PRELIMINARY STUDIES ON THE EFFECT OF DEFICIENCY IN POTASSIUM OR MAGNESIUM ON CONCENTRATION OF CHLOROGENIC ACID AND SCOPOLIN IN TOBACCO

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Roots, stems, and leaves of tobacco plants grown on complete or on potassium-deficient or magnesium-deficient nutrient solutions were analyzed quantitatively for content of scopolin and of chlorogenic, neochlorogenic, and 4-O-caffeoylquinic acids. As previously found for nitrogen deficiency, magnesium and potassium deficiencies increased the scopolin content of tobacco leaves. The increase correlated approximately with appearance of deficiency symptoms. Magnesium deficiency decreased appreciably the amounts of chlorogenic acid, neochlorogenic acid, and 4-O-caffeoylquinic acid in leaves and of chlorogenic acid in stems and roots after appearence of deficiency symptoms. Changes in concentrations of chlorogenic acids due to potassium deficiency were minor and inconsistent.

Results of several quantitative investigations concerning effects of mineral deficiences on the concentration in tobacco of scopolin (7-glucoside of scopoletin) and chlorogenic acid (3-O-caffeoylquinic acid, abbreviated here as CGA) have appeared in the literature. Except for one report from our laboratories, however, they did not include quantitative analyses for neochlorogenic acid (5-O-caffeoylquinic acid, neoCGA) and 4-O-caffeoylquinic acid (4-CQA, sometimes called "Band 510"). Watanabe and coworkers (1, 2) reported increases in scopolin in boron-deficient tobacco and sunflower leaves. Fowler (3) showed that increased chloride in tobacco leaves of the same physiological age was associated with increased scopoletin (6-methoxy-7-hydroxycoumarin). In addition, he also reported that other factors affecting the nutrient balance, such as low potash content, appeared to favor the accumulation of scopoletin. Loche and Chouteau (4) found increases in scopolin and decreases in chlorogenic acid in tobacco leaves which were deficient in magnesium, calcium, or phosphorus. Chouteau and Loche (5) noted a decreased concentration of CGA in potassium-deficient tobacco leaves, but an increased concentration of CGA in nitrogen-deficient tobacco leaves, stems, and roots as compared with those in the corresponding parts of control tobacco plants. The increases correlated approximately with the time of first observable deficiency symptoms. In the preliminary study reported here, a similar comparison was made of scopolin, CGA, neoCGA, and 4-CQA concentrations in leaves, roots, and stems of magnesium-deficient potassium-deficient and of untreated (control) tobacco plants over a 5-week period.

METHODS

Tobacco plants (*Nicotiana tabacum*, One-Sucker variety) were grown in pure quartz sand in Percival growth chambers on a daily cycle with a 16 hr light period (1300 ft-c) at 28.8 C and an 8 hr dark period at 16.6 C. Plants were watered with Fe-EDTA double strength Hoagland's (7) nutrient solution until treatment began. Approximately 70 days after germination, plants were selected for uniformity and the pots were leached thoroughly with distilled water. Control plants were watered, thereafter, with the double strength nutrient solution. Treated (deficient) plants were watered with a similar nutrient solution made deficient in either magnesium or potassium by a procedure similar to that outlined by Machlis and Torrey (8). Soil jars were leached every 7 days with 3,000 ml of distilled water. Deficient plants were raised on blocks to keep apices of deficient and control plants at the same illumination level.

A control plant and a treated plant were

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harvested at the end of 1, 3, and 5 weeks. Leaves at various developmental stages were harvested from each plant; sampling was kept uniform. The top 1 cm of the plant was removed before the stem was harvested, whereas the entire root system was harvested. These separate harvests were ground and extracted by the procedure used by Wilson et al. (9). Quantitative analysis of scopolin and the three caffeoylquinic acids was performed by the method described by Koeppe, Rohrbaugh, and Wender (10).

RESULTS

Stunting and interveinal chlorosis due to magnesium deficiency became evident after 2 weeks of treatment. It was not until nearly the end of the 5-week treatment period, however, that the potassium deficiency symptoms of chlorosis and "cupping under" of leaves became evident. Slight stunting was observed somewhat earlier.

Concentrations of CGA and of 4-CQA in leaves of potassium-deficient tobacco plants were higher one week after start of the treatment and were lower at the end of 3 and 5 weeks of treatment as compared with those of control plants at corresponding time periods (Tables 1 and 2). Leaves of such treated plants showed lesser amounts of neoCGA than did corresponding control plants.

Compared to that of control samples taken at the corresponding time period, the CGA concentration in stems of potassium-deficient tobacco plants was lower at the end of week 1 and higher at weeks 3 and 5 (Table 1). The CGA concentration in roots of the same treated plants was very slightly lower at the end of weeks 1 and 5, but higher at week 3, as compared with corresponding controls. Concentrations of 4-CQA and neoCGA in stems and roots of all control and treated plants were too low, if present, to be quantitated by the procedure used.

TABLE 1. *Concentrations of chlorogenic acid in magnesium- and potassium-deficient and control tobacco plants.*

Treatment	Micrograms/gram fresh weight Week 1	Week 3	Week 5
		Leaves	
Control	504	811	989
Mg-deficient	640	525	593
K-deficient	716	701	860
		Stems	
Control	481	179	143
Mg-deficient	220	94	102
K-deficient	452	270	297
		Roots	
Control	257	206	509
Mg-deficient	255	176	238
K-deficient	240	444	463

TABLE 2. *Concentrations of 4-O-caffeoylquinic acid and neochlorogenic acid in magnesium- and potassium-deficient and control tobacco leaves.*[a]

Treatment	Micrograms/gram fresh weight Week 1	Week 3	Week 5
		4-O-Caffeoylquinic Acid	
Control	293	460	522
Mg-deficient	369	274	322
K-deficient	326	380	458
		Neochlorogenic Acid	
Control	174	331	438
Mg-deficient	215	220	266
K-deficient	152	286	343

[a] Concentrations of neochlorogenic and 4-O-caffeoylquinic acids if present in roots or stems were too low to determine by the procedure used.

TABLE 3. *Concentrations of scopolin in magnesium- and potassium-deficient and control tobacco plants.*

Treatment	Micrograms/gram fresh weight Week 1	Week 3	Week 5
		Leaves	
Control	3.2	4.8	9.9
Mg-deficient	2.2	7.8	12.9
K-deficient	2.5	5.0	15.5
		Stems	
Control	42.0	23.6	23.2
Mg-deficient	10.3	14.2	17.8
K-deficient	30.3	9.6	24.6
		Roots	
Control	67.9	65.1	138.8
Mg-deficient	40.0	64.7	93.5
K-deficient	51.1	110.8	144.4

The scopolin content of leaves of potassium-deficient plants differed little, if any, from that of control plants at weeks 1 and 3 (Table 3). By the end of 5 weeks, however, the scopolin concentration in leaves of the treated tobacco was definitely higher than in corresponding controls. This increase in scopolin coincided approximately with the appearance of deficiency symptoms in the potassium-deficient plants. Compared to controls, potassium-deficient stems contained less scopolin at weeks 1 and 3, but had a slightly higher concentration by week 5 (Table 3). Roots of these plants had a lower concentration of scopolin than did controls only at week 1; relatively higher concentrations were present at weeks 3 and 5.

The concentration of CGA in leaves of magnesium-deficient tobacco plants was higher at the end of week 1, but lower at the end of weeks 3 and 5 than in corresponding controls (Table 1). Concentrations of neoCGA and 4-CQA paralleled these changes, although on a lower scale. The CGA concentration of stems of mag-

nesium-deficient tobacco plants was lower at weeks 1, 3, and 5 than in corresponding controls (Table 1). Compared with controls, there was a very minor decrease of CGA in roots after one week of treatment; a slightly greater reduction at week 3 was followed by a much greater reduction at week 5.

The concentration of scopolin in leaves of magnesium-deficient tobacco plants dropped slightly at week 1, but increased at weeks 3 and 5 compared with that of corresponding control plants (Table 3). The increases in scopolin occurred at approximately the time that deficiency symptoms appeared in the magnesium-deficient plants.

Further studies are needed to explain the significance of these observations.

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