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Micropropagated dwarf off-type Cavendish bananas (*Musa* spp., AAA) show improved tolerance to suboptimal temperatures

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Abstract. The responses of micropropagated normal plants and dwarf off-types of Cavendish (*Musa* spp. AAA) bananas to suboptimal temperatures were evaluated under field and controlled environmental conditions. Compared with bananas grown at 30/25°C (day/night), leaf production at 18/14°C was inhibited by 51% in normal plants and 18% in dwarf off-types. The emergence of the first leaf that developed at low temperature was delayed by 11 days for normal plants and 5 days for the dwarf off-types. Photoinhibition of lamina, measured by decrease in the chlorophyll fluorescence variable Fv/Fm, occurred in all banana plants growing in the field during the winter months. The extent to which the plants were photoinhibited was significantly greater for the normal plants than dwarf off-types. Under controlled environmental conditions, photoinhibition was similarly greater in normal plants than dwarf off-types. After 153 h at 18/14°C and a 9-h photoperiod of photon flux density (PFD) of 380 μmol/m$^2\cdot$s, Fv/Fm was reduced by 22 and 13% for normal and dwarf off-types, respectively. When plants were exposed to 18°C and a continuous PFD of 380 μmol/m$^2\cdot$s for 20 h, Fv/Fm was reduced by 50% for normal plants and 36% for dwarf off-types. The results of the study indicate that dwarf off-types generated from banana micropropagation showed improved tolerance to low temperature and light, showing better growth and lower susceptibility to low-temperature induced photoinhibition than normal plants.

Additional keywords: photoinhibition, photosynthesis, chlorophyll fluorescence, cold tolerance.

Introduction

The banana (*Musa* spp.) is a tropical plant best suited for growth in climates where the mean annual temperature does not fall below 20°C (Rehm and Espig 1991). In subtropical conditions, plant growth potential is seasonally limited (Stover and Simmonds 1987). The mean minimum monthly air temperatures in winter at a number of subtropical, banana-growing sites may fall to 17 to 9°C, or even lower (Stover and Simmonds 1987). Low temperatures can have some drastic effects on bananas. Growth rates are reduced, leaves yellow, and plants show disturbed physiological activities such as reduced and lowered rates of photosynthesis, transpiration, and stomatal conductance (Turner and Lahav 1983; Eckstein and Robinson 1995). Schmueli (1960) monitored the effects of an ambient temperature of −1°C on the leaves of 'Dwarf Cavendish' growing in the field in Israel. Leaves of plants bearing fruits turned pale green 4–5 days after experiencing the low temperature, and after 7–9 days leaves became chlorotic and water-soaked and 3 weeks later died. Leaves on young suckers turned yellow 14–20 days after exposure but regreened 5–6 weeks later. Low temperatures during fruit development also affect cell differentiation and result in deformed fruits (Fahn *et al.* 1961).

Another widely occurring form of low temperature injury is photoinhibition of photosynthesis in the leaves exposed to the sun (Bongi and Long 1987; Demmig and Björkman 1987; Greer and Laing 1992). Long-term

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exposure of leaves to the cold under high light results in photooxidative destruction of the chlorophyll (Van Hasselt and Van Berlo 1980). Photoinhibition can be detected visually in banana by comparing the colour of leaves exposed to full sun with that of leaves kept in the shade. Where chlorophyll photooxidation occurs, the leaves take on a bleached appearance, while the shaded sections of the lamina remain green (Turner 1994). In the deciduous kiwifruit vine (*Actinidia deliciosa* A. Chev), the onset of photoinhibition occurs when air temperatures fall to 14–16°C (Greer and Laing 1992). Photoinhibition in field-grown *Monstera deliciosa* Liebm., papaya (*Carica papaya* L.), and banana has been recorded in winter (Smillie *et al.* 1988). Cold-induced photoinhibition has also been recorded in plants grown in their natural habitat. Photoinhibition occurred during winter in needles of *Pinus sylvestris* L. (Leverenz and Öquist 1987) and in leaves of juvenile snow gums (*Eucalyptus pauciflora* Seibb. Ex. Spreng; Ball *et al.* 1991) during winter. In the major subtropical banana-growing area in south-east Queensland (27°S, 153°E) and northern New South Wales (30°S, 153°E), mean minimum temperatures of 7–11°C are typically recorded in winter (Bureau of Meteorology 1991*a*, 1991*b*). Banana growers in south-east Queensland who have planted micropropagated Cavendish bananas in the field have observed that the leaves of the normal (true to type) plants showed symptoms of yellowing, whereas the leaves of dwarf off-types, a major micropropagation-induced variant (Smith and Drew 1990), remained pale green to green during the winter months (M. K. Smith, unpublished data). This reaction to winter conditions, which apparently discriminated dwarf off-types from normal plants in the field, warrants further investigation.

The main aim of this study was to characterise the response of micropropagated normal plants and dwarf off-types to low temperature and light. The specific aims were to compare the growth performances of recently established micropropagated normals and dwarf off-types at temperatures thought to be optimal and suboptimal for growth and to measure tolerance to low-temperature induced photoinhibition both in the field and under controlled environmental conditions.

Materials and methods

Plant materials and growing conditions

Suckers from normal plants and dwarf off-types generated from micropropagation of Cavendish (*Musa* spp. AAA) cv. New Guinea Cavendish were obtained from the banana evaluation block of Queensland Department of Primary Industries (QDPI) field site at Wamuran, south-east Queensland (27°S, 153°E). Shoot tips from the suckers were cultured *in vitro* following the procedure of Drew and Smith (1990). After 6 cycles of multiplication, shoots were rooted, deflasked, and planted in 13-cm-diameter pots containing University of California potting mix B and fertiliser II (Matkin and Chandler 1957). The plants were maintained in a controlled growth cabinet set to 28°C day and 25°C night and a 10-h photoperiod. The photosynthetic photon flux density (PFD) at the top of the canopy was 200 μmol/m$^2\cdot$s. The plants were watered twice daily to field capacity and fertilised 2 weeks after deflasking with slow release fertiliser (Osmocote Plus 15 : 11 : 13 NPK; Sierra Grace, Nottingham, UK) at the rate of 2 g/pot. Fertiliser application was repeated every 4 weeks. Four weeks after deflasking, plants that had developed 3–4 fully expanded leaves were either used in experiments or transferred to 20-cm pots for a further 4 weeks of growth in a controlled environment glasshouse set to 30/25°C day/night and irradiated with natural daylight for 10 h/day. The PFD at the top of the canopy was 800–1200 μmol/m$^2\cdot$s. Plants remained under these conditions until they had reached the 6–7 leaf stage.

Plant growth measurements

Four-week-old established plants were transferred from 28/25°C temperature growing conditions to 2 controlled environmental glasshouses. Half of the plants were transferred to 30°C day/25°C night, and the remaining plants were placed at 18°C day/14°C night. In both growth rooms the photoperiod was 10 h. The PFD at the top of the canopy was 800–1200 μmol/m$^2\cdot$s. At the start of the treatment, plants had 4 fully expanded leaves and the next emerging leaf was still enclosed in the leaf-sheath. Leaf number, length, and width were measured weekly up to 5 weeks on all fully expanded leaves that had developed during the temperature treatments. Leaf length was measured from the base of the lamina to the tip of the lamina and the width was taken from the widest portion of the lamina. Leaf area was calculated from the length and width measurements. For plant height measurements, the base of the youngest petiole was labelled with a permanent marker at the start of the experiment. Height of the plant was measured weekly from this point to the base of the next youngest petiole.

Assessment of winter temperature damage

Plant ability to resist low temperature and light damage in the field was determined on cvv. New Guinea Cavendish and Williams grown in QDPI evaluation blocks at Wamuran in August 1992 (mean minimum temperature of 9·9°C, mean daily photon receipt of 26·8 mol/m^2, total rainfall 23·4 mm, Table 1). Plants had been grown using standard commercial cultural practices (Gall and Vock 1994). At the time the measurements were taken, the plants were bunching and the percentage bunch filling ranged from 70 to 90%. Normal plants were recognised by being substantially taller than dwarf off-types. The mean height of normal New Guinea Cavendish and Williams was 2·3 and 2·2 m, respectively; the height of dwarf off-types was 1·6 m for both cultivars. Leaf samples were collected from either the most recently expanded or the second most recently expanded leaf from the top of mature and bearing plants. Two leaf samples per plant were taken, one from a portion of the lamina facing north to west (called sunny side) and one from a portion facing south to east (called shaded side). At both sampling points on each leaf a lamina strip approximately 5–10 cm wide was cut from the leaf, from petiole to leaf margin. Immediately after harvest, leaf strips were placed in a moistened plastic bag, sprayed with distilled water to prevent desiccation, and kept in darkness. Measurements were made on leaves 3–4 h after harvest. The

degree of yellowing of the leaf sample was rated using the following scale: 1, entire leaf sample yellow; 2, 75% yellow; 3, 50% yellow; 4, 25% yellow; 5, entire leaf sample pale green; 6, dark green. Fluorescence measurements were taken along each strip of lamina starting 2 cm from the midrib and repeated at 2-cm intervals to the margin; 6–8 measurements were recorded from each leaf sample.

Table 1. Mean monthly maximum (T_{max}), minimum (T_{min}), and average (T_{ave}) air temperature, mean daily photon receipt (DPR), and total monthly rainfall at Wamuran, south-east Queensland (27°S 153°E), during winter (June–August) 1992

Values are means±s.d. (n = 30–31)

	T_{max}	T_{min} (°C)	T_{ave}	DPR (mol/m^2)	Total rainfall (mm)
June	19·1±1·5	9·3±2·6	14·5±1·8	19·2±5·9	24·4
July	19·7±2·1	9·5±2·6	14·8±2·2	22·0±5·1	49·8
August	21·1±2·4	9·9±2·4	15·7±1·9	26·8±7·3	23·4

Chlorophyll fluorescence measurements

Chlorophyll fluorescence of leaves dark-adapted for 15 min was measured using a portable pulse amplitude modulated (PAM-2000) chlorophyll fluorometer (Heinz Walz, GmbH, Effeltrich, Germany). The minimal fluorescence yield (Fo), with all photosystem II reaction centres fully open, was first measured by exposing the dark-adapted tissue to a weak modulated measuring light. The maximal fluorescence yield (Fm), with all photosystem II reaction centres fully closed, was then recorded while exposing the tissue to a saturating pulse of PFD of 4800 μmol/m^2 for 0·8 s. The variable fluorescence (Fv) was calculated from the difference between Fm and Fo. Photoinhibition was measured by a decrease in variable to maximal fluorescence yield (Fv/Fm).

Chlorophyll extraction

Chlorophyll was extracted from 1-cm-diameter leaf discs cut from the same spots where fluorescence measurements were made. Chlorophyll extracts were made by placing discs in N,N-dimethylformamide at a ratio of 1 : 10 (w/v) and kept in the dark at 4°C for 48 h (Moran 1982). The absorbance of the extracts was measured on a spectrophotometer (GBC UV/VIS 916, GBC Scientific Equipment Pty Ltd, Dandenong, Vic.) at wavelengths 647 and 664 nm. The chlorophyll concentration was calculated using the formula of Moran (1982).

Susceptibility to low-temperature induced photoinhibition under controlled environmental conditions

Established normal plants and dwarf off-types with 6–7 fully expanded leaves were taken out of the 30/25°C chamber 4 h after dawn. The youngest fully expanded leaf was laid flat on a frame (50 cm by 30 cm) strung with 7 equidistant lines of nylon thread, 0·18 mm diameter. The abaxial surface was kept flat against the frame by nylon thread tightened across the middle of adaxial leaf surface. The desired experimental light level, i.e. a moderate PFD of 220 or 380 μmol/m^2·s provided by eight 160 W fluorescent tubes, was achieved by adjusting the distance of the experimental leaf to the light source. For dark control treatment, a portion of the lamina for each leaf was covered with aluminium foil. Initial fluorescence measurements were taken 1 h after placing the plants in the chamber and allowing them to equilibrate to 18°C in the dark. After 15 h of darkness at 14°C, plants were irradiated using moderate PFD of either 220 or 380 μmol/m^2·s for a 9-h photoperiod. Measurements of Fv/Fm were recorded each day 15 min before dawn, at midday, and at the end of day, for 7 days. Fluorescence measurements were made on the adaxial surface of the lamina, 2 cm from the midrib on both sides of the lamina. Yellowing and necrosis ratings on the leaves exposed to light were taken after 175 h of exposure to low temperature. The yellowing on the leaf margin was rated using the following scale: 1, entire margin yellow; 2, 75% yellow; 3, 50% yellow; 4, 25% yellow; 5, whole leaf green. Leaf necrosis on the complete lamina was rated using the scale: 1, no necrosis; 2, very slight necrosis (1–3 necrotic spots, a necrotic spot approximately 10–20 mm); 3, slight necrosis (4–5 necrotic spots); 4, severe necrosis (>5 necrotic spots or the necrotic spots coalesced to form long lesions approximately 50 mm); 5, whole leaf necrotic.

Photoinhibition in detached and attached leaves

The youngest fully expanded leaves were harvested from established plants 4 h after dawn. Leaves were kept in plastic bags and sprayed with distilled water. Leaf pieces (2 by 2 cm) were cut from the middle of the lamina and placed on a wet filter paper supported on an aluminium plate (45 cm by 30 cm by 0·03 cm). The leaf pieces were covered with thin polyethylene film. The plate was then placed in a controlled growth cabinet. Specimens of still-attached, youngest fully expanded leaves were laid flat on the other side of the plate and covered with polyethylene film. For the dark control treatment, a portion of attached leaf and leaf pieces laid on the same plate were covered with aluminium foil. Initial fluorescence measurements were taken after 1 h of temperature equilibration at 18°C in the dark, then the leaves were irradiated at a PFD of 380 μmol/m^2·s. Photoinhibition, measured by a decrease in Fv/Fm, was taken after 20 h of exposure at 18°C.

Experimental design and statistical analysis

All experiments were carried out using a completely randomised design. The experiment comparing growth at 2 temperature regimes was conducted using a 2 × 2 factorial with temperature and plant type as the 2 factors. There were 15 replications per treatment and each plant was a replicate. For assessment of winter temperature damage in the field, 2 leaf samples were taken from 8 normal and dwarf plants. For photoinhibition experiments involving intact leaves, 8 replications were used, with 1 leaf per plant as a replicate. For the detached leaf experiment, 20 replications were used with 2 leaf pieces obtained per plant. Data were analysed using the Statistical Analysis System (SAS Statistical Institute, Cary, NC). Where appropriate, comparisons of means were undertaken using the least significant difference (l.s.d.) and t-test at $P = 0·01$ and 0·05.

Results

Growth response at two growing temperatures

Normal plants and dwarf off-types grown at 18/14°C showed lower leaf production and mean leaf area per plant than normal and dwarf plants grown at 30/25°C (Fig. 1). After 5 weeks of growth at 18/14°C, leaf production was reduced by 51% in normal plants, compared with 18% in dwarf off-types (Table 2). The emergence of the first leaf that developed at low temperature was delayed by 11 days in normal plants and 5 days in dwarf off-types (Table 2). The second

Table 2. Mean number of leaves, days to leaf emergence, leaf area, leaf length/width ratio, and plant height for normal plants and dwarf off-types grown at 18/14°C and 30/25°C (day/night)

Measurements were taken on all new growth that occurred during temperature treatments. Number of leaves and plant height were determined after 5 weeks. Leaf measurements were taken at full leaf expansion

Means are from 15 plants, and means within columns followed by the same letter are not significantly different (l.s.d. at $P = 0{\cdot}05$).

Plant type	No. of leaves	Days to leaf emergence		Leaf area (cm^2)		Leaf length/width ratio		Plant height (cm)
		Leaf 1	Leaf 2	Leaf 1	Leaf 2	Leaf 1	Leaf 2	
				18/14°C				
Normal	2·0c	18·1a	16·3a	26·1a	37·1b	2·4a	2·3a	3·95c
Dwarf	3·9b	12·6b	12·6b	22·2b	30·5c	1·9b	2·0b	3·66c
				30/25°C				
Normal	4·1ab	7·0c	7·9c	29·8a	49·6a	2·6a	2·3a	8·27a
Dwarf	4·8a	7·5c	6·8c	20·8b	33·6bc	2·1b	2·0b	7·16b

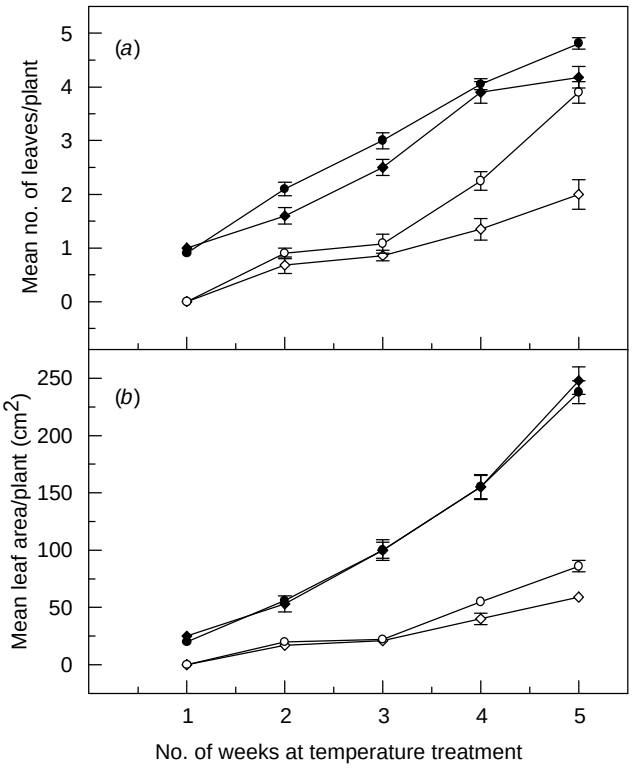

Fig. 1. (*a*) Mean number of leaves per plant and (*b*) mean total leaf area per plant for normal plants (◇ ◆) and dwarf off-types (○ ●) grown at 18/14°C (open symbols) and 30/25°C (closed symbols). Values represent means of 15 replications ± s.e.

leaf of the normal plants emerged 3 days earlier than the first leaf, whereas for dwarf off-types leaf 1 and 2 showed the same rate of emergence. The mean leaf area was significantly lower at 18/14°C than at 30/25°C (Table 2). Normal plants had higher mean leaf area than dwarf off-types at either temperature. Mean total leaf area per plant after 5 weeks at 18/14°C was significantly higher in dwarf off-types than in normal plants (Fig. 1*b*), and this was due to greater numbers of new leaves in the dwarf off-types. Leaf index (leaf length/width) was not affected by the growing temperature (Table 2). Significant differences in plant height between normal plants and dwarf off-types occurred at 30/25°C, but not at 18/14°C (Table 2).

Table 3. Leaf yellowing rating, chlorophyll content, and Fv/Fm measured from field-grown normal plants and dwarf off-types during winter

Means are from 8 normal plants (2 New Guinea Cavendish and 6 Williams) and 8 dwarf off-types (6 New Guinea Cavendish and 2 Williams). Leaf yellowing was rated using the scale 1 (yellow) to 6 (green) as described in **Materials and methods**

Means within columns followed by the same letter are not significantly different (l.s.d. at $P = 0{\cdot}05$)

Plant type	Leaf rating	Chlorophyll content (mg/g fw)	Chlorophyll a/b ratio	Fv/Fm
		Sunny side		
Normal	3·4b	2·18b	3·52a	0·67b
Dwarf	4·2ab	2·37a	2·67b	0·72a
		Shaded side		
Normal	4·6a	2·11b	3·17a	0·70a
Dwarf	4·8a	2·37a	2·52b	0·73a

Photoinhibition in field-grown normal plants and dwarf off-types

Micropropagated normal plants and dwarf off-types grown in the field during winter (mean minimum temperature 9·6°C, Table 1) showed typical yellowing of the leaves, especially on the section of the lamina directly exposed to sun, the north to west facing side. The yellowing of the leaves due to photooxidation of the chlorophyll was more severe in normal plants than in dwarf off-types (Table 3). At either side of the lamina the chlorophyll content of the leaves was significantly higher for dwarf off-types, while the chlorophyll a/b

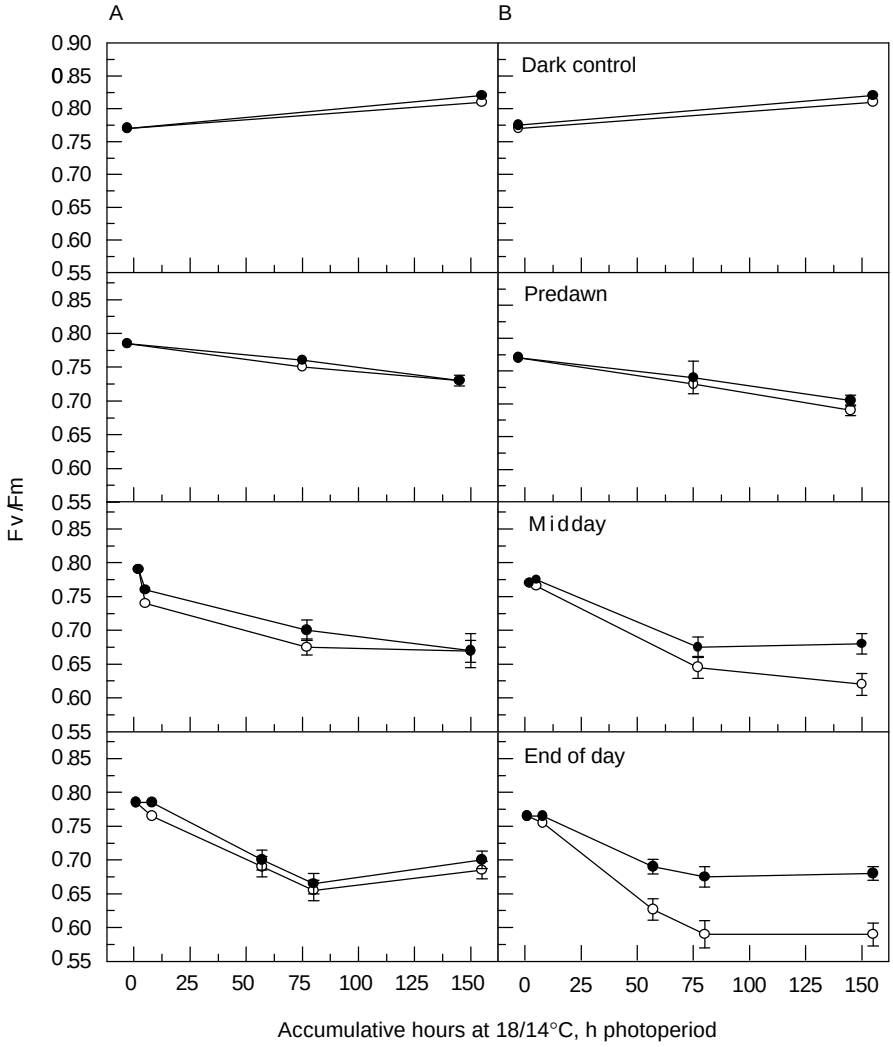

Fig. 2. Change in Fv/Fm in normal plants (○) and dwarf off-types (●) kept at 18/14°C and irradiated at PFD of (*a*) 220 or (*b*) 380 μ mol/m^2·s. Initial Fv/Fm measurements at time 0 were taken before the start of the photoperiod. Values represent means of 8 replications ± s.e.

ratio was significantly higher for normal plants (Table 3). No significant differences in chlorophyll measurements were observed between the sunny and shaded side of lamina. Photoinhibition of the lamina, as measured by the decrease in Fv/Fm, occurred in both normal plants and dwarf off-types (Table 3). The extent of photoinhibition on lamina from the sunny side was significantly greater in normal plants than in dwarf off-types.

Photoinhibition in normal plants and dwarf off-types under controlled environmental conditions

Normal plants and dwarf off-types kept at 18/14°C and irradiated at PFD of 220 or 380 μmol/m^2·s for 9-h photoperiods showed progressive decline in Fv/Fm (Fig. 2). Irradiation at a PFD of 220 μmol/m^2·s did not show significant differences between normal and dwarf plants (Fig. 2*a*). Increasing the PFD to 380 μmol/m^2·s, however, caused significant differences (Fig. 2*b*). After 153 h of exposure, the end-of-day Fv/Fm was reduced by 22·5% for normal plants and 12·9% for dwarf plants. The Fv/Fm measurements of the dark control plants did not change after 153 h of low temperature treatment.

Yellowing and necrosis in the lamina were observed after prolonged exposure of the plants to 18/14°C day/night temperatures. Yellowing occurred along the margins of lamina. For the plants irradiated at a PFD of 220 μmol/m^2·s, 25% of normal and dwarf plants showed yellowing of leaves (Table 4). At PFD of 380 μmol/m^2·s, 62·5% of normals and 25% of the dwarf off-types showed yellowing of leaves. Necrosis of leaves was observed only in the normal plants (Table 4).

Table 4. Percentage of plants showing severe yellowing and necrosis in normal and dwarf off-type plants grown at 18/14°C and irradiated at PFD of 220 or 380 μmol/m^2·s

Visual assessments are from 8 plants and taken after 175 h of exposure to low temperature and light. Plants showing severe yellowing include those with ratings of 1–3 and plants showing necrosis include those with ratings of 3 and 4 as described in **Materials and methods**

PFD (μmol/m^2·s)	Plants showing severe yellowing (%)		Plants showing necrosis (%)	
	Normal	Dwarf	Normal	Dwarf
220	25·0	25·0	37·5	0
380	65·2	25·0	37·5	0

Photoinhibition in attached leaves and detached leaf pieces

Significant reduction in Fv/Fm ratio occurred in the attached and detached leaves after 20 h of exposure at 18°C and a PFD of 380 μmol/m^2·s (Fig. 3). Fv/Fm was decreased by 50% for the attached leaves of normal plants and 36% for attached leaves of dwarf off-types. For the detached leaves, the reduction in Fv/Fm was higher, 62% for the normal plants and 51% for dwarf off-types. In both attached and detached leaves, the decrease in Fv/Fm was significantly ($P = 0·01$) higher in normal plants than in dwarf off-types. The Fv/Fm of the dark control treatment did not decrease after 20 h of exposure to low temperature.

Discussion

Dwarf off-types from micropropagated Cavendish bananas showed improved tolerance to suboptimal temperatures in both the field and controlled environments. The emergence of leaves in recently established micropropagated bananas was significantly inhibited at 18/14°C day/night temperatures (Table 2, Fig. 1). Leaf emergence was inhibited by 51% in normal plants and 18% in the dwarf off-types. Total leaf area per plant was reduced in normal plants but not in dwarf off-types. Earlier emergence of leaves and higher total leaf area would contribute to higher photosynthetic capacity of the dwarf off-types at low temperatures.

In southern Queensland, banana plants growing in the field during winter (mean temperature about 15°C, Table 1) showed both yellowing of leaves (Table 2) and evidence of photoinhibition as indicated by low Fv/Fm ratios (Table 2). Photoinhibition and yellowing of the leaves were more severe in normal plants than in dwarf off-types. Under controlled conditions of low temperature and light (18/14°C, 380 μmol/m^2·s, 9-h photoperiod), significant reduction in Fv/Fm was observed: 22% for normal plants and 13% for dwarf off-types (Fig. 2). At 18°C and continuous illumination of PFD of 380 μmol/m^2·s for 20 h, Fv/Fm declined in both attached and detached leaves. The reduction was significantly higher for normal plants than dwarf off-types (Fig. 3).

Reduced photosynthetic efficiency at low temperatures would also affect overall photosynthetic capacity. Leaves may become photoinhibited at low temperatures, resulting in decreases in both photosynthetic CO_2 fixation rates and quantum yields (Demmig and Björkman 1987). The extent of photoinhibition can be measured by the decrease in Fv/Fm, as Björkman

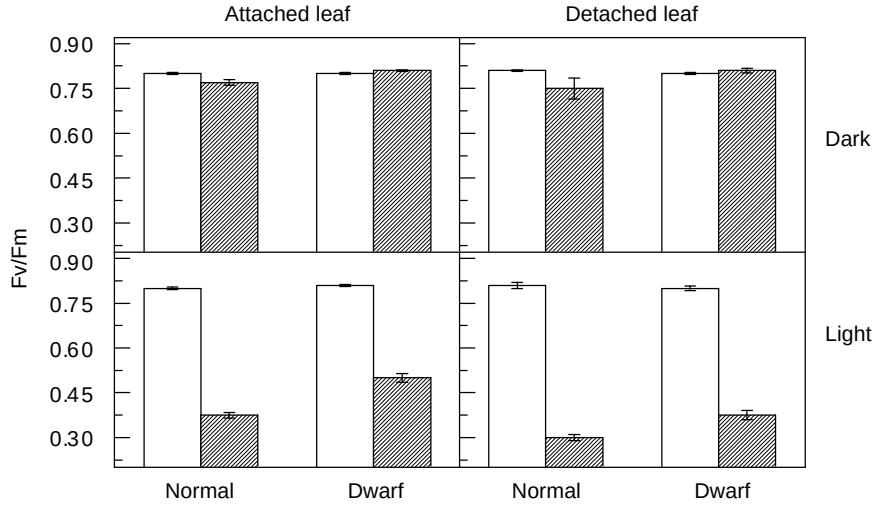

Fig. 3. Change in Fv/Fm in attached leaf and detached leaf pieces of normal plants and dwarf off-types kept at 18°C and irradiated at PFD of 380 μmol/m^2·s. Initial Fv/Fm measurements (unshaded) were taken before irradiation and final Fv/Fm measurements (shaded) were taken after 20 h of irradiation. Values are means of 8 (attached leaf) and 20 (detached leaf) replications ± s.e.

and Demmig (1987) showed that for a wide range of plants whose leaves were photoinhibited, the reduction in Fv/Fm and decrease in quantum yield were highly correlated. Severe episodes of light and cold can also result in photooxidative destruction of photosynthetic pigments (Van Hasselt and Van Berlo 1980), thus further reducing photosynthetic capacity.

Crops introduced from the tropics to subtropical or temperate growing climates are especially at risk to low-temperature induced photoinhibition. In maize growing in southern England in June (mean air temperatures about 16°C) leaves become photoinhibited during sunny mornings (Long *et al*. 1992). Significantly, the incidence of low-temperature photoinhibition corresponded closely to decreased efficiency of dry matter production. Those authors concluded that photoinhibition may severely limit accumulation of dry matter during, and, because recovery took several days, also following, periods of low temperature. Micropropagated Cavendish bananas grown in southern Africa (25°S) during winter (mean minimum temperature about 8°C) showed typical yellowing symptoms on the adaxial leaf surfaces, especially those exposed to the sun in the west (Eckstein and Robinson 1995). Photosynthetic rate on the adaxial side of leaves showing photooxidation was reduced by 59% compared with the adaxial side of normal green leaves in winter.

The results of the present study indicate that dwarf off-types were less susceptible to low-temperature induced photoinhibition both in the field and in controlled environments. The earlier emergence of leaves, higher leaf area per plant, and higher Fv/Fm (less photoinhibition) would contribute to higher photosynthetic capacities in the dwarf off-types at low temperatures. Dwarf Cavendish, similar in stature to the dwarf off-type, also tolerates low temperatures and is better adapted to cool climates than other commercial Cavendish cultivars (Samson 1986). Dwarf Cavendish is the main banana cultivar grown in the more temperate regions (e.g. Canary Islands, 28°N). Dwarf Parfitt, a naturally occurring extra dwarf Cavendish cultivar and much smaller in stature than the dwarf off-type, has also been shown to have higher tolerance to low temperature (Moore *et al*. 1993). Dwarf Parfitt maintained higher chlorophyll concentrations, photosynthetic rates, and Fv/Fm ratios than Williams during winter in southern Queensland. The results of this study agree with previous reports on the low temperature tolerance of other dwarf banana cultivars, and suggest that dwarf stature is a characteristic often associated with tolerance to low temperature. Indeed, dwarfing, either as a permanent feature or plastic response, appears to be an adaptive mechanism of the plant in response to a range of stresses (Fitter and Hay 1987).

Tolerance to low temperature is an important trait for banana improvement for the subtropical regions (Persley and DeLanghe 1987). Somaclonal variation induced from banana micropropagation may be utilised for the improvement of this trait, as the results of this study suggest that dwarf off-types have increased resistance to low temperature, although the exact mechanism is unknown. Somaclonal variation has similarly been used to increase the low temperature tolerance of watermelon (*Cucumis melo* L.) so that seeds germinate at temperatures as low as 15°C, thus allowing earlier spring planting (Ezura *et al*. 1995). The likely close association between dwarf stature and low temperature tolerance could be a useful selection parameter for utilising somaclonal variation to enhance low temperature tolerance in banana, although further studies are needed to establish the precise relationship between the 2 characters. Also, the relationship between low temperature tolerance and yield and fruit quality needs to be established when making decisions on the suitability of new banana cultivars in the subtropics. Chlorophyll fluorescence, which has been used as a rapid and non destructive method to screen for cold tolerance in many plants (Smillie *et al*. 1983; Hetherington *et al*. 1989) should also be useful for routinely checking the susceptibility of new off-types generated from banana micropropagation to low-temperature induced photoinhibition.

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