Characterization of biomass production, cytology and phenotypes of plants regenerated from embryogenic callus cultures of *Pennisetum americanum* × *P. purpureum* (hybrid triploid napiergrass)

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Summary. Five hundred and twenty-four plants of a triploid, sexually sterile hybrid napiergrass (*Pennisetum americanum* × *P. purpureum*; 3x = 21) were regenerated from embryogenic callus cultures obtained from segments of young inflorescences. Replicated field trials were conducted for two consecutive years to compare the biomass yield, phenotype and cytology of tissue culture regenerants (TC) and vegetatively propagated (V) plants. In the first year total biomass yield of TC plants was significantly greater than V plants but there was no significant difference in the second year. TC plants had more tillers compared to V plants. V plants did not show any morphological variability. The TC population also exhibited a high degree of phenotypic stability (96%). There were 23 phenotypic variants in the TC population of 524, most of them being more dwarf and late-flowering. Detailed morphological analysis of the TC-variant plants suggests that they very likely arose from only a few variant cell lines. Cytological analysis indicated stability of the triploid status in randomly selected regenerants. Two of the morphological variants were hexaploids (6x = 42). It is concluded that embryogenic callus cultures can provide useful alternative for the rapid propagation of hybrid napiergrass which is commonly propagated by cuttings.

Key words: Biomass – Gramineae – Genetic variability – Hybrid triploid napiergrass – *Pennisetum* – Plant tissue culture – Somatic embryogenesis

Introduction

Plant tissue cultures, including those of gramineous species, are prone to chromosomal (Orton 1980; McCoy et al. 1982; Nakamura and Keller 1982), genetic (Edallo et al. 1981; Larkin et al. 1984) and molecular (Gengenbach et al. 1981; McNay et al. 1984) changes. In addition to commonly observed transient and epigenetic changes, plants regenerated from such cultures show heritable variation for morphological and physiological traits (Larkin et al. 1984). In all the above studies, plant regeneration was achieved by organogenesis.

More recently, plants regenerated from embryogenic callus cultures of some grass species have been shown to be relatively uniform and cytogenetically stable. Examples include *Panicum maximum* (Hanna et al. 1984), *Pennisetum americanum* (Swedlund and Vasil 1985), *Zea mays* (Armstrong and Green 1985) and others (see Vasil 1985). These observations have led to the suggestion that plants regenerated from embryogenic callus cultures are cytologically normal and stable because somatic embryos are derived from single cells and because embryogenic cells appear to have a selective advantage in morphogenesis (Vasil 1983, 1985; Swedlund and Vasil 1985).

Regeneration through somatic embryogenesis of *Pennisetum* spp, including pearl millet and napiergrass has been demonstrated previously using a variety of explants (Vasil and Vasil 1981; Haydu and Vasil 1981; Wang and Vasil 1982; Chandler and Vasil 1984; Chandler et al. 1984). Napiergrass and pearl millet-napiergrass hybrids are both efficient biomass species, and are currently undergoing extensive testing for use as animal feed as well as an important, renewable source of energy through production of methane by fermentation (Smith and Frank 1985).

In this paper we report biomass yields and cytological and morphological analyses of a large number
of plants regenerated from inflorescence derived embryogenic callus cultures of a semi-dwarf, sexually sterile, triploid napiergrass hybrid.

**Materials and methods**

**Callus initiation, maintenance and plant regeneration**

Six young inflorescences (10–30 mm long) were collected from field-grown plants of a triploid (3x = 21), sterile *Pennisetum F;* hybrid: dwarf *P. americanum* cv. 'Tf23-DA' (2x = 14) × dwarf *P. purpureum* cv. 'N-75' (4x = 28); selection No. 3. Callus cultures were initiated from inflorescence explants according to the methods of Chandler et al. (1984) on agar-solidified (8.0 g l⁻¹ Bactoagar, Difco) medium (Murashige and Skoog 1962; MS) supplemented with 0.5 mg l⁻¹ benzyladenine, 2.0 mg l⁻¹ 2,4-dichlorophenoxyacetic acid and 5% (v/v) coconut milk and incubated at 27°C in the dark. Embryogenic callus cultures were maintained by subculture at 3–4 week intervals. Germination of somatic embryos was accomplished by growing in the light (160 μmol m⁻² s⁻¹; 27°C) on MS medium devoid of growth regulators. Plantlets were first grown on semi-solid agar (6.0 g l⁻¹) medium in test tubes containing half strength MS inorganic nutrients for 7–10 days to promote root proliferation and erect growth. After transfer to soil (MetroMix® 300, Grace Horticultural Products, Cambridge, MA) in plastic tubes (“Conetainers”, Ray Leach Conetainer Nursery, Canby, OR), plantlets were maintained in a growth cabinet illuminated with fluorescent and incandescent light (300 μmol m⁻² s⁻¹; 27°C) for 7–10 days.

**Field experiments**

Individual plantlets in “Conetainers” were transferred to a greenhouse (26±2°C) and were grown under sun light for 30–40 days before planting in the field. An equal number of control plants were grown from single node cuttings of vegetatively propagated clones of Selection No. 3. Care was taken to keep the age of the tissue culture-derived (TC) and vegetatively propagated (V) plants uniform. Both TC and V plants were repeatedly trimmed at 50 mm above soil level to keep the age of the tissue culture-derived (TC) and vegetatively propagated control (V) plants uniform. Both TC and V plants were repeatedly trimmed at 50 mm above soil level to promote uniform growth in the greenhouse. The first planting was made on 13 June 1984 with 224 each of TC and V plants in a randomized, replicated field trial (Field 1; 8 replications; 28 plants per replication). The second planting was on 17 July 1984 with 300 each of TC and V plants (Field 2; 12 replications of 25 plants each).

Morphological characters including height of the plants at flowering, length and width at the broadest point of uppermost expanded leaves and number of tillers were recorded from a minimum of 80 plants of TC and V populations at the end of the first growing season in 1984 and the second growing season in 1985. Stem diameter was measured at the 5th internode from soil level. Biomass yield was recorded from each harvest and expressed in kg dry matter ha⁻¹. Two harvests were made in 1984; 80 days after planting and 75 days after regrowth. In 1985 the first and the second harvests were made 108 and 102 days after regrowth, respectively. Percentage dry matter was determined from samples of biomass after drying at 60°C for 72 h.

Data were subjected to analysis of variance using Statistical Analysis System (SAS Inc., Cary, NC). Duncan’s Multiple Range Test (*P* = 0.05) was used to study the significance of morphological variation among the TC and V populations.

**Cytological studies**

Root tips were collected from a minimum of 40 randomly selected plants in each of TC and V population for chromosome staining according to the procedures of Swedlund and Vasil (1985). All the plants showing one or more variant characters, as compared to the normal TC regenerants, were also included for cytological analyses. At least 3 root tips were examined from each plant and a minimum of 25 cells were counted in each root tip. Meiotic chromosomes were also counted by staining pollen mother cells with aceto-orcein.

**Results**

More than 80% of the inflorescence segments produced embryogenic callus within two weeks of culture initiation. Five hundred and twenty-four plants were regenerated in two batches of 224 and 300 plants after six subcultures. All the regenerated plants survived the transfer from aseptic conditions to soil.

**Morphology of tissue culture regenerated (TC) and vegetatively propagated (V) plants**

Several morphological characters including leaf length, stem diameter, height of plants at flowering and leaf width were measured from 80 randomly selected plants in each of the TC and V populations. V plants were morphologically uniform. TC regenerants, when compared to V plants, exhibited a high degree of morphological uniformity except that they had significantly more tillers (50% in the first field trial; 115% in the second field trial; Tables 1, 3). Among the TC regenerants there were 13 morphological variants in the first field (out of 224 plants) and 10 in the second (out of 300 plants). Two of the variants in the first field were hexaploid. Most of the variants were dwarf with either narrow and erect or broad leaves and were late-flowering (Fig. 1) as compared to normal TC and V plants. The two hexaploid plants were also shorter and one of them had broader leaves and thick internodes compared to the normal TC and V plants (Figs. 2 and 3). The leaves of this hexaploid were longitudinally split near the midrib. A few basal tillers of one regenerant in the first field trial had variegated leaves in the second regrowth in 1985.

Morphological characters recorded from the variants and from randomly selected normal plants of TC origin were plotted on a graph using two parameters at a time (for example: plant height and leaf width or leaf length and stem diameter; Figs. 2 and 3). Variant plants from the first field trial fell into six significantly distinct morphological groups (according to Duncan’s Multiple Range Test, *P* = 0.05; Figs. 2 and 3), and there were three groups from the second field trial (data not shown), irrespective of the parameters.