J. Phytol. Res. **8** (2): 119-122, 1995

POLYEMBRYONY AND *IN VITRO* SEED CULTURE IN *SYZYGIUM ALTERNIFOLIUM* (WIGHT) WALP.

P.S. SHA VALLI KHAN, G. SUDARSANAM AND K. R. RAO
Plant Tissue Culture Lab, Department of Botany, S.V. University, Tirupati - 517 502, A.P., India.

Under natural conditions 30-35% of mature seeds of *Syzygium alternifolium* (wight) walp. were indicated polyembryony by the presence of pluricotylity and uneven germination. All the seeds (both tricotyledonous and dicotyledonous) cultured *in vitro* were triggered greatly and showed various responses such as formation of multiple shoots and roots, multiple shoots only, single shoot with multiple roots, callus, callus with roots due to the effect of exogenous supply of BA and IAA.

Keywords: *In vitro* seed culture; Polyembryony; *Syzygium alternifolium.*

Introduction

Polyembryony has been defined as the occurrence of more than one embryo in a seed[1]. It has great significance in horticulture and plant breeding[2,3]. High frequency of naturally occurring polyembryony in some tropical forest and fruit trees (eg. Citrus, Mangifera, *Syzygium* sps) have been reported[4]. Some polyembryonate species of *Syzygium* (*Eugenia)* such as *S.cumini*[5], *E. Jambos* and *E. malaccnse*[6] have already been cultured *in vitro* for different studies. So far there has been no report on the occurrence of polyembryony and *in vitro* culture of seeds of *Syzygium alternifolium* (Wight) Walp., an endangering, endemic deciduous forest, fruit tree of medicinal and economic importance. It is restricted to certain forest tracts in Kurnool, Cuddapah, Chittoor districts in Andhra Pradesh, North Arcot district in Tamil Nadu and Nagari hills in Assam[7,8]. Here we report our preliminary observations on expression of polyembryony and *in vitro* seed culture in *S.alternifolium.*

Materials and Methods

Mature fruits of *S. alternifolium* were collected during May and June from trees situated in Tirumala hills of Chittoor district, A.P. The seeds from fruits were taken out and their morphological features were noted.

Dicotyledonous seeds and tricotyledonous seeds were separated and washed thoroughly under tap water for 1 h. Afterwards the seeds were surface sterilized with 0.05% (w/v) aqueous mercuric chloride solution for 5 min followed by thorough washings with sterile distilled water. Then the disinfected seeds were planted in plastic trays containing sterile moist sand for germination. For *in vitro* culture one sterile seed was inoculated in each test tube containing 15ml of culture medium.

Modified Murashige and Skoog's[9] medium supplemented with 0.2% (W/V) sucrose and 0.8% (W/V) agar was used as a culture medium. The growth regulators employed were BA (Benzyl adenine) and IAA (Indole-3-acetic acid). The BA and IAA were either used singly or in various combinations. The pH of medium was adjusted to 5.8 using 0.1 N HCl or 0.1N NaOH before autoclaving at 1.06 kg cm^2 for 15 min.

The plastic trays and culture tubes were incubated at 16h light / 8h dark photoperiod under light intensity of about

2000 lux provided by cool white fluorescent lamps in combination with incandiscent bulbs at 25 ± 2°c with 55% relative humidity. For each experiment 40 seeds were used and all experiments were performed thrice. Data have been compiled from all observations.

Results and Discussions

Seed Morphology : Mature fruits of *S. alternifolium* were dark purple in colour, oblong to ovoid in shape and measure 1.8 to 2 cm in length. Each fruit contains more or less spherical seed, occassionally double seeds also. The seeds are 1 to 1.5 cm in length with thick testa. Seeds consists of whitish, shapeless, minute embryos and large, fleshly, cotyledons which resemble the cotyledons of pea. Most of them were associated with normal two cotyledons but some seeds (about 15%) are tricotyledonary, rarely pentacotyledonary also. The presence of odd cotyledonous condition was explained as an instance of polyembryony and attributed to fusion of two cotyledons or suppression of one cotyledon with its fellow cotyledon[10,11].

Germination Studies : Both dicotyledonous seeds and tricotyledonous seeds planted in moist sand were germinated after 15 days. Approximately 80% of dicotyledonous seeds have produced normal plantlets while the others have been shown formation of shoots or roots only or more than one shoot and root. Pijil[12] ascertained the presence of plural embryos in a seed and their incomplete development due to imbalance of growth

Table 1. *In vitro* effect of BA and IAA on *S. alternifolium* mature seeds.

Hormonal Concentration (mg/l)	Type of seed	
	Dicotyledonous	Tricotyledonous
BA		
1.0	MS + R	MS + R
2.0	MS + R	MS + R
4.0	MS	MS
IAA		
1.0	SS + R	MS + R
2.0	C	C
4.0	C + R	C
BA + IAA		
1.0 + 1.0	MS + R	MS + R
1.0 + 2.0	C	C
2.0 + 1.0	MS + R	MS + R
2.0 + 2.0	MS + R	MS + R

M S : Multiple shoots, SS : Single Shoot, R : Roots formation, C : Callus

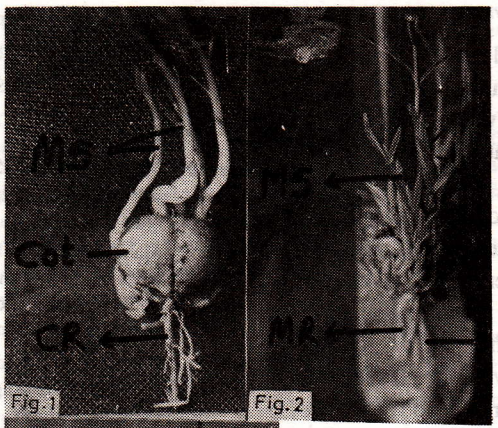

Fig. 1 Tricotyledonous seed with multiple shoots and common root.
2. *Invitro* cultured dicotyledonous seed with multiple shoots and roots.
3. *Invitro* cultured dicotyledonous seed with callus.
MS: Multiple shoots, Cot: Cotyledon, CR: Common root, MR: Multiple roots, Ca: Callus

regulators. All the tricotyledonous seeds on germination produced 2-3 shoots with a common root (Fig.1). Similar condition was reported in highly polyembryonic citrus and ascribed due to varicius degrees of plural embryos fusion at their radicular end[13]. The *in vitro* culture study was conducted with dicotyledonous as well as tricotyledonous seeds to see their responses to exogenous supply of plant growth regulators (BA and IAA).

***In vitro* seed culture** : The two types of seeds cultured on medium with no growth regulator revealed responses similar to seeds germinated in moist sand. Whereas the seeds cultured on media containing BA and IAA either singly or incombinations were shown varied responses according to the media formulation used (Table 1). In presence of BA (1 to 2 mg/1), BA + IAA (1 mg/1 + 1 mg/1; 2 mg/1 + 1 mg/1 and 2 mg/1 + 2 mg/1) dicotyledonous seeds (Fig.2) as well as tricotyledonous seeds have produced shoots and roots simultaneously from their embryonal region. At higher concentrations of BA (4 mg/1) both types of seeds formed only multiple shoots and depressed root growth. A loose, yellow friable callus was induced from the embryonal region of dicotyledonous (Fig.3) and tricotyledonous seeds at 2 mg/1 IAA and 1 mg/1 BA and 2 mg/1 IAA. In dicotyledonous seeds formation of single shoot with multiple roots and callus with roots have also been observed at 1 mg/1 IAA and 4 mg/1 IAA respectively. Similar responses have been reported by Litz[6] in the adventitious embryo culture of two polyembryonic *Eugenia* species, namely *E.*

Jambos and *E. malaccense.*

In current study, under natural conditions 30-35% of mature seeds indicated polyembryonic nature while the others exhibited monoembryonate behaviour. The expression of less percentage of polyembryony than its actual frequency in mature seeds was attributed due to arrest of additional embryos at very early stages or their degeneration during the course of seed development[3]. All the seeds (both dicotyledonous and tricotyledonous) cultured *in vitro* were triggered greatly to the effect of exogenous supply of BA and IAA and showed responses accordingly. This is possibly due to the fact that the presence of endogenous hormone levels in seeds is balanced by the exogenous supply, which has lead the morphogenesis and expression of polyembryony.

These preliminary results on expression of polyembryony and *in vitro* seed culture makes *S. alternifolium*, a rewarding system for experiments on embryology, somatic embryogenesis and organogenesis.

Acknowledgements

The authors acknowledge the Department of Non-conventional Energy sources (DNES), New Delhi, for financial support. One of us (PSVK) wishes to thank the UGC for the award of Junior Research Fellowship.

References

1. Maheshwari P 1950, *An introduction to the embryology of Angiosperms* McGraw-Hill, New York.
2. Maheshwari P and Sachar R C 1963, *In* : Recent advances in the embryology of Angiosperms pp 265-296 (P Maheshwari ed.) Intl. Soc. Pl. Morphol, Delhi (India).
3. Bhojwani S S and Bhatnagar S P 1984, *In* : Embryology of Angiosperms, pp 185-189 Vikas Publishing House, New Delhi, India.
4. Kaur A, Jong Co Ha K, Sands V E, Chan H T, Padmo E Soc and Ashton P S 1978, *Nature* **271** 440
5. Yadav U, Madan Lal and Jaiswal V S 1990, *Plant Cell Tissue and Organ Culture* **21** 87
6. Litz R E 1984, *Hort.Science* **19** 720
7. Hooker J D 1876, The flora of British India, Vol.II pp 497 Bishen Singh Mahendra Pal Singh, Rep. Ed. Dehradun.
8. Madhava Chetty and Rao K N 1990, *Vegetos* **3** 12
9. Murashige T and Skoog F 1962, *Physiologia Plantarum* **15** 473
10. Tiwary N K 1926, *J.Indian Bot.Soc.* **5** 124
11. Roy S K and Sahai R 1962, *J.Indian Bot.Soc.* **41** 45
12. Pijil L V 1934, *Rec. Trav.Bot.Neerl*, **31** 113
13. Maheshwari P and Rangaswamy N S 1958, *Indian J. Hortic.***15** 275