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In-vitro Clonal Propagation of an Indian Medicinal Plant- *Justicia procumbens*

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Abstract

In vitro cultures of an Indian native medicinal plant *Justicia procumbens* was established on MS and low cost KFAplus medium for its clonal propagation and assess the efficiency of the two media pertaining to the growth and multiplication. High percentage of shooting was observed in MS medium supplemented with IBA (0.5mg/L) and FAP (1.0mg/L) in about 21days and in KFA plus medium in about 20 days. Healthy and long roots were obtained using both the media. Hardening of the tissue cultured plants were carried out in soil under artificial conditions and 100% survivability was recorded. This was the first report of rapid multiplication of *Justicia procumbens* in *in-vitro* condition using MS and Low cost KFAplus medium.

Keywords: Medicinal plant, *Justicia*, KFAplus, in vitro, Clonal propagation

INTRODUCTION

Since ancient times, mankind has been dependent on plants for food, feed, flavours, medicine and many other uses. Ancient written records of many civilizations including India, give strong evidence regarding use of medicinal plant. In majority of the developing countries, people of rural areas use folk medicine made from plants and plant parts for the treatment of various diseases and ailments. The World Health Organisation (*WHO*) reported that 80% of people in the developing world use medicinal plants for their primary health care. At present there are many well established herbal and plant medicine practices which are popular in many parts of the world as complementary and alternative medicine (CAM) therapy[1].Many plant based remedies are back in use and find increasing applications as source of direct

therapeutic agents, as a raw material base for deriving more complex semi-synthetic chemical compounds, as models for new synthetic compounds, and as taxonomic markers for the discovery of new compounds.

Most of these above applications are based on phytochemicals like alkaloids, phenols, flavones, flavonoids, tannins, coumarins, and essential oils, which are mainly plant's secondary metabolites. These metabolites work either independently or in coherence with other metabolites. It is an established fact that there is an enhancement of secondary metabolites when plants are cultured *in vitro*[2,3]. Keeping in view the enormous plant resource which can be judicially exploited for medicinal purposes we have attempted *invitro* multiplication of one such native Indian plant *Justicia procumbens* using plant tissue culture technique. The genus Justicia belongs to family Acanthacae comprising of large number of species with known medicinal properties. *Justicia procumbens* is used in the southern parts of India as folk medicine and highly valued for its uses in treating cold, cough, asthma and for snake bites. For the *in vitro* multiplication of *J. procumbens* we used Murashige and Skoog[4]medium (MS) as well as a low cost Kakoli Fly Ash medium (KFA and KFAplus)[5]. Various medicinal and ornamental plants *Mentha sp., Lilium asiatic, Bacopa monnereii, Petunia hybrida, Dianthus caryophylus* have been well established on KFA media and have been successfully transferred to soil[6-8].

MATERIALS AND METHOD

Source of plant material: *Justicia procumbens* plant was obtained from Andhra Pradesh, India.

Establishment of culture: Aseptic cultures were established from the nodal explants with axillary buds. The explants were washed thoroughly with water to remove the dirt and surface sterilized with 70% ethanol for 30 seconds followed by 0.1% (w/v) mercuric chloride for 7 minutes followed by rinsing with sterile distilled water thrice and inoculated in KFA, KFA plus (KFA+) which had 10% Flyash (FA) as the main source of inorganic constituent and MS 11 medium which was used as control. KFA plus was supplemented with nitrogen source comprising of Glycine 2mg/l, Nicotinic acid 0.5 mg/l, Pyridoxine-HCl 0.5mg/l, Thyamine-HCl 0.1mg/l while KFA was devoid of nitrogen source. All the three media were supplemented with 3% sucrose (w/v) as carbon source, 0.8% agar (w/v) and plant growth regulators (PGRs) in the combinations/concentrations ofIBA-0.5mg/L, FAP (Kinetin)-1.0mg/L. The pH of all media was set to 5.7. All cultures were set in ten replicates for each combination and the experiments were repeated thrice. The cultures were maintained at 25±2^{o}C, 1500 lux intensity, photoperiod of 16hrs light/8hrs dark regime and 75% humidity. The cultures were routinely observed for any contamination. Different growth parameters like bud break with respect to number of days after inoculation; % shoot growth,

average bulblets/explants, % rooting and morphological characters of regenerated plantlets were recorded routinely. Statistical analysis was carried out by calculating Mean, Standard Deviation and Standard Error for induction frequencies of the explants and % shooting. Analysis of Variance (ANOVA) and Student t-test was performed for the media type and their response to culture of plants. All statistical analysis was performed by using SPSS software program.

RESULTS AND DISCUSSION

Nodal explants from *Justicia procumbens* were cultured on MS medium supplemented with IBA(0.5mg/L) and FAP (1.0mg/L) in which 98.33% shooting was observed in about 21days. When cultured on KFA plus medium 81.66% shooting was observed in around 20 days (Table 1, Figs: 1, 4 and 5).

Table 1: Student t-Test for percent shooting, mean shoot/explants and percentage rooting in MS and KFA+ medium

	Media	Test Value = 0					
		T	df	Significant	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
percent shooting in MS and KFA+ medium	MS	59.00	5	.000	98.33	94.049	102.617
	KFA+	26.57	5	.000	81.66	73.766	89.566
Mean shoot/explants in MS and KFA+ medium	MS	6.693	5	.001	1.72	1.057	2.376
	KFA+	11.311	5	.000	1.18	0.914	1.452
percentage rooting	MS	7.416	5	.001	73.33	47.914	98.751
	KFA+	4.332	5	.007	28.33	11.520	45.146

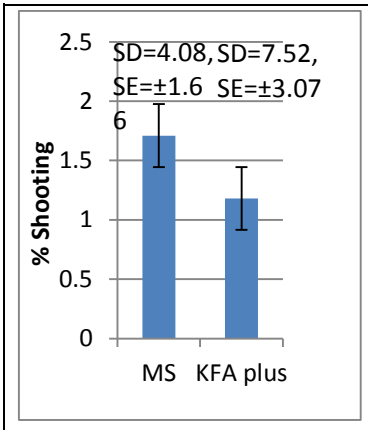

Figure 1: Percentage of shooting in *Justicia procumbens* on MS and KFA+ media.

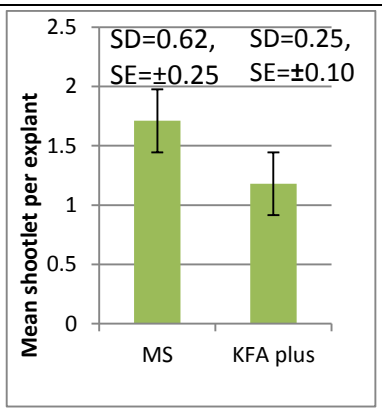

Figure 2: Mean shootlet/explant in *Justicia procumbens* on MS and KFA+ media.

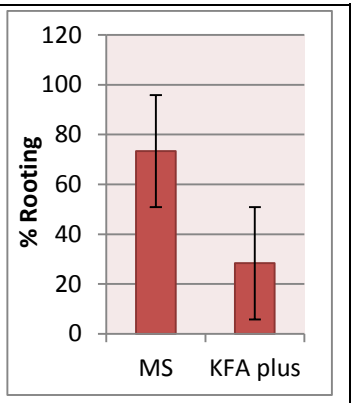

Fig 3: Percentage of rooting in *Justicia procumbens* on MS and KFA+ media.

Fig 3: Shootlets of *J. procumbens* Developed on MS medium supplemented IBA (0.5mg/L) and FAP (1.0mg/L).

Fig 4: Shootlets of *J. procumbens* Developed on KFA+ medium supplemented IBA (0.5mg/L) and FAP (1.0mg/L).

Fig 6: Rooting of *J. procumbens* on MS medium supplemented with IBA (0.5mg/L) and FAP (1.0mg/L

Fig 7: Rooting of *J. procumbens* on KFA + medium supplemented with IBA (0.5mg/L) and FAP (1.0mg/L

Fig8: Hardening of *J. procumbens* in pots.

Mean shootlet/explant: Plants were subcultured and mean shootlet/explant with SD±SE were calculated. Mean shootlet/explant of 1.71 was observed in MS. Similarly, mean shootlet/explant of 1.18 was observed in KFA+ (Table1, Figs 2).

Percentage Rooting: Extensive fibrous roots were obtained in each plantlet in the medium supplemented with IBA (0.5mg/L) + FAP (1.0mg/L). Roots obtained were long and healthy. The percentage rooting was 73.33% in MS medium supplemented with PGR's and 28.33% rooting was observed in KFA+ medium (Table 1, Figs 3, 6,

7).

Hardening: Hardening was carried out in soil under artificial conditions (Fig: 8). Plants were given high to low humidity and low to high light intensity from sun. On hardening in proper conditions of light and humidity each and every plantlet survived. Therefore, the net survivability was 100%.

In the present study high percentage shooting was obtained after 21 days in both MS and KFA+ medium (Table:1) supplemented with IBA (0.5mg/L) + FAP (1.0mg/L) as compared to the previous study on *Justicia adhatoda* where only 72% shooting was observed from nodal explants in MS medium containing after 20-25 days in the presence of coconut water (15% v/v) and BAP (5mg/L) while MS supplemented with BAP (1mg/L) with 10% coconut milk gave 80% response within 3 weeks[9]. A maximum of 86% response was reported in *J. gendarussa* in MS with BAP (2mg/l)[10]. An efficient and optimal plant regeneration of *J. prostrata* through direct organogenesis from nodal explants was obtained in different concentrations of various growth regulators (2, 4-D, BAP, NAA and IAA). It was observed that nodal explants showed growth response by direct regeneration, enlargement and initiation of callus[11]. Initially mean shootlet per explant obtained were 1.71±0.62 and subsequently each shoot provided 4-5 explants which is less as compared to obtained from the callus, as reported by Agastian *et al*[12]. However, the advantage of our study was the maintenance of homogeneity as they were developed from axillary buds while callus tends to show somaclonal variation thereby causing genotypic as well as phenotypic variation in the final in vitro produced plants[13]. Direct organogenesis and multiplication is always favourable to maintain the genetic makeup (clones), in comparison to organogenesis via callus where the chances of somaclonal variation more. The less number of shoots as compared to previous reports may also be due to species variation, as well as absence of coconut water which was added in the other plants. In this study, MS medium gave high mean shootlet/explants as compared to KFA+ medium. Statistically MS medium proved to be slightly better than KFA+ medium when the data for both % shooting and mean shoots/explants were analyzed using Student t-test.

We obtained 73.33% in MS medium and 28.33% rooting in KFA+ medium both supplemented with IBA (0.5mg/L) and FAP (1.0mg/L) after 21 days. Rooting was observed in the same hormone concentration and combination as that of shooting media. However basal MS medium proved to be competent to induce 100% *in vitro* rooting within 20-22 days. Similar results were reported in *J. adhatoda* and *J. gendarussa*[9,14]. Basal medium devoid of growth regulators create a stress condition to the shoots thereby forcing the shoots develop more roots. In MS medium the per cent rooting was found to be high in comparison to KFA+ as determined statistically. In our previous studies for assessing the efficiency of low cost KFA+ medium on other plant species on the basis of per cent shoot production, rooting and shoots/explant we

found KFA+ to be statistically more efficient as compared to widely used MS medium[6-8]. This could be attributed to the variable response of this particular plant to different medium.

The objective of the present work was to establish the culture of *Justicia procumbens* on MS, and KFA+ media for *in vitro* clonal propagation and to assess the efficiency of the two media pertaining to the growth and multiplication of this plant. Rapid multiplication of whole plants was achieved in short time period in both the medium. KFAplus medium can very well be used to micropropagate this plant thereby reducing the cost of in vitro propagated plants.

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