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In vitro propagation of a *Saccharum officinarum* (L.) and *Sclerostachya fusca* (Roxb.) A. Camus Hybrid

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Summary. Callus induction and plant differentiation were obtained in an intergeneric hybrid of *Saccharum officinarum* and *Sclerostachya fusca.* The sub clones showed morphological variation. Chromosome numerical variation was not observed but structural aberrations were noticed in some sub clones. The study indicates the use of tissue culture technique for inducing intergeneric gene transfer in *Saccharum* hybrids.

Key words: *Saccharum officinarum* – *Sclerostachya fusca* – Callus Sub clones – Dicentric chromosome

Introduction

Several intergeneric hybrids have already been produced between *Saccharum* and such related genera as *Narenga* and *Sclerostachya* (Parthasarathy and Venketraman 1942) *Sorghum* (Thomas and Venketraman 1930) *Imperata, Erianthus* and *Zea* (Janaki Ammal 1941). However, a survey of the parentage of the man-made commercial sugarcane varieties reveals that none of the varieties produced so far have any genes from genera outside *Saccharum.* Many of the genera mentioned above have got highly desirable characteristics, such as waterlogging resistance and profuse tillering in *Sclerostachya,* drought and soil salinity resistance and heavy stooling nature in many species of *Erianthus* and early maturity in the case of *Sorghum.* Sugarcane workers all over the world are now much concerned about the very narrow genetic base of the present day breeding populations and efforts are being made to utilise the hitherto untapped genetic resources available. Tissue cultue techniques may prove useful in overcoming many problems in intergeneric gene transfer.

The occurrence of chromosomal numerical and structural variations are common in plant tissue culture. This phenomenon has been utilised successfully in creating genetic variability for selecting better genotypes in many crop plants, especially in sugarcane (Larkin and Scowcroft 1981). Because of the recovery of a large number of aneuploids, this technique may also have potential in gene transfers through addition and substitution lines (Nakamura et al. 1981).

Material and methods

The material used in the present study is an intergeneric hybrid between *Saccharum officinarum* (2 n = 80) and *Sclerostachya fusca* (2 n = 30). The hybrid has 2 n = 55 chromosomes. In morphology the hybrid is more towards *S. fusca* with a bushy profuse tillering habit, thin stalks and low sucrose. This hybrid flowers very early, at six and a half months, as does the male parent. It is male sterile and produces a few seedlings only very rarely when pollinated with *Saccharum* and *Sclerostachya.*

Developing leaves and leaf sheaths surrounding the apical meristem of shoots, harvested from field grown plants, were used as explants. The bits of materials, excised under sterile conditions, were inoculated in 100 ml conical flasks with 20 ml medium. Murashige and Skoog (1962) basal medium was supplemented with 100 mg/l meso inositol, 2 mg/l 2,4-D, 10% by volume coconut milk and 20 g/l sucrose. The medium was solidified with 0.8% Difco Bacto agar with a pH of 5.8. The inoculated flasks were incubated in very low intensity light at 26 ° ± 1 °C. The callus was subcultured once or twice.

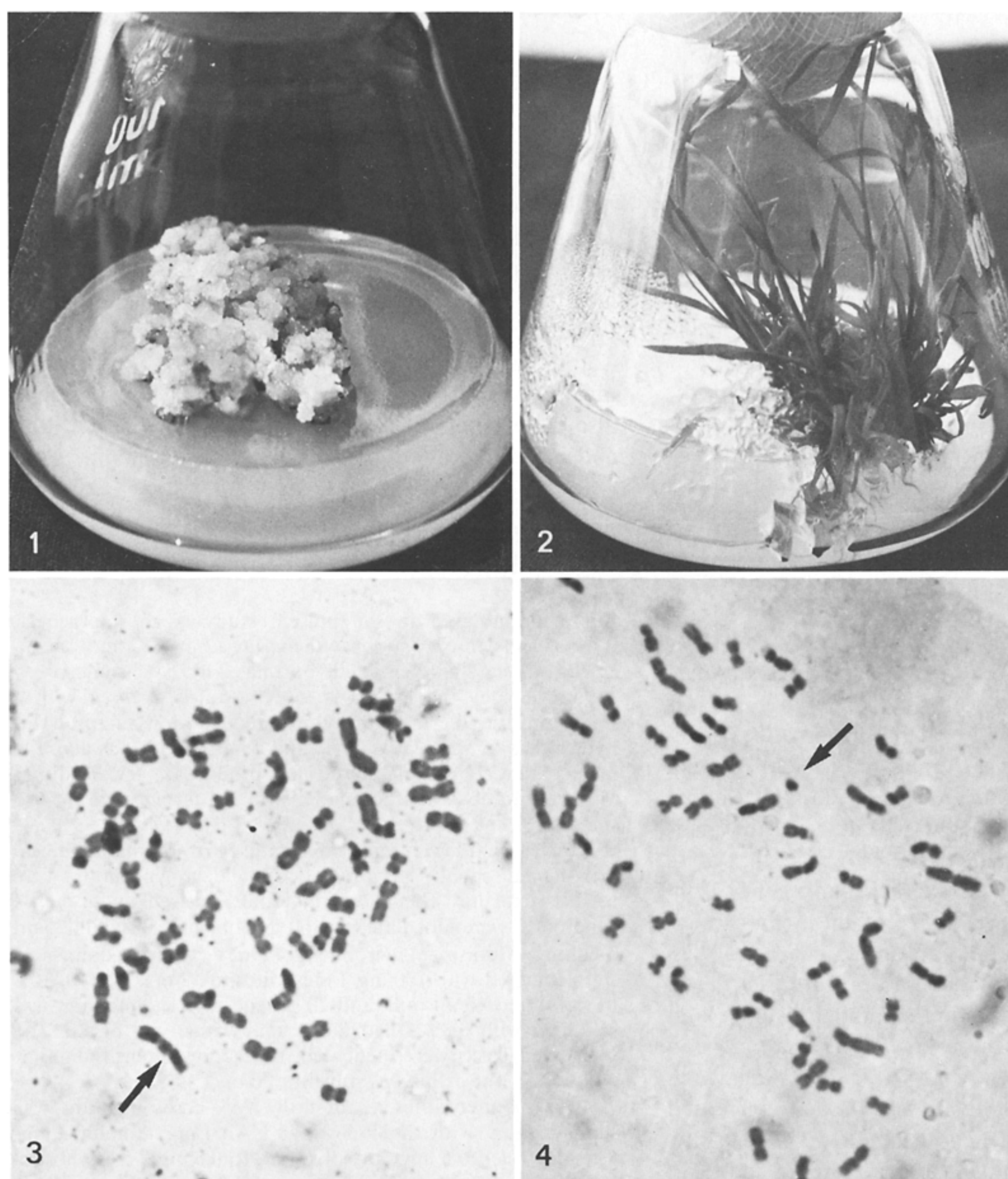
For plantlet differentiation the MS basal medium was supplemented with the following: NAA 1 mg; kinetin 2 mg; thiamine Hcl 0.5 mg; pyridoxine Hcl 0.5 mg; mesoinositol 100 mg/l.

Cytological studies were carried out on root tip cells of plants established in pots, utilizing the standard procedures.

Results

Callus initiation was noticed after 10–15 days. The production of callus was not uniform from all the explants. The growth of the callus was rapid and within 30 days after initiation the callus was ready for subculture (Fig. 1). Initially the callus was pale yellow but certain patches remained white. The callus showed variation in morphology, some of the callus was rough and friable whereas others were smooth and shiny. Callus could be maintained for a long time, but in some segments differentiation of root-like structures occurred even in callussing medium.

A differentiation of shoots was observed eight to ten days after the transfer of the callus to the differentiating medium. Initially the callus showed numerous pink spots, later these spots turned into light green areas. Within 20–25 days numerous small leaved shoots emerged and the whole callus surface was greenish in colour. Growth of these shoots was very quick and soon several shoots emerged from each callus (Fig. 2). Simultaneous callus proliferation was also observed for a time in all the flasks. Shoots differentiated from these newly formed callusses but their fast growth required the removal of larger plantlets from the callus. Root primordia were visible about the time when plantlets



Figs. 1 Callus obtained from expanding leaf bits; **2** Plant differentiation from callus; **3** Chromosomes from root tip cells of a subclone with one dicentric chromosome; **4** Root tip cell chromosomes of the same subclone with an acentric fragment

produced five to six leaves and could be found developing from the base of the plantlets. When plantlets with small roots were transferred to White's basal medium, root development was very fast and a well developed root system could be obtained. The leaves were trimmed either two or three times, which made the plants sufficiently thick and hardy at the time of transfer to mildly sterilised soil. Fifty plants were transferred to the soil but only 32 plants ultimately survived. Further maintenance of the plants was done through stem cuttings.

The general morphology of the sub clones obtained through culture was similar to that of the parent. Variation was noticed with respect to tillering, stalk diameter, leaf angle and erectness and stem epidermal pattern. In ten plants where sufficient stalks were available for sucrose analysis, sucrose percent in the sap ranged from 5.88 to 10.39. Brix percent ranged from 9.34 to 12.94. The parent had a brix value of 10.68% and sucrose 5.39%.

Cytological studies were done for 20 plants at random. Every plant showed $2n=55$ chromosomes in their root tip cells. One plant was exceptional with one dicentric chromosome which could be identified in 60% of the cells studied (Fig. 3). In 10% of the cells an acentric fragment was also noticed (Fig. 4). Looking at the size of the chromosome it is suspected that one of the *Sclerostachya* chromosomes is involved in this translocation as most of the larger chromosomes are those of *Sclerostachya*.

Discussion

As in the case of commercial sugarcane varieties, callus induction and differentiation of plants could be induced in *Saccharum* × *Sclerostachya* intergeneric hybrids from leaf explants without any modification of the medium. In sugarcane tissue culture, obtaining a good root system is slightly difficult (Heinz et al. 1977). A profuse root system generally develops only when plantlets are transferred to White's basal medium (Sreenivasan and Jalaja 1982). In this hybrid, rooting was very profuse even in the differentiating medium. This probably reflects the inherent capacity of *Sclerostachya* to produce an extensive fibrous root system under 0.75 to 1.0 m stagnant water in their natural habitat, making the species highly resistant to waterlogging.

Interspecific and intergeneric hybrids of *Saccharum* are characterised by autosyndetic pairing of chromosomes at meiosis. Hence, genetic recombination through meiotic chromosome exchange is practically limited. Price (1965) indicated that even the evolution of modern commercial sugarcane varieties must have been through segregation and recombination of whole chromosomes only. To achieve segmental interchanges, the use of mutagenic agents has been suggested.

Variation among subclones due to gross changes in chromosome numbers such as aneuploidy or polyploidy have been reported in many plants, including sugarcane cultivars (Murashige and Nakano 1967; Kao et al. 1970; Bayliss 1973, 1980; Skirvin 1978; Roy 1980; Larkin and Scowcroft 1981; Heinz et al. 1969, 1977; Krishnamurthy and Tlaskal 1974; Liu and Chen 1976; Liu et al. 1977). It is clear that the variation noticed in the present study is not due to chromosome numerical variation. It is quite probable that cryptic chromosome rearrangements plays an important role in bringing out genetic variation in cultured cells and differentiated plants (Foroughi-Wehr et al. 1979; Orton 1980; Ahloowalia 1976, 1978). The isolation of one sub clone with a dicentric chromosome from a small population indicates that the variation noticed between sub clones may be due to chromosome structural alterations. The observation is important in that it will be now possible to induce segmental exchange between *Saccharum* and *Sclerostachya* chromosomes which otherwise is possible only by reducing the chromosome number of one of the genus to its basic set (Parthasarathy 1948) through repeated backcrosses. The variation noticed in economically important characters such as tillering and sucrose percent will also facilitate selection and utilisation of the best genotypes for further hybridisation. Incorporation of such genotypes into the breeding programme may help the sugarcane breeder in achieving intergeneric gene transfer.

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