FACTORS AFFECTING MICROPROPAGATION OF ANOGEISSUS ACUMINATE. A TREE OF STRESSED ECOSYSTEM.

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ABSTRACT

Various Experiments viz. Types of Seedling Explants (epicotyledonary nodal segments, cotyledonary nodal segments, hypocotyl, cotyledon etc.) types of growth regulators (KN, BAP,IAA,NAA) and its various concentration were conducted to standardized the methods of in vitro micropropagation of A. acuminata. Out of Various types of explants used, the cotyledonary nodal shoot segments found the best for shoot induction. Highest number of shoot bud proliferation i.e. 15-20 shoots was found on MS medium supplemented with IAA 0.1mg/l + BAP 1.5 mg/l, while only 4-6 shoots were produced from epicotyledonary nodal shoot segments .Poor response i.e. only callusing was observed on hypocotyl and cotyledon explants. On higher concentration of BAP i.e. more than 2.5 mg/l, although number of shoot were increased but they remain dwarf. On Higher concentration of IAA and NAA, callusing was observed.

Keywords: explants , in vitro, medium ,callus. Shoot induction, seedling

INTRODUCTION

Anogeissius acuminata (Roxb.ex Dc) Guill & Perr. is belongs to Family Combretaceae a majestic hardwood tree of a stressed ecosystem with special reference to Rajasthan .It grows upto a height of 15-20 meter with cylindrical bole and 1meter of girth .It provides fuel . fodder, gum, tannins and timbers. The timbers of A. acuminata has a great potential values because of its strength and working qualities. The plant is extensively used for making energy rich charcoal (Sharma and Tiagi 1979).

In nature it is mainly propagate through seeds but it has very poor seed viability i.e. 0.5-1.0% only . This is a common problem with other species of Anogeissus (Joshi et al 1991). Due to over exploitation and poor propagation the population of the plant is declining with alarming rate . Conventional method of propagation like grafting , cutting, air layering etc are not reported in Anogeissius species .Therefore non-conventional method i.e. plant tissue culture offers a great potential to propagate this plant at large scale (Thorpe 1985,Boulay 1985,Chalupa1988).

MATERIALS AND METHODS

Extensive field survey has been made in order to collect seeds. The fruits were collected during Feb –April from A. acuminata growing in various site like Parshuram ,Ranakpur(Pali),different foot heals area of Aravlis ,Mt. Abu. Kaylana (Jodhpur) etc. The seeds were collected by mechanical dissecting out of the fruit. These seeds were washed in tap water followed by tween 80. Then these seeds were surface sterilized with 70% of ethanol for 40-60sec. followed by 0.1% of mercuric chloride for3-4 minutes. Then these seeds are washed with sterile distilled water for 4-6 times. These surface sterilized seed were Inoculated on hormone free MS (Murashiege and Skoog 1962) medium for germination.

The explants viz. epicotyledonary nodal seqments ,cotyledonary nodal seqments hypocotyl, cotyledon etc were taken from four week old in vitro grown seedling .

These explants were further cultured on various concentration (0.1 to 2.5 mg/l) and types of cytokinin (KN & BAP) on MS medium supplemented with 0.1mg/l of IAA+ additives(ascorbic acid 50 mg/l, citric acid 25 mg/l,arginine25 mg/l, adenine sulphate 25 mg/l).

RESULTS AND DISCUSSION

Viable seeds were collected by mechanically removal of fruit wall. These viable seeds were surface sterilized and inoculated on hormone free MS medium for germination. Different types of explants viz. cotyledon, epicotyledonary node, cotyledonary node,

hypocotyl were taken from in vitro grown seedling. Out of Various type of explants were used , the best result were recorded from cotylenonary nodal segments on MS medium supplemented with IAA 0.1mg/l + BAP 1.5mg/l +additives (Rathore et al 1993).Highest number of shoots i.e. 15-20 were produce on cotyledonary nodal segments compare to epicotyledony nodal segment (6-10short/node). No shoot induction were observed from hypocotyl region and cotyledon (Table-1) .

Different types of cytokinin (KN, BAP) with various concentration (0.1-2.5mg/l) along with auxin (IAA & NAA) were tested for shoot induction(Table 2,3) .Highest number of shoots 15-20/node were produced on MS medium supplemented with BAP 1. 5mg/l+ IAA 0.1mg/l compare to KN . On same combination of KN with IAA lesser number of shoots i.e. 6-10 shoot per nodal segment were produced. The high callus intensity were found on higher concentration of IAA and NAA. It was further observed that higher temperature i.e. 28±2 C under 12h of photoperiods enhance shoot bud proliferation. Callusing was observed when culture kept under dark condition (Shekhawat N S et al 1993). Each experiment were consisted of fifteen replicate and repeated thrice.

Table 1. Effect of explants type on multiple shoot induction on MS medium containing 0.1mg/l IAA + additives +various concentration of BAP

Explant Used	BAP mg /l	Number of shoots/explants ± SD	Shoot length (cm) ±SD	Callus intensity
Epicoty ledonary nodal segments				
	0.1	2.3±0.4	2.2±0.3	++
	0.5	3.4±0.4	2.2±0.2	-
	1.0	5.6±0.6	2.4±0.4	-
	1.5	6.2±0.7	2.4±0.6	-
	2.5	7.6±0.8	1.8±0.5	-
Cotyledonary nodal segments				
	0.1	1.9±0.2	1.6±0.5	+
	0.5	5.9±0.8	1.9±0.4	+
	1.0	11.4±1.6	2.2±0.6	-
	1.5	16.8±2.2	3.5±0.8	-
	2.5	22.5±2.8	1.6±0.4	-
Hypocotyl				
	0.1	-	-	+++
	0.5	-	-	++
	1.0	-	-	++
	1.5	-	-	+
	2.5	-	-	-
cotyledon				
	0.1	-	-	+++
	0.5	-	-	++
	1.0	-	-	++
	1.5	-	-	+
	2.5	-	-	-

- =No callusing ; + =Little callusing ; ++ moderate callusing, +++ = vigorous callusing

Table 2 . Effect of BAP 1.5mg/l + auxins (IAA & NAA) on multiple shoot induction from cotyledonary nodal explant of A. acuminata on MS medium +additives.

Types of auxins	Number of shoots ±SD	Length of Shoots (cm)± SD	Callusing
Control	6.18±0. 83	1.83±1. 29	
IAA			
0.05	8.87± 0.78	3.62±0.30	
0.1	16.44±1.94	3.91 ±0.28	
0.5	8.55±0.68	2.76±0.22	+
1.0	6.71±0.45	2.54±0.59	++
2.5	4.25±0.82	1.83±0.59	+++
NAA			
0.05	6.90±0.89	7.07±0.18	
0.1	7.50±0.50	4.80±0.41	+
0.5	5.00±0.80	2.98±0.39	++
1.0	4.60±0.89	1.77±0.29	++
2.5	1.54±0.49	1.45±0.53	+++

- =No callusing ; + =Little callusing ; ++ moderate callusing; +++ = vigorous callusing

Table 3 . Effect of cytokinin (BAP & KN) + 0.1mg/l IAA on multiple shoot induction from cotyledonary nodal explant of A. acuminata on MS medium + additives.

Types of cytokinins	Number of shoots ± SD	Length of Shoots (cm) ± SD	Callus intenasity
Control	1. 4±0. 2	2 . 2± 0. 4	+++
KN			
0.1	2 . 8± 0 .4	1 .2±0 . 4	++
0.5	4 .6±0.6	2.1±0.3	+
1.0	6.2±0.6	2.1±0.3	-
1.5	10.7±0.8	1.4±0.4	-
2.5	14.6±0.8	0.9±0.1	-
BAP			
0.1	1.9±0.2	1.6±0.5	+
0.5	5.9±0.8	1.9±0.4	+
1.0	11.4±1.6	2.2±0.6	-
1.5	16.8±2.2	3.5±0.8	-
2.5	22.5±2.8	1.6±0.4	-

- = No callusing ; + = Little callusing ; ++ moderate callusing; +++ = vigorous callusing

Abbreviations: IAA-Indole-3-acetic acid; BAP-6-Benzyleaminopurine; KN-Kinetin

NAA-Napthalene acetic acid; MS-Murashige and Skoog ; SD-Standard deviation

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