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In vitro propagation of crossandra (*Crossandra infundibuliformis* (L.) Nees.) var. Arka Shravya and Pondicherry local through direct organogenesis

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Abstract
Investigation on "*In vitro* propagation of crossandra (*Crossandra infundibuliformis* (L.) Nees.) var. Arka Shravya and Pondicherry local through direct organogenesis" was carried out at the Tissue Culture laboratory of the Horticultural College and Research Institute of TNAU, Coimbatore. In both the varieties shoot tips and nodal segments were used as explants. Of these, nodal segments were found to be the most suitable explants with respect to response for *in vitro* regeneration and multiplication. Nodal segments pre-treated with 0.2% Bavistin (30 min.) + 0.5% Streptomycin (45 min.) + 70% Ethanol (30 sec.) + 0.5% NaOCl (5 min.) registered the highest survival per cent. Shoot tips pre-treated with 0.2% Bavistin (30 min.) + 0.3% Streptomycin (45 min.) + 70% Ethanol (30 sec.) + 0.5% NaOCl (5 min.) registered the highest survival per cent. MS medium + BAP (2 mg l^{-1}**)** was found to be best for nodal segment cultures with respect to the maximum percentage response to shoot proliferation, days required for shoot emergence and number of shoots per explant. MS medium + BAP (2 mg l^{-1}) + GA_3 (1 mg l^{-1}**)** was found to be best with response to highest shoot elongation percentage of shoots and average length of shoots. Efficient rooting was achieved with ½ MS medium + IBA (1 mg l^{-1}**),** gave the maximum *in vitro* rooting percentage. Among the two varieties compared in crossandra, Arka Shravya proved to be more potential than Pondicherry Local, in respect of time taken for *in vitro* regeneration and rooting.

Keywords: Crossandra, sterilization, *in vitro* regeneration and multiplication, *in vitro* shoot elongation, *in vitro* rooting

Introduction
Crossandra (*Crossandra infundibuliformis*) (L.) Nees is an important horticultural plant and belongs to the family Acanthaceae. It is native to Southern India and Sri Lanka. It consist of about 40 – 50 species. It is an erect, evergreen shrub growing to 1 m (3 ft 3 inch) with glossy, wavy-margined leaves and fan-shaped flowers, which may appear at any time throughout the year. Flower colours range from common orange to salmon-orange or apricot, coral to red, yellow and even turquoise. The flowers are of great demand for use in hair adornments and in the form of garlands, venis and gajras. Though not fragrant, crossandra flowers are very popular because of their attractive bright colour, light weight and good keeping quality. Traditional tetraploid, triploid cultivars of crossandra and present day hybrids have a great demand among the farmers for the want planting materials. As their seeds provide segregation and lack uniformity of plants, the only way of propagation is through cuttings. Propagation by conventional methods necessarily limits the rate of output and makes the end product expensive. Micropropagation is being used extensively for the rapid clonal propagation of many fruits, nuts and ornamental trees (Zimmerman, 1985) [6]. The present study includes both traditional variety and popular hybrid recently released by IIHR - Bangalore, which is widely under cultivation. In order to meet the demand an attempt has been made to evolve mass multiplication methods through *in vitro* culture.

Materials and Methods
The Study was conducted at the Tissue Culture Laboratory of the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2015-2016. Pondicherry Local is a widely growing local type collected from Crossandra Innovation Centre (CIC), Pondicherry. Arka Shravya is a F_1 hybrid released by IIHR, Bangalore.

Sterilization

Both shoot tips and nodal segments have the potential to regenerate new plantlets under *in vitro*. Initially the shoot tips and nodal segments were taken from the mother plants. The collected explants were thoroughly washed many times with running water to remove the dirt particles which were adhered to them. Then the leaves are removed from shoot tips and nodal segments further washed with running tap water. Then the shoot tips and nodal segments were subjected to the following sterilizing agents. 0.1% Bavistin (30 min.) + 70% Ethanol (30 sec.) + 0.2% NaOCl (3 min.), 0.1% Bavistin (30 min.) + 70% Ethanol (30 sec.) + 0.2% and 0.5% NaOCl (5 min.), 0.1% Bavistin (30 min.) + streptomycin 0.1%, 0.3% and 0.5% (45 min.) + 70% Ethanol (30 sec.) + 0.5% NaOCl (5 min.), Control (Washing with sterile distilled water).

***In vitro* shoot regeneration and multiplication**

The surface sterilised explants were inoculated for shoot regeneration and multiplication in full strength MS media with different concentration of BAP (1, 2, 3, 4 mg l^{-1}) and control- Basal MS.

***In vitro* shoot elongation**

After three subcultures at the interval of 20 days in the same appropriate concentration of BAP medium, the regenerated explants were transferred to shoot elongation medium with BAP (2 mg l^{-1}) + GA_3 (0.5, 1, 1.5 and 2 mg l^{-1}) and Control (Basal MS without BAP and GA_3).

***In vitro* rooting**

After the shoots were elongated (40days), they are transferred to half strength MS medium with different concentrations of IBA (0.5, 1, 1.5 and 2 mg l^{-1}) and Control- (Basal ½ MS) for *in vitro* rooting.

Rescue of contaminated cultures

Attempts were made to develop techniques to rescue cultures at various stages of *in vitro* culture namely, culture establishment, shoot regeneration and proliferation and *in vitro* rooting. The details of rescue treatments adopted in the present investigation were 70% Ethanol (30 sec.) + (0.1, 0.2, 0.3 and 0.4%) Streptomycin (15 min.).

Statistical analysis

All experiments were arranged in a Factorial Completely Randomised Design (FCRD). The obtained data were compared according to the method described by Snedecor and Cochran (1989) [5].

Results and Discussion

Effect of sterilants on the explants

The data in the table (1) indicate that nodal segments pre-treated with 0.2% Bavistin (30 min.) + 0.5% Streptomycin (45 min.) + 70% Ethanol (30 sec.) + 0.5% NaOCl (5 min.) registered the highest survival per cent. Shoot tips pre-treated with 0.2% Bavistin (30 min.) + 0.3% Streptomycin (45 min.) + 70% Ethanol (30 sec.) + 0.5% NaOCl (5 min.) registered the highest survival per cent with minimum per cent of contamination and mortality in both the varieties. Reduction in contamination is due to the mode of action of streptomycin (antibiotic) which acts inhibitors of bacterial cell wall synthesis, bacterial protein synthesis and DNA replication blockers (Quesnel and Russell, 1983) [4].

Effect of growth regulators on shoot regeneration and multiplication

The results from the table (2) explain that MS medium + BAP (2 mg l^{-1}**)** was found to be best for nodal segment cultures with respect to the maximum percentage response to shoot proliferation, days required for shoot emergence and number of shoots per explant. It may be due to the fact that cytokinins promote cell division and axillary shoot formation by decreasing apical dominance. BAP is a well known cytokinin for its stimulatory effect on shoot induction in tissue culture studies (Khanna, 2008) [3].

Table 1: Effect of growth regulators on shoot regeneration and multiplication

Treatments	Arka Shravya						Pondicherry Local					
	Shoot tips			Nodal segments			Shoot tips			Nodal segments		
	days taken for shoot emergence	shoot regeneration (%)	number of shoots plantlets $^{-1}$	days taken for shoot emergence	shoot regeneration (%)	number of shoots plantlets $^{-1}$	days taken for shoot emergence	shoot regeneration (%)	number of shoots plantlets $^{-1}$	days taken for shoot emergence	shoot regeneration (%)	number of shoots plantlets $^{-1}$
BAP (1 mg l^{-1})	7.67	76.67	18.63	6.63	83.33	19.63	7.83	56.67	17.33	6.97	70.00	18.63
BAP (2 mg l^{-1})	7.23	80.00	21.83	5.83	93.33	23.57	8.23	66.67	19.57	6.37	86.67	22.17
BAP (3 mg l^{-1})	8.23	56.67	15.47	7.03	73.33	16.83	8.73	46.67	15.23	7.33	63.33	15.23
BAP (4 mg l^{-1})	8.73	43.33	9.47	7.83	53.33	10.13	9.23	30.00	8.47	8.03	43.33	9.13
Control (Basal MS)	9.23	20.00	4.47	8.27	26.67	5.27	9.97	16.67	4.27	8.97	13.33	4.83

Note: Mean Values were calculated at CD(P= 0.05) by FCRD

Effect of growth regulators on shoot elongation

The data in the table (3) reveal that MS medium + BAP (2 mg l^{-1}) + GA_3 (1 mg l^{-1}**)** was found to be best with response to highest shoot elongation percentage of shoots and average length of shoots. It might be due to the fact that GA_3 had a greater influence in cell elongation and thereby increase the cell size. It results in elongation, especially in the internodal regions of the plants (Deepa *et al.,* 2011) [1].

Table 2: Effect of growth regulators on shoot elongation

Treatments	Arka Shravya				Pondicherry Local			
	Shoot tips		Nodal segments		Shoot tips		Nodal segments	
	Shoot elongation (%)	Shoot length (cm)	Shoot elongation (%)	Shoot length (cm)	Shoot elongation (%)	Shoot length (cm)	Shoot elongation (%)	Shoot length (cm)
BAP (2 mg l^{-1}) + GA_3 (0.5 mg l^{-1})	63.33	3.13	70.00	3.32	53.33	2.92	63.33	3.18
BAP (2 mg l^{-1}) + GA_3 (1 mg l^{-1})	73.33	3.26	80.00	3.53	66.67	3.12	70.00	3.33
BAP (2 mg l^{-1}) + GA_3 (1.5 mg l^{-1})	46.67	2.96	60.00	3.22	40.00	2.77	53.33	2.96
BAP (2 mg l^{-1}) + GA_3 (2 mg l^{-1})	36.67	2.56	50.00	2.73	30.00	2.43	43.33	2.59
Control (without BAP and GA_3)	23.33	2.13	33.33	2.26	20.00	2.00	26.67	2.01

Note: Mean values were calculated at CD (P= 0.05) by FCRD.

Effect of growth regulators on *in vitro* rooting

The data in the table (4) explain that efficient rooting was achieved with ½ MS medium + IBA (1 mg l^{-1}**),** gave the maximum *in vitro* rooting percentage. The role of auxins in inducing roots in plants both *in vitro* and *in vivo* has been demonstrated beyond doubt. Investigations in the field of plant tissue culture have shown that *in vitro* rooting could be successfully achieved by reducing salt concentration in the media, particularly in high salt media like MS and its derivatives (Gupta, 1986) [2].

Effect of sterilizants on rescue of contaminated cultures

The data in the table (5) reveal that contaminated cultures of crossandra treated with 70% Ethanol (30 sec.) + 0.3% Streptomycin (15 min.) gave the highest rescue percentage. Reduction in contamination is due to the mode of action of streptomycin (antibiotic), which acts as inhibitors of bacterial cell wall synthesis, bacterial protein synthesis and DNA replication blockers (Quesnel and Russell, 1983) [4].

Table 3: Effect of sterilants on the explants

Treatments	Arka Shravya						Pondicherry Local					
	Shoot tips			Nodal segments			Shoot tips			Nodal segments		
	Survival (%)	Contamination (%)	Mortality (%)	Survival (%)	Contamination (%)	Mortality (%)	Survival (%)	Contamination (%)	Mortality (%)	Survival (%)	Contamination (%)	Mortality (%)
0.1% Bavistin (30 min.) + 70% Ethanol (30 sec.) + 0.2% NaOCl (3 min.)	33.33	63.33	3.33	33.33	66.67	0.00	23.33	63.33	6.67	30.00	76.67	0.00
0.1% Bavistin (30 min.) + 70% Ethanol (30 sec.) + 0.2% NaOCl (5 min.)	43.33	50.00	6.67	46.67	50.00	3.33	33.33	53.33	6.67	43.33	60.00	3.33
0.1% Bavistin (30 min.) + 70% Ethanol (30 sec.) + 0.5% NaOCl (5 min.)	50.00	43.33	6.67	53.33	43.33	3.33	46.67	43.33	10.00	50.00	43.33	6.67
0.1% Bavistin (30 min.) + streptomycin 0.1% (45 min.) + 70% Ethanol (30 sec.) + 0.5% NaOCl (5 min.)	63.33	23.33	13.33	66.67	26.67	6.67	43.33	30.00	13.33	63.33	30.00	10.00
0.2% Bavistin (30 min.) + streptomycin 0.3% (45 min.) + 70% Ethanol (30 sec.) + 0.5% NaOCl (5 min.)	76.67	10.00	13.33	78.21	10.00	10.00	70.00	10.00	16.67	72.21	10.00	13.33
0.2% Bavistin (30 min.) + streptomycin 0.5% (45 min.) + 70% Ethanol (30 sec.) + 0.5% NaOCl (5 min.)	73.33	3.33	23.33	80.00	6.67	10.00	66.67	6.67	26.67	73.33	6.67	16.67
Control- Washed with sterile distilled water.	0.00	100.00	0.00	0.00	100.00	0.00	0.00	100.00	0.00	0.00	100.00	0.00

Note: Mean values were calculated at CD (P= 0.05) by FCRD.

Table 4: Effect of growth regulators on *in vitro* rooting

Treatment	Arka Shravya								Pondicherry Local							
	Shoot tips				Nodal segments				Shoot tips			Nodal segments				
	Days taken for root emergence	Root regeneration (%)	Number of roots plantlets $^{-1}$	Root length (cm)	Days taken for root emergence	Root regeneration (%)	Number of roots plantlets $^{-1}$	Root length (cm)	Days taken for root emergence	Root regeneration (%)	Number of roots plantlets $^{-1}$	Root length (cm)	Days taken for root emergence	Root regeneration (%)	Number of roots plantlets $^{-1}$	Root length (cm)
IBA (0.5 mg l^{-1})	19.17	46.67	2.43	2.45	17.53	73.33	2.53	2.53	19.83	40.00	2.13	1.96	18.17	53.33	2.33	2.24
IBA (1 mg l^{-1})	17.43	76.67	2.83	2.67	16.27	86.67	2.93	2.78	18.93	70.00	2.63	2.16	17.67	80.00	2.73	2.45
IBA (1.5 mg l^{-1})	21.17	33.33	2.03	2.06	19.53	56.67	2.37	2.24	22.93	26.67	1.83	1.63	20.53	43.33	2.07	1.85
IBA (2 mg l^{-1})	24.17	26.67	1.83	1.80	23.13	36.67	2.17	2.03	25.13	20.00	1.43	1.32	23.97	30.00	1.63	1.63
Control-(Basal ½ MS)	27.57	16.67	0.93	1.54	26.37	23.33	1.33	1.74	29.83	6.67	0.73	1.03	28.17	13.33	0.87	1.24

Note: Mean values were calculated at CD(P= 0.05) by FCRD.

Table 5: Effect of sterilizants on rescue of contaminated cultures

Treatments	Rescue percentage (%)			
	Arka Shravya		Pondicherry Local	
	Shoot tips	Nodal segments	Shoot tips	Nodal segments
70% Ethanol (30 sec.) + 0.1% Streptomycin (15 min.)	0.00	3.33	0.00	3.33
70% Ethanol (30 sec.) + 0.2% Streptomycin (15 min.)	10.00	16.67	0.67	13.33
70% Ethanol (30 sec.) + 0.3% Streptomycin (15 min.)	30.00	43.33	23.33	33.33
70% Ethanol (30 sec.) + 0.4% Streptomycin (15 min.)	16.67	36.67	10.00	26.67

Note: Mean Values were calculated at CD (P= 0.05) by FCRD.

Shoot regeneration and multiplication

In vitro rooting

Conclusion

The present study led to the inference that, among the varieties Arka Shravya performed better than Pondicherry Local in response to *in vitro* shoot regeneration, multiplication, elongation and rooting. Among the explants, nodal segments performed better than the shoot tips in all the growth characters. Hence, micropropagation of crossandra using nodal segments as explants could be a viable and rapid means of producing disease free true-to-type plants without segregation.

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