged on a 2-cm diam column containing Florisil (30 g, 100-200 mesh), and eluted column with 50-ml each of chloroform and chloroform:methanol in 95:5, 90:10, 85:15, 80:20, 70:30, and 60:40 v/v proportions. We collected and reduced 10-ml fractions to 1/2 ml under flash evaporation, then spotted 50 μl from each fraction on thin layer plates.

We then attempted gas chromatographic (GLC) analysis using pure cucurbitacins and the cotyledon extracts; derivation of cucurbitacins using various temp and time combinations with 2 columns, 3% SE-30 and 1% OV-1 on Gas chromatography.

peaks in the OH/NH stretch region. The cotyledon extract gave no precise peak maxima matching the peaks from the known cucurbitacin B and E, or B or E. The derivation of either pure, or BZ cucurbitacins was not successful so identification of cucurbitacins by GLC was not possible.

Relative abundance of major positive fragments for cucurbitacin B, E, and for BZ cotyledon extracts are shown in Fig. 2. All major peaks in 300 to 500 m/e region for known cucurbitacin B also appeared in cotyledon extract, the largest (relative abundance 5%) in the high mass region was at 499 m/e. These data indicate that the cotyledons of 'Black Zucchini' contained cucurbitacin B.

We also compared a commercial source of cucurbitacin I with the cucurbitacin I standard. Although peaks obtained for the major fragment ions in the high mass region (300-540 m/e) were distinct for cucurbitacin I, additional peaks indicated the presence of B and E as an impurity.

Mass spectra of pure cucurbitacins were similar to those reported previously (1). Information obtained from the other analytical methods was less conclusive than that obtained by mass spectrometry, which, therefore, appears to be appropriate for characterizing unknown mixtures of cucurbitacins.

**Literature Cited**


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**Interactions in Source of Nitrogen Fertilizer and Liming Procedure in the Control of Fusarium Wilt of Tomato**

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*Abstract.* Symptoms of fusarium wilt of tomato were less severe in plants supplied with nitrate-N and greater in those supplied with ammonium-N. Liming with calcium hydroxide decreased disease severity but this effect was negated by high ammonium-low nitrate fertilization. The combination of high nitrate, low ammonium, and lime reduced disease development additively. *Fusarium oxysporum* f. sp. *lycopersici* race 2 was more virulent when grown in liquid culture with ammonium than with nitrate as the sole N source.

Liming certain soils to a pH of about 7.0 to 8.0 reduced the severity of fusarium wilt of tomato (3, 4). Albert (1) using nutrient solution culture, showed that 2 mutually dependent factors, pH and nitrate-N, significantly increased fusarium wilt resistance and decreased wilt injury of cotton. This study evaluates the individual and interrelated effects of NO₃ versus NH₄-N, lime, and soil pH upon the severity of fusarium wilt of 'Manapal' tomato incited by *Fusarium oxysporum* f. sp. *lycopersici* race 2.

The pathogen was cultured in liquid media with NO₃ or NH₄ as the sole N source (5). Mycelial material was separated from solution cultures by filtration, and washed 3 times with deionized water. Inoculum was prepared by a brief blending of 5 mg of fresh mycelial material in each ml of deionized water. Virulence of inoculum was tested by root inoculation of 'Manapal' tomato seedlings. Fifteen seedlings were inoculated with each of the 24 cultures. Disease ratings indicated that inoculum grown on the ammonium form of N was more virulent than that grown on nitrate-N (Table 1). This observation on the direct effect of