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Cytokinin activity in *Citrus* seedlings colonized by mycorrhizal fungi

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Introduction

The level of cytokinin-like substances is greater in the leaves of the vesicular–arbuscular mycorrhizal monocotyledon *Bouteloua gracilis* compared to non-mycorrhizal plants (Allen *et al.*, 1980). This increase may be associated with colonization of roots by vesicular–arbuscular mycorrhizal fungi or the result of improved mineral nutrition (Van Staden and Davey, 1979). The objectives of this research were to: 1) establish the presence of cytokinins in leaves of *Citrus jambhiri* Lush. seedlings inoculated with vesicular–arbuscular mycorrhizal fungi; and 2) isolate cytokinins with demonstrated biological activity.

Materials and Methods

Five vesicular–arbuscular mycorrhizal fungi including *Glomus caledonium* (Nicol. and Gerd.), *G. epigaeum* (Dan and Trappe), *G. etunicatum* (Becker and Gerd.), *G. fasciculatum* Thaxt. (Gerd. and Trappe) and *G. mosseae* (Nichol. and Gerd.) were used to inoculate seedlings using methods described by Bethenfalvay and Yoder (1981). Plants were grown in a glasshouse for 105 d under previously described culture conditions (Menge *et al.*, 1978).

Following harvest, seedling dry weight and vesicular–arbuscular mycorrhizal colonization of inoculated and non-inoculated plants were measured. Seedling leaf subsamples were analyzed for phosphorus content (Menge *et al.*, 1978).

Leaf samples were collected for cytokinin analysis before final harvest of seedlings. The partially purified cytokinin extracts of fully expanded leaves were separated by high performance liquid chromatography using procedures described by Horgan and Kramers (1979).

Results

All *C. jambhiri* seedlings inoculated with fungal symbionts exhibited abundant vesicular–arbuscular mycorrhizal development after 105 d. Control seedlings were non-mycorrhizal. Inoculation of *Citrus* seedlings with vesicular–arbuscular mycorrhizal fungi significantly influenced total dry

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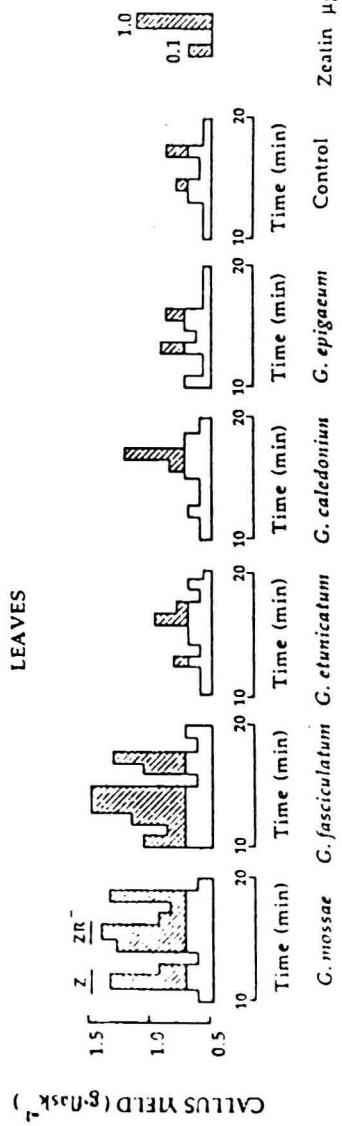

Fig. 1. Cytokinin activity of leaf tissue extracts of vesicular–arbuscular mycorrhizal and non-mycorrhizal *C. jambhiri* seedlings as determined by the soybean callus bioassay after high performance liquid chromatography. Cross-hatched areas represent activity significantly different from that of the control at the 0.95 confidence level. Z, zeatin; ZR, zeatin riboside. The analysis was performed after 105 d.

weight and phosphorus content of leaves. Seedlings inoculated with *G. caledonium*, *G. fasciculatum* and *G. mosseae* were significantly larger than the non-inoculated control plants. Similarly, leaf phosphorus levels were significantly greater in the vesicular–arbuscular mycorrhizal seedlings compared to the non-mycorrhizal plants.

Inoculation with vesicular–arbuscular mycorrhizal fungi significantly increased cytokinin activity in leaves of *C. jambhiri*. Colonization by *G. fasciculatum* and *G. mosseae* resulted in a significant increase in seedling leaf cytokinin activity. Several cytokinins, including zeatin and zeatin riboside, were detected in seedling leaves.

Discussion and Conclusion

These results demonstrate the presence of cytokinins in leaves of *C. jambhiri*. Endogenous cytokinin activity in *C. jambhiri* leaves has not been reported previously. The cytokinin activity in leaves may be attributable to acropetal transport from roots and/or regulation to cytokinin oxidase enzymes (Van Staden and Davey, 1979).

This study established significant changes in cytokinin activity in leaves attributable to colonization of seedlings with different vesicular–arbuscular mycorrhizal symbionts. Changes in cytokinin-like activity in leaves of other *Citrus* species due to vesicular–arbuscular mycorrhizal colonization was also reported by Edriss *et al.* (1984). The significantly greater cytokinin activity in the leaves of vesicular–arbuscular mycorrhizal *C. jambhiri*, relative to non-mycorrhizal seedlings, is consistent with the report of zeatin and zeatin riboside production by mycorrhizal fungi *in vitro* (Barea and Azcon-Aguilar, 1982).

Elevated cytokinin activity of seedlings inoculated with *G. fasciculatum* and *G. mosseae* was associated with concomitant improvements in total dry weight, phosphorus nutrition and vesicular–arbuscular mycorrhizal colonization. A minimum level of phosphorus nutrition is requisite for cytokinin activity. Significant improvements in the growth and phosphorus nutrition of other *Citrus* species due to vesicular–arbuscular mycorrhizal colonization have been reported (Menge *et al.*, 1978). Cytokinins may facilitate phosphorus utilization and affect cell growth by attracting nutrients to developing tissue (Van Staden and Davey, 1979).

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