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In Vitro Propagation of *Miscanthus sinensis*

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Abstract. Immature inflorescences of *Miscanthus sinensis* Andress. 'Gracillimus', 'Variegatus', and 'Zebrinus' were cultured on modified MS medium with 9.0 μM 2,4-D, 20 g sucrose/liter, 2.0 g Gelrite/liter, and 0.75 g MgCl_2 /liter. Organogenesis was observed 8 to 12 weeks after callus initiation. Shoots were rooted on half-strength MS medium without growth regulators. After rooting, tillers were initiated. When transferred to soil, plants matured to flowering quickly and retained their variegation patterns. Propagation through in vitro tillering is suggested. Chemical name used: 2,4-dichlorophenoxyacetic acid (2,4-D).

Interest in new, low-maintenance landscape plants has increased in recent years (Weatherly, 1988). *Miscanthus sinensis* 'Gracillimus', 'Variegatus', and 'Zebrinus' are ornamental grasses that are low maintenance, drought tolerant, adaptable to poor soils, and hardy through zone 4 (Corley, 1983; Gibson, 1986). Each of these cultivars exhibits a unique variegation pattern: 'Gracillimus'—green blade with white midrib, 'Variegatus'—vertical variegation, and 'Zebrinus'—horizontal variegation. These cultivars are sterile and must be propagated through division (Gibson, 1986) and, therefore, have restricted commercial availability. More efficient propagation could enhance the availability and use of *M. sinensis*. This report describes an efficient procedure for in vitro regeneration of these cultivars.

In preliminary regeneration experiments,

immature inflorescences, mature leaves, immature leaves, nodal segments, internodal segments, meristematic regions, and ovules were tested for regeneration (Gawel et al., 1987). Explants were cultured on Murashige and Skoog (1962) (MS) medium containing a wide range of auxin types and concentrations. The only explant types to produce callus were immature inflorescences and, therefore, were used as the sole explants in this study. Only media containing 4-amino-3,5,6 trichloropicolinic acid (Picloram) or 2,4-D with no cytokinin produced the regeneration response.

All explants were harvested from 7-year-old, field-grown plants. Surface sterilization consisted of a 20-sec dip in 95% (v/v) ethanol and 20 min in 0.525% (v/v) sodium hypochlorite followed by three rinses in sterile, distilled water. Explants were surface-sterilized as intact stems containing unelongated inflorescences, then dissected to remove the immature inflorescence. Unless otherwise noted, the basal medium consisted of MS salts and vitamins supplemented with 20 g sucrose, 100 mg myo-inositol, 2.0 g Gelrite (Scott Laboratories, Atlanta), and 0.75 g MgCl_2 (all per liter). The MgCl_2 was added to replace divalent cations sequestered by the

Gelrite during gelling. Growth regulators were added, and pH was adjusted to 5.5 with 1 N NaOH before sterilization. The media were dispensed as 8-ml aliquots into 14-ml glass vials and autoclaved for 20 min at 121°C. Cultures were maintained at 27 to 30°C under 16 hr of light provided by 110-W wide-spectrum fluorescent bulbs (70 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$).

Auxin type and concentration for optimum callus initiation were determined by placing 2-cm-long sections of immature inflorescences on basal medium supplemented with either 4.5, 9.0, 18.1, or 36.2 μM 2,4-D or 4.1, 8.3, 16.6, or 33.2 μM picloram. Each auxin type/concentration/cultivar combination was replicated 10 times. Cream-colored callus began to form within 10 to 14 days. Cultures were scored for callogenesis after 6 weeks. The most efficient treatment was 9.0 μM 2,4-D (Table 1). Overall, 93% of the explants produced callus with this treatment.

Callus initiation was influenced by the developmental stage of the inflorescences. Im-

Fig. 1. Highly meristematic shoot primordia of *M. sinensis* 'Variegatus' callus ($\times 17$).

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Table 1. Percent of *M. sinensis* cultures initiating callus by incubation with 2,4-D or Picloram.

Cultivar	2,4-D (μM)				Picloram (μM)			
	4.5	9.0	18.1	36.2	4.1	8.3	16.6	33.2
	Percent							
Gracillimus	90	100	70	60	50	50	80	80
Variegatus	70	100	50	40	40	20	30	30
Zebrinus	80	80	60	40	40	20	0	70
Mean	80	93	60	60	46	30	36	60

Fig. 2. In vitro tillering of *Miscanthus sinensis* 'Gracillimus'.

mature inflorescences 15 cm or shorter (3 to 4 weeks before flowering) produced the best results in 'Gracillimus' and 'Zebrinus'. 'Variegatus' initiated callus best from inflorescences <5 cm long (4 to 5 weeks before flowering); explants from inflorescences longer than this either died or produced a thin layer of callus and thick mats of roots. Inflorescences of any cultivar that had begun to emerge from the stem produced

an insignificant (<1% of cultures) callogenic response.

Callus initiation was also influenced by explant density within the culture vessel. If more than 3 to 5 inflorescence sections were placed in a vial (25 mm in diameter), explants turned black and died. This may have been due to an excessive accumulation of phenolic-like compounds in the culture medium.

Once initiated, callus was subcultured every 4 to 6 weeks. At the time of subculture, phenolics were visible in the medium at the point of contact between the callus and media. Callus from all cultivars grew best on basal medium devoid of growth regulators, but could also be maintained on medium supplemented with 9.0 μM 2,4-D. Callus usually died after 8 to 12 months in culture; however, long-term callus maintenance was not necessary for plant regeneration.

Within 8 to 12 weeks from initiation, organogenic calli differentiated. Nonorganogenic callus was cream-colored and friable. Two types of organogenic callus were distinguishable: cream-colored and semi-friable, and yellow-green and nodular. Occurrence and type of organogenic/nonorganogenic development was independent of the growth regulator treatment used for callus initiation and maintenance. Each of the organogenic callus types produced shoots, although in different ways. The semi-friable callus developed localized regions of green cells that eventually formed single shoots. Each region produced only one to three shoots before losing meristematic activity. This pattern of organogenesis has been described in oat by

Heyser and Nabors (1982). The yellow-green nodular callus turned dark green and produced cores of highly meristematic shoot primordia (Fig. 1). These cores initiated 20 to 30 shoots before losing their meristematic activity.

When shoots from either callus type were 2 to 5 cm long, most (>90%) spontaneously initiated roots. Root formation could be induced on the remaining nonrooted shoots by placing them on basal medium containing half-strength salts. About 1% of the shoots produced would not initiate roots, regardless of root induction treatment. During root formation, shoots also formed tillers. These tillers could be separated and rooted and subsequently produced more tillers (Fig. 2). On average, each shoot was capable of producing one to three tillers every 6 to 8 weeks. Tillering continued throughout the duration of the study of ≈ 1 year.

When shoots had formed two to three roots in vitro, they were transferred to potting soil (Pro Mix BX, Premier Brands, New Rochelle, N.Y.). Plants were acclimatized under shade and intermittent mist (10 sec mist/5 min, 24 hr-day⁻¹) for 6 to 9 days; mist cycles were gradually decreased to zero during the last 3 days of acclimatization. About 80% of the plants survived acclimatization. Alternative acclimatization regimes may lead to greater survival rates. Acclimatized plants were grown without shade and fertilized with Peter's (W.R. Grace, Fogelsville, Pa.) 20N-20P-20K (100 ppm) weekly. When moved to the field, the plants matured to flowering within 4 months (Fig. 3). The variegation pattern of each cultivar was conserved during the tissue culture process.

As a means of large-scale propagation, we recommend exploiting the in vitro tillering behavior of *M. sinensis*, rather than relying on the production of meristematic cores from callus. From the five to 15 explants obtained from each immature inflorescence, only one to three would be expected to form meristematic cores. However, when the 20 to 30 shoots produced by each meristematic core are multiplied by the tillering ability of each shoot, hundreds of plants can be obtained within a relatively short period, i.e., 20 to 24 weeks. Also, plant production through tiller harvesting keeps the callus stage to a minimum, thus reducing the risk of somaclonal variation.

The regeneration/propagation technique outlined here produces many more plants than standard whole-plant division procedures. It is efficient and relatively simple. We recommend its use when many *M. sinensis* plants are desired.

Fig. 3. Mature, regenerated *Miscanthus sinensis* plants, 6 months postacclimatization.

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Nitrogen, Shading Duration, Gas Exchange, and Growth of Cassava

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Abstract. The effects of applied N and shading duration on net gas exchange and growth of two southern Florida cassava (*Manihot esculenta* Crantz.) cultivars grown in containers were determined. Both cultivars responded similarly to shading and N with respect to the measured variables. There were no interactions between shading duration and N application rate for any of the variables measured. Tissue dry weights, total leaf N and chlorophyll concentrations, net CO₂ assimilation (A), transpiration (E), water-use efficiency (WUE), and stomatal conductance (g_s) were quadratically related to the concentration of N applied to the soil. The optimum N application rate for maximum growth of both cultivars was 60 mg/plant per day. Increased shading duration reduced A, E, g_s, WUE, storage root number, and weight and increased the shoot : root ratio.

Cassava is traditionally cultivated in association with other crops in many developing countries (Okigbo and Greenland, 1976). Reduced light interception by cassava when associated with over-story crops can depress yield (IITA, 1981). Bhagsari and Harmon (1982) suggested that crop yield is largely determined by net photosynthetic potential and partitioning of photosynthates to the harvested portion of the plant. Some studies have suggested that the effect of reduced solar input on cassava depends on the plant's growth stage (Fukai et al., 1984).

Nitrogen utilization is conditioned by the light environment in which plants develop. Evans (1989) observed that species that flourish in the shade have lower net CO₂ assimilation (A) per unit of N. Leaf N has been positively correlated with A (Evans, 1989; Sage and Pearcy, 1987). Excess N accumulates as proteins and nitrates in some

species and accounts for excess top growth at the expense of root growth. In cassava, excess N results in accumulation of cyanogenic glucoside (Bruijn, 1971).

Shading duration may affect the photosynthetic efficiency, growth, and yield of cassava, as well as N utilization. Therefore, determining the optimum N application rate for cassava grown with various shading durations may assist in designing intercropping systems that efficiently use this crop. The purpose of this study was to determine the effects of N application rate and shading duration on leaf gas exchange and growth of cassava.

Cuttings (25 cm in length) of 'Manti-queira' cassava, a profusely branching cultivar, and 'Senorita', a cultivar with an upright growth habit and little branching, were planted in 1 sand : 1 vermiculite (v/v) in 7.6-liter plastic pots. The experiments were conducted in Homestead, Fla., from Feb. to Aug. 1989.

Nitrogen application (Expt. 1). This experiment was designed to determine the optimum N application rate for A and growth. Plants were divided into four N treatments: 0, 60, 120, or 180 mg of NH₄NO₃/plant per day applied to the soil. This corresponded to

a total of ≈0, 50, 100, and 150 kg N/ha available to the plant during the growth period. Plants were fertilized twice weekly with the appropriate amount of N, along with an N-free nutrient solution previously described by O'Hair (1979). The experimental design was a randomized complete block with four replications and three single-plant samples per replication per cultivar.

Nitrogen and shading (Expt. 2). This experiment was designed to test the effects of N and shading duration on gas exchange and growth of cassava. Four 6.1 × 1.5 × 1.5-m metal frames were constructed and covered with woven polyethylene cloth, blocking 30% of the incoming radiation [maximum photosynthetic photon flux (PPF) 1300 μmol·m⁻²·s⁻¹, as determined with a 190SA quantum sensor (LI-COR, Lincoln, Neb.)]. Plants were arranged in a split plot design, with shading duration as the main plot and N application rate and cultivar as subplots. The treatments were the same as in Expt. 1 with the addition of shading. The four shading durations were 0, 1, 2, and 3 months beginning at time of planting. At the end of

Table 1. Effect of N application on number of storage roots per plant and root dry weight of cassava 90 DAP (Expt. 2).

Nitrogen (mg/plant per day)	Roots (no.)	Root wt (g)
0	0.1	0.5
60	4.4	55.4
120	4.8	50.2
180	2.9	24.3
Contrast		
Linear	NS	NS
Quadratic	**	**

**, NS Significant at $P < 0.01$ or nonsignificant, respectively, according to orthogonal contrast analysis.

Table 2. Effect of shading duration on number of storage roots per plant, root dry weight, and shoot : root ratio (dry-weight basis) 90 DAP (Expt. 2).

Shading duration (days)	Roots (no.)	Root wt (g)	Shoot : root ratio*
0	4.5	56.5	1.42
30	3.3	33.2	1.90
60	2.5	24.8	2.10
90	1.8	14.3	2.81
Contrast			
Linear	*	*	**
Quadratic	NS	NS	NS

*Shoot : root ratio calculated as shoot dry weight/total root dry weight.

***, NS Significant at $P < 0.05$ or 0.01 or nonsignificant, respectively, according to orthogonal contrast analysis.

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