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Research Article

RAPID *IN VITRO* PROPAGATION OF *ACACIA AURICULIFORMIS* ON SOLID AND LIQUID MEDIA: ROLE OF ORGANIC ADDITIVE, ANTIOXIDANT AND PLANT GROWTH REGULATORS

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ABSTRACT
Acacia auriculiformis, one of the short rotation and fast growing tree species is known for its pharmacological properties notably anti-helminthic, antifilarial, microbicidal, spermicidal activity etc. Conservation of *Acacia auriculiformis* is essential via ex situ plantation as the seed germination rate of this plant species has been reported very poor around 20-28%. Clonal multiplication forms an alternative means of propagating elite trees by preserving their desirable characters intact. Cotyledon explants of *Acacia auriculiformis* were cultured on MS solid and liquid media supplemented with 15% CW, ascorbic acid and different cytokinins like (Δ^2 isopentenyl) adenine, benzylaminopurine and kinetin in varying concentrations for shoot bud regeneration. Among the different PGRs tested, (Δ^2 isopentenyl) adenine (2iP) proved best in terms of the shoot bud regeneration directly from de-embryonated cotyledon. Napthalene acetic acid at lower level combined with various levels of 2iP was applied for differentiation of shoot buds from intermediary callus phase. The response of the explants was always higher in liquid cultures compared to semi-solid medium. More than 90% of the transplanted plants were survived. Transplanted plants showed normal seedling morphology and growth.

Key Words: *Acacia auriculiformis, Cotyledon, Organogenesis, (Δ^2 isopentenyl) Adenine*

Abbreviations: 2iP- (Δ^2 isopentenyl) adenine, ANOVA- Analysis of variance, BAP- N^6-benzylaminopurine, CW- Coconut water, DMRT- Duncan's multiple range test, KN- Kinetin, MS- Murashige and Skoog medium, NAA- Napthalene acetic acid, PGR- Plant growth regulator, SE- Standard error

INTRODUCTION
Plantation forestry is important to the world economy which provides renewable energy, fibre and timber as well as maintains and preserves the ecological balance. Forest trees pose a long generation time and this has been the major obstacle in traditional breeding. In addition, the high heterozygosity of forest trees especially those, which are mainly propagated by seeds hamper improvement by conventional breeding techniques. Thus large scale clonal propagation of superior clones along with accelerated tree improvement programs are necessary for successful reforestation and forest management programs. *Acacia auriculiformis*, one of the short rotation and fast growing tree species is known for its pharmacological properties notably anti-helminthic, antifilarial, microbicidal activity etc. (Mandal *et al.*, 2005; Ghosh *et al.*, 1993). The seeds of this tree are rich source of triterpinoid saponins especially, Acaciaside-A (Ac-A) and Acaciaside-B (Ac-B). Earlier investigations have demonstrated that Ac-B isolate from seed extracts of *A. auriculiformis* possess spermicidal activity (anti-fertility) even at significantly lower concentrations (Pakrashi *et al.*, 1991; Pal *et al.*, 2009) especially safe for vaginal epithelia (Pal *et al.*, 2009) and most importantly inhibits transmission of HIV (Kabir *et al.*, 2008) without any mutagenic effect (Pal *et al.*, 2009). Trees producing high concentrations of desirable secondary metabolites need to be screened and propagated clonally; because seed is a product of genetic recombination and can results in segregation of alleles leading to loss of desirable traits (Fenner and Thompson, 2005). On the contrary, clonal multiplication forms an alternative means of propagating elite trees by preserving their desirable characters intact (Mittal *et al.*, 1989).

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The natural resurgence of *Acacia auriculiformis* is through seeds. However, their germination rate has been reported very poor around 20-28% (Udaykumar et al 1995), which was attributed in part to the impervious seed coat (Bonga and Aderkas, 1997). Therefore, conservation of Acacia is essential via ex situ plantation, for which large scale planting materials would be required. In view of the problems of conventional propagation of *Acacia auriculiformis* and a high demand of planting material in addition to maintain the superiority of the elite germplasm, the large scale multiplication of this tree species in a short span of time using various in vitro techniques is the need of time.

Although micropropagation of different species of *Acacia* has been reported by various researchers (Dawan *et al.,* 1992; Beck *et al.,* 2000; Xie and Hong 2001; Vengadesan *et al.,* 2002; Maity *et al.,* 2007), high frequency rapid *in vitro* propagation of *Acacia auriculiformis* through direct and indirect organogenesis on solid and liquid media supplemented with various cytokinins like 2iP- (Δ^2 isopentenyl) adenine (2iP), N^6- benzylaminopurine (BAP) and kinetin (KN) in varying concentrations, coconut water (CW) and antioxidant has not been done yet. Therefore, the objective of the present investigation was rapid in vitro propagation through different ways.

MATERIALS AND METHODS

Seeds of *Acacia auriculiformis* were collected from the forests of Santiniketan, Birbhum, West Bengal, India. They were washed in 2% (V/V) detergent solution "Teepol" (Qualigens, India) and subsequently surface sterilized in 0.1% (w/v) aqueous mercuric chloride solution for 12 minutes. After rinsing 4 times with sterile distilled water seeds were transferred to moist cotton beds in 250 ml Erlenmeyer flask and incubated in the culture room for 7 days under 10 hours of photoperiod of 37.5 µ mol. $m^{-2}s^{-1}$ light intensity. Cotyledons were excised from 7-day old axenic seedlings and were inoculated onto MS basal media (Murashige and Skoog 1962) supplemented with 15% CW with or without ascorbic acid (10 $mg.dm^{-3}$) and various concentrations of different cytokinin like BAP, KN and 2iP (0.5 to 4 $mg.dm^{-3}$) either singly or in combination for *de novo* formation of shoot buds. Shoot buds produced at higher concentrations of cytokinin were sub-cultured on MS solid and liquid media supplemented with lower levels of cytokinin (0.1 - 0.5 $mg.dm^{-3}$) or without cytokinin for subsequent leafy shoot emergence. Excised cotyledon explants were inoculated onto MS basal media supplemented with various concentrations and combinations of napthalene acetic acid (NAA) (0.2 to 4.0 $mg.dm^{-3}$) and 2iP (0.2 to 4.0 $mg.dm^{-3}$) with or without 15% CW and ascorbic acid (10 $mg.dm^{-3}$) for initiation of callus and subsequent shoot bud regeneration through organogenesis. Friable callus of 45 days old, were sub-cultured on MS media with various concentrations of 2iP (0.2 to 4.0 mg dm^{-3}) in combination with a constant level of NAA (0.2 $mg.dm^{-3}$) with or without 15% CW and ascorbic acid (10 $mg.dm^{-3}$) for regeneration of shoot buds. Shoot buds were transferred to both on the solid and liquid media with lower levels of 2iP (0.1-1.0 $mg.dm^{-3}$) and NAA (0.2 $mg.dm^{-3}$) for emergence of leafy shoots.

Twenty five replicates were used per treatment. The pH of the media was adjusted to 5.8 prior to autoclaving. The media were solidified with 0.8% w/v agar. Routinely, 25 ml molten medium was dispensed into culture tube (25 x 150mm), plugged with non-absorbent cotton and subsequently sterilized at 121ºC and 102 x 10^{-6} $kg.m^{-2}$ pressure for 15 min. Cultures were incubated at 25 ± 2ºC under a light intensity 37.5 µ $mol.m^{-2}s^{-1}$ under a photoperiod of 10 hours.

The regenerated shoots were excised from the parent culture and transferred to half strength MS semi-solid medium with different concentrations of NAA (0.5to 4 $mg.dm^{-3}$) for induction of roots. *In vitro* grown plants with well developed root systems were first washed under running tap water and transferred to plastic cups containing sterile sand-soil mixture (1:1) with adequate water. Surviving plants were finally transplanted to the soil on earthen pots. The process of transfer and gradual acclimatization to the natural conditions were stringently monitored.

The experimental units were assigned to "randomized complete block design" with single replicate per block. The mean values of different morphogenetic responses were shown along with their respective standard errors (SE) and were analyzed by analysis of variance (ANOVA). After obtaining a significant F value ($\alpha = 0.05$) the treatment means were separated by Duncan's Multiple Range Test (DMRT) in case of single Factor ANOVA only. Statistical analyses were performed according to Little and Hills (1978).

RESULTS AND DISCUSSION

Direct organogenesis

In vitro response of cotyledon explant of *Acacia auriculiformis* cultured on MS solid and liquid media supplemented with 15% CW, ascorbic acid and different cytokinins in varying concentrations after 60 days of incubation has been presented in Table 1. Direct differentiation of shoot buds (Fig. 1A) was monitored in cotyledon explants both on the PGR-free and PGR supplemented media. PGR-free control did not exhibit shoot bud formation. Compared to control, the use of cytokinins (2iP, BAP and KN) exhibited marked morphogenetic changes, although very high concentration suppressed the shoot bud regeneration potential of the explant. Direct regeneration of shoot buds has also been reported in a number of tree legumes viz., *Albizia lebbeck, Dalbergia paniculata, Leucaena leucocephala and Acacia auriculiformis using* different cytokinins at various levels (Raju *et al.,* 1984; Goh *et al.,* 1990; Maity et al 2005 and 2007).

Although swelling of cotyledon explant occurred on different culture media, *de novo* differentiation of shoot buds and further development of plantlets did not occur easily and prudent application of PGRs was necessary to achieve that. Thus it was observed that exogenous PGRs were crucial for improved development of shoot buds as well as for plantlet formation which is in conformity with the observation of Banerjee *et al.,* (2007 and 2011) in another leguminous species.

Among the different PGRs tested, 2iP proved best in terms of the shoot bud regeneration directly from de-embryonated cotyledon. However, considerable enhancement in response was achieved with the application of an antioxidant, ascorbic acid (10 mg/l) combined with PGRs. In all the instances the explants that failed to initiate shoot bud differentiation remained in a swollen green condition throughout the culture period. The best response in terms of number of shoot buds regenerated per cotyledon explant was observed in presence of 2mg dm^{-3} 2iP combined with CW (15% V/V) and ascorbic acid (10 mg/l).

In the experimental time frame, less than half of the explants developed swelling in the control, indicating a dormant stage of the explants. However, formation of shoot buds was triggered by the application of exogenous cytokinins. Therefore, it appears that the inactiveness of explants in the control could be due to lack of adequate endogenous growth regulators (Roy and Banerjee 2000). Although shoot bud development was common for each of the treatments supplemented with different kinds of cytokinins, the differential response of the explants to the media supplemented with different kinds of exogenous cytokinins was apparent from the differences in the number of shoot buds developed per explant on the media supplemented with different cytokinins. Previous reports on the culture of trees exhibited the effectiveness of BAP but the present investigation clearly demonstrated the supremacy of 2iP. The response of the explants in terms of the number of shoot bud development is enhanced with the addition of CW and ascorbic acid. CW is an undefined complex mixture of organic substances that has been successfully employed for culturing different plant species (Skolmen and Mapes 1976; Arditti and Ernst 1993; Suttle1996).

However, ascorbic acid (10 mg/l) is very much effective as anti-oxidants and adsorbents to prevent the tissue browning as well as the media, due to exudation of phenolics from the cultured tissue which is in conformity with the observation made by (Rao *et al.,* 1989; Singh *et al.,* 1993; Gupta et al.1994; Das *et*

al., 1996; Bhaskar et al.1996; Kaur et al.1998; Vengadesan *et al.,* 2000). Exudation of phenolics is a natural mechanism in plants. Furthermore, the number of shoot buds induced per explant was increased upon supplementation of ascorbic acid (10 mg/l) in the media.

Table 1: Direct regeneration of shoot buds on cotyledon explant of *Acacia auriculiformis* cultured on MS solid and liquid media supplemented with 15% CW, ascorbic acid and different cytokinins in varying concentrations (Response recorded after 60 days of incubation)

PGR ($mg.dm^{-3}$)	Mean number of shoot buds ± SE	
2iP	Solid media	Liquid media
0.0	0.0±0.0	0.0±0.0
0.5	$54.98±5.21^{g}$	$95.25±4.84^{i}$
1.0	$76.23±7.03^{f}$	$120.44±4.22^{g}$
2.0	$185.12±4.02^{b}$	$234.62±3.63^{b}$
2.0*	$196.68±2.44^{a}$	$245.36±2.82^{a}$
3.0	$128.71±6.97^{d}$	$178.87±3.28^{d}$
4.0	$94.06±6.32^{e}$	$122.48±3.66^{g}$
BAP		
0.5	$48.34±6.01^{h}$	$87.23±3.25^{ij}$
1.0	$69.53±9.32^{fg}$	$113.02±7.45^{gh}$
2.0	$135.26±7.014^{d}$	$188.36±6.68^{d}$
2.0*	$162.43±4.02^{c}$	$211.33±5.28^{c}$
3.0	$130.97±3.88^{d}$	$180.62±2.68^{d}$
4.0	$102.46±5.66^{e}$	$131.54±4.02^{f}$
KN		
0.5	$28.41±2.33^{i}$	$52.38±3.68^{l}$
1.0	$37.25±3.24^{h}$	$66.23±3.24^{k}$
2.0	$44.38±6.12^{h}$	$71.64±5.34^{k}$
3.0	$58.82±4.66^{g}$	$82.52±5.33^{j}$
3.0*	$79.26±10.28^{f}$	$104.26±4.28^{h}$
4.0	$51.61±4.34^{gh}$	$80.22±6.42^{j}$
2.0BAP+0.5KN	$165.74±3.54^{c}$	$221.38±6.68^{c}$
2.0BAP+1.0KN	$178.33±12.62^{c}$	$227.44±3.46^{c}$
2.0BAP+2.0KN	$124.42±6.56^{d}$	$168.82±2.66^{e}$

Mean values followed by same letter within a column are not significantly different at 0.05 levels (DMRT) *Media contain ascorbic acid 10 $mg.dm^{-3}$

Table 2: Emergence of leafy shoots from tiny shoot buds of *Acacia auriculiformis* on MS solid and liquid media supplemented with 15% CW and different PGRs (Response recorded after 60 days of incubation)

PGR ($mg.dm^{-3}$)	Percentage of leafy shoot emergence ±SE	
2iP	Solid media	Liquid media
0.0	$52.06±1.89^{c}$	$64.68±2.65^{c}$
0.1	$64.28±3.22^{a}$	$85.21±1.31^{a}$
0.2	$56.62±1.42^{b}$	$63.64±1.88^{c}$
0.4	$44.56±2.33^{d}$	$57.24±0.66^{d}$
0.5	$28.11±1.25^{f}$	$31.42±2.64^{f}$
BAP		

0.1	61.22±2.42[a]	82.26±1.33[a]
0.2	58.64±2.12[ab]	63.56±2.21[c]
0.4	47.64±2.88[d]	49.55±1.68[e]
0.5	33.46±1.64[e]	34.21±1.28[f]
KN		
0.1	60.33±2.24[a]	71.26±2.42[b]
0.2	67.54±1.88[a]	83.62±1.06[a]
0.4	58.53±2.66[ab]	71.44±1.28[b]
0.5	44.62±1.22[d]	55.62±2.32 [d]

Mean values followed by same letter within a column are not significantly different at 0.05 levels (DMRT)

Table 3: Induction of callus using cotyledon explant of *Acacia auriculiformis* on MS solid media supplemented with 15 % CW, ascorbic acid (10 mg.dm^{-3}) and varying concentrations of NAA and 2iP (Response recorded after 45 days of incubation)

PGR (mg.dm^{-3})		Frequency of callus formation (%)	**Nature of callus	*Amount of callus
NAA	2iP			
0.0	0.0	No response	-	-
0.0	0.2	No response	-	-
0.0	0.5	No response	-	-
0.0	1	No response	-	-
0.0	2	No response	-	-
0.0	4	No response	-	-
0.0	8	No response	-	-
0.2	0	No response	-	-
0.5	0	No response	-	-
1	0	No response	-	-
2	0	No response	-	-
4	0	No response	-	-
8	0	No response	-	-
1.0	0.2	100	Friable white callus	++
1.0	0.5	100	Friable white callus	++
1.0	1.0	100	Friable white callus	+
2.0	0.2	100	Friable white callus	+++
2.0	0.5	100	Friable white callus	+++
2.0	1.0	100	Friable white callus	++
4.0	0.2	100	Friable white callus	++++++
4.0	0.5	100	Friable white callus	++++++
4.0	1.0	100	Friable white callus	++++++
8.0	0.2	100	Callus with profuse rooting	++++
8.0	0.5	100	Callus with profuse rooting	++++
8.0	1.0	100	Callus with profuse rooting	++++
8.0	2.0	100	Callus with profuse rooting	++++

** '+' sign denotes relative amount of callus (eye-estimated); '-' sign denotes no callus formation*

***Callus grown on the media without CW and ascorbic acid tend to be brown and necrotic*

Table 4: Regeneration of shoot buds from callus through organogenesis in *Acacia auriculiformis* on MS solid media supplemented with varying concentrations of different PGRs in presence or absence of 15% CW and 10 mg/l ascorbic acid (Response recorded after 60 days of incubation)

PGR ($mg.dm^{-3}$)			Mean no. of shoot buds ± SE
2iP	NAA	CW and Ascorbic acid	
0.0	0.0	+	Callus growth arrested*
0.0	0.2	+	Soft, white and friable callus*
0.2	0.0	+	Soft, white and friable callus*
0.5	0.0	+	Soft, white and friable callus*
1.0	0.0	+	Soft, white and friable callus*
2.0	0.0	+	Soft, white and friable callus*
4.0	0.0	+	Green nodular callus
0.2	0.2	+	36.33 ± 5.47^{f}
0.2	0.2	-	18.32 ± 3.27^{g}
0.5	0.2	+	74.60 ± 9.22^{d}
0.5	0.2	-	71.33 ± 6.42^{d}
1.0	0.2	+	128.5 ± 6.42^{b}
1.0	0.2	-	117.5 ± 4.42^{bc}
2.0	0.2	+	162.1 ± 5.48^{a}
2.0	0.2	-	123.1 ± 7.22^{b}
4.0	0.2	+	61.88 ± 4.37^{e}
4.0	0.2	-	65.60 ± 5.97^{de}

*Mean values followed by same letter within a column are not significantly different at 0.05 levels (DMRT) '+' sign denotes presence of CW and ascorbic acid and '-' sign denotes absence *Callus grown on the media without CW and ascorbic acid tend to be brown and necrotic*

Table 5: Emergence of leafy shoots from tiny shoot buds (regenerated through organogenesis from callus) of *Acacia auriculiformis* on MS solid and liquid media supplemented with 2iP and NAA (Response recorded after 60 days of incubation)

PGR($mg.dm^{-3}$)		Percentage of leafy shoot emergence ±SE	
2iP	NAA	Solid media	Liquid media
0.0	0.0	38.56 ± 1.58^{b}	47.35 ± 2.97^{b}
0.1	0.2	53.28 ± 1.46^{a}	67.66 ± 2.45^{a}
0.2	0.2	34.44 ± 1.64^{c}	48.6 ± 1.58^{b}
0.5	0.2	32.51 ± 2.88^{c}	41.4 ± 2.74^{c}
1.0	0.2	26.64 ± 1.62^{d}	33.64 ± 1.86^{d}

Mean values followed by same letter within a column are not significantly different at 0.05 levels (DMRT)

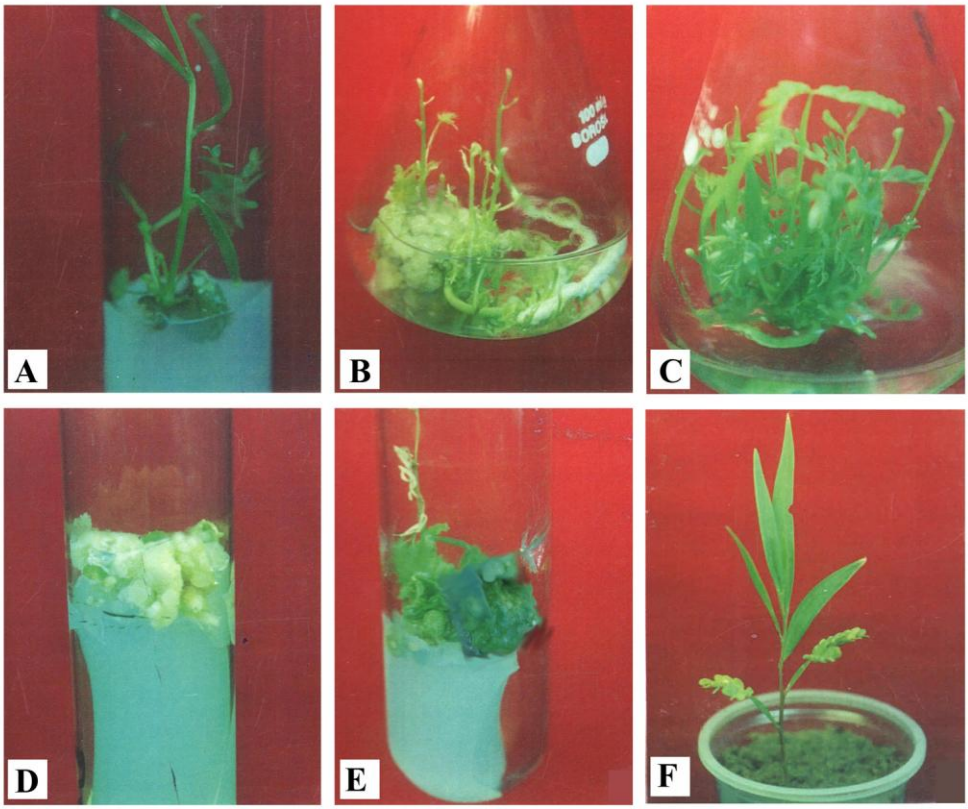

Figure-1

Figure: 1 A- F Direct and indirect regeneration of shoot buds of *Acacia auriculiformis*

A. Direct shoot bud regeneration with shoots from cotyledon explant on MS agar medium
B. Direct shoot bud regeneration in liquid culture
C. Emergence of leafy shoots from tiny shoot buds
D. Friable organogenic callus
E. Numerous shoot buds with micro shoots developed on the surface of organogenic callus
F. Plantlet transferred to plastic cup

It is interesting to note that mean number of shoot buds regenerated at BAP (2 mg/l) alone was 135.26±7.014 whereas, in combined application of BAP (2 mg/l) and KN (1 mg/l) was 178.33±12.62; which were significantly different at 0.05 levels of DMRT (Table 1). Therefore, a synergistic effect of BAP and KN has been found as reported earlier (Maity *et al.*, 2004).

Cultures grown in liquid media showed higher number of shoot bud regeneration compared to semisolid media having the same PGR levels (Table 1 and Fig. 1 B), which conforms the earlier observations of Maity *et al.*, (2007). In general, a very high number of shoot buds were regenerated in liquid cultures in all the treatments and the best response was observed at 2iP 2 $mgdm^{-3}$ combined with CW and ascorbic acid as on the semisolid media. The possible reason for this could be that the agar has high adsorptive capacity and hinders the uptake of cytokinin and other chemicals (Bonga *et al.*, 1987). The inhibitory response at higher concentration of agar was probably due to the accumulation of invertase immediately below the explants (Romberger and Tabor 1971). Comparative assessment of liquid and semi-solid

cultures revealed that the former system was more economic in terms of saving of time and media requirements. Besides it was easy to retrieve uniform plantlets from liquid media than from the solid ones.

Emergence of leafy shoots (Table 2 and Fig. 1 C) from tiny shoot buds primarily depended upon the applied PGRs both in semi-solid and liquid systems. Complete omission of PGRs exhibited lower percentage of leafy shoot development compared to low levels of PGRs (0.1mg.dm^{-3}) which is in agreement with Maity *et al.,* (2005, 2007). Similar responses have been recorded in *Arachis hypogaea*, *Vanilla sp*. (McKently *et al.,* 1990; Mary Mathew *et al.,* 1991; Banerjee *et al.,* 2011) and thus considered as a common phenomenon among the herbs and trees as well.

Callus mediated organogenesis

Formation of callus was not observed in PGR free medium as well as in the media supplemented with only NAA or 2iP (Table 3). Combined application of NAA and 2iP showed considerable amount of callus formation (Fig. 1D). Growth of the callus was faster when the 2iP level was kept lower than NAA. The growth pattern has clearly demonstrated that a combination of auxin and cytokinin was absolute prerequisites for the induction and development of callus in *Acacia auriculiformis*. Growth of the callus was enhanced when the amount of auxin was kept higher in the medium compared to cytokinin (Figure d). The present investigation has clearly demonstrated that higher ratio of exogenous NAA and 2iP induced rooting which marked the end of morphogenetic development (Narayanswamy 1994). It is reasonable to conclude that improved efficiency of auxin in association with cytokinin is attributed to their role in DNA synthesis and mitosis (Skoog and Miller, 1957). Callus grown on the media without CW and ascorbic acid tend to be brown and callus necrosis occurred. High level of NAA differentiated roots from the callus.

NAA at lower level (0.2 mg.dm^{-3}) combined with various levels of 2iP (0.2-4 mg.dm^{-3}) were applied for differentiation of shoot buds from callus. The shoot bud differentiation (Fig. 1E) from callus was found optimum at 2iP 2 mg.dm^{-3} combined with NAA (0.2 mg.dm^{-3}) while very high concentration of 2iP suppressed this augmentative effect (Table 4). However, the treatment supplemented with CW and ascorbic acid in addition to 2iP and NAA showed significant increment in the number of regeneration of shoot buds. Such response corroborated with earlier observations (Ghosh and Bandyopadhyay 1984; Gharyal and Maheswari 1990; Banerjee *et al.,* 2011) in other plant species. 2iP alone at higher concentration induced the development of greenish nodular callus while stimulation of shoot bud differentiation was achieved by supplementing the medium with NAA (0.2 mg dm^{-3}). Auxins and cytokinins might have acted synergistically to promote either cell division or cell expansion depending upon other factors within the cell, which react with other hormones. Thus the presence of NAA was crucial for the regeneration of shoot buds from callus (Mitra and Chaturvedi, 1970).

Shoot buds regenerated at relatively higher concentrations of 2iP were developed into small plantlets upon subculture on the media either with very low levels of 2iP or free of 2iP (Table 5). It is evident from the table that 0.1 mg/l 2iP gave better response than the media supplemented with higher concentration of 2iP and without 2iP which is in accordance with the observation made by Banerjee et al (2011) in *Arachis hypogaea*. The emergence of leafy shoots from organogenic callus mass was always higher in liquid cultures (67.66±2.45) compared to semi-solid medium (53.28±1.46) as discussed by Maity *et al.,* (2004, 2007) in two other leguminous species viz. *Acacia auriculiformis* and *Leucaena leucocephala*.

Induction of roots on the regenerated shoots, excised from the parent culture, was observed at all the concentrations of NAA tested (0.5 to 4 mg.dm^{-3}). Best response was obtained at 1 mg.dm^{-3}. It could be mentioned that the roots developed at the base of the isolated shoots did not turn into callus, which is very

important for successful transplantation of the regenerated shoots. *In vitro* grown plants with well developed root systems were successfully transplanted to the sand-soil mixture (1:1) with adequate water on plastic cup. More than 90% of the transplanted plants were survived. Transplanted plants showed normal seedling morphology and growth (Fig. 1F).

The *in vitro* protocol to obtain the regenerated plantlets of *Acacia auriculiformis* could therefore be used for the improvement of individual clones of this species through direct and indirect organogenesis. The techniques described here for the propagation of this commercially important species could be effectively applied for conservation purposes and is important for future programs of genetic transformation of this species.

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