ACKNOWLEDGMENT

The author wishes to thank Frederick J. Francis for the critical review of the experimental results.

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Received for review March 27, 1978. Accepted June 5, 1978. This work was supported by funds from Hatch Project No. 346.

Thin-Layer Chromatographic System for Identification and Quantitation of Potato Tuber Glycoalkaloids

A system involving thin-layer chromatography and densitometry is described for quantitating the individual glycoalkaloids of potato tubers. The procedure is simple and inexpensive and could easily be used to assay individual glycoalkaloids in a large number of tuber samples.

While several methods are known for the determination of total glycoalkaloids in potato tissue (Fitzpatrick and Osman, 1974; Smittle, 1971), quantitation of individual glycoalkaloids has been accomplished only by rather lengthy and complicated gas (Herb et al., 1975) and high-pressure liquid (Hunter et al., 1976) chromatographic procedures. These methods are not well-suited for the screening of large numbers of potato varieties for the levels of individual glycoalkaloids.

We developed the methods described in this paper for the routine analysis of potato glycoalkaloids in our breeding program.

EXPERIMENTAL SECTION

Extraction of Tissues. Potato tubers were washed and sliced, and the outer 5 mm, including the peel, was extracted according to the method of Shih and Kúc (1974). The extracted glycoalkaloids were dissolved in the solvent used for thin-layer chromatography (TLC) at a level of 2 mL/10 g fresh tuber weight.

Total Glycoalkaloid Assay. Glycoalkaloids were assayed by a modification of the method of Wang et al. (1972). A 0.5-mL aliquot of each glycoalkaloid extract was placed in a test tube, evaporated to dryness, and then dissolved in 0.5 mL of 5% acetic acid. Analogous tubes containing up to 0.5 mg of standard α-solanine were also prepared. Each tube then received 1.5 mL of 85% H_3PO_4 with mixing and 1 mL of paraformaldehyde reagent (0.2 g of *p*-formaldehyde dissolved in 15 mL of H_2O and then diluted to 100 mL with 85% H_3PO_4). The tubes were incubated at 60 °C for 5 min, and the absorbance was then read at 600 nm in a Hitachi Model 124 spectrophotometer.

Assay of Individual Glycoalkaloids. The identities of the glycoalkaloids isolated in this study were confirmed using standard 20 cm × 20 cm silica gel TLC plates in several solvent systems (Shih and Kúc, 1974). For quantitative assays, the glycoalkaloid extracts and solutions containing various amounts of standard α-solanine were spotted on Kontes 1 in. × 3 in. Q6F silica gel plates and developed in the organic layer of $CHCl_3$–95% ethanol–1% NH_4OH (2:2:1, v/v). The developed plates were air-dried, dipped in $CHCl_3$ saturated with $SbCl_3$, and then heated at 150 °C for 4 min. The intensities of the colored spots which appeared were determined with a Kontes densitometer using a single-beam mode, reference head, medium light intensity, and a long-wavelength ultraviolet light source.

Table I. Glycoalkaloid Contents of Tubers from Three Cultivars of Potato[a]

cultivar	glycoalkaloid content, mg/100 g fresh weight		
	α-solanine	α-chaconine	total[b]
B 5141-6	22.9 ± 0.8	42.9 ± 2.7	60.8 ± 0.3
B 6039-WV6	16.4 ± 0.3	25.0 ± 0.5	46.8 ± 0.3
B 6039-WV9	21.3 ± 0.7	24.9 ± 0.7	40.6 ± 0.5

[a] Each value is the average of three assays ± the standard error of the mean. [b] Total glycoalkaloid values were determined by the *p*-formaldehyde–H_2SO_4 method.

RESULTS AND DISCUSSION

The levels of total glycoalkaloids in the tubers of three potato cultivars are shown in Table I. The amounts of α-solanine and α-chaconine, the only glycoalkaloids isolated from these tissues, are also summarized in Table I. It can be seen that the sums of the concentrations of the individual glycoalkaloids are in close agreement to the values for total glycoalkaloid determined by the paraformaldehyde method. Thus the TLC method reported here can be used as a quantitative assay for total as well as individual glycoalkaloids.

The R_f values for α-solanine and α-chaconine were considerably different on the small TLC plates used in this study than they were on standard silica gel TLC plates. Apparently the hardness of the adsorbent and the length of the Kontes microplates contributed to significant variations from the recorded R_f values.

The small TLC plates used in this study were convenient because of their cost and the speed of the development step. Standard plates could have been used and quantitated with equal ease, and they may be desirable in some studies. For example, other tubers or potato foliage may contain glycoalkaloids that are more difficult to separate than the two obtained in this study. In addition, if a densitometer with lower sensitivity than the Kontes instrument is used, greater sample sizes might be needed and it is unlikely that the microplates used in this study would completely resolve the individual alkaloids. It is also possible that the distribution of glycoalkaloids in foliage or tubers of different cultivars of potato might necessitate

0021-8561/78/1426-1453$01.00/0

the use of a developing solvent other than the one used to generate the data of this report.

In some preliminary studies the glycoalkaloids were extracted with 5% trichloroacetic acid in 75% methanol (Smittle, 1971). It was discovered that under these conditions a spot corresponding to the aglycon solanidine was present on the developed TLC plates. Since the amount of this acid hydrolysis product varied from one extraction to another, the milder extraction method of Shih and Kûc (1974) was utilized. It is worth noting that although the extraction medium containing trichloroacetic acid cannot be used if individual glycoalkaloids are to be quantitated, the total glycoalkaloid assay results were the same for identical samples extracted with this method and with the gentler method of Shih and Kûc (1974). This was true for the total glycoalkaloid assays using the paraformaldehyde reagent and the total glycoalkaloid assay involving the sum of the individual glycoalkaloid spots obtained by TLC.

The concentration of glycoalkaloids in potato tubers is typically much greater near the peel than it is in the center of the tuber. Therefore, the level of total or individual glycoalkaloids obtained in an assay depends markedly on the sampling techniques used. Thus, Deahl et al. (1973) reported 7.8 mg of glycoalkaloid/100 g of whole tuber of potato cultivar B6039-WV6. In the present study we found 41.3 mg of total glycoalkaloid/100 g of thick peel of this same cultivar, and we undoubtedly would have found even higher levels if thin peels had been used.

The techniques described in this paper offer an accurate and sensitive method that is preferential to other methods for routine analysis of individual glycoalkaloids in a large number of samples.

ACKNOWLEDGMENT

The authors wish to thank J. W. White, USDA-ARS, Eastern Regional Research Laboratory, Philadelphia, Pa., for an authentic sample of α-solanine.

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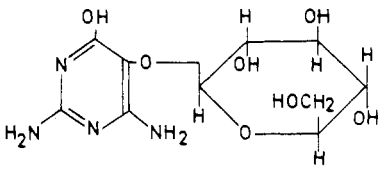
Received for review June 29, 1977. Accepted July 21, 1978. Manuscript published with the approval of the Director of the West Virginia Agricultural Experiment Station, Morgantown, as Scientific Paper No. 1495.

Variation in Vicine Concentration during Pod Development in Broad Bean (*Vicia faba* L.)

Changes in the concentration of vicine in broad bean seeds at various stages of pod development were determined. It was found that the very young seeds from pods 1–2 cm long contained about 1140 mg of vicine/100 g of dry tissue. This value was raised to about 2460 mg when pods were 5–6 cm long and dropped to about 900 mg at pod maturity. A similar pattern of variation in vicine concentration was noted for whole seeds, cotyledons, and seed coats. Statistical analyses revealed highly significant correlations between the vicine content of cotyledons, seed coats, and whole seeds.

Vicine [2,4-diamino-5,6-dihydroxypyrimidine-5-(β-D-glucopyranoside)], a pyrimidine glycoside, was first isolated from vetch (*Vicia sativa*) seeds (Ritthausen and Kreusler, 1870) and later found to occur in broad beans (*Vicia faba*) (Winterstein and Somlõ, 1933). Its structure, as shown here, was established two decades later by Bendich and



Clements (1953). The pyrimidine moiety of this compound, presumably resulting from enzymatic breakdown of vicine in the digestive tract after ingestion, was later implicated as one of the factors responsible for the hemolytic disease called favism (Mager et al., 1965). A comprehensive review of favism has been reported by Mager et al. (1969).

Jamalian et al. (1977a) developed a routine method for the estimation of vicine in leguminous seeds. The concentration of vicine in seed coats and cotyledons of mature broad bean seeds of a large number of Iranian and foreign cultivars was determined by this method (Jamalian, 1978).

Brown and Roberts (1972) studied the formation of vicine and its analogue, convicine, in the developing seeds of *Vicia faba* L. (cv. Mammoth Windsor). They found no vicine in 10-cm long pods or the seeds within. The 13–15 cm pods were also devoid of vicine; however, the seeds in such pods did contain vicine. The investigations of Jamalian et al. (1977a) confirmed the absence of vicine in pods. However, contrary to the findings of Brown and Roberts (1972), the vicine concentrations in the immature seeds of two Iranian broad bean cultivars, Hendu Kola and Rud Pish, were as high as 2.22 and 1.81%, respectively.

0021-8561/78/1426-1454$01.00/0