Multiple shoot Induction in *Abrus precatorius* L Var W S C (white seed coat).

Nagamani.S & Aniel Kumar.O and Snikdha.V
Department of Botany,Andhra University,Visakhapatnam, Andhra Pradesh

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ABSTRACT *An efficient in vitro multiplication protocol was developed through excised shoot tip, explants of Abrus precatorius L. The effect of different growth regulators alone or in combination on multiple shoots production from different explants of A. precatorius L was studied Multiple shoots were obtained from excised shoot tip, explants on MS medium supplemented with BAP (3.0 mg/L) and NAA (1.0 mg/L). Maximum number of shoots (16-18) per explants were obtained from excised shoot tips. Effective in Vitro shoot elongation and rooting was achieved on 10 mg/l GA_3 and 60 mg/ L IBA respectively. The rooted plants were hardened and transplanted into the soil and showed 60-76% survival during transplantation.*

Keywords: *Abrus precatorius L, shoot tip, explants, plant growth regulators, In vitro multiplication.*

Introduction

Medicinal plants are the richest source of drugs for traditional medicines, nutraceuticals and food supplements, folk medicines, Pharmaceutical intermediates etc (Harmer et al, 1999). For many years, tissue culture techniques are being applied for germplasm conservation, mass propagation and disease free plant production of medicinal plants. With the help of tissue culture it is possible to produce a huge number of plantlets from single explants within shortest span of time (Bajaj 1995). Micropropagation has been used to conserve the germplasms of many rare and endangered species of medicinal plants (Fay, 1992; Mikulik, 1999). India has a great natural resource of medicinal plants and plant based health remedies are in practice here for long since. To harvest proper benefits from medicinal plants measures need to be taken regarding conservation and management of medicinal plants. Pharmaceutical industries can also utilize this technique for drug development approach from medicinal plants and gain economic success.

The demand for medicinal plants is therefore very high leading to their over-exploitation from the wild. In fact the collection medicinal plants has become a form of rural self-employment in India, which is generating a lot of income for the rural poor.

India has the richest plant diversity in the world, many of which are medicinally useful. The rich resource is decreasing at an alarming rate as a result of over-exploitation. Plant in vitro regeneration is a biotechnological tool that offers a potential solution to the problem of medicinal plants decimation in India.

Among the traditional system of medicine *Abrus precatorius* L. is one of the important herb commonly known as Indian liquorice belonging to family Fabaceae, Ratti - in Hindi and Gurivinda – in Telugu. It is a vine originally native to India that is now commonly distributed throughout tropical and subtropical regions of the world (choi *et. al*. 1989). This plant can be easily identified with different colored hard seed coated seeds. The unique characteristic of this plant is that it has toxic red seeds with black mark at the base (Mensah, 2011). Plant parts such as leaf extracts is used for leucoderma, the seed having abrin A and B is used as a purgative and abortive and the root extract used against coughs in the ayurvedic system of medicine. The seeds of this plant exhibit potent HIV-I PR inhibitory activity (Marshall, 1998). Medicinally *A. precatorius* is well reputed for its antitumar properties in Ayurvedic Medicine (Indian indigenous system & Medicine). Two toxic antitumor proteins Abrin A and B were isolated from seeds (Prakash and Nain Wal, 2013). Seeds are containing several chemical constituents and promise in treatment to several diseases (Rajaram and Janardhanan,1992 and Mohan & Janardhanan 1995) Indian goldsmiths used its seeds as weights in ancient times (Nadakarni 1976).

In nature the propagation of *A. precatorius* through seeds is difficult because of their hard seed coat – a trait which explains its sparse distribution. It is therefore important to develop a protocol for in vitro Propagation to save this medicinally important taxon from further depletion of its population, at the same time to meet up the demand of the traditional medicine industry.

In vitro propagation has proven as a potential technology for mass scale production of medicinal plant species (Lui and Li, 2001, Wawrosch et al 2001, Martin 2002, 2003, and Azad et al 2005). Unfortunately, conventional propagation through seeds is rather slow and not very efficient. Hence, there is

an obvious need to develop an efficient regeneration system for its effective conservation and rapid multiplication 'Micropropagation methods can often be the most efficient way to clonally propagate a species without depleting wild resources and can be an important part of an integrated program for the conservation of medicinal and aromatic plants worldwide (Reed et. al. 2011). So our knowledge goes, no report has been published on direct micro propagation of *A.precatorius*. The present investigation reports the in vitro propagation technique that can be used as a potential tool for large scale production of this medicinal plant.

Materials and Methods

Explant Source:-

Mature seeds of *Abrus precatorius* L. White seed coat colored genotype were collected from Addateegala, East Godavari, District, Andhra Pradesh. The hard seed coats were scarified by a mechanical method at a low speed for 5-15 minutes and at a high speed 2-7 minutes, separately. Only the sacrified undamaged seeds were selected and treated with Bavistin. The seeds were sown in the polythene bags with sterilized soil at Botany experimental farm, Andhra University, Visakhapatnam for raising seedlings. Juvenile twigs from one year old mature plants were used as source of explants.

The shoot tip explants were washed thoroughly in running tap water for 30 min, immersed in 1% (m/v) Bavistin solution and later treated with 5%(v/v) Teepol solution for 20 min. After thorough washing in running tap water, the explants were surface sterilized with 0.1/(m/v) $HgCl_2$ for 3 min, followed by 70% ethanol and thorough washing with sterile distilled water in a laminar airflow before implantation.

Culture Media:-

Ms basal medium (Murashige and Skoog 1962) supplemented with 3% (m/v) sucrose and 0.8%/(m/v) agar was used in all the experiments. The medium was adjusted to PH 5.8 and sterilized by autoclaving at 121^0C and 1.06 Kg Cm^{-2} Pressure for 20 min Cultures were incubated at 25 ± 2^0C, a 16-h photoperiod, photo n flux density (PFD) at 50 $\mu^{mol\ m-2}\ s^{-1}$ (Cool florescent tubes) and relative humidity of 55-60%.L Shoot tip explants were cultured on MS medium fortified with various concentrations of BAP, and Kinetin either individually (1.0-5.0 mg/1) or in combination with auxin NAA (2.0-3.0 mg/1) with an adsorbent polyvinyl Pyrolidone (PVP)/Charcoal for bud break. After wards, the optimum concentration of each cytokinin and 120 mg dm^{-3} PVP were used in a combination with NAA at different concentrations for multiplication and proliferation of shoots. Sub culturing was performed on the same fresh media after every 4 weeks. Data on the frequency of explants producing shoots, shoot number per explant and shoot length were recorded after 8 weeks of incubation.

Shoots with fully expanded leaves were excised from the shoot clusters and transfer to different strength of MS medium (Ms ½) singly for root induction. Afterwards, the optimized half strength MS medium was tried incombination with either IBA or NAA at various concentrations for better rhizogenesis. Observations on rooting percentage, number of roots per shoot and root length were recorded after 4 weeks.

Acclimatization and field experiment:-

Well rooted plantlets were allowed to grow on rooting medium for further 2 weeks and then they were removed from the culture tubes, washed gently with running tap water, transferred to plastic cups containing growing medium (autoclaved garden soil, farmyard mannure and sand mixture (2:1:1) and grown in the culture room. Poly cups/poly bags were opened gradually and removed after 21days to acclimatize plantlets under the culture room conditions. After 28days, the plantlets were then transplanted to the field at Botany experimental farm, Andhra University, Visakhapatnam.

Results and Discussion

Multiple shoot induction through shoot tip explants:

The plant regeneration from shoot tip segments are considered to be one of the most promising ways from multiplying a selected variety true to its type showing the same agronomic characteristics the shoot tip explants cultured on Ms basal medium without any growth regulator did not show any morphogenetic response and failed to produce shoots, Generally, a cytokinin is required for shoot induction and proliferation. However its effective type and optimal concentration varies with the system. Similar results were also reported in Salix sps (Park et al 2008).

A differential response with regard to shoot bud induction was observed when shoot tip explants were cultured on MS medium supplemented with BAP or Kinetin alone (1.0-5.0 mg/l) alone or in combination with auxins NAA Swelling of the shoot tips took place, within 7 days followed by differentiation multiple shoot induction after 6 weeks of the culture Table-1 fig A-D. BAP was found more effective than

KIN as seen in the number of shoot bud formation after 6 weeks of culture. (Table 1: Fig.D) The reason for effectiveness of the BAP may be because of its ability to stimulate the plant tissues to metabolize the natural endogenous hormones or alternatively can induce the production of natural hormone system for the induction of shoot organogenesis. Among the various concentration of cytokinesis tested BAP (3.0 mg/L) showed the highest shoot regeneration frequency 91 ± 1.90) and number of shoots (16 ±0.05) with maximum shoot length (58 ± 0.83) mm after 6 weeks of culture (Table-1). This concentration was considered as the optimal growth regulator for shoot regeneration in *Abrus precatorius L.* among the treatments tested. The potential of BAP on multiple shoot bud differentiate on has been demonstrated in a number of plant species using variety of explants (Usha et al, 2007, Jain and Bashir 2010,and Ahmed et al 2013), The regeneration frequencies and number of shoots declined with an increasing concentration of cytokinin beyond the optimal level. Similar results were also reported in *Vitex* species (Balaraju et al, 2008 and Ahmed et al 2013). When MS medium fortified with Kinetin, was observed with a low regeneration frequency (Table-1), A maximum of (6.0 ± 0.03) shoots with the highest percentage (37%) of responding explants was produced at 3.0 mg/l Kin in after 6 weeks of Inoculation. These results clearly showed that presence of BAP on shoot multiplication was more prolific with BAP over KIN.

MS medium supplemented with cytokinins alone BAP or Kinetin produced shoots in shoot tip explants. But by supplementing the medium with cytokinins (BAP or Kinetin) was noticed slightly increased the regeneration frequency number of shoots and shoot length and the combined effects of cytokinins along with an auxin (NAA) was also studied for their ability to affect the shoot multiplication rate by taking the optimized concentration (3.0 mg/l) of different Cytokinins incombination with 1.0 mg/1 (NAA) (Table-1), both the percentage of shoot regeneration as well as number of shoots per culture was increased, which shows that auxins (NAA) also plays significance role in multiple shoot induction. The enhanced rate of multiple shoot induction in cultures supplemented with BAP and NAA may be largely ascribed due to increased rates of cell division induced by cytokinin (BAP) in the terminal and auxiliary meristematic zone of explant tissues. Cells in this zone divide with the faster pace and thus, produced large number of shoots. This positive effect of BAP+NAA on differential morphogenetic response may be due to apical dominance. The increased multiple shooting in excises shoot tip explants may be due to rapid division of cells in the excised shoot tip and production of several primordial out growth, which eventually develop into shoots. Thus, it can be inferred from the above results that the interactive effect of BAP and NAA could ensure better in vitro regeneration and their synergism in proper concentration was extremely favorable for multiplication.

Variation in the activity on explants, cytokinins can be attributed to their differential uptake rates, as reported for some species varied translocation rates to meristematic regions and certain metabolic processes in which the cytokinin may be degraded or conjugated with sugars or amino acids to form biologically inert compounds .In addition, it was suggested that Cytokinin efficiency may be affected by different affinities of cytokinin receptors involved in shoot induction process Similar observations were reported by Blakesey,1991: Tran Thanhvan and Trinh,1990 and Kaminek, 1992).

Elongation of shoots was higher i.e., (4.6 cm) on MS medium containing 0.10 mg 1 GA_3. This may due to the action of GA_3 on cell elongation Similar observation was reported in pepper Qin et al 2005. The elongated shoots were excised and implanted on MS medium agumented with different concentration of IBA (0.20-1-0 mg/1). The optimum rooting frequency 76%, root number (8 ± 0.16) and root length (12 ± 0.10) per shoot was on Ms Medium fortified with IBA (0.60 mg/1) Table-2; Fig-1 E Similar results were also reported in capsicum (Aniel kumar and Subba Tata 2010) The present study it was proved that BAP + NAA could make the explants more responsive to induce shoot bud proliferation. The regenerated Plants showed 60-76% survival during hardening and acclimatization and there were no observable differences between the parent plant and *in-vitro* raised plants. The transplanted plantlets established well in pots and in the field.(Fig-1.F)

Table 1:- Multiple shoot induction in excised shoot tip explants of *A. precatorius* L in MS medium supplemented with different concentrations of BAP, KIN, and NAA in combination or alone.

Growth Regulators (mg/l)	% shoot formation of excised shoot tip	Average shoot per culture of excised shoot tip	Average length of shoot (cm) of excised shoot tip
MS	-	-	-
MS + BAP			

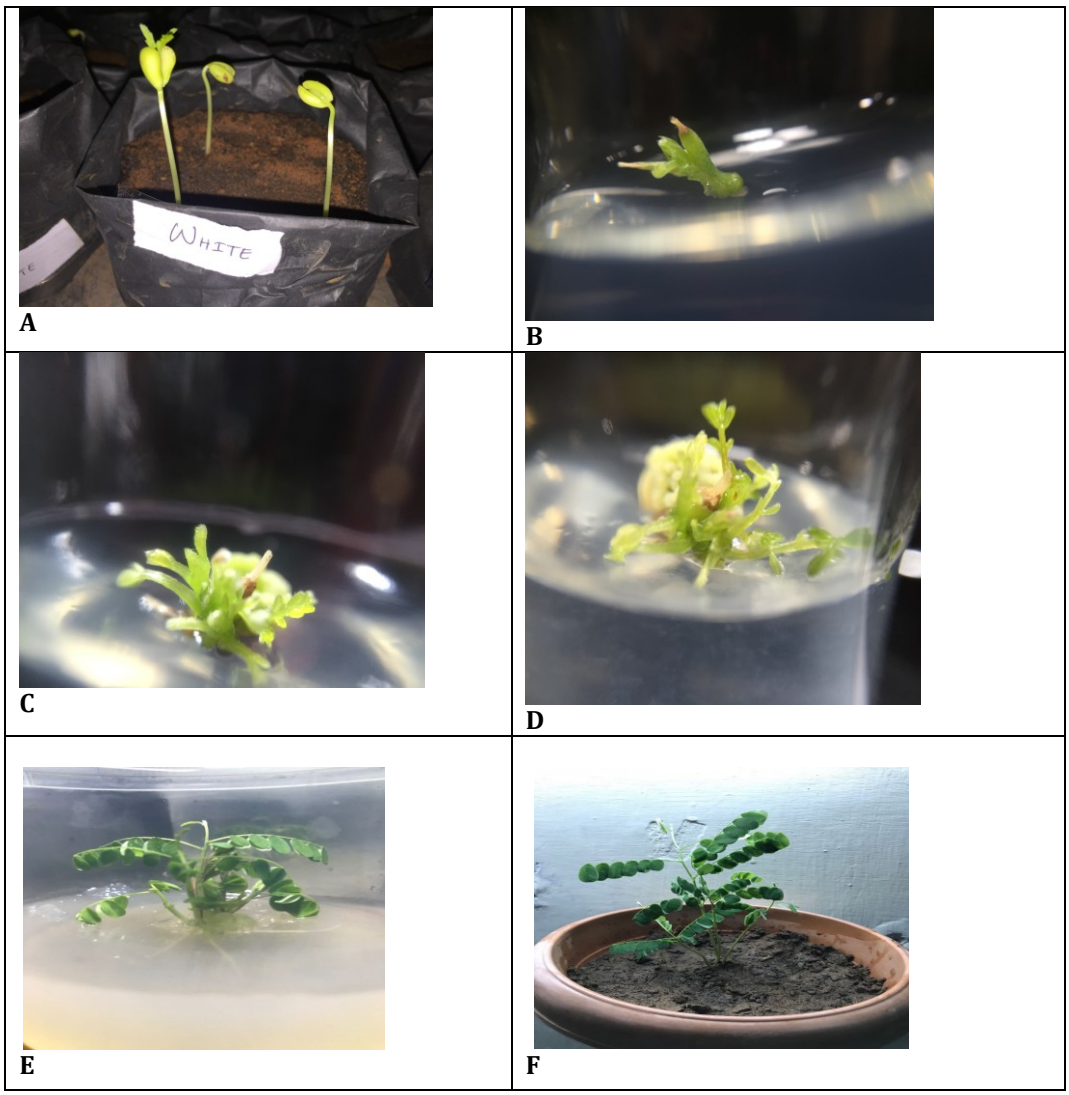
Growth Regulators (mg/l)	% shoot formation of excised shoot tip	Average shoot per culture of excised shoot tip	Average length of shoot (cm) of excised shoot tip
1.0	51.0 ± 0.91	6.0 ± 0.02	33.0 ± 0.28
2.0	72.0 ± 1.32	13.0 ± 0.13	45.0 ± 0.33
3.0	91.0 ± 1.90	16.0 ± 0.05	52.0 ± 0.83
4.0	68.0 ± 0.72	11.0 ± 0.10	41.0 ± 0.19
5.0	64.0 ± 0.17	8.0 ± 0.03	30.0 ± 0.85
MS + KIN			
1.0	18.0 ± 0.54	1.0 ± 0.02	11.0 ± 0.09
2.0	29.0 ± 0.33	3.0 ± 0.01	13.0 ± 0.15
3.0	37.0 ± 0.19	6.0 ± 0.03	12.0 ± 0.31
4.0	33.0 ± 0.09	5.0 ± 0.03	10.0 ± 0.28
5.0	30.0 ± 0.43	2.0 ± 0.05	7.0 ± 0.06
MS + BAP + KIN			
1.0 + 1.0	38.0 ± 0.12	5.0 ± 0.06	29.0 ± 0.75
2.0 + 1.0	46.0 ± 0.43	6.0 ± 0.13	40.0 ± 0.17
3.0 + 1.0	60.0 ± 0.51	8.0 ± 0.05	46.0 ± 0.26
4.0 + 1.0	53.0 ± 0.33	4.0 ± 0.02	36.0 ± 0.04
5.0 + 1.0	42.0 ± 0.15	3.0 ± 0.01	30.0 ± 0.07
MS + BAP + NAA			
1.0 + 1.0	57.0 ± 0.18	8.0 ± 0.14	38.0 ± 0.19
2.0 + 1.0	76.0 ± 0.91	14.0 ± 0.04	47.0 ± 0.37
3.0 + 1.0	95.0 ± 0.79	18 ± 0.13	55.0 ± 0.76
4.0 + 1.0	73.0 ± 0.28	13 ± 0.15	48.0 ± 0.62
5.0 + 1.0	69.0 ± 0.16	10.0 ± 0.12	36.0 ± 0.23
MS + KIN + NAA			
1.0 + 1.0	26.0 ± 0.10	2.0 ± 0.01	11.0 ± 0.04
2.0 + 1.0	30.0 ± 0.60	4.0 ± 0.04	14.0 ± 0.13
3.0 + 1.0	39.0 ± 0.08	7.0 ± 0.01	22.0 ± 0.16
4.0 + 1.0	36.0 ± 0.16	6.0 ± 0.11	18.0 ± 0.20
5.0 + 1.0	33.0 ± 0.12	3.0 ± 0.12	15.0 ± 0.13

Results are mean of 20 replicates Mean **±** SE

Table-2 Effect of IBA on rooting in vitro regenerated shoots

SNO	Plant hormone (mg/l)	Rooting (%)	Root No/shoots	Root length (cm)
1	MS	-	-	-
2	MS+IBA(0.20)	47.0 ±0.06	3.0 ±0.13	6.0 ± 0.14
3	MS + IBA(0.40)	53.0 ±0.14	5.0 ±0.11	8.0 ± 0.05
4	MS + IBA(0.60)	76.0 ±0.12	8.0 ±0.16	12.0 ± 0.10
5	MS + IBA(0.80)	63.0 ±0.18	6.0 ±0.19	10.0 ± 0.07
6	MS + IBA(1.00)	60.0 ±0.10	4.0 ±0.07	7.0 ± 0.03

Results are mean of 20 replicates Mean ±SE



Legends: Figure 1.Multiple shoot induction of Abrus precatorius.L var WSC using shoot tip explants.

A. Seedling of *Abrus precatorius* var white seed coat (WSC).
B. Shoot induction after 2 weeks
C. Multiple shoot induction on MS medium fortified with BAP(3mg/l) after 4 weeks
D. Optimum shoot multiplication MS medium supplemented with 3.0 mg/l (BAP) +1.0 mg/l(NAA) after 6 weeks.
E. Rhizogenesis on MS medium fortified with 0.60 mg/l (IBA).
F. Regenerated plant established in 2:1:1. (garden soil:farm-yard, manure:sandmixture)

Conclusion

In the present investigation higher number of multiple shoots (16-18 shoots for explant) were obtained from excised shoot tip explants of *Abrus precatorius* L. There are no earlier reports on direct organogenesis, in *A. precatorius L*. Thus, this protocol can be suitably exploited for the mass multiplication on a large scale for commercial and may be worth full in conservation of natural reserves of *A. precatorius* L.

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