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Gibberellic acid detection of dwarf offtypes in micropropagated Cavendish bananas

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Summary. Detection of dwarf offtypes produced by micropropagation of Cavendish bananas (*Musa* spp.) cultivars New Guinea Cavendish and Williams was achieved by spraying gibberellic acid (GA_3) solution (289 μmol/L) onto deflasked plants and measuring various plantlet responses. The most useful identification criterion was elongation of the sheath of the first leaf to form after GA_3 application. Elongation of this structure was about 2-fold greater in normal plants than observed in dwarfs. Similar measurements taken earlier during *in vitro* culture or later during plant establishment in soil were not as useful in discriminating between normals and dwarfs as the measurements made at deflasking. The similar GA_3-induced elongation response of the dwarf offtype and that of the naturally occurring dwarf cultivar Dwarf Parfitt suggests that the mechanism for dwarfism could be the same in the 2 cases.

Introduction

Micropropagation offers a rapid method of producing large quantities of disease-free bananas (*Musa* spp.) for commercial production. However, widespread use of such materials is hindered by the high percentages of tissue culture-induced dwarf variants (hereafter called offtypes), especially with Cavendish bananas (Smith 1988; Israeli *et al.* 1991). The dwarf offtype is characterised by short stature, reduced fruit length, closely packed hands and poor fruit exsertion (choking) under adverse conditions (Smith and Drew 1990*a*; Israeli *et al.* 1991). Consequently dwarfs have no commercial value. Early detection and elimination from planting materials is highly desirable but not easy to undertake. Dwarfs are not visibly detected *in vitro* and can only be observed in the later stages of nursery production when plants are taller than 20 cm and growing vigorously (Smith and Hamill 1993). When plants are grown under less than ideal conditions, dwarf offtypes often escape detection, hence other methods of dwarf detection are desirable.

Gibberellic acid (GA_3) is a major growth regulator controlling many plant responses including stem and petiole elongation. In terms of response to exogenous GA_3, dwarf plants have been classified as GA_3-responsive or GA_3-non-responsive (Graebe 1987). GA_3-responsive dwarfs show greater elongation responses to GA_3 applications than the corresponding normal or tall forms and have been described in a wide range of species, including rice (*Oryza sativa*, Murakami 1972), maize (*Zea mays*, Phinney 1957) and pea (*Pisum sativum*, Reid *et al.* 1983). GA_3-non-responsive dwarfs show a reduced or no elongation response to GA_3 application and have been described in wheat (*Triticum aestivum*, Gale and Marshall 1973), maize (Phinney 1957) and rye (*Secale cereale*, Jlibene and Gustafson 1992). Dwarfs of Cavendish banana, induced by micropropagation, are often GA_3-non-responsive and this includes the cultivars Williams (Reuveni 1990) and Grand Naine (Cote *et al.* 1993) dwarf offtypes.

The aims of the present study were to: (i) characterise the GA_3-response in dwarf banana offtypes generated originally by micropropagation, (ii) determine if different growth responses induced by exogenous GA_3 can be used as selection criteria to differentiate plant type early in the tissue culture procedure, and (iii) determine parameters of the GA_3 test which would result in the greatest discrimination between normal and dwarf plants.

Materials and methods

Plant materials

Three Cavendish (*Musa* spp. AAA group) cultivars, differing in plant stature, were used: Williams (tall), New Guinea Cavendish (medium–tall) and Dwarf Parfitt (extra-dwarf). In addition, dwarf offtypes (originally produced through micropropagation) of Williams and New Guinea Cavendish were used. All plant materials were obtained from Queensland Department of Primary Industries' Maroochy Horticultural Research Station, Nambour, Queensland. All 5 plant types were used to establish *in vitro* cultures using the procedure of Smith and Drew (1990*b*). This system used Murashige and

Skoog's (MS) basal nutrients and organics (Murashige and Skoog 1962) supplemented with 30 g/L sucrose and 0.7% agar (Agar Agar, Sigma, St Louis, Missouri, USA). Shoots were multiplied on MS medium supplemented with 6-benzylaminopurine (BAP; 11 μmol/L, Sigma, St Louis, Missouri, USA) and subcultured every 4–6 weeks. Shoots developed roots when placed on MS medium without growth regulators. All cultures were placed in a growth room [26 ± 1°C; 14 h photoperiod; and photosynthetic photon flux density (PPFD) of 17–35 μmol/m^2.s].

Dwarf screening

Gibberellic acid (90% pure; Sigma, St Louis, Missouri, USA) was applied to normal and dwarf plants at 3 stages of growth: during *in vitro* culture, at deflasking, and following establishment in soil.

In vitro *culture.* For the screening of plants during *in vitro* culture, leaves and roots of single-shoot cultures of cultivars New Guinea Cavendish, Williams and Dwarf Parfitt, were trimmed and the explants placed on a solid (0.7% agar) MS medium containing filter-sterilised GA_3 at final concentrations of 0, 3, 15, 29, 59 and 145 μmol/L. Shoot cultures were incubated at 26 ± 1°C under a PPFD of 17–35 μmol/m^2.s (Sylvania Gro-Lux F40 fluorescent tubes) for 30 days and the leaf–sheath (base of shoot to start of leaf lamina) length measured.

Deflasking. For the screening of plants at deflasking, single-shoot cultures of cultivars New Guinea Cavendish, Williams and Dwarf Parfitt were rooted on MS medium, deflasked and planted in pots (10 cm diameter) containing a steam-sterilised sand and peat (2:1, v/v) mixture. These plants were immediately sprayed with GA_3 (289 μmol/L) solution or distilled water. A concentration of 289 μmol GA_3/L was chosen because it resulted in a maximum response for plants established in soil (data not shown). Each plant received 5 mL applied evenly as a spray (droplet size about 200 μm) to the whole plant. Following spraying, plants were covered with clear plastic bags to help their establishment in soil and placed in a growth cabinet (28/25 ± 1°C day/night; 14 h photoperiod; PPFD about 200–220 μmol/m^2.s provided by 40 W fluorescent tubes). The plastic bags were removed 2 weeks later and leaf–sheath measurements taken on the newly emerged leaves as previously described following a further 2 weeks of incubation. The newest leaf to form was called leaf 1 and the one after that, leaf 2.

Establishment in soils. For the screening of established plants in soil, established micropropagated plants of cultivar New Guinea Cavendish growing in soil (4 weeks from deflasking) were sprayed with 1 or 2 applications of 289 μmol GA_3/L. Several applications of GA_3 were tried since preliminary results showed that established plants were not as sensitive as newly deflasked plants. For each application 8 mL of solution was applied evenly to each plant. The time interval between applications was 5 days. Plants were incubated in a controlled temperature glasshouse (30/25°C day/night; about 14 h photoperiod; PPFD>500 μmol/m^2.s) for 5 weeks, after which leaf–sheath measurements were taken on newly emerged leaves as previously described.

Experimental design and statistical analysis

All experiments were carried out in a completely randomised design with 10 replications for *in vitro* culture, 14 replications for deflasking, and 7 replications for established plant stage. Data were analysed using the MicroQuasp (The University of Queensland, St Lucia, Qld) analysis of variance. Where appropriate, comparisons of means were undertaken using the l.s.d. test at $P = 0.05$.

Results

In vitro *culture stage*

Micropropagated, but normal plants of New Guinea Cavendish and Williams produced leaf–sheaths in response to GA_3 (3–145 μmol/L) that were significantly longer than those produced by dwarf plants of the same cultivar (Table 1). Addition of 3 μmol GA_3/L induced a 3-fold elongation in normal plants compared with a 2-fold elongation in dwarf plants. Concentrations above 3 μmol/L had no further effect on the mean response. The best separation of the normal and dwarf plant population was seen at 59 μmol GA_3/L (Fig. 1) where only 10% of normal plants fell into the same leaf–sheath category as dwarf plants. With lower (3–29 μmol/L) or higher (145 μmol/L) concentrations more than 15% of normals fell into the same leaf–sheath category as dwarf plants. Dwarf Parfitt plants responded to the various concentrations of GA_3 in a similar fashion to New Guinea Cavendish and Williams dwarf plants, exhibiting a 2-fold elongation (Table 1).

Table 1. Effect of gibberellic acid (GA_3) on leaf–sheath length (cm) of normal and dwarf plants of New Guinea Cavendish and Williams, and normal plants of Dwarf Parfitt taken at the time of *in vitro* culture

Values within rows followed by the same letter are not significantly different at $P = 0.05$

GA_3	New Guinea Cavendish		Williams		Dwarf Parfitt
(μmol/L)	Normal	Dwarf	Normal	Dwarf	Normal
0	1.1a	1.2a	1.1a	1.0a	1.0a
3	3.5a	1.9b	3.6a	2.0b	2.1b
14	4.0a	2.2b	4.0a	2.1b	1.9b
29	3.6a	2.2b	4.2a	1.9b	1.9b
59	3.7a	2.1b	3.7a	2.2b	2.3b
145	5.4a	4.2b	4.2b	3.1c	3.0c

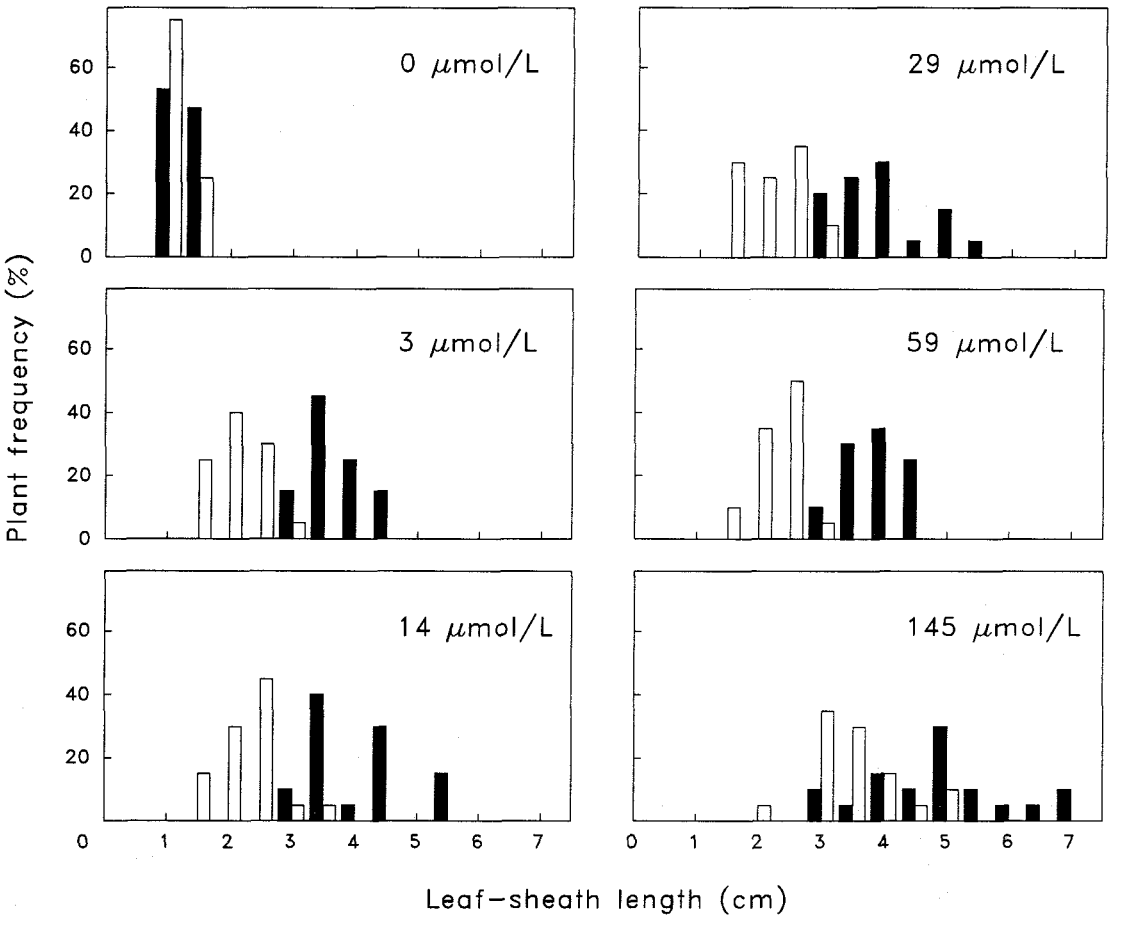

Figure 1. Effect of gibberellic acid concentration on frequency distribution for leaf–sheath length of normal (open bars) and dwarf (solid bars) plants at time of *in vitro* culture. Leaf–sheath lengths are for 20 plants (10 plants each of cultivars New Guinea Cavendish and Williams) for each GA_3 concentration.

Deflasking stage

Deflasked normal plants of New Guinea Cavendish produced leaf–sheaths in response to 289 μmol GA_3/L that were significantly longer than those produced by dwarf plants of the same cultivar (Table 2). Measurements taken on leaf 1 showed a 6- to 7-fold increase in response to GA_3 in normal plants but only a 3- to 4-fold increase in dwarf plants. Leaf 2 produced a 3-fold increase in response to GA_3 in normal plants but only a 2-fold increase in dwarf plants. The best separation of the normal and dwarf plant populations was seen in leaf 1 where no overlap was observed. When leaf 2 was studied (Fig. 2), 46% of normal plants fell into the same category as dwarf plants. Dwarf Parfitt plants responded to GA_3 in a similar

Table 2. Effect of gibberellic acid (GA_3) on leaf–sheath length (cm) of normal and dwarf plants of New Guinea Cavendish and Williams, and normal plants of Dwarf Parfitt taken at deflasking

Values with rows followed by the same letter are not significantly different at $P = 0.05$

GA_3 (μmol/L)	Leaf number	New Guinea Cavendish		Williams		Dwarf Parfitt
		Normal	Dwarf	Normal	Dwarf	Normal
0	1	1.2a	1.1a	1.6a	0.9a	0.9a
0	2	2.9a	2.5a	3.0a	2.5a	2.8a
289	1	7.6a	2.9d	6.4b	4.0c	2.1e
289	2	8.8a	5.6b	8.2a	6.6b	4.7c

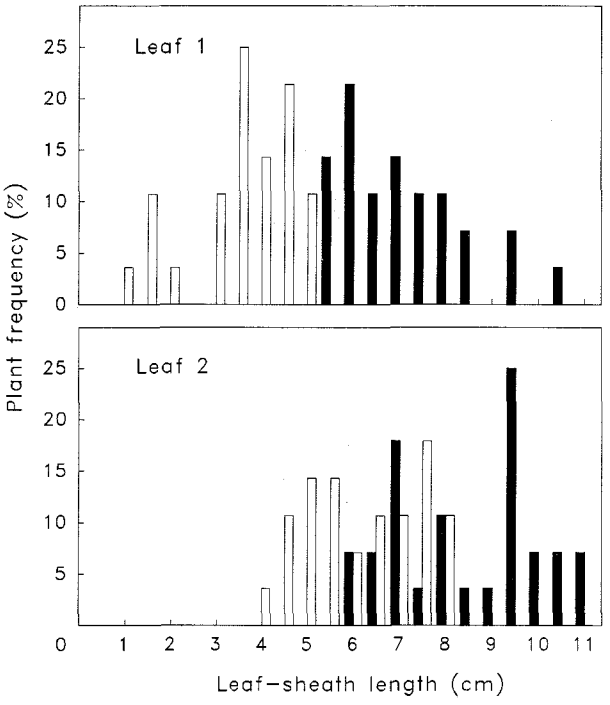

Figure 2. Effect of a single spray application of gibberellic acid (289 μmol/L) on the frequency distribution for leaf–sheath length of normal (open bars) and dwarf (solid bars) plants at deflasking. Leaf–sheath lengths are for 28 plants (14 plants each of cultivars New Guinea Cavendish and Williams) for each leaf–sheath studied.

fashion to New Guinea Cavendish and Williams dwarf plants exhibiting a 2-fold increase for both leaf 1 and 2 (Table 2).

Established plant stage

Established normal plants of New Guinea Cavendish produced leaf–sheaths in response to 289 μmol GA_3/L that were significantly longer than those produced by dwarf plants (Table 3). Measurements taken following GA_3 application showed a 2-fold increase in normal but less than a 2-fold increase in dwarf plants. After a second application of GA_3 no greater differences between normal and dwarf plants were observed. Good separation between normal and dwarf plants was not obtained with either 1 or 2 GA_3 applications (Fig. 3). In both cases 30% of the normal plant population fell into the same leaf–sheath length category as dwarf plants (Fig. 3). Additional studies using 3 or 4 GA_3 applications (data not shown) did not show any additional improvements in dwarf detection.

Table 3. Effect of gibberellic acid (GA_3) on leaf–sheath length (cm) of normal and dwarf plants of New Guinea Cavendish taken at the time of plant establishment

Values within rows followed by the same letter are not significantly different at $P = 0.05$

GA_3 (μmol/L)	Dose	Leaf–sheath length (cm) Normal	Dwarf
0	0	3.3a	3.3a
289	1	6.0a	4.2b
289	2	6.9a	4.6b

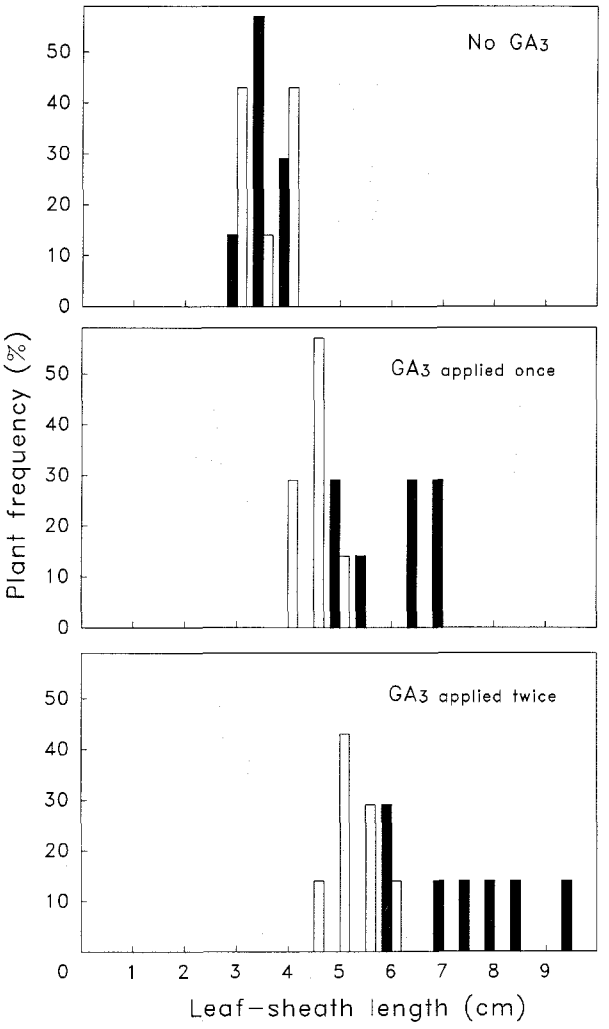

Figure 3. Effect of 0, 1 or 2 spray applications of gibberellic acid (GA_3) on frequency distribution for leaf–sheaf length of normal (open bars) and dwarf (solid bars) plants at the time of plant establishment in soil. Leaf–sheath lengths are for 7 plants of cultivar New Guinea Cavendish for each GA_3 application.

Discussion

Whether tested *in vitro* (Table 1), at deflasking (Table 2) or following establishment in soil (Table 3), micropropagated normal plants exhibited a significantly

greater leaf–sheath elongation in response to GA_3 than micropropagated dwarf plants. In all cases, when compared with dwarf plants, elongation in normal plants was 2-fold greater. When the screen was undertaken on the first leaf produced after GA_3 application taken from plants at deflasking, separation of normal and dwarf plants was complete (Fig. 2). For the second leaf produced after GA_3 application (Fig. 2), or for leaves taken from *in vitro* (Fig. 1) and established (Fig. 3) plants, there was at least 10% overlap with dwarf plants, indicating that 10% of normal plants would have to be discarded before it was clear that only normal plants remained.

This differential growth induced by GA_3 classifies Cavendish banana micropropagation-induced dwarfs GA_3-non-responsive similar to that reported by Reuveni (1990) and Cote *et al.* (1993). This differential growth response could be the basis for a selection method for detecting and eliminating dwarfs from micropropagated plantlets. The earliest detection of dwarfs with a 90% success rate (Fig. 1) is at the *in vitro* stage. However, to be absolutely sure offtypes have been eliminated, selection tests should be applied to plantlets at deflasking (Fig. 2). Plants established in soil are the least responsive to GA_3 and can only be detected at a 70% success rate (Fig. 3). The leaf–sheath length of the first leaf that emerged after GA_3 treatment was the best discriminating parameter in identifying dwarf and normal plants (Fig. 2) and repeated applications of GA_3 did not improve the response of plants established in soil (Fig. 3).

GA_3-treated plants, at all stages of growth, exhibited only temporary etiolation. These plants resumed normal growth by the time the 4th or 5th leaf after GA_3 application had been produced (data not shown). It is not thought that the short-term, GA_3-induced changes in physiology and morphology will bring about poor yield performances in established banana plants.

The GA_3 screening method applied to plantlets at deflasking using the elongation response of the first leaf–sheath could be developed for commercial use for the early detection of dwarfs. This would achieve greatest discrimination still allowing a sufficient period for normal plants to resume regular growth. The method would be simple and cheap, and could prevent the waste of resources on non-productive dwarf plants.

The similar GA_3 response of the dwarf offtypes or the naturally occurring extra-dwarf cultivar suggests that the mechanism for dwarfism in Cavendish bananas could be the same. However, further studies are underway to elucidate the mechanism and characterise the dwarf offtypes using molecular techniques.

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