

TISSUE CULTURE IN CASHEW: POTENTIAL, PRIORITIES AND APPLICATIONS

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Abstract

Although conventional methods of vegetative propagation of cashew are available, such as layering, patch-budding, side-grafting, veneer grafting and epicotyl grafting, which give varying degrees of success, these are not adequate to meet the large quantum of 8-10 million planting materials that are required for gap-filling, replanting and new planting in the country. Hence, there is an urgent need for supplementing these methods through tissue and meristem culture for: (a) generating more scion material for use in grafting; (b) obtaining rooted plants directly from elite trees; (c) developing an *in vitro* system for screening germplasm, hybrids, and other induced variability for resistance/tolerance to diseases and pests, and (d) exploring possibility of inducing mutations of somaclonal variations for higher productivity and quality, as well as resistance/tolerance to diseases, pests and stress environments. The potential applications of tissue culture in cashew improvement and some preliminary results obtained are discussed in this paper.

INTRODUCTION

CASHEW (*Anacardium occidentale* L.), a member of the mango family Anacardiaceae, and native of tropical America, somewhere in S.E. Brazil, has now become naturalized in India since its introduction by the Portuguese during the 16th century, as well as in some African countries like Tanzania, Kenya and Mozambique. It was in India where the scientific processing and export of cashew were started in early 1900's. There has been a steady increase in area from 110,000 ha in 1955-56 to 481,000 ha in 1981-82, and the production from 79,000 tonnes to 196,000 tonnes during the same period (Das, 1985).

However, India is facing a deficit of 95,000 tonnes of raw nuts per annum, which was being met until recently by imports from African countries. This import has steadily dwindled after 1970's when these countries started their own mechanized processing of raw nuts, resulting in decline of our exports. Hence, the urgency is not only to increase area but also the production and productivity of cashew. This calls for the rapid planting of new areas as well as replanting senile, unproductive trees with high-yielding selections and hybrids.

Although cashew is both seed-propagated as well as vegetatively multiplied through veneer grafting, budding, air-layering (Madhava Rao, 1958) and epicotyl grafting (Nagabhushanam, 1982, 1984), the rate of multiplication and field establishment possible are by no means commensurate with the demand for superior planting materials. Being a highly cross-pollinated crop, seed-propagation gives a highly variable progeny resulting in high instability in yields. Hence the urgent need has been felt to standardize more rapid and prolific rate of vegetative multiplication, using modern techniques of cell, tissue and organ culture, in order to generate adequate clonal planting materials of high-yielding hybrids and selections. Thus, tissue culture techniques are expected to supplement the conventional clonal propagation methods in cashew in order to meet the ever-increasing demand for superior planting materials.

Plant tissue culture is now a well-established technology which has been commercialized in several crops, both in dicots and some monocots in several countries (Murashige, 1974). Although success with tree crops and woody perennials has been comparatively limited, this technique has been employed for rapid propagation of certain gymnospermous trees like pines, fir, and hemlock, and angiospermous trees like teak, eucalyptus, poplar, apple oil-palm and so on. More recently even coconut clonal plantlets have been produced through tender leaf cultures (Raju *et al.*, 1984). Attempts on cashew and its fellow species mango have been rather limited mainly because of the problem of polyphenols in the vegetative tissue which rapidly arrest the growth of the explants *in vitro*. Some of the attempts on cashew tissue culture, the problems thereof and the potentialities for future are discussed in this paper.

MATERIALS AND METHODS

For standardizing an effective clonal multiplication method for cashew, the obvious choice of material is the meristematic bud present in the leaf axils. These meristem cultures may either be induced to give multiple shoots directly, or to first induce a callus from which shoots or somatic embryoids could be generated subsequently. For obtaining the meristem explant, the nodal segments were first surface-sterilized in 0.05-0.1% mercuric chloride solution for 10-15 minutes, and rinsed thrice in sterilized distilled water. The meristems were then dissected out in an aseptic chamber and transferred to a modified Murashige and Skoog's (1962) medium containing 2% sucrose, auxins like α -naphthalene acetic acid (NAA, 0.5-2 mg/litre), or 2,4-dichlorophenoxyacetic acid (2,4-D, 0.05-2 mg/litre) if a callus type of development is desired, and cytokinin, such as benzylaminopurine (BAP 1-2 mg/litre) or kinetin. The medium was gelled with 0.8% agar, after adjusting the pH to 5.8-6.0, dispensed into culture tubes (Corning 25×150 mm) 15-20 ml in each, and autoclaved at

121°C and a pressure of 1.1 kg/cm² for 15 minutes. For calli, 125 ml Erlenmeyer flasks were used with 30-50 ml medium in each. To prevent browning of the explants, they were dissected in 0.1% cysteine hydrochloride or in a 1:1 mixture of 0.1% citric acid + 0.15% ascorbic acid solution, and/or 100 mg/litre of ascorbic acid was incorporated in the medium. Liquid media used with filter-paper supports also helped in reducing the browning, if frequent transfers are made to fresh media.

Another favourite explant used in cashew is the cotyledon and hypocotyl segments of the kernel (Philip and Unni, 1979, 1984; Philip, 1984). Here, the kernel was surface-sterilized in 0.05% mercuric chloride, and the hypocotyl and cotyledonary segments of 3-5 mm were inoculated in a modified MS medium of Lin and Staba (1961) supplemented with 0.5 mg/litre each of IAA and kinetin, at a pH of 6.2. The cultures were incubated at 26° ± 1°C and given 16 hour photoperiod of 1000 lux illumination from white fluorescent tubes. Philip and Unni (1984) reported favourable effect of initial dark incubation of cultures for 24 hours, followed by continuous illumination of 7000-8000 lux intensity with a temperature of 24° ± 2°C. The plantlets obtained in culture were initially transferred to liquid media containing lower auxin levels, with filter-paper supports, and when the roots were hardened sufficiently and became vigorous they could be planted in pots containing 1 : 1 mixture of sterilized sand and vermiculite. After achieving vigorous growth and when new leaves started emerging, the potted plants were taken out of the growth room or mist-chamber and planted in the field. To maintain high humidity, if mist-chamber is not possible, the plants in pots were covered with a polythene hood, leaving some space below for aeration, and watering the pots daily to prevent drying. Once the plants have recovered from the transplantation shock, the hood can be removed to acclimatize the plants to normal humidity for 2-3 weeks prior to final planting in field.

RESULTS AND DISCUSSION

Meristem Culture

Both apical meristems measuring 3-5 mm and axillary buds in nodal segments excised from adult trees were tried earlier in this laboratory (Kuruvinashetti and Iyer, unpublished). The shoot showed considerable elongation but the cut-ends developed a compact, white or creamish callus which could be separated and grown. Dark incubation was favourable both for the induction and rapid multiplication of this callus, and medium containing 2,4-D (2 mg/litre) was most effective. However, for rooting of the shoots, NAA at 1 mg/litre or lower levels was more suitable, although rooting was very sparse. In a subsequent attempt, nodal segments containing axillary buds gave rise to multiple shoots but no rooting (Raju *et al.*, unpublished).

Browning of explants could be reduced considerably by the use of cysteine hydrochloride, or a mixture of citric and ascorbic acid solutions while dissecting out the explant, and also incorporating 100 mg/litre ascorbic acid in the medium. Use of activated charcoal (1 g/litre) also helped in preventing browning of explants to a considerable extent.

Cotyledon and Hypocotyl Explants

The first report on the use of embryonal tissue of cashew to produce clonal plantlets was by Philip and Unni (1979), who traced their origin to single cells in epidermal layer without callus formation. The authors had used fragments of hypocotyl and cotyledons measuring 3-5 mm, which were cultured on MS medium as modified by Lin and Staba (1961) supplemented with IAA and kinetin (0.5 mg/litre of each) at a pH of 6.2. The cultures after an initial dark incubation for 24 hours were transferred to 7000-8000 lux illumination at a temperature of $24^{\circ} \pm 2^{\circ}\text{C}$. After 6 weeks, the hypocotyl explant produced a shoot bud but no roots in 5% of the cultures, whereas the cotyledonary explants produced a number of shoot buds earlier than roots in 20% cultures, and roots earlier than shoot buds in 80% of the cultures (Philip and Unni, 1984). Explants from basal portion of cotyledons responded faster and produced healthy plantlets, and there was no sign of callus formation in any. The shoot buds arose superficially without any vascular connection with mother tissue, and the root vasculature was discontinuous with shoot, thus indicating their adventitious origin. These plantlets can, therefore, be separated and transferred to soil without injury.

More recently, Philip (1984) reported formation of complete plantlets with a shoot-bearing leaves, and a vigorous root system in 90% of the cotyledonary explants cultured for 5 weeks on Lin and Staba's (1961) medium containing 0.5 mg/litre each of IAA and kinetin. Histological studies showed direct organogenesis originating from single epidermal cells on the adaxial side of cotyledonary explants. These embryogenic cells can be recognized by their larger size and densely stained RNA in cytoplasm and nucleoli with pyronin-B, and also by the accumulation of starch grains in and around these plantlet-forming cells. In about 10 days, the shoot apex with a whorl of leaf-primordia is organized, and, after formation of the first pair of leaves, the root apex gets organized on the abaxial side of the mother tissue. The formation of complete plants from single epidermal cells without the intervention of a callus phase is a good sign, since it avoids the problem of diplontic selection and chimera formation (Broertjes *et al.*, 1968), respectively, in a highly heterozygous tree crop like cashew. Further, since shoots and roots developed simultaneously from single epidermal cells in the same medium, the author felt that the system was an improved method for production and screening of mutants.

The only other report so far of somatic embryogenesis in another member

of the Anacardiaceae, namely, mango (*Mangifera indica* L.) is by Litz *et al.* (1982), on ovule cultures of 5 polyembryonic cultivars, on a modified MS medium comprising half strength major salts, 6% sucrose, 400 mg/litre glutamine, 100 mg/litre ascorbic acid, supplemented with filter-sterilized coconut water (20% v/v) or 1-2 mg/litre benzyladenine. The nucellar tissue was excised after 1-3 weeks of culture and transferred to fresh media of same composition. Proliferating cultures were transferred to liquid MS medium containing 20% CW in 125-ml Erlenmeyer flasks kept at 100 rpm in a rotary shaker at 25°C with 16-hr photoperiod (1000 lux). They obtained most efficient somatic embryogenesis in liquid media containing CW in 5 out of 9 naturally polyembryonic cultivars or seedling lines tried.

Among other woody dicot trees tried, growth and proliferation of axillary buds and shoot tips were obtained in apple (Abbott and Whiteley, 1976; Zimmerman, 1984), papaya (Litz, 1984), nucellar cultures of *Citrus* (Rangan *et al.*, 1969; Kochba *et al.*, 1972), and ovule cultures of grape (Mullins and Srinivasan, 1976; Srinivasan and Mullins, 1980; Krul and Mowbray, 1984).

Potential Applications in Cashew

The national average yield of cashew is only 2.1 kg/tree/year, but we have some high-yielding selections like 'Vengurla-2' giving an average yield of 43 kg from a tree in a regularly spaced 20-year-old plantation, certain exceptional yielders like a 45-year-old tree at Bapatla giving 90 kg raw nuts/year, and a record yield of 125 kg from a single tree at Kottarakkara in Kerala (Nair *et al.*, 1979). Such high-yielding elite trees would form ideal starting material for the tissue culturist for rapidly multiplying them, thereby conserving these rare genotypes for future exploitation in breeding programmes. The highly cross-pollinated nature of cashew, combined with the scope it offers for clonal propagation using both conventional as well as modern methods of tissue culture, makes it an ideal system for reaping full benefit from additive gene action and heterosis (Nambiar, 1977). There is ample genetic potential for breaking the yield barrier as evident from the work done under the All-India Co-ordinated Cashew Improvement Project of ICAR (Nambiar, 1985).

Another interesting aspect is the possibility of producing haploids and homozygous diploids through anther/pollen culture, which needs to be exploited in cashew, for getting inbred lines for heterosis breeding and genetic analysis (Bajaj, 1984).

A third important area where tissue culture can be used in cashew is the *in vitro* screening of germplasm and hybrids for resistance/tolerance to pests, diseases and environmental stresses such as salinity and drought. The immediate problem is to locate resistance/tolerance to tea-mosquito which is a major pest in cashew. A callus system or a meristem culture system needs to be developed for screening against toxins released by the pest, since field level

screening is not feasible in such perennial tree crops (Evans *et al.*, 1983).

Thus, it is evident that tissue culture holds tremendous possibilities not only as a potent tool for rapid clonal propagation to supplement conventional methods but also for generating additional variability through mutations, haploids and their detection through *in vitro* screening procedures.

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DISCUSSION

S. Nagabhushanam : With regard to the success of soft-wood grafting in cashew tried at the CPCRI, clarification is given that soft-wood grafting in containers as well as under *in situ* situations was successful.

V. N. Madhava Rao : Both cashewnut and coconut should be considered together. The delay in obtaining results in coconut can be offset by the success in cashewnut. If there is one crop that requires priority, it is cashewnut in regard to tissue culture.

R.D. Iyer : We have taken up tissue culture work on cashew also to supplement conventional methods of clonal multiplication.

A.R. Mehta : In the light of the problems in cashew posed by Dr M.C. Nambiar, I have two suggestions to make: (1) Has any root stock been found to impart resistance to pathogens or dwarfism, or to raise more female flowers, etc.? (2) The Baroda Tissue Culture Conference in 1978 had emphasized tissue culture work on cashew. I suggest that those who have already done some work should describe their results, even if they be negative.

R.D. Iyer : Our preliminary efforts have given callus formation from the cut-end (lower one only) in nodal explants, and axillarybuds have elongated to give shoots.