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# Somatic embryogenesis and plantlet formation in *Santalum album* and *S. spicatum*

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## Abstract

**A reproducible system for somatic embryogenesis and plantlet formation of sandalwood has been developed. A high frequency (100%) of somatic embryos were induced directly from various explants in MS (Murashige and Skoog, 1962) medium with thidiazuron (1 or 2 $\mu$M) or indirectly in medium containing 2,4-D plus thidiazuron. Within 8 weeks, white globular somatic embryos or friable embryogenic tissue developed on cultured explants. In *S. album* the globular somatic embryos were transferred to MS medium supplemented with IAA (6 $\mu$M) and kinetin (1 $\mu$M) where they developed further, multiplied and maintained friable embryogenic tissue. After 15–30 d, mature somatic embryos (1–2 mm) with well-developed cotyledons were separated and subcultured on to medium containing $GA_3$ (6 $\mu$M) for germination. Once germinated, elongated somatic embryos (10–20 mm long) grew further in MS supplemented with lower $GA_3$ (3 $\mu$M). In *S. spicatum*, the addition of casein hydrolysate and coconut milk was necessary for plantlet development from somatic embryos. From histological studies, it appeared that primary somatic embryos arose from single cells or had a multicellular origin from the epidermis or cortical parenchyma. Secondary somatic embryos and friable embryogenic tissue differentiated from groups of proembryogenic cells from a superficial layer of the primary somatic embryos.**

Key words: *Santalum album*, *Santalum spicatum*, somatic embryogenesis, histological studies.

## Introduction

Western Australian sandalwood (*S. spicatum* R.Br A.DC.) and Indian sandalwood (*S. album* L.) are commercially important species because they produce essential oil in the heartwood which is used extensively in the incense and perfumery industry (Loneragan, 1990; Rao and Bapat, 1995). *Santalum spicatum* natural stands are depleted since the rate of regeneration cannot keep pace with the rate of harvesting. Sandalwood harvesting, which usually involves removing the entire tree, may have resulted in a critical loss of genetic diversity and valuable agronomic characters. Techniques for vegetative propagation of WA sandalwood are not yet available.

In addition to conserving and regenerating the indigenous species, Indian sandalwood, which contains a higher oil content, is being evaluated as a potential plantation species in the Ord river region of Western Australia (Applegate and McKinnell, 1993). However, *S. album* is susceptible to many pests, such as fungi, nematodes (Rai, 1990), and spike diseases caused by micoplasma (Parathasarathy and Venkatesan, 1982).

Conventional breeding of sandalwood for introgression of new genetic information can be an expensive and difficult task because of their long generation time, sexual incompatibility and heterozygous nature (Rugkhla, 1997). *In vitro* regeneration techniques can be used to clone superior lines, and are needed for *Agrobacterium*-mediated gene transfer techniques and for protoplast fusion. Regeneration via somatic embryogenesis of *S. album* can be achieved from hypocotyl, nodal and endosperm explants (Bapat and Rao, 1979; Lakshmi Sita *et al*., 1979; Rao and Bapat, 1992).

Preliminary results on somatic embryogenesis of *Santalum album* obtained following the published methods, were unreliable, particularly for the two steps of inducing embryogenic callus using 2,4-D and subculturing into $GA_3$ ( Lakshmi Sita *et al*., 1979) or IAA and BAP (Rao and Bapat, 1992). A low frequency (0.5–1%) of embryogenic callus occurred from culturing nodal

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explants for at least a year. In preliminary tests with combinations of CPPU (2 or 4 $\mu$M) and 2,4-D (2.5 $\mu$M) on the induction of sandalwood somatic embryo, it took 8–10 months to obtain somatic embryos and the frequency was low (5–10%). These results could not be reproduced easily.

Confirmation of somatic embryogenesis is based on histological evidence that the structure is bipolar and has no vascular connection to the explant (Haccius, 1978). The most suitable regeneration systems for transformation are direct or repetitive production of somatic embryos or *de novo* shoot organogenesis, which originate from single cells of the epidermal layer (Marcotrigiano, 1986; Raemakers *et al*., 1995).

In this study, a new method for more efficient and reliable induction of somatic embryos and recovery of plants via somatic embryos of *S. album* and *S. spicatum* is described. The histology of the origin of the somatic embryo was also studied.

## Materials and methods

### *Explant source*

Nodal and seed explants were taken from superior trees grown in the field trial area of Curtin University, Perth, Western Australia. Nodal segments were taken from new sprouting branches of mature trees of *S. album* and *S. spicatum*. Physiologically mature seed was harvested from a tree when the fruit exocarp was changing from a green to a reddish colour. The pericarp of *S. album* seeds were removed under aseptic conditions.

Single nodal segments and seed without pericarp were soaked in commercial soap solution (1%, v/v) for 5–10 min and surface-sterilized in 1% sodium hypochlorite for 15 min. The explants were rinsed in sterile distilled water 3–5 times and placed individually in 20 ml of MS (Murashige and Skoog, 1962) basal medium (see below). To determine if the explants from tissue culture would respond similarly to explants from the field, leaves and nodal segments were taken from axillary shoots multiplied in basal MS media (Murashige and Skoog, 1962) with MS vitamins supplemented with 5 $\mu$M BAP (Rao and Bapat, 1992) and alternately subcultured into the same basal medium supplemented with 2 $\mu$M $GA_3$ to increase the leaf size. The leaves were cut in half before use as explants. All experiments were repeated at least twice.

### *Somatic embryo induction*

All basal media contained MS salts and vitamins, 20 g $l^{-1}$ sucrose and 2 g $l^{-1}$ gelrite (Phytagel, Sigma). The pH was adjusted to 5.8 before autoclaving at 121 °C, 15 psi for 15 min. The effects of thidiazuron (TDZ) on somatic embryo induction was tested using nodal segments from mature trees (genotype 614) and seed. Media containing 2.5 $\mu$M 2,4-D and 2.5 $\mu$M kinetin (Lakshmi Sita *et al*., 1979) was also included. The effect of 2,4-D and TDZ, or TDZ alone, on somatic embryo induction in leaf explants from *in vitro* culture was examined. Levels of TDZ were varied from 0, 1, 2, and 4 $\mu$M which are reported to be suitable for many other woody species (Bates *et al*., 1992; Huetteman and Preece, 1993). Cultures were maintained at 25 $\pm$ 2 °C under filtered light (2 $\mu$mol $m^{-2}$ $s^{-1}$) during somatic embryo induction and maturity then transferred to a 16 h photoperiod of 50 $\mu$mol $m^{-2}$ $s^{-1}$ during plantlet development. Subcultures were routinely performed at 6 week intervals during somatic embryo induction and 3 week intervals during maintenance of embryogenic tissue. Morphological development of somatic embryo induction was observed under a binocular microscope each week. Somatic embryos were classified as direct or green somatic embryos, secondary or white somatic embryos and friable embryogenic tissue based on their morphology.

The amino acids, proline or arginine, and the polyamine, putrescine, were included in embryo induction medium or multiplication medium to test their effect on the production of spontaneous white friable embryogenic tissue or on the maintenance of embryogenic tissue. These substances were chosen as they were reported to involve somatic embryo induction, multiplication and development in a number of plant cell cultures (Feirer *et al*., 1984; Armstrong and Green, 1985; Meijer and Simmonds, 1988; Emons *et al*., 1993).

### *Embryo maturation and repetitive somatic embryos*

In *S. album*, somatic embryos and friable embryogenic tissue were transferred to embryo multiplication medium (EMM) which consisting of basal medium supplemented with IAA (6 $\mu$M) and kinetin (1 $\mu$M), either for maintenance of embryogenic tissue or further development and maturation of somatic embryos. The types and percentages of gelling agents were optimized for both purposes.

### *Germination and plant development*

In *S. album*, for embryo germination and conversion, somatic embryos at the cotyledonary stage (1–2 mm) were separated and transferred individually to plantlet development medium (PDM) consisted of MS medium with $GA_3$ (3 and 6 $\mu$M) or a combination of $GA_3$ and BAP or $GA_3$ with IAA and BAP or IAA and kinetin for germination and plant development (Table 5). After 15 d, embryo germinations were scored as having normal germination when the root had elongated at least 2 mm and two green cotyledons were present. The elongated embryos (10–20 mm) were further grown in medium with a lower $GA_3$ concentration (3 $\mu$M). To obtain plantlets with well-established root systems, semi-solid or liquid media were tested. To regenerate plantlets of *S. spicatum*, the medium was modified by further addition of myo-inositol, glutamine, casein hydrolysate (CH), and coconut water (CW) (Table 6).

### *Histological studies*

Histological studies were carried out on embryogenic tissues which were cultured for 30 and 60 d. The explants were fixed in FAA (formalin 5% (v/v): acetic acid 5% (v/v): ethanol 90% (v/v)), dehydrated in an ethanol series and embedded in paraffin wax. Sections, 10–16 $\mu$m thick were cut and stained with toluidine blue-O (Feder and O'Brien, 1968).

## Results

### *Somatic embryo induction*

About 85% of explants from recent flushes and using 2nd to 5th internodes from the apical meristem gave sterile explants. On culture, the frequency of direct somatic

embryogenesis (Fig. 1A) and white embryogenic tissue (Fig. 1B) did not vary significantly with the level of TDZ (Table 1). However, seed explants produced more friable embryogenic tissue than nodal segments. The frequency of somatic embryo induction in *S. spicatum* was lower than that of *S. album* and this species only produced globular proembryo structures, not friable embryogenic tissue (Table 1).

### *Genotypic effect on somatic embryo induction*

The genotypes had an effect on the frequency, the speed of development and morphology of somatic embryos (Table 2). Genotype A13 spontaneously formed friable embryogenic tissue at the cut surface and around axillary buds within 6 weeks of culture. Unlike the other four genotypes tested, explants of this particular genotype turned brown, but this did not have an adverse effect on embryogenic tissue development. Spontaneous initiation of friable embryogenic tissue appeared to be controlled by genotype and could not be induced or increased by the addition of proline, arginine, or putrescine (data not shown).

### *Effect of induction media on* in vitro *explants*

Although not the case for field-derived explants of both species, leaf and nodal explants derived from *in vitro* culture became bleached after a few days of culture in induction medium. At the end of primary culture, leaf explants of *S. album* and *S. spicatum* produced white globular embryogenic tissue in medium containing 1 $\mu$M TDZ (Fig. 1E; Table 3). This tissue was confirmed to be proembryogenic masses by microscopic examination. The tissue was composed of isodiametric small cells, rich in cytoplasmic content, some of which developed into globular embryos.

In medium containing a combination of TDZ and 2,4-D, leaf explants of *S. album* expanded and became brown, then produced a small amount of brown callus (Fig. 1C). Within a week of the first subculture (6 week interval), white friable embryogenic tissue developed all over the primary callus surface.

### *Multiplication of repetitive embryogenesis and maturation*

When white globular embryos of *S. album* were transferred to new medium, they produced a number of secondary somatic embryos (Fig. 1D). A mixture of somatic embryos and friable embryogenic tissue was produced in EMM. However, a low concentration of gelrite (2%) was used for maintenance of friable embryogenic callus while a higher concentration of gelrite (4%) was found suitable for somatic embryo development and maturation (Table 4). Somatic embryos could be maintained in this medium without losing their embryogenic potential for at least 2–3 years, provided that the white friable embryogenic tissue was subcultured to new media every 3 weeks. However, friable embryogenic tissue obtained from media containing the mixture of TDZ and 2,4-D often became necrotic.

### *Germination and plantlet development*

When shoot and root apices of somatic embryos reached 1–2 mm, they were separated and germinated (Fig. 1F) in PDM. $GA_3$ at 6 $\mu$M gave a higher number of embryos within 15 d than $GA_3$ at 3 $\mu$M (Table 5). The inclusion of BAP or BAP and IAA in the medium caused higher abnormal germination; usually lack of root or shoot apices or fused cotyledons. Subculture of elongated plantlets in MS liquid media was superior to semi-solid media for shoot height, leaf expansion and lateral root formation (Fig. 1G).

The medium used for plantlet regeneration (PDM) of *S. album* was not effective for *S. spicatum*. In *S. spicatum*, the proembryos were difficult to regenerate and took 4–5 subcultures to form shoots with stunted roots (Fig. 1H). The combination of CH and CW increased the total number of regenerated shoots (Table 6).

### *Histological observation*

The initial green translucent globular structure originated from epidermal and sub-epidermal layers of the explant. They were clearly bipolar, with shoot and root poles as well as continuous procambial strands and distinct epidermis. They had no vascular connections with the explant and fulfilled the anatomical requirement for direct somatic embryos (Fig. 2A, C). A proembryo was found which appeared to develop from a single cell of subepidermal cells (Fig. 2B). The section also showed a secondary globular structure which grew on the surface layer of primary somatic embryos (Fig. 2E). As division in superficial cells progressed, the cortical parenchyma cells actively divided in periclinal and anticlinal directions, which partly led to the nodular appearance; they later became the multicellular suspensor of the somatic embryos (Fig. 2G).

While sections at 30 d showed that the epidermal and parenchymatous cortex were vacuolated with a small nucleus, longitudinal sections at 60 d revealed a new distinct type of dark-stained epidermal cell which had a dense cytoplasm and appeared scattered in the epidermal and subepidermal cells (Fig. 2I). A differentiated embryogenic mass was observed on top of a single row of dark-stained cells which suggested a unicellular origin (Fig. 2F). It is likely that a single epidermal cell had undergone anticlinal division before the uppermost cell differentiated into the embryogenic mass. It was not clear if secondary somatic embryos originated from single cells. As superficial cells formed somatic embryos, new proembryos developed at adjacent deeper zones of the

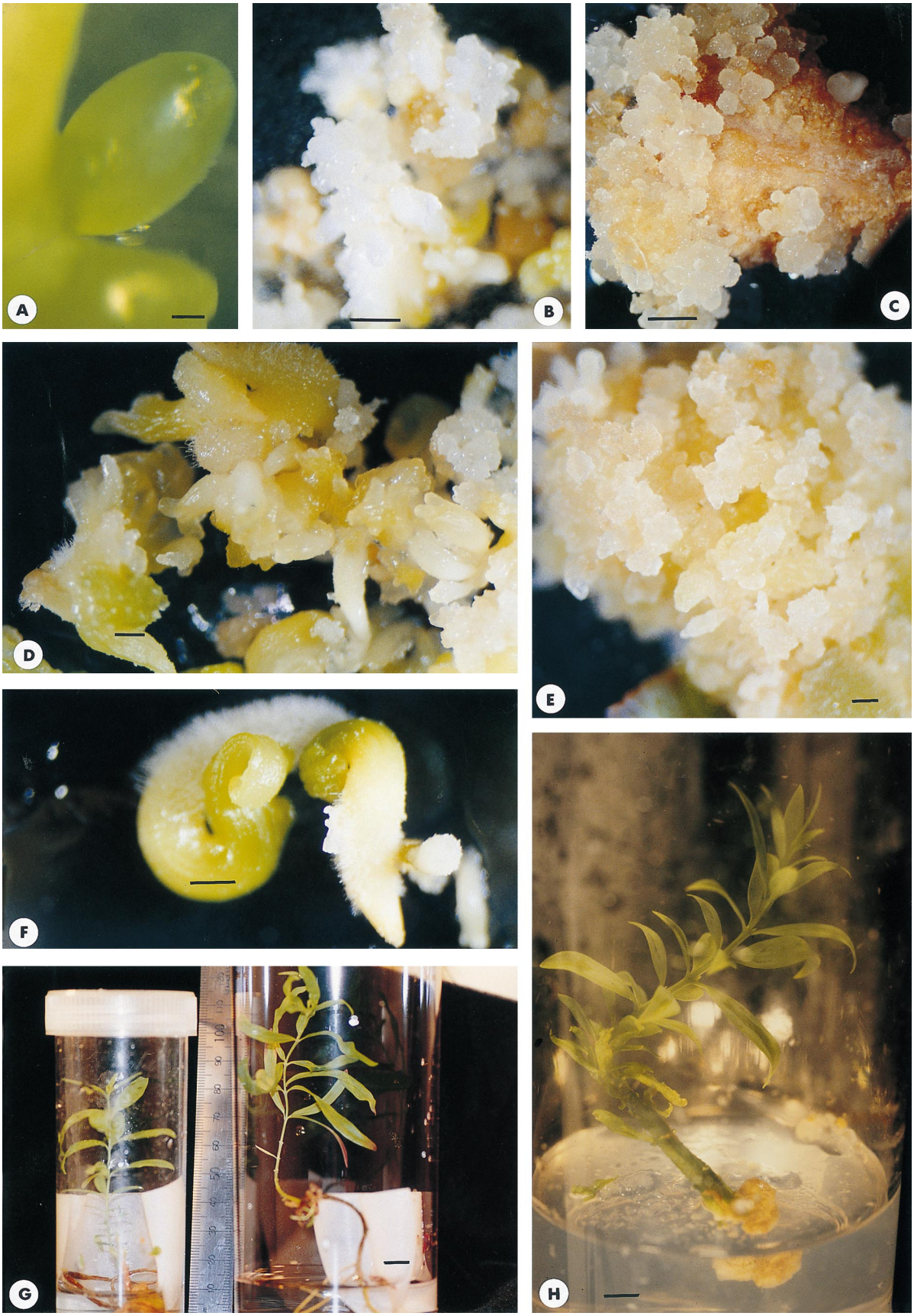


**Table 1.** *Frequency of direct somatic embryo induction, secondary somatic embryo and friable embryogenic callus of* S. album *genotype 614 and* S. spicatum *genotype S107 in response to TDZ levels at the end of the third subculture*

The number of explants was 25 for TDZ treatment and 150 for 2,4-D treatment. NT: not tested.

| Treatment | Non-embryogenic callus (%) | *S. album* (% explant) | | | *S. spicatum* (% explant) | | |
|---|---|---|---|---|---|---|---|
| | | Green somatic embryos | White somatic embryos | Friable embryogenic tissue | Direct somatic embryos | Secondary somatic embryos | Friable embryogenic tissue |
| Nodal segment | | | | | | | |
| TDZ (1 $\mu$M) | 0 | 100 | 64 | 56 | 20 | 20 | 0 |
| TDZ (2 $\mu$M ) | 0 | 100 | 72 | 64 | 16 | 16 | 0 |
| TDZ (4 $\mu$M) | 0 | 100 | 68 | 52 | 0 | 0 | 0 |
| 2,4-D (2.5 $\mu$M)+Kin (2.5 $\mu$M) | 100 | 0 | 0 | 0 | 0 | 0 | |
| Seed | | | | | | | |
| TDZ (1 $\mu$M) | 0 | 0 | 92 | 92 | NT | NT | NT |
| TDZ (2 $\mu$M) | 0 | 0 | 96 | 96 | NT | NT | NT |
| TDZ (4 $\mu$M) | 0 | 0 | 84 | 84 | NT | NT | NT |
| 2,4-D (2.5 $\mu$M)+Kin (2.5$\mu$M) | 100 | 0 | 0 | 0 | NT | NT | NT |

**Table 2.** *Frequency of somatic embryogenesis formed on nodal segments derived from various genotypes of* S. album *cultured on medium containing 2 $\mu$M TDZ*

Total number of explants in each treatment was 25.

| Genotypes | Green somatic embryo (%) | White somatic embryo (%) | Friable embryogenic tissue (%) |
|---|---|---|---|
| A0 | 100 | 32 | 32 |
| A4 | 100 | 72 | 60 |
| A13 | 0 | 100 | 84 |
| A14 | 100 | 64 | 12 |
| A16 | 100 | 0 | 0 |

**Table 3.** *Frequency of proembryo formation in explants derived from* in vitro *culture of* S. album *and* S. spicatum *in response to TDZ at the end of the third subculture*

Total number of explants in each treatment was 25.

| TDZ ($\mu$M) | *S. album* (% explant) | | | *S. spicatum* (% explant) | | |
|---|---|---|---|---|---|---|
| | A0 | A3 | A9 | S1 | S2 | S3 |
| Nodal segment | | | | | | |
| 1 | 78 | 56 | 76 | 56 | 44 | 72 |
| 2 | 74 | 64 | 80 | 48 | 48 | 68 |
| Leaf | | | | | | |
| 1 | 100 | 100 | 92 | 100 | 80 | 100 |
| 2 | 84 | 64 | 48 | 78 | 74 | 68 |

**Table 4.** *Effect of gelling agents in EMM on morphogenesis and development of embryogenic tissue (+: low; ++: medium; +++high)*

| Gelling agent (%) | Friable embryogenic tissue | Mature somatic embryos |
|---|---|---|
| Gelrite (2) | +++ | + |
| Gelrite (3) | ++ | + |
| Gelrite (4) | + | ++ |
| Gelrite (5) | + | +++ |
| Gelrite (6) | + | +++ |
| Gelrite(2)+Agar (2.5) | ++ | + |
| Agar (5) | ++ | + |

**Table 5.** *Effect of growth regulators on the germination of somatic embryo*

Total number of mature somatic embryos was 25 in each culture. Values are the mean number of 10 cultures ($\pm$standard error).

| Plant growth regulators ($\mu$M) | No. normal germination | | | No. abnormal germination |
|---|---|---|---|---|
| | <3 mm | 3–5 mm | >5 mm | |
| $GA_3$ (3 $\mu$M) | 10$\pm$3 | 7$\pm$1 | 3$\pm$2 | 5$\pm$1 |
| $GA_3$ (6 $\mu$M) | 7$\pm$2 | 10$\pm$3 | 6$\pm$4 | 2$\pm$2 |
| $GA_3$(6 $\mu$M)+BAP (1 $\mu$M) | 2$\pm$3 | 3$\pm$3 | 1$\pm$2 | 19$\pm$3 |
| $GA_3$ (6 $\mu$M)+BAP (1 $\mu$M)+IAA (1 $\mu$M) | 1$\pm$1 | 4$\pm$2 | 3$\pm$1 | 17$\pm$2 |
| IAA (6 $\mu$M)+kinetin (1 $\mu$M) | 2$\pm$1 | 3$\pm$2 | 1$\pm$1 | 19$\pm$3 |

**Fig. 1.** Somatic embryogenesis and plantlet formation of *S. album* and *S. spicatum* (bar=0.5 mm.). (A) Somatic embryo of *S. album* growing directly on nodal segments cultured in MS medium with TDZ. (B) White globular somatic embryo of *S. album* forming on top of primary green somatic embryos derived from nodal segments. (C) White friable embryogenic tissue of *S. album* developed on leaf segment cultured in MS basal medium containing 2,4-D and TDZ. (D) Various stages of repetitive somatic embryos of *S. album* cultured in medium containing IAA and kinetin with 4% gelrite. (E) Friable embryogenic tissue growing on top of leaf explant of *S. spicatum* cultured in MS medium with TDZ. (F) Germinated somatic embryos of *S. album* obtained by choosing mature, uniform-sized cotyledonary somatic embryos before transferring to germination medium. (G) Plantlet formation derived from somatic embryos of *S. album*, the plant on the right has been cultured in liquid medium while the one on the left has been growing in semi-solid medium. (H) Plantlet with stunted root derived from somatic embryo of *S. spicatum*.

**Table 6.** *Effect of casein hydrolysate (CH), coconut water (CW) and glutamine on plantlet regeneration of* S. spicatum *somatic embryos*

Each culture contained 200 mg of somatic embryo. The basal media contained 6 $\mu$M $GA_3$ and 200 mg $l^{-1}$ myo-inositol. Values are mean number of 10 cultures ($\pm$ standard error).

| Compound | Concentration (mg or ml $l^{-1}$) | No. plantlets/200 mg somatic embryos | | |
|---|---|---|---|---|
| | | Subculture 1–3 | Subculture 4 | Subculture 5 |
| 0 | 0 | 0 | 0 | 0 |
| CH | 250 | 0 | 0 | 2$\pm$2 |
| CW | 50 | 0 | 3$\pm$2 | 4$\pm$3 |
| CH + CW | 250 + 50 | 0 | 7$\pm$3 | 6$\pm$2 |
| CH + CW + glutamine | 150 + 50 + 500 | 0 | 3$\pm$1 | 4$\pm$2 |

explant and subsequently, parenchymatous cells of cortex also formed proembryos (Fig. 2H).

## Discussion

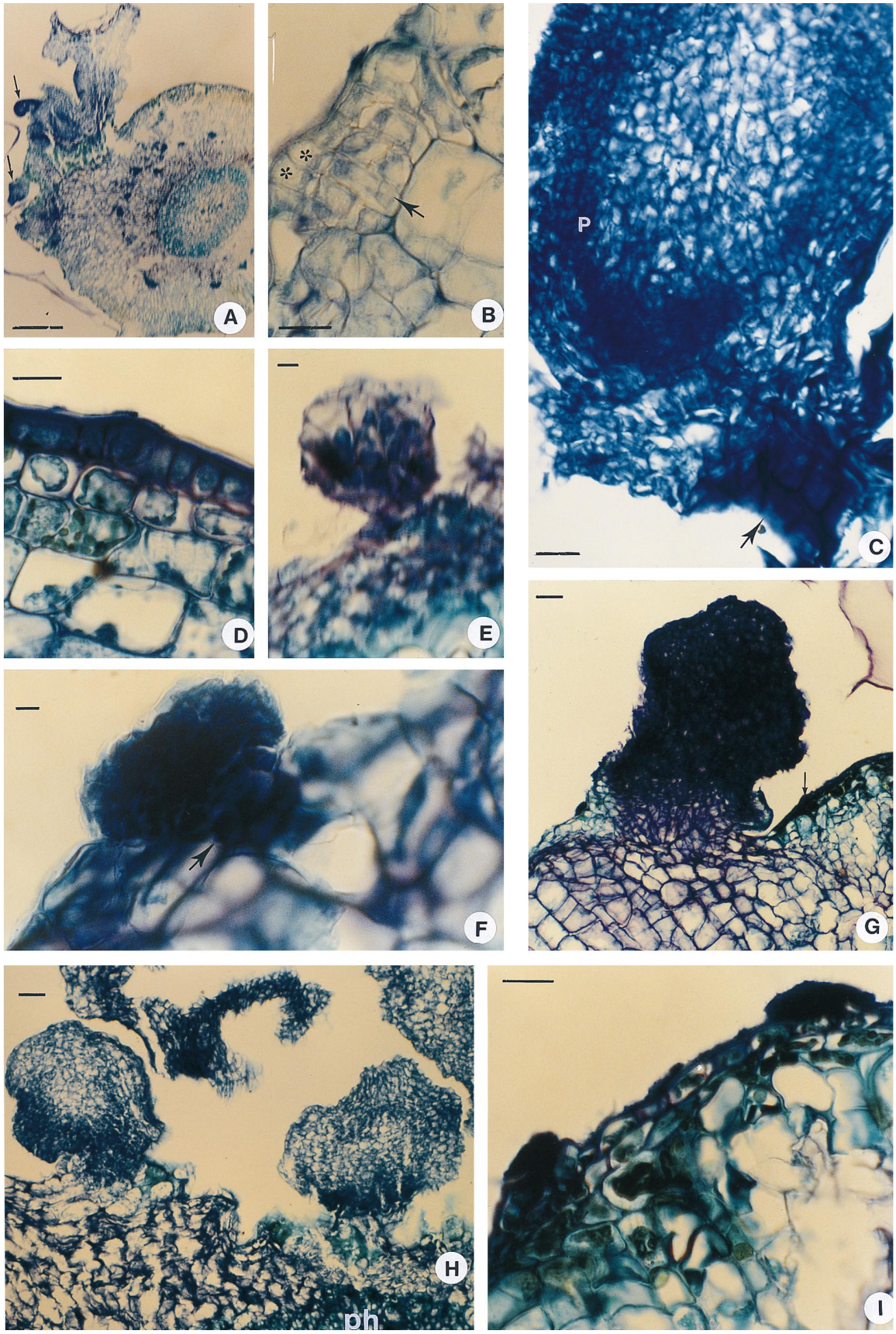
This study has firstly shown that *S. album* and *S. spicatum* can spontaneously produce direct somatic embryos from cells of several types of explants. Morphological and anatomical observations confirmed that somatic embryos developed directly from epidermal, subepidermal or cortical parenchyma without an intervening callus phase. The somatic embryos formed were bipolar without a vascular connection to the maternal explant. These embryos could multiply as repetitive somatic embryos and convert to plantlets. However, the conversion process in *S. spicatum* was more difficult than for *S. album*, and requires refinement of conditions to improve root elongation.

It has been reported that 10–20% of explants produced callus in media containing 2,4-D and kinetin or BAP, and that subculturing to media with other hormones was then necessary to produce embryogenic callus (Lakshmi Sita *et al*., 1979; Rao and Bapat, 1992). In the present study, TDZ alone or with 2,4-D can spontaneously induce embryogenesis at a frequency ranging from 50–100% and with greater reproducibility. The system described here required different hormone combinations and concentrations from those previously reported to obtain the most normal somatic embryos and their best germination. Rao and Bapat (1995) used media containing IAA and BAP for multiplication and conversion to plantlets and noted the problem of low conversion frequency. In this study, matured somatic embryos needed to be germinated in media containing $GA_3$, otherwise many abnormal somatic embryos were obtained and this resulted in a low conversion to plantlets.

Somatic embryogenesis of white ash (Bates *et al*., 1992) walnut, watermelon, muskmelon, geranium, grape (Lu, 1993; Huetteman and Preece, 1993), and peanut (McKently, 1995) has been found in media containing TDZ. In sandalwood, the optimum concentration of TDZ for somatic embryo induction was quite low compared to other species. TDZ at low concentrations (1–10 $\mu$M) has been reported to be more efficient in inducing organogenesis or somatic embryogenesis than other cytokinins, particularly in recalcitrant woody species (Huetteman and Preece, 1993). The reasons for the high activity of TDZ in woody species has not been investigated at the physiological or molecular level. A carbon isotope study showed that TDZ was very stable in the culture media and persistent in plant tissue (Mok and Mok, 1985). It has been suggested that TDZ helps to establish the internal optimum balance of cytokinin and auxin required for induction and expression of somatic embryogenesis (Saxena *et al*., 1992; Lu, 1993).

The results reported here indicate that most cells in sandalwood, which were still actively dividing, were embryogenically competent and could serve as the starting point in the developmental pathway. Two general patterns of *in vitro* embryogenic development, direct or indirect have been recognized. It has been suggested that direct embryogenesis occurs from pre-embryogenic determined cells, while indirect somatic embryogenesis requires the induction of embryogenically determined cells (Sharp

**Fig. 2.** Sections of somatic embryo forming on nodal segment of *S. album* cultured in media containing TDZ at 30 and 60 d. (A) Transverse section of nodal segment with primary and secondary somatic embryo (arrows) at 30 d after culture (bar = 0.5 mm). (B) Proembryo structures formed by irregular division of subepidermal cell often found in section at 30 d after culture, note periclinal division of epidermal cell (asterisks) (bar = 50 $\mu$m). (C) Longitudinal section through green globular somatic embryo showing bipolarity by developing procambial strand (p) with a close radicular end without vascular connection with the mother tissue, the narrow row of cells connected to the explant is similar to suspensor (arrow) normally found in zygotic embryo (bar = 0.1 mm). (D) Section at 60 d after culture, the epidermal layer showing intense staining indicating high cytoplasmic content (bar = 50 $\mu$m). (E) Secondary globular structure formed on primary somatic embryo (bar = 50 $\mu$m). (F) Proembryogenic mass differentiated from single epidermal cell, note the single row of dark stained cells (arrows; bar = 50 $\mu$m). (G) Longitudinal section through white somatic embryo on nodal segment showing that the embryo is composed of small cells high in cytoplasmic content, the multicellular suspensor at the base derived from periclinal and anticlinal division of cortical parenchyma cells (bar = 20 $\mu$m);. (H) Some somatic embryos differentiated from cortical parenchyma, ph: phloem (bar = 20 $\mu$m). (I) Embryogenic mass scattered along epidermal layer, note the greenish content in the surrounding cells indicating the accumulation of phenolic compounds (bar = 20 $\mu$m).



*et al*., 1980; Williams and Maheswaran, 1986). However, in a more recent review, these terms were considered inaccurate in describing embryogenic cells because of the extremely plastic response to the external environment (Carmen, 1990). Embryogenically competent cells is probably a more suitable term than embryogenic determined cells. The term refers to cells that could access the embryogenic programme *in vitro* and is transient. Carmen (1990) suggested that the manner of these types of cells are analogous to genes that are transcriptionally competent, but are not expressed.

Histological observations indicated that differentiation of maternal tissue led to a suspensor-like structure of primary somatic embryos. The presence of a narrow suspensor indicated a single cell origin and a broad suspensor area indicated a multicellular origin of somatic embryos (Williams and Maheswaran, 1986). Both narrow and broad suspensors were found in the present study. Somatic embryos of single or multiple cell origin derived from the epidermal layer were found in *Medicago sativa* (Dos Santos *et al*., 1983), and from epidermal and cortical cells in *Trifolium repens* (Maheswaran and Williams, 1985). The single cell origin of secondary somatic embryos was noted in walnut (Polito *et al*., 1989)and used successfully in developing solid transgenic plants (McGranahan *et al*., 1988).

The combination of high induction frequencies, many embryogenically competent cells and relative uniformity of regeneration responses across genotypes and explants of *S. album* make them attractive starting materials in initiating efforts to develop a reliable *Agrobacterium*-based transformation. However, care needs to be taken during early post-transformation selection. It appears that primary somatic embryos formed without physiological isolation from the neighbouring cells, even when originated from a single cell. To avoid losing transgenic cells or producing chimeric transgenic plants, the selection criteria for transgenic cells probably requires a stepwise selection procedure, to eliminate non-transgenic cells gradually as used successfully in mango (Mathews *et al*., 1992). The multiplication of friable embryogenic tissue or repetitive embryogenesis was more independent of the surrounding cells and could also be used as starting materials for *Agrobacterium*-mediated transformation (Scorza *et al*., 1990).

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