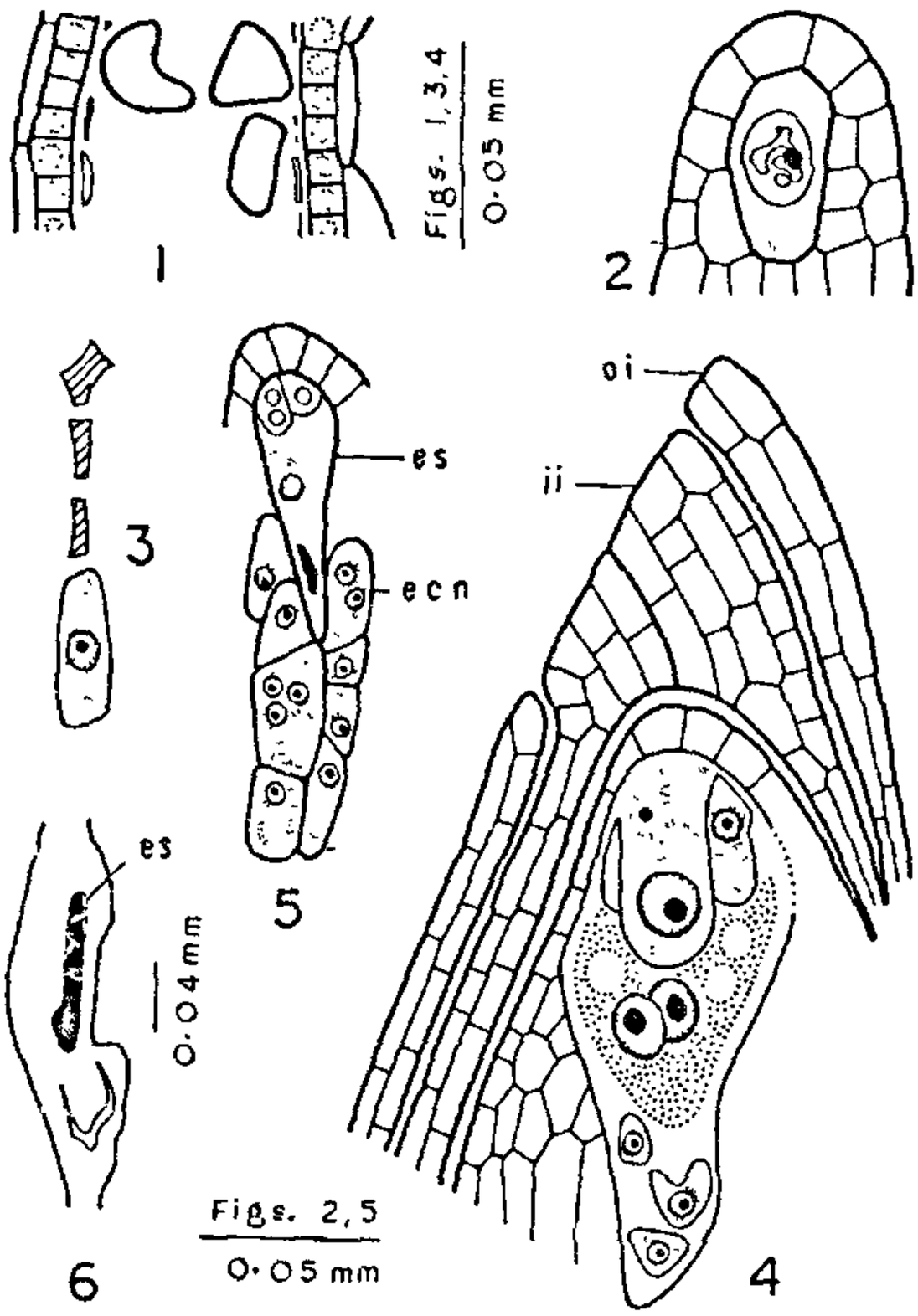

Figures 1–6. Sterility in *Phragmites communis* (Retz.) Trin. **1.** L.s. of an anther lobe. **2–4.** Development of the ovule and embryo sac. **5.** Enlarged nucellar cells surrounding the degenerating embryo sac. **6.** L.s. of ovary showing degenerating ovule and embryo sac. (ecn, enlarged cells of the nucellus; es, embryo sac; ii, inner integument; oi, outer integument)

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REDUCTION IN COST OF TISSUE CULTURE OF *LEUCAENA LEUCOCEPHALA* (LAM) DE WIT BY REPLACING AR GRADE SUCROSE BY SUGAR CUBES

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TISSUE culture techniques are being increasingly applied in industry and agriculture. A major consideration in any commercial operation is the cost of the product. In the developed countries the main factor that contributes to the fixation of price of a tissue culture product is the cost of labour[1]. However, in India equipment, glassware and importantly the media constituents escalate the cost of tissue culture operations. Sucrose is a necessary ingredient of all plant tissue culture media and is used in the range of 2–4%[2] but sometimes at much higher levels[3,4]. Whereas for critical analytical studies on growth and development a highly purified sucrose is needed, for large scale micropropagation commercial grades of sucrose should be adequate as energy sources.

AR grade surcrose which now costs Rs 150 per kg is used in most laboratories in India. To minimize the cost of nutrient media we have tried two inexpensive sources of sucrose and have compared them with AR grade sucrose (BDH) for *in vitro* shoot multiplication and embryo culture of *Leucaena leucocephala* cv K-8. The two experimental systems are described below.

(1) *In vitro shoot multiplication*: The cultures were initiated from single node and terminal cuttings (1 cm long cuttings consisting of shoot-tip and one node) derived from aseptically raised 14-day old seedlings. On MS[5] medium (with 4% BDH sucrose) supplemented with 6-Benzylaminopurine (BAP; 3×10^{-6} M) the explants developed an unbranched shoot which after three weeks provided 4 nodal and terminal cuttings (propagules) for further shoot multiplication. Comparative effects of sucrose from different sources were studied in the third passage of shoot multiplication, and the growth parameters considered were: (a) per cent cultures showing bud break, (b) shoot length, and (c) rate of shoot multiplication, which refers to the number of culturable cuttings obtained after three weeks growth.

(2) *Embryo Culture*: Seedling development in the cultured decotylated embryos on MS medium was studied. Sucrose samples were tested at 3% level.

Growth was assessed on the basis of several seedling characteristics (table 2).

For raising aseptic cultures seeds were scarified in concentrated HCl for 30 min, washed in running tap water for 1 hr followed by surface sterilization in 1% sodium hypochlorite solution for 15 min. These seeds were transferred to sterile distilled water at 80°C. After soaking for 48 hr under laboratory conditions, they were sterilized once more in 1% sodium hypochlorite solution for 15 min and planted on culture medium. For embryo culture, the seed coat and cotyledons were removed under aseptic conditions and the embryo axis was used.

The composition of the medium was after Murashige and Skoog[5]. It was gelled with 0.8% 'Centron' agar (Centron Research Laboratories, Bombay). All ingredients of the medium, including sucrose, were dissolved in glass distilled water and the pH of the medium adjusted to 5.8 before autoclaving at 121°C for 15 min. Besides AR grade sucrose, sugar cubes manufactured by Daurala Sugar Works, Daurala (Distt. Meerut, U.P.) and a market sample of sugar (henceforth referred to as commercial sugar) were tried. Cultures were stored in continuous diffuse light at 30°C.

Quantitative data on the response of the two systems to various sucrose samples are given in tables 1 and 2. In both the systems responses were poor when commercial sugar was used. It was especially inhibitory for epicotyl growth, lateral root formation and fresh weight of seedlings in embryo culture (table 2). However, sugar cubes provided a satisfactory source of sucrose for shoot multiplication (table 1) as well as embryo culture (table 2); for most growth parameters it compared well with AR grade sucrose (tables 1 and 2).

Since the cost of AR grade sucrose is over 10 times higher than that of sugar cubes, we recommend the use of the latter source of sucrose for tissue culture of *L. leucocephala* and suggest that its suitability be tested for tissue cultures of other plants under investigation in various Indian laboratories. For one litre of medium with 3% sucrose, change over from AR grade sucrose to sugar cubes would cut down the cost of this ingredient from Rs 4.35 to Rs 0.40 resulting in substantial reduction in the cost of media for micropropagation and other applications. In situations where sucrose is used at higher concentrations (6–10%)[3,4] the use of sugar cubes would be even more economical.

Table 1 *Effect of different sources of sucrose on shoot multiplication in the cultures of nodal segments of Leucaena leucocephala*. Culture medium. MS + BAP ($3 \times 10^{-6} M$); Growth period 3 weeks*

Source of sucrose	Shoot length (cm)	Number of propagules**
AR grade (BDH)	4.53 ±0.23	4.20 ±0.09
Sugar cubes (Daurala)	4.36 ±0.34	4.20 ±0.17
Commercial sugar	3.82 ±0.79	3.41 ±0.14

Percentage cultures showing bud break-100.
* All values are average of 24 cultures.
± = standard error.
** Refers to number of culturable nodal/terminal cuttings.

Sugar cubes are manufactured out of refined sugar and their quality conforms to British Pharmacopoeia and I.S.I. specifications. According to the analysis supplied by Daurala Sugar Works, the sugar cubes contain 99.80% sucrose, 0.05% moisture, 0.03% reducing sugars, 0.02% ash sulphated and < 25 ppm sulphur dioxide. In comparison, the AR grade sucrose (BDH) contains 99.92% sucrose, 0.05% moisture, 0.01% reducing sugars, 0.005% ash sulphated and 0.0078% other impurities.

The shoots multiplied in medium containing sugar cubes readily rooted *in vitro* and the plants were as

Table 2 *Effect of different sources of sucrose on seedlings developing from cultured decotylated embryos of Leucaena leucocephala*. Culture medium. MS; Growth period: 3 weeks*

Source of sucrose	Length of hypocotyl (cm)	Length of epicotyl (cm)	Length of main root (cm)	Number of lateral roots	Fresh weight of the seedling (mg)
AR grade (BDH)	1.28 ±0.04	2.57 ±0.18	13.36 ±0.28	6.70 ±0.6	71.81 ±1.69
Sugar cubes (Daurala)	1.01 ±0.02	2.76 ±0.28	14.41 ±0.21	6.45 ±0.45	71.51 ±2.03
Commercial sugar	1.04 ±0.03	1.73 ±0.13	14.83 ±0.21	4.40 ±0.76	51.08 ±1.62

* All values are average of 24 cultures. ± = standard error.

vigorous as those raised on medium with AR grade sucrose. Attempts to transplant them are in progress.

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7 August 1984

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POLYPLOIDY IN *ROTTBOELLIA EXALTATA* LINN. COMPLEX

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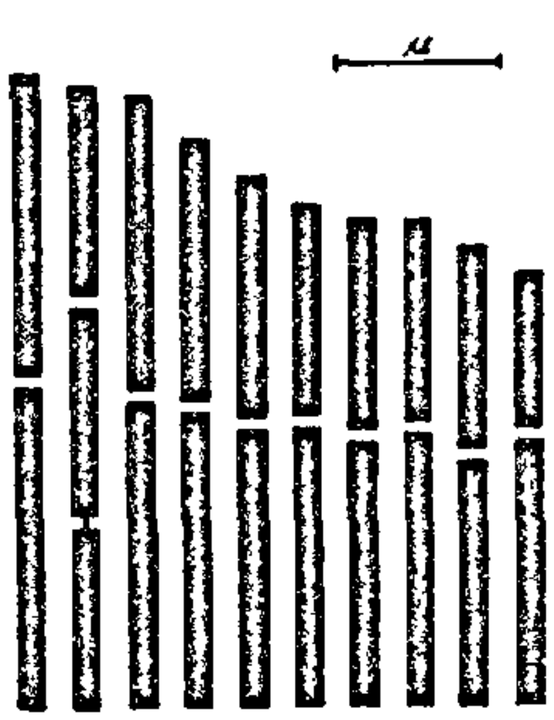
THE genus *Rottboellia* consists of about 30 species which are annual or perennial grasses, distributed in the tropical and sub-tropical regions of the world, of which only one species, *R. exaltata* Linn. occurs commonly in India[1,2]. The vegetative characters such as plant height and length and breadth of leaf are highly variable in this species, and all taxonomists have recognised two varieties, a "short" variety (1–3 ft) and a "tall robust" one (1–12 ft). Several chromosome numbers are reported[3] for this species such as $2n = 20$, 36, 40 and 60. It is not known whether these reports pertain specifically to the "short" morphotype or "tall" morphotype. In the present study detailed karyomorphological studies of both the varieties have been made to find out if there is any chromosomal difference between the two morphotypes.

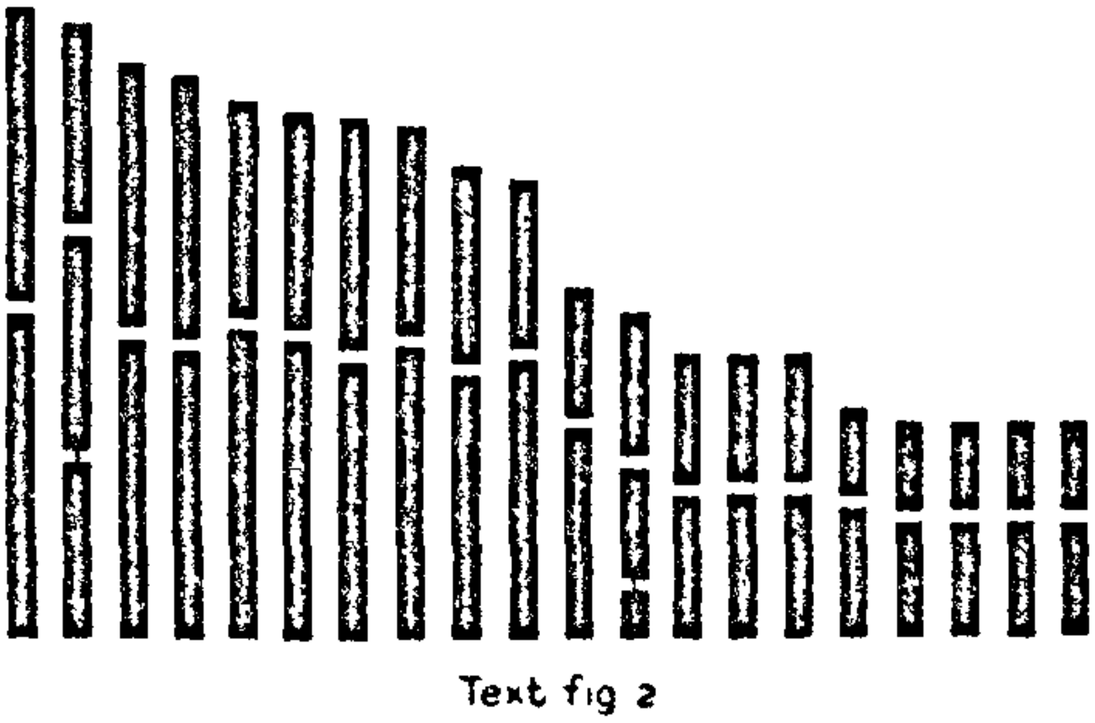
Materials of the species were collected from different localities of Kerala State such as Trivandrum, Kallikkad, Quilon, Kottayam, etc. Chromosome studies were made from PMCs and root tip cells fixed in 1:3 acetic alcohol and stained in aceto-carmine.

The "short" variety showed $2n = 20$ chromosomes which ranged in length from 2.66–3.91 μ. The karyotype analysis[4] showed 9 pairs of m-type and one pair of sm-type of chromosomes and it comes under 1A category[5]. The second pair possesses secondary constriction in the long arm (figure 1). The "tall" variety however showed $2n = 40$ chromosomes ranging from 1.33–3.97 μ in length. The karyotype consists of 19 pairs of m-type and one pair of sm-type and belongs to the category 2B. The second and the 12th pair possess secondary constrictions in the long arm (figure 2).

Ten chromosomes (1–10) of the "tall" variety are found to be almost similar in size and morphology to the 10 chromosomes of the "short" variety, and the remaining 10 chromosomes (11–20) of the "tall" variety are distinctly smaller and their counterparts are not found in the short variety. The tall variety showed 10 large and 10 small bivalents at diakinesis,



Text fig.1


Text fig 2

Figures 1, 2. 1. Idiogram of *Rottboellia exaltata* ($2n = 20$). **2.** Idiogram of *R. exaltata* ($2n = 40$).