and that bulblet size and/or soil temperature may affect leaf emergence.

Bulbs of 'Hinomoto' (14- to 16-cm in circumference), produced in Okino-Erabu Island, Japan, were received on 15 July 1983, and were dark-stored at 25°C. On 19 Sept. the bulbs were hot-water treated (45° for 30 min), and the middle scales (3) were removed from the parent bulbs and disinfected in a 0.5% solution of 3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thio]-1-*H*-isoindole-2,3(2*H*)-dione (captan) for 15 min. After air-drying, the scales were packed in polyethylene bags filled with moist peatmoss and placed in a temperature sequence of 25° for 10 weeks, 17° for 4 weeks, and 4° for 4 weeks, as modified from van Tuyl (4). On 23 Jan. 1984, scale bulblets were separated into 2 size groups, large (L) and small (S). The L bulblets were 8 to 11 mm in diameter and the S were 6 to 7 mm in diameter. None of the bulblets had emerged leaves. Half of each group had their roots removed. All were planted 1 to 1.5 cm deep in plastic pots filled with sand. Bulblets (80 to 100) for each of the 4 lots were grown without fertilizer at 25°, 20°, or 15° in darkness. Leaf emergence was checked every 5 days for 50 days.

Root removal delayed and reduced leaf emergence at temperatures of 25° and 20°C. At the 15° temperature, root removal delayed leaf emergence but did not affect the percentage of leaf emergence at the end of experiment (Fig. 1).

Leaf emergence was delayed and percentage of leaf emergence was reduced with S bulblets (except bulblets with roots at 15°C) when compared to L bulblets. Accordingly, independent of bulblet size, high temperatures (25° and 20°) and root removal reduced the leaf emergence percentage more than at 15°. Leaf emergence appears to be partially dependent on size or maturity of newly formed scale bulblets, as L- and S-size bulblets were removed from the parent scales on the same date. The large bulblets may be more mature or sensitive to high temperature treatment, i.e., more vernalized than smaller bulblets.

These results clearly show that the large scale bulblets are more desirable than small, and that the roots from small bulblets should neither be cut off nor broken before planting.

At 25° and 20°C, the percentage of leaf emergence was reduced but peaked more rapidly when compared to the 15° treatment (Fig. 1). In contrast, the 15° treatments allowed almost 100% leaf emergence, but required 40–50 days. Such a high percentage of leaf emergence at low temperatures has been observed by Matsuo (2). At 15°, leaf emergence from L bulblets without roots initially was delayed when compared to L bulblets with roots.

Our present data and those from Matsuo (2) also indicate that 1) 15°C temperature acted as the additional low temperature for vernalization, resulting in a high percentage of leaf emergence as also observed in the Netherlands; 2) 25° and 20° temperatures inhibited vernalization, except for those bulblets fully vernalized during the temperature sequence, and resulted in lower percentage leaf emergence; 3) high temperatures after planting bulblets in Japan (mean temperature during September to October, 20° at Fukuoka), when compared to the Netherlands (mean temperature in April, 10°), promotes leaf emergence of previously vernalized bulblets, and gradual lowering of the temperature increases the amount of vernalized bulblets and results in nonuniform leaf emergence, as observed in "Rinpen-Kiribana-Saibai".

Literature Cited

1. Kondo, H., T. Matsukawa, S. Mamezuka, and Y. Kobayashi. 1985. *Lilium longiflorum* cut flower production by scale propagation 1 (in Japanese). 1985 Abstr. Jpn. Soc. Hort. Sci. Spring Meeting. 542.
2. Matsuo, E. 1975. Studies on the growth and development of bulbs in the Easter Lily (*Lilium longiflorum* Thunb.): IV. Effect of temperature and light conditions on leaf emergence of scale bulblet (in Japanese with English summary). J. Jpn. Soc. Hort. Sci. 44(3):281–285.
3. Matsuo, E., K. Arisumi, and H. Kawashima. 1982. Cultural practices influencing premature daughter leaf and/or shoot emergence in scale-propagated Easter lily. HortScience 17:196–198.
4. van Tuyl, J.M. 1983. Effect of temperature treatments on the scale propagation of *Lilium longiflorum* 'White Europe' and *Lilium* x 'Enchantment'. HortScience 18:754–756.

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Rapid TLC and HPLC Quantification of Cucurbitacin C in Cucumber Cotyledons

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Additional index words. *Cucumis sativus*, cucumber bitterness, seedling insect resistance

Abstract. **Two rapid methods for estimating cucurbitacin C (Cuc C) in cucumber cotyledons are described. Both methods require a single cotyledon extracted with methanol. The filtered extract is injected into a C18 HPLC column with methanol–water (55:45) solvent, and quantitative estimates of Cuc C are obtained by UV detection at 254 nm. In the 2nd method, the extract is concentrated for spotting on silica gel TLC plates, the TLC plates are developed with ethylacetate : benzene (75:25), and visualized with vanillin-orthophosphate to give a semiquantitative estimate of Cuc C. The 2 methods are quick and suited for screening seedlings for cucurbitacin levels in segregating populations to select for cucumber beetle resistance and for nonbitter fruit.**

Cucurbitacins (Cucs) are a group of bitter secondary plant substances that occur mainly in the Cucurbitaceae (4, 5), but also in other families (7, 8). These compounds are very toxic (13) and are responsible for the bitter flavor of fruit. Occasional poisonings have been reported in people eating bitter squash (9, 12); however, such poisonings are rare because of the extremely bitter flavor of fruits. Different Cucs are present in various species of the Cucurbitaceae, and they have chemotaxonomic value (5).

Andeweg and de Bruyn (1) reported that most cucumber cultivars have bitter foliage, and their fruit have the potential to be bitter, depending on the environment. A few cultivars never develop bitter fruit, but their vegetative parts are bitter. These workers found a spontaneous mutant, recessive for the *bi* gene, that had nonbitter foliage as well as fruit. This gene is the basis for the nonbitter cultivars that have become popular in recent years. Barham (2) determined that a single dominant gene (*Bt*) was responsible for the very bitter fruit of PI 173889, a cucumber introduced from India.

The presence of Cuc C in cucumber cotyledons is associated with susceptibility to cucumber beetles (3, 6, 11). The seedlings containing no Cuc C are insect-resistant and never produce bitter fruits. Testing for the lack of Cuc in cucumber cotyledons, due to *bi*, is a means of simultaneously selecting for cucumber beetle resistance and for nonbitter fruit.

Andeweg and de Bruyn (1) reported a rapid micromethod for Cuc C, but they found it was not suitable for analyzing cucumber fruit. We found this test unreliable for cucumber cotyledons also. Other methods employing TLC and spectrophotometry and/or TLC and HPLC are too slow for screening large numbers of plants in segregating populations (6).

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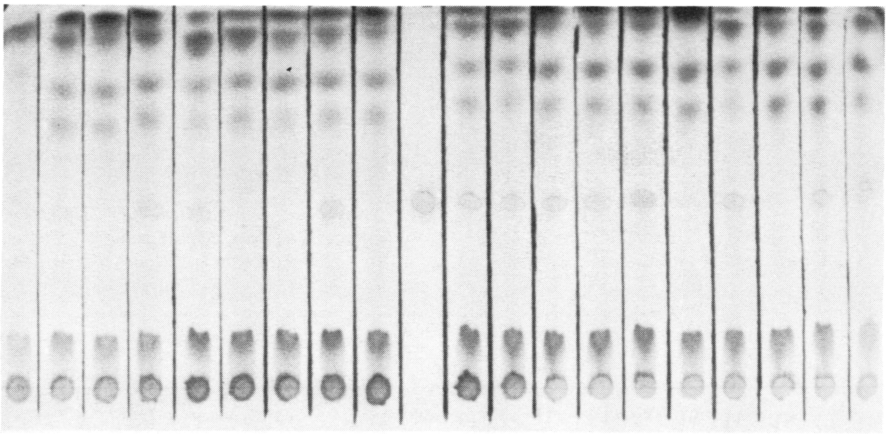

Fig. 1. TLC chromatogram of methanol extracts from cotyledons of 19 F_2 seedlings showing the segregation of 9 high : 4 medium : 6 zero cucurbitacin C. Pure cucurbitacin C was spotted at the center of the plate.

In this paper we report rapid TLC and HPLC methods for estimating Cuc C in cucumber cotyledons.

Seeds of bitter (PI 173889, *Bt Bt bi⁺ bi⁺*), and nonbitter ('Spartan salad', *Bt⁺ Bt⁺ bi bi*) cucumbers, and the F_2 generation of a cross of these parents were planted in pots in the greenhouse. One cotyledon was taken from each seedling 3–5 days after germination, when the cotyledons were fully expanded. The cotyledons were cut into small pieces, placed in 1.5-ml centrifuge tubes, and ground in 0.5 ml methanol with a cone-shaped pestle. The tubes were closed, shaken, and allowed to stand for one hour.

TLC analyses. Tubes were centrifuged and the methanol extract was transferred to a new tube and left overnight at 40°C until dry. Thirty microliters of methanol was added to each tube, which was then shaken and left for one hour. Twenty-five microliters of the extract was applied to a 10 × 20-cm silica gel plate. Twenty samples were placed on the plate along the long base, using a heat gun (hair dryer) to speed methanol evaporation. The plates were developed in ethyl acetate : benzene (75:25), allowed to dry, and sprayed with vanillin-orthophosphoric acid in ethanol (14). Heating the plates at 120° for 5 min made the areas containing Cuc C visible.

HPLC analyses. The methanol extracts were filtered through 0.45-μm, 9-mm diameter nylon membrane filters in a Schleicher and Schuell microfiltration apparatus. Fifteen microliters of methanol extract was injected in a Waters Model 45 HPLC, using methanol: water (55 : 45) solvent at 2.0 $ml \cdot min^{-1}$. Cuc C was separated from UV-absorbing impurities on a 150 × 4.6-mm C18 column and detected at 254 nm with a Waters Model 440 detector. Cuc C was estimated by peak height measurements and comparison with Cuc C standards isolated from bitter cucumber fruits. Purity of the standard was established by TLC (10) and MS (11).

The difference in concentrations of Cuc C in the cotyledons of bitter and nonbitter cucumbers was easily observed by TLC. Pure standard of Cuc C can be detected at 1 μg per spot. Analysis of the F_2 population of bitter × nonbitter (Fig. 1) permitted selection of nonbitter individuals, as well as those seedlings containing medium and high amounts of Cuc C. Thirty-four F_2 seedlings contained high amounts of Cuc C, 14 seedlings medium amounts, and 16 seedlings were free of Cuc C. This does not differ significantly from an expected 9:3:4 ratio, based on segregation for *bi* and *Bt*.

HPLC analyses of Cuc C in the cotyledons confirmed the TLC results. The HPLC chromatograms show that Cuc C is well-separated from the UV-absorbing contaminants that are present in methanol extracts. The HPLC analysis is a quick, quantitative method; only 3 to 4 min is needed for each sample injected. We found that the cotyledons of the bitter cucumber parent contained 30–60 $\mu g \cdot g^{-1}$ fresh weight of Cuc C, and there was no detectable Cuc C in the nonbitter parent (Fig. 2).

The weight of the cotyledons was very uniform (0.4 ± 0.03 g), so the whole cotyledon was used for the analyses. Due to the large differences in Cuc C content, the small differences in cotyledon weight did not affect the classification.

Both methods are quick and well-suited for screening for Cuc C-free seedlings, as well as for classification for Cuc C concentration in segregating populations. Because only one cotyledon is needed for analysis, the seedlings may be grown to produce fruit for additional analyses and seed for breeding purposes.

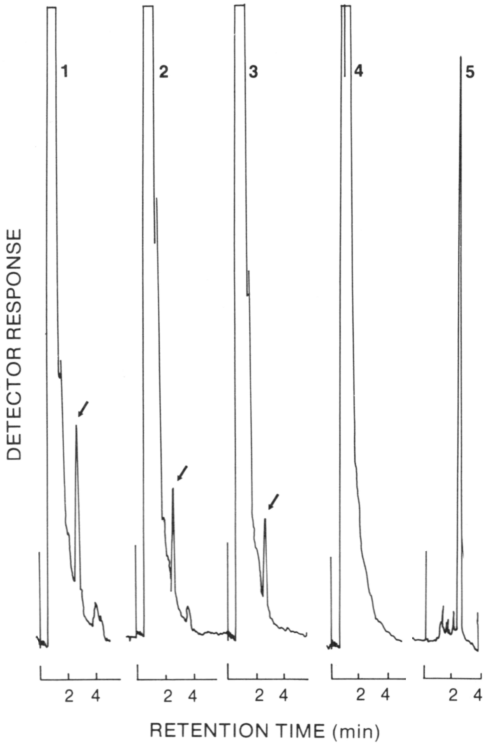

Fig. 2. HPLC chromatogram of methanol extracts of bitter (**1, 2,** and **3**), nonbitter (**4**), and pure cucurbitacine C (**5**). Arrows show cucurbitacin C peaks.

Literature Cited

1. Andeweg, J.M. and J.W. de Bruyn. 1959. Breeding of nonbitter cucumbers. Euphytica 8:13–20.
2. Barham, W.S. 1953. The inheritance of a bitter principle in cucumbers. Proc. Amer. Soc. Hort. Sci. 62:441–442.
3. DaCosta, C.P. and C.M. Jones. 1971. Resistance in cucumber, *Cucumis sativus* L. to three species of cucumber beetles. HortScience 6:340–342.
4. Enslin, P.R. 1954. Bitter principles of the Cucurbitaceae: I. Observations on the chemistry of cucurbitacin A. J. Sci. Food Agr. 5:410–416.
5. Enslin, P.R. and S. Rehm. 1956. The distribution and biogenesis of the cucurbitacins in relation to the taxonomy of the Cucurbitaceae. Proc. Linnean Soc. London 169:230–238.
6. Ferguson, J.E., E.R. Metcalf, R.L. Metcalf, and A.M. Rhodes. 1983. Influence of cucurbitacin content in cotyledons of Cu-

curbitaceae cultivars upon feeding behavior of Diabrotica beetles (Caleoptera: Chrysomelidae). J. Econ. Ent. 75:47–51.
7. Gmelin, R. 1966. Bitterstoffe in der familie *Cruciferie*. Planta Med. 14:119–127 (Suppl.)
8. Gmelin, R. 1967. Analysis of active compounds in *Gratida officinalis*. Arch. Pharm. 30:234–240.
9. Herrington, M.E. 1983. Intense bitterness in commercial zucchini. Cucurbit Genet. Coop. Rpt. 6:75–76.
10. Pohlman, J. 1975. Die Cucurbitacine in *Bryonia alba* and *Bryonia dioica*. Phytochemistry 14:1587–1589.
11. Rice, C.A., O.L. Chambliss, and F.A. Johnson. 1981. Chromatographic and mass spectral analysis of cucurbitacin of three *Cucumis sativus* cultivars. J. Agr. Food Chem. 29:194–196.
12. Rymal, K.S., O.L. Chambliss, M.D. Bond, and D.A. Smith. 1984. Squash containing toxic cucurbitacin compounds occurring in California and Alabama. J. Food Protection 47:270–271.
13. Stoewsand, G.S., A. Jaworski, S. Shannon, and R.W. Robinson. 1985. Toxicologic response in mice fed *Cucurbita* fruit. J. Food Protection 48:50–51.
14. Zielinski, J. and J. Konopa. 1968. Thin-layer chromatography of cucurbitacins- a group of tetracyclic triterpenes. J. Chrom. 36:540–542.

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Effects of Gene *B* in *Cucurbita moschata*

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Additional index words. pumpkin, squash, *Cucurbita pepo,* pleiotropy, vegetable breeding

Abstract. **The effects of gene *B* in *Cucurbita moschata* Poir. were studied by comparing 2 near-isogenic lines, PI 165561 (B^+B^+) and Precocious PI 165561 (*BB*) at 2 locations in Israel. *B* significantly increased femaleness and number of fruits, but decreased fruit size and yield and numerically decreased seed yield at one or both locations. Fruits of B^+B heterozygotes were distinctly bicolor. There was a deficiency in the number of bicolor-fruited plants in BC_6 progeny, the deviation from the expected 1:1 ratio of green-fruited to bicolor-fruited being significant. Overall, the effects of *B* in *C. moschata* were no different from its effects in *C. pepo* L.**

Gene *B* of *Cucurbita pepo* L. is pleiotropic. Its primary effect is yellow or bicolor yellow and green (instead of B^+B^+ normal green) color of the ovary, long before opening of the flower. Among the manifold secondary effects of *B* are yellow spotting of foliage, reduced growth rate of the fruit, reduced fruit size and productivity, increased fruit quality, increased cracking of maturing fruits, and decreased seed production. Which of these secondary effects are manifested is dependent on the genetic background. Gene *B* originated as an unstable gene in the bicolor ornamental gourds of *C. pepo* (13).

Besides the ornamental gourds, *C. pepo* includes a host of cultivars grown for their immature fruits (summer squash) as well as numerous cultivars grown for their mature fruits (pumpkins and winter squash). Among the summer squash cultivars, gene *B* has proven useful because it can impart intense golden-yellow fruit coloration (11), slower growth rate of the fruit (7), and slim fruit shape (12), these characteristics are important for increasing the yield quality (4) and speed and efficiency of the harvest (6). Among the pumpkin and winter squash cultivars, gene *B* has proven useful because it imparts attractive intense orange fruit coloration of the rind and flesh (3) and increases carotene content of the flesh (9).

Cucurbita moschata Poir. includes a wide range of cultivars grown for their mature fruits. Fruits of this species are considered in many cases to be superior in quality to those of *C. pepo* (16). Gene *B* was transferred recently, through 6 backcross generations, to *C. moschata* as a first step in determining if *B* could be useful in improving cultivars of this species (5). Our purpose was to study the effects of *B* in the genetic background of *C. moschata* into which it was first transferred, PI 165561.

Seeds of *C. moschata* PI 165561, B^+B^+, were kindly provided by the USDA North Central Plant Introduction Station, Ames, Iowa, and maintained by self-pollination. A *BB* line of PI 165561, designated "Precocious PI 165561," was developed by crossing a *C. pepo* accession carrying gene *B* with PI 165561, followed by 6 generations of backcrossing to PI 165561 and 2 generations of self-pollination and selection for *BB* (5).

A 2-generation inbred of PI 165561, designated PI 165561-1-6 (B^+B^+), and Precocious PI 165561 (BB) were compared in Spring–Summer 1985 at Hazera Seeds, Mivhor Farm (near Qiryat Gat, southcentral Israel) and at Newe Ya'ar (near Qiryat Tiv'on, northern Israel) using standard cultural practices at each location. Seeds were sown at Mivhor on 15 Apr. and at Newe Ya'ar on 9 Apr. in groups 1.0 m apart within single rows on raised beds, with 1.8 m between bed centers at the former location and 2.0 m at the latter. The plants were thinned to one per group after expansion of the first true leaf, to give densities of 5556 plants·ha^{-1} and 5000 plants·ha^{-1} at each of the respective locations. A paired experimental design with 4 replicates was employed at each location, with 5 plants per plot at Mivhor and 4 plants per plot at Newe Ya'ar.

The node at which the first female flower appeared on the main stem was recorded for each plant and averaged for each plot. Fruit yields and their components were determined for each plot. Seed yields and their components were determined for each plot only at Newe Ya'ar. Each of these variables was subjected to a paired *t* test.

Remnant seeds of the BC_6 accession used in the development of Precocious PI 165561, which had been obtained by crossing PI 165561 (B^+B^+) with a BC_5 bicolor (B^+B) segregate, were sown singly, 0.5 m apart, at Newe Ya'ar on 9 Apr. All of the resulting plants were scored for fruit color, and the data subjected to χ^2 analysis.

The first female flower was produced earlier, on a developmental basis, by Precocious PI 165561, *BB*, than by the inbred of PI 165561, B^+B^+ (Tables 1 and 2). Node number is a reliable indicator of female tendency in cucurbits: the lower the node at which the first female flower appears, the stronger the female tendency (10). The increased female tendency of the precocious PI is in ac-

Table 1. Effects of gene *B* on pistillate flowering and yield of *Cucurbita moschata* at Mivhor, Israel.

Genotype	Node of first female flower on main stem	No. fruits per plot	Fruit wt (kg)	Yield of fruits (t·ha^{-1})
PI 165561, $B+B+$	33.3	8.0	4.96	42.7
Precocious PI 165561, *BB*	30.9	12.3	3.20	43.2
t	1.42	3.24	5.22	0.11
P	0.30–0.20	0.05–0.02	0.02–0.01	>0.50

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