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Endogenous Levels of Cytokinins, Indoleacetic Acid, Abscisic Acid, and Pigments in Variegated Somaclones of Micropropagated Banana Leaves

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Abstract. The banana (*Musa* spp. AAA) micropropagation shows a high incidence of off-types, among whose variegated plants are very common. Endogenous levels of growth regulators and pigment content were measured in normal and variegated leaves of the micropropagated banana plants growing in a greenhouse. Growth regulators were separated by high pressure liquid chromatography and submitted to enzyme-linked immunosorbent assay for quantification. Pigment content was measured using the colorimetric method. Green leaves contained 1.9 and 10 times more cytokinins compared with green and yellow sectors of variegated leaves, respectively. The levels of indoleacetic acid in normal leaves were significantly higher than those found in green and yellow sectors of variegated leaves; however, the levels of abscisic acid were lower in normal leaves. The lower content of chlorophylls in variegated leaves coincided with decreased endogenous levels of cytokinins, which indicated that variegation in banana leaves may be associated with alterations in the metabolism of this growth regulator.

Key Words. Banana—Growth regulators—Pigments—Variegation—Somaclonal variation

Micropropagation of banana cultivars from the Cavendish subgroup enables the production of good quality plants. However, for unknown reasons, the use of shoots tips for in vitro micropropagation of these cultivars often results in severe genetic defects (Hwang and Ko 1987, Reuveni et al. 1986, Stover 1987), including plant nanism and leaf variegation. It is believed that these mutations are caused by the growth regulators used in the culture media, mainly cytokinins, as well as the duration in tissue culture (Reuveni et al. 1986).

The occurrence of variegated off-types, a defect in the formation of photosynthetic pigments, seems to be associated with high levels of cytokinins in the tissue culture medium (Reuveni et al. 1986). One of the possible effects of cytokinins on plants cultivated in culture medium is the increase in the level of photosynthetic pigments (Caers et al. 1985) because these growth regulators seem to influence chloroplast metabolism (Parthier 1979). Therefore, the low level of chlorophyll in variegated leaves of banana may be associated with alterations in the metabolism of cytokinins and/or the hormonal balance of these leaf tissues.

In the present paper we report on the endogenous levels of four cytokinins as well as the indoleacetic acid (IAA), abscisic acid (ABA), chlorophyll, and carotenoid pigments in normal and variegated leaves harvested from micropropagated banana plants.

Materials and Methods

Three-month-old micropropagated banana plants (*Musa* spp. AAA) cv. Grand Naine were used. The plants were grown in a greenhouse, under a 16 h photoperiod at 25 ± 3°C. Fully expanded mature leaves were

Abbreviations: IAA, indoleacetic acid; ABA, abscisic acid; Ck(s), cytokinin(s); Z, zeatin; [9R]Z, zeatin riboside; iP, isopentenyladenine; [9R]iP, isopentenyladenosine; HPLC, high pressure liquid chromatography; ELISA, enzyme-linked immunosorbent assay.

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harvested; in variegated leaves, the green and yellow sectors were separated. Pigments were extracted and measured as described by Lichtenthaler (1987). Endogenous levels of IAA, ABA, and the cytokinins (Cks), zeatin (Z), zeatin riboside ([9R]Z), isopentenyladenine (iP), and isopentenyladenosine ([9R]iP) were measured in 1 g of leaf tissue by an immunoenzymic method according to Maldiney et al. (1986), Pelèse et al. (1989), and Sotta et al. (1987), which allowed the determination of these hormones in the same extract. Freeze-dried powdered tissues were stirred up and extracted with cold 80% methanol containing 80 mg · dm^{-3} butylhydroxytoluene as antioxidant for 60 h at 4°C in darkness. Tritiated radiolabeled standards (IAA and ABA, specific activity Gbq · $mmol^{-1}$ and Gbq · $mmol^{-1}$, respectively Amersham) were added to the samples for recovery estimation after purification. The methanolic extracts were filtered and then passed through Sep-Pak C-18 cartridges. After filtration, the eluates were reduced to dryness in vacuo with a rotary evaporator and the residues resuspended in 500 μL of acid water, pH 3.0. Growth regulators were then separated over 80 min by HPLC using a reverse phase semipreparative μBondapak 19 × 300 mm column at a flow rate of 5 mL · min^{-1} with a 5–50% methanol gradient in phosphoric acid buffered to pH 3.0 with triethylamine (Sotta et al. 1987). Fractions were collected at 1-min intervals and reduced to dryness in a Speed-Vac concentrator. They were then methylated with ethereal diazomethane for IAA and ABA analysis before being submitted to ELISA with anti-IAA and anti-ABA antibodies. Ck measurements were carried out by ELISA with anti-[9R]Z and anti-[9R]iP antibodies. Each treatment consisted of five replications. The hormone level in each sample was measured four times, and the values were corrected for recovery. The results were subjected to an analysis of variance to determine the significance of the difference among the three types of leaves.

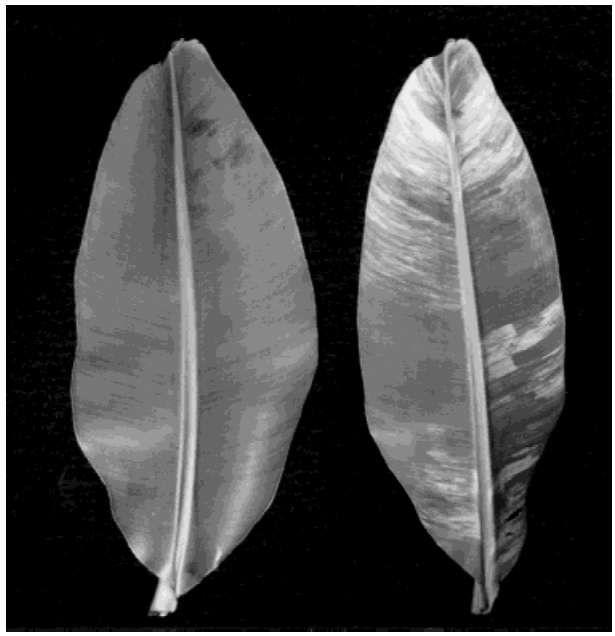

Fig. 1. Normal (left) and variegated leaves (right) of micropropagated banana plants cultivar Grand Naine 3 months after transfer to greenhouse conditions.

Results and Discussion

Leaf variegation did not seem to influence the normal size and development of banana leaves at the 3rd month after tissue culture (Fig. 1). Even profoundly variegated leaves developed normally. Endogenous levels of IAA and Cks in normal leaves were significantly higher than those observed in variegated leaves; however, the levels of ABA were higher in variegated leaves (Table 1). Because IAA and Cks are produced mainly in meristematic tissues and are transported to the mature fully expanded leaves, a defect in the transport system for these hormones would lead to a decrease in the levels of IAA and Cks and an increase in the levels of ABA in variegated leaves.

The concentration of Z and [9R]Z in normal leaves and in both green sectors and yellow sectors of variegated leaves, contributed 90, 60, and 80% of the total free Ck pool, respectively. Green leaves presented 1.9 and 10 times more Cks compared with green and yellow sectors of variegated leaves, respectively. Van Staden et al. (1994) found that the levels of Cks in green leaves of *Schefflera arboricola* were ten times higher than those in yellow leaves. The authors postulated that the movement of Cks in yellow leaves was slower, but the metabolism was faster. In banana plants, the lower amounts of Cks found in green and yellow sectors of variegated leaves could be attributed to a limitation in the xylem transport of the Cks produced in the roots of mutant plants.

The levels of IAA in normal leaves were significantly higher than those found in green and yellow sectors of variegated leaves; however, the levels of ABA were lower in normal leaves (Table 1).

The ratio of IAA to Cks in normal leaves and in green sectors of variegated leaves was 2.80 and 2.02, respectively, whereas in yellow sectors the ratio was 8.67. This seems to indicate a substantial correlation between the Ck levels and pigment content in the variegated sectors.

The content of chlorophyll *a* and *b* and carotenoids was significantly higher in normal leaves compared with both green and yellow sectors of variegated leaves (Table 2). These results suggest a positive correlation between the content of photosynthetic pigments and the endogenous levels of IAA and Cks and a negative correlation with the levels of ABA. Longo et al. (1979) suggested that the Cks produced in the roots were transported to leaves and would influence the synthesis of chlorophylls and/or the keeping of the photosynthetic apparatus, aided by light. The influence of kinetin on the development of protoplastids, particularly in the grana formation of cultured tobacco cells, was observed by Stetler and Laetsch (1965). The lower content of chlorophyll in variegated leaves was parallel with a decrease in the endogenous levels of Cks, which indicates the possibility that variegation in banana leaves could be associated with genetic alterations in the metabolism of this growth regulator. Further work currently under way in this laboratory, dealing with biosynthesis of Cks and

Table 1. Endogenous concentrations of cytokinins, IAA, and ABA (pmol · g FW^{-1}) and IAA/cytokinin ratio in normal and variegated leaves of micropropagated banana plants cv. Grand Naine 3 months after transfer to greenhouse conditions.

		Variegated leaf	
Hormone	Normal leaf	Green sector	Yellow sector
Cytokinins			
Zeatin	34.60 a	21.67 b	2.99 c
Zeatin riboside	32.28 a	11.11 b	1.47 c
Isopentenyladenine	4.78 a	3.48 ab	1.80 b
Isopentenyladenosine	0.73 b	1.56 a	0.70 b
Total	72.39	37.82	6.96
Auxin			
IAA	203.09 a	76.69 b	60.38 b
Auxin/cytokinins	2.80	2.02	8.67
ABA	45.57 b	75.18 a	64.03 a

Note. Mean in a horizontal line followed by the same letter are not significantly different at the 5% level according to Tukey's test. FW, fresh weight.

Table 2. Content of chlorophylls and carotenoids (μg · g FW^{-1}) in normal and variegated leaves of micropropagated banana plants of the cv. Grand Naine 3 months after transfer to greenhouse conditions.

		Variegated leaf	
Pigment	Normal leaf	Green sector	Yellow sector
Chlorophylls			
Chlorophyll *a*	373.95 a	287.26 b	235.14 c
Chlorophyll *b*	151.00 a	122.45 b	97.77 c
Total	530.20 a	412.95 b	332.79 c
Carotenoids			
Xanthophyll + carotene	161.94 a	125.15 b	107.49 c

Note. Means in a horizontal line followed by the same letter are not significantly different at the 5% level according to Tukey's test. FW, fresh weight.

auxins as well as with the activity of Ck-oxidase and auxin-oxidase in variegated banana leaves, could account for a better understanding of the effects of genetic traits on the metabolism and endogenous levels of these growth substances.

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