

The main root preserved is 5.5 cm in length and 6.5 mm in breadth; bearing horizontally spreading laterals of 0.5 mm width, with numerous root nodules. Lateral rootlets are opposite to those suboppositely attached; nodules are spherical to oval in shape measuring 1 mm × 1 mm to 2.5 mm × 2 mm; internal structure of nodule is not preserved. Whether the nodulated roots belong to Araucariaceae or Podocarpaceae is yet to be deciphered, as plant fossils of both the gymnospermous families are frequently present in the megafloreal assemblage.

Recognition of corolloid root in Araucarian plant⁴ and the present record on the occurrences of gymnospermous root system with evidence of the presence of nodules, from the Rajmahal Basin indicate that symbiotic N₂ fixation or nutrient

mobilization in the palaeo-community/ecosystem, possibly existed during the Early Cretaceous period in the Rajmahal Basin.

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Somatic embryogenesis of Sturt's Desert pea (*Swainsona formosa*)

Sturt's pea (*Swainsona formosa*) is an ornamental landscape plant of Western Australian desert origin. The plant belongs to the family Fabaceae, order Leguminosae. It is the most attractive wild flower of South Australia. It has good potential as ground cover, a container-grown ornamental, and as a cut-flower plant. It blooms late in winter and throughout early summer. *Swainsona* species grows mainly in arid parts of southern and central Australian sandy soil over limestone^{1,2}.

Natural propagation of this species is through seeds, which requires some scarification treatments for good germination. *S. formosa* is often grafted onto *S. puniceus*, a New Zealand native perennial plant. Cutting propagation from axillary buds produces plagiotropic plant (prostrate creeping plants) forms that are not suitable for pot-grown or cut-flower plants. In addition to the plagiotropic nature of rooted cuttings, they also have a poor root system resulting in inferior anchorage and low root to shoot ratios (Jusaitis, unpublished results).

Variations in growth form and flowering have been observed in seedling-populations¹. Lack of uniform plant material was identified as the main problem in cut-flower production. Clonal propagation, through which plants similar to seedlings can be propagated, is necessary for the introduction of selected clones into the ornamental plant industry.

Several tissue-culture attempts were tried on this species using seedling-derived explants^{1,3–5}. Recently, Jusaitis² reported the effects of auxins and cytokinins on proliferation and growth of *S. formosa* cultures initiated from adult lateral buds via axillary bud growth. However, a successful somatic embryogenesis method for the mass propagation of selected clones of this species has not been reported. Hence, we undertook research on somatic embryogenesis pathway for the regeneration of true-to-type selected clones.

Recently, *S. formosa* plants were successfully introduced in the Kuwait Institute for Scientific Research campus, Kuwait from Australia. Axillary shoots were removed from the healthy adult plant for the *in vitro* experiments. Stem segments, petiole and leaflets were surface sterilized with 20% commercial Chlorox[®] containing 1.05% sodium hypochlorite and a drop of Tween-20[®] for 15 min. After thorough washing in sterile distilled water, the tissues were treated with 0.1% mercuric chloride solution for 3 min. After rinsing in sterile distilled water three times, 5 mm segments of leaflet, petiole and stem were isolated using a sterile surgical knife and used as explants for the study.

The explants were placed on the surface of the Murashige and Skoog (MS) medium⁶ containing different concentrations of 2,4-D. The pH of the medium was adjusted to 5.6 and gelled with 0.13% Phytigel[®]. The medium was dis-

pensed into 25 mm × 150 mm Pyrex[®] culture tubes (12 ml/tube) and was autoclaved at 121°C temperature and 15 lb pressure. All cultures were incubated in a growth room at 25 ± 2°C for 16 h light and 8 h darkness photoperiods, and 1000 lux light intensity. Cultures were subcultured once every 20 days.

Each treatment contained 40 explants and the experiments were repeated twice. Cultures were observed once every five days to record the callus initiation and morphogenetic responses. Embryogenic calluses were isolated and subcultured in growth regulator-free MS basal medium repeatedly for embryogenesis, embryo maturation and germination.

Embryolings were carefully removed from the medium without damaging the root system and washed in running water to remove traces of the medium. Clean embryolings were carefully transplanted into small pots filled with soil mixture after dipping in an aqueous solution of 0.5% Benlate[®]. The soil mixture was prepared by mixing sand, peat moss and humus at 1 : 1 : 2 ratio and aired after autoclaving at 121°C and 15 lb pressure for 45 min. Embryolings, after planting in the soil mix, were kept in the growth room at 25 ± 2°C and with 3000 lux light intensity under 16 h photoperiod for 20 days prior to the greenhouse transfer. Later, they were gradually acclimatized to the greenhouse environmental conditions by reducing the relative humidity in the incubating containers.

The healthy explants that were excised from adult flowering plants without any contamination were enlarged after one week of incubation in all the treatments. After 15 days, white calluses were initiated at the cut end of the explants. The stem explants turned pinkish in colour before initiating the callus as a result of anthocyanin pigment accumulation (Figure 1 *a*). Stem, petiole and leaflet explants showed 100% callus initiation response to all the treatments with 2,4-D concentrations of 1, 3, 5 and 10 mg/l in MS medium (Table 1). From leaf tissues, callus was first initiated at the leaf edges, generally near the vein or midrib, and was soft and whitish. The growth of the callus was enhanced when it was subcultured on fresh medium. The callus was very soft and whitish in colour when it was in the medium with 2,4-D. After 2–3 subcultures, the calluses turned light greenish in colour and were friable in nature (Figure 1 *b*). At this stage, the calluses that were transferred to the growth regulator-free MS basal medium produced

green-coloured globular nodules. These green nodules multiplied by the adventitious budding method. The callus that was initiated and maintained on 10 mg/l 2,4-D medium slowly turned brown and finally died after 2–3 subcultures. The calluses that were isolated from these cultures and transferred onto growth regulator-free medium immediately after initiation produced embryogenic callus. Callus developed at low concentrations (1–3 mg/l), grew and multiplied in the same medium.

The pale greenish-coloured callus grew on growth regulator-free MS medium and produced globular green nodule-like structures after 30 days. These globular nodules multiplied in the same medium through the adventitious budding method. After repeated subculture in the same medium, some of the greenish globular nodules produced somatic embryos (Figure 1 *c*). Nodular callus explants transferred to medium with 2,4-D or BA did not produce somatic embryos. The average percentage of cultures that produced

somatic embryos in the hormone-free medium was found to be 40.2. From each 5 mg nodular callus explant, an average of 9.5 mature somatic embryos were obtained (Table 2) after 30 days. Mature somatic embryos were isolated and transferred to the same medium. Transferred somatic embryos germinated and produced embryolings within 20 days (Figure 1 *d* and *e*). Most of the somatic embryos planted upright germinated, and the ones placed horizontally on the surface of the medium showed secondary adventive embryogenesis.

Each single germinated embryoling was transferred into a test tube and inserted vertically into growth medium con-



Figure 1. *Swainsona formosa*. *a*, Callus growth from cultured explant on medium containing 2,4-D (1 ×); *b*, Growth of friable callus on a growth regulator-free medium (1 ×); *c*, Multiplication and maturation of somatic embryos (1 ×); *d*, Stages of embryo germination (1 ×); *e*, Embryoling in culture medium (1 ×); and *f*, Growth of embryoling on a medium devoid of growth regulators and containing 1.5% activated charcoal (0.5 ×).



Figure 2. *S. formosa*. *a*, Acclimatized plantlets; *b*, Plant in the field; and *c*, *In vitro* plant with flowers in the field (0.5 ×).

Table 1. Morphogenetic response of different explants of *Swainsona formosa* after 30 days in culture

Medium	Explant	Morphogenetic response
MS basal	Stem, petiole and leaflet	Turned brown
MS + 1 mg/l 2,4-D	Stem, petiole and leaflet	Soft greenish callus
MS + 3 mg/l 2,4-D	Stem, petiole and leaflet	Soft greenish callus
MS + 5 mg/l 2,4-D	Stem, petiole and leaflet	Soft and nodular callus
MS + 10 mg/l 2,4-D	Stem, petiole and leaflet	Soft and nodular callus
MS basal	Soft greenish callus	Globular nodules
MS basal	Nodular callus	Somatic embryos
MS basal	Somatic embryos	Germination and growth

Table 2. Effect of growth regulators on 5 mg nodular callus explants of *S. formosa*

Medium	Percentage of cultures with SE	No. of SE/culture (average)
MS basal	40.2 ± 0.14	9.5 ± 0
MS + 1 mg/l 2,4-D	6.6 ± 2.2	0
MS + 3 mg/l 2,4-D	0	0
MS + 5 mg/l 2,4-D	0	0
MS + 1 mg/l BA	0	0

SE, Somatic embryos; (±), Standard error; Data from 40 replicates; Experiment was repeated twice.

taining 1.5% activated charcoal and 30 g/l sucrose (Figure 1f). Embryolings were acclimatized in the growth chamber and transferred to the greenhouse. Hardened plantlets (Figure 2a) which were transferred to the field showed normal growth and development (Figure 2b). More than 90% of the plants survived in the field and produced flowers (Figure 2c) similar to the mother plant.

S. formosa exhibits vegetative dimorphism. The orthotropic shoots with flowers are preferred by the cut-flower industry. Plantlets regenerated from the nodal explants of the adult plant showed plagiotropic shoots². Adventitious organ formation and somatic embryogenesis have been suggested as techniques for the possible reduction or elimination of plagiotropy⁷. The results of the present study on this species may solve the problem of plagiotropism, which could help the cut-flower industry.

Auxin 2,4-D was the only hormone that significantly stimulated callus production in *S. formosa*² and *S. galegifolia*⁸. The results indicate that 2,4-D induces callus with embryogenic potential. Concentrations from 1 to 10 mg/l initiated embryogenic callus. However, low concentration of 2,4-D (1–3 mg/l) was found to be suitable for the normal growth and multiplication of the callus. At higher concentrations (5–10 mg/l), callus initi-

ated earlier than at low concentration, but the callus showed browning, which leads to its death after 30 days in culture.

All callus pieces that were transferred to the MS basal medium showed the formation of greenish, globular nodule-like structures after the second subculture. Globular nodules multiplied and produced somatic embryos within 30 days. These somatic embryos multiplied by repeated secondary adventitious somatic embryogenesis. Germination of the embryos was affected by the position when they were subcultured in fresh medium. Isolated individual embryos when planted vertically, cotyledon facing up, in the basal medium germinated into embryolings. The embryos placed horizontally on the surface of the medium showed secondary somatic embryogenesis.

Vitrification (hyperhydration) is a major problem in the tissue culture industry since it can affect shoot multiplication and culture vigour⁹. Vitrification of the embryolings was one of the major problems that affected the mass production of plantlets in this species. Vitrified shoots of *Swainsona* could be restored to normality by keeping them at 5°C for 10 days followed by transfer to room temperature for 30 days¹. In our study, we found better results by desiccating the embryos at high humidity for 1 or 2 days

to avoid vitrification and enhance the germination.

In conclusion, a protocol for tissue culture propagation via embryogenesis of *S. formosa* was developed, which consisted of a three-stage medium: (1) MS basal medium with 1–3 mg/l 2,4-D for callus initiation and induction of embryogenesis; (2) Growth regulator-free MS medium for embryogenesis, multiplication and embryo germination; (3) MS medium containing 1.5% activated charcoal for plantlet growth. The clonal nature of the plants was ascertained from the field results. This technique of mass propagation can be used to propagate selected clones of *S. formosa* and produce propagules similar to seedlings in growth habits. Further studies related to mass production and field evaluation of this species are in progress in our laboratory.

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