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Ex-situ conservation of an endangered medicinal plant *Andrographis paniculata* by plant tissue culture

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ABSTRACT

An effective and rapid *in vitro* regeneration protocol of Kalmegh (*Andrographis paniculata*) was established by investigating the factors like combinations of plant growth regulators and explant types (stem, leaf and midrib). To find out the effective medium for callus induction and shoot regeneration, different explants of *A. paniculata* were cultured on MS media enriched with several concentrations of 6-benzylaminopurine (BA), á-naphthalene acetic acid (NAA) and 2, 4-dichlorophenoxy acetic acid (2,4-D). Stem explant was noticed more responsive than leaf and midrib explant both in callus initiation and shoot regeneration. The ranges of callus initiation from stem, leaf and midrib explants were 26.67 - 100%, 20 - 93.33% and 13.33 - 73.33%, respectively. The calli obtained from midrib explants were not used in shoot initiation because of its poor size. The stem explant exhibited the maximum 73.33% shoot regeneration frequency in a comparison with leaf explants (60%). The maximum callus induction (100%) and shoot regeneration (73.33%) from stem explants were noticed in MS medium strengthened with 0.5 mg/L NAA and 2.0 mg/L BA and half strength MS media complemented with 0.1 mg/L NAA and 3.0 mg/L BA respectively. The highest shoot regeneration from the stem explant may be due to presence of more active parenchymatous cells than that of leaf explant. Half MS medium fortified with 2.0 mg/L IBA considered as best root initiation medium as it resulted in maximum rooting (93.33%). After acclimatization, the plants were transferred to field and found identical to the mother plant.

Keywords: Callus, Kalmegh, medicinal plant, organogenesis, stem explants.

INTRODUCTION

Kalmegh (*Andrographis paniculata*) is a medicinal plant of immense worth, which has immeasurable uses in Ayurveda and Unani medicines for treating many ailments like as common cold, fever, asthma, cough, urinary tract infections,tuberculosis, bronchitis, acute diarrhea, dysentery, acidity, skin diseases, snake bites *etc*. (Wang *et al.,*2014, War *et al.,* 2018). It is placed at 17th position among the 32 prioritized medicinal plants which is ranked by the Indian National Medicinal Plants Board (INMPB) (Verma *et al.,* 2019) and it is the major ingredients of at least 26 Ayurvedic compositions stated by the Indian Pharmacopoeia (Mishra *et al.,* 2007; Verma *et al.,* 2019). It is also included in Pharmacopoeia of People's Republic of China as a "cold property herb" utilized to get recovery from fever and eliminate toxins (Abhilasha and Arpita, 2017). Thailand's Ministry of Public Health has also recorded this plant in "National List of Essential Drugs A.D. 1999" (Pholphana *et al.,* 2004; Abhilasha and Arpita, 2017). The plant carries a wide range of curative properties like anti-oxidant (Trivedi and Rawal, 2001), anti-viral (Wiart *et al.,* 2005), anti-fungal (Sule *et al.,* 2012), immune enhancement and anti-HIV activities (Calabrese *et al.,* 2000), cardio-protective (Ojha *et al.,* 2012), anti-cancer and immune-non stimulatory (Kumar *et al.,* 2004), hepatoprotective (Nagalekshmi *et al.,* 2011), anti-hypertensive and anti-microbial (Jindal *et al.,* 2015).

Approximately half of the death throughout the world caused by communicable diseases and it is reported that more than 50% of hospital deaths are occurred due to communicable diseases (Tandon *et al.,* 2015). And these numbers are increasing rapidly due to the occurrence of bacterial resistance to some specific

antibiotics and introduction of new infectious diseases like COVID-19. In the meanwhile, in this pandemic situation almost 3.7 million excess deaths occurred across all the countries and the situation isgetting worsen constantly (Karlinsky and Kobak, 2021). So, there is a dire need for research to investigate the alternative source of existing drugs to combat different diseases. *A. paniculata* can be an alternative source of existing drugs due to its high medicinal value.

In recent years, a noticeable amount of medicinal plant species is rapidly dissipating in the world and are under serious risk of extinction as a result of anthropogenic habitat destruction (Ayuso *et al.*, 2019). The declining rate of natural habitats coupled with the over-exploitation as a source of pharmaceutical drugs make this species endangered that cause genetic diversityshrinkage and demolition of biological communities, that are very essential for ecosystem functioning as well as human well-being (Silveira *et al*., 2016). International Union for Conservation of Nature (IUCN) included this species in the list of threatened plants. This species was also classified as vulnerable by CAMP (Conservation Assessment and Management Prioritization) exercise in India in 2003 (Gowthami *et al.,* 2021). Again, Bangladesh National Herbarium (BNH) has already identified about 97 of such over-used and threatened plants in Bangladesh and *A. paniculata* is one of them (Khan *et al.,* 2001). Nevertheless, the traditional vegetative propagation of *A. paniculata* can't meet the ever-increasing requisite for pharmaceutical industries because of its slower propagation rate. Hence, divergent ordinated programs are direly necessary to protect and conserve the existence of these precious plant resources. Recently in India, INMPB emphasized 32 medicinal plants for conservation and *A. paniculata* have also been on that list (Gowthami *et al.,* 2021).

The intense pressure on this species will rise continuously unless proper initiative for conservation and sustainable use are taken. To protect threatened plants, appropriate management of wild populations as well as natural habitats protection are urgentthrough several *in situ* conservation strategies (Ayuso *et al.,* 2019). But in case of *A. paniculata*, very low seed production and delay in root initiation from seedlings reduce its production from seeds (Martin, 2004) and make a great obstacle for the *in-situ* conservation of this species. Conversely, *in situ* conservation is getting troublesome day by day because the natural habitats are utilized to meet the ever-increasing requirements of human beings as well as its natural habitats cannot be expanded. However, in critical situations, *ex situ* strategies play a vital role in the preservation of endangered plants and offer some scopes of saving wild resources against the loss of their natural habitats (Nam off *et al.,* 2010; Ayuso *et al.,* 2019). Plant i*n vitro* culture has arisen as a promising biotechnological technique for expeditious production of enormous number of individuals from limited number of plant material and conserving the species within a short period and confined space, which hardly disturbs wild populations. The popularity of *in vitro* culture has developed in recent years for the protection of endangered, endemic and rare species (Bunn *et al.*, 2007). Moreover, *in vitro* regeneration provides easy access to the re-introduction of plants to their natural habitats and offers some distinct advantages over alternative strategies. Furthermore, to do some improvements on the drawback side of conventional plant breeding, like reduced growing rate, very lower seed production, delayed rooting of seedlings and lower production of secondary metabolites, *in vitro* culture of plant becomes a powerful biotechnological technique to study the plant secondary metabolism and appears as an effective system to produce bioactive compound (Rusedski *et al.,* 2017; Isiah *et al.,* 2018; Hu *et al.,* 2019).

Considering these points, plant tissue culture can provide an additional strategy for mass scale production of this species which can overthrow the constrains of extracting valuable metabolites and can act as a 1st step for the *ex-situ* conservation of this plant's germplasm. Although several reports were found worldwide, few studies (Al-Mamun *et al.,* 2015; Roy, 2014) with limited success were found on the regeneration of *A. paniculata* in Bangladesh. In Bangladesh, its medicinal properties are not well known, hence no activities are in placetoconserve this valuable plant. However, *ex-situ* strategies also help for the conservation of this species as well as give additional conservation options. Therefore, the present study is undertaken to develop as an economical and reliable regeneration protocol for its large-scale production to meet the increasing demand of phyto-pharmaceutical industries and to protect this highly valued medicinal plant from extinction.

MATERIALS AND METHODS

Plant materials

Healthy cuttings of *A. paniculata* were collected from Moulvibazar and Sunamganj district of Bangladesh. Collected cuttings were planted in the research field and rooftop of the Department of Genetics and Plant Breeding for explant preparation.

Explant and media preparation, and culture method

For explant (stem, leaf and midrib) preparation, at first a shoot was excised from healthy plant of *A. paniculata* and swabbed with 70% alcohol-soaked cotton and the shoot was cut into small pieces. After that the small pieces were washed in running tap water for 20 min followed by washing with household detergent for 5 to 7 min. They were brought to laminar air flow after washing with distilled water. Then the shoot pieces were treated with 70% ethyl alcohol (MERCK, Germany) for 2 min for surface sterilization and rinsed in sterile double distilled water 3 to 4 times. Again, the shoot bits were surface sterilized with 10% Chlorox (Sodium hypochlorite, The Clorox Company, Oakland, USA) for 8 minutes, and were rinsed with sterile double distilled water 3 to 4 times to remove the effect of surface sterilizing agents. The explants were then split into little pieces and used as explants after being surface sterilized. Finally, the segment of stem, leaf and midrib (5-7 mm in length) from excised shoot were used as explant for the callus induction.

Stem, leaf and midrib explants (5-7 mm in length) were inoculated in MS (Murashige and Skoog, 1962) media complemented with several concentrations of BA (99%, Duchefa Biochemie, the Netherlands) (0.5, 1.0, and 2.0 mg/L), NAA (98%, Duchefa Biochemie, the Netherlands) (0.1, 0.5 and 1.0 mg/L) and 2,4-D (96%, Duchefa Biochemie, the Netherlands) (0.1, 0.5 and 1.0 mg/L) to find the best medium for callus initiation. The process was carried out entirely in a laminar airflow cabinet. In each culture jar containing 50 ml callus induction media, five explants were put. On the surface of the medium, the stem, leaf, and midrib segments were inoculated horizontally (Fig. 1a, b & c) and implanted in culture room with high light intensity (16/8 hours light/dark) produced by 144 W white fluorescent lamp, a temperature of 25±2°C and a relative humidity of 70%.

After attaining a convenient size, the calli were re-cultured on freshly prepared shoot induction media (Fig. 1 d & e) containing MS salts and vitamins, 3% sucrose, 1% agar and different strengths of 2,4-D (0.5 and 1.0 mg/L), NAA (0.1, 0.5 and 1.0 mg/L) and BA (1.0, 2.0 and 3.0 mg/L). When in *vitro* raised shoots grew about 2-3 cm in height was aseptically transferred into full strength and half strength MS media separately supplemented with different strengths of NAA (0.5, 1.0 and 2.0 mg/L) and IBA (0.5, 1.0 and 2.0 mg/L). A permanent marker was used to mark the cultivated vessels after being sealed with parafilm to denote each treatment. They were then placed in a culture room.

After development of adequate root system, 4-6 cm plantlets were removed cautiously from the cultivated vessels without damaging roots and rinsed smoothly in running tap water to eliminate agar medium and sucrose trace elements to avoid fungal infection. Then the complete rooted plantlets were transferred into plastic pots containing wet soil and provide a glassware cover (beaker) to prevent dehydration (Fig. 1k). When the plantlets were successfully adapted to the natural environment then they were transferred to field condition (Fig. 1l).

Statistical analysis

The experiment was designed following completely randomized design (CRD) with three replications. The recorded data on the percentage of callus initiation, shoot regeneration and root regeneration and statistically analyzed to ascertain the significance of the experimental results. MS Excel 2010 was used for calculating the mean and standard deviation for all treatments. Dunkan's Multiple Range Test (DMRT) was used for evaluating the significance and difference between means by utilizing R software (version Rx64 3.4.3).

RESULTS& DISCUSSION

The optimal medium for callus induction

Callus initiation was started within a week from the stem, leaf and midrib explants of *A. paniculata* after incubation of explants on MS medium containing several combinations of BA (0.5, 1.0 and 2.0 mg/L), 2, 4-D (0.1, 0.5 and 1.0 mg/L) and NAA (0.1, 0.5 and 1.0 mg/L). Among the combinations tested, MS medium supplemented with 0.5 mg/L NAA and 2.0 mg/L BA showed the highest callus initiation frequency for all three types of explants with significant heterogeneity among the explants (Fig. 2 and Table 1). The highest callus formation was 100%,

93.33% and 73.33% for stem, leaf and midrib explants respectively in 0.5 mg/L NAA and 2.0 mg/L BA combination, while the lowest recorded callus formation for stem (26.67%), leaf (20%) and midribexplant (13.33%) found in MS medium supplemented with 0.1 mg/L 2,4-D and 0.5 mg/L BA combination (Fig. 2). However, the callus initiation frequency of stem, leaf and midrib explantson rise with the increase of NAA and 2,4-D up to 0.5 mg/L individually in a combination with BA up to 2mg/L. Afterword, with the increase of NAA and 2,4-D the callus initiation frequency has decreased. The phytohormone2,4-D in a combination with BA produced 80%, 66.67% and 53.33% callus for stem, leaf and midrib explants respectively, whereas, NAA along with BA induced 100%, 93.33% and 73.33% for stem, leaf and midrib explants respectively. So, it is clear that callus initiation frequency is mostly regulated by NAA and 2, 4-D and NAA was more responsive compared to 2, 4-D (Fig. 2). On the other hand, higher concentration of NAA induced excessive rooting in the callus (Fig. 4a) and eventually reduced the shoot forming capacity of the callus. However, in hormone free MS media, none of the explants produced callus.

In this study, stem and leaf explants showed better response for callus induction than midrib explants are in conformity of previous finding of War *et al.,* 2018. Jindal *et al.,* 2016 reported that best callus induction was obtained as MS medium containing 1 mg/L 2, 4-D and 1 mg/L NAA by using leaf explant. War *et al.,* 2018 suggested that optimal callus induction medium for leaf explant was MS medium containing 2, 4-D (1.0 mg/L) and Kinetin (0.5 mg/L) followed by 2,4-D (1.5 mg/L) and Kinetin (1.0 mg/L) and optimal callus induction medium for stem explant is MS medium supplemented with 2,4-D + Kinetin (1.5 + 1.0 mg/L). Sharma and Jha (2012) reported that, highest callus initiation was achieved on MS media consist of 1 mg/L 2,4-D and 1 mg/L NAAas well as combinations of 1 mg/L 2,4-D and 0.5 mg/L Kin and1 mg/L BAP and 1 mg/L NAA.

The physical appearance of a callus is influenced by the type of explants and the composition of the culture media (Sharma and Nautiyal, 2009). In this study, several types of distinguishable callus in terms of consistency and colour were generated from different types of explants (Table 1) in different auxin (NAA and 2, 4-D) and cytokinin (BA) combinations (Table 2). When the concentration of auxin (NAA and 2, 4-D) and cytokinin (BA) were increased (above 1 mg/L) very compact, brownish, brownish white or white calli were found. Whereas, at low NAA, 2,4-D and BA (below 0.5 mg/L) concentrations fragile, brownish or whitish colour calli were produced (Table 2). Again, in the presence of NAA and BA, leaf explants generate compact, green-colored callus. On the other hand, 2,4-D and BA combinations generated friable white callus from leaf explant.Therefore, compact, fragile, greenish, whitish and brownish calli derived from various explants in various combinations and concentrations of growth regulators. However, these types of callus morphology were also found in some other plants like *Ipomoea obscura* L. (Mungole *et al.,* 2009), *Gynura procumbens* (Nurokhman *et al.,* 2019). A green compact callus was found in *Orthosiphonsta mineus* from a balanced concentration of NAA and BA (Elangomathavan *et al.,* 2017). In contrast, higher concentration of NAA and BA provided yellowish white compact callus in *Dianthus caryophyllus* (Arif *et al.,* 2014).

However, it is revealed that, varied concentration and combination of plant growth regulators also influenced the callus morphological responses. Differences in plant growth regulator concentrations and combinations have a significant impact on morphogenic responses of callus and the morphogenic response is affiliated with the mineral nutrition that is applied (Avilés *et al.,* 2009). Additionally, color of callus can change due to changes in chlorophyll as a result of reaction among different plant growth regulators; explant types and environmental culture factors like light exposure and temperature (Elias *et al.,* 2015).

The optimal medium for shoot regeneration

In this experiment, stem and leaf explants had a stronger callus induction response and produced robust, vigorous calli then the midrib explants. Hence, calli derived from stem and leaf explants were employed in shoot regeneration.Two types of auxins (2,4-D and NAA) and cytokinin (BA) were used tofind out the best medium for shoot regeneration.21 days old healthy calli were cultured on half strength MS medium containing a variety of BA, 2,4-D and NAA combinations and concentrations (Fig. 3). After three weeks of callus culture, shoot bud formation started from the calli. Both the calli and calli with the shoot

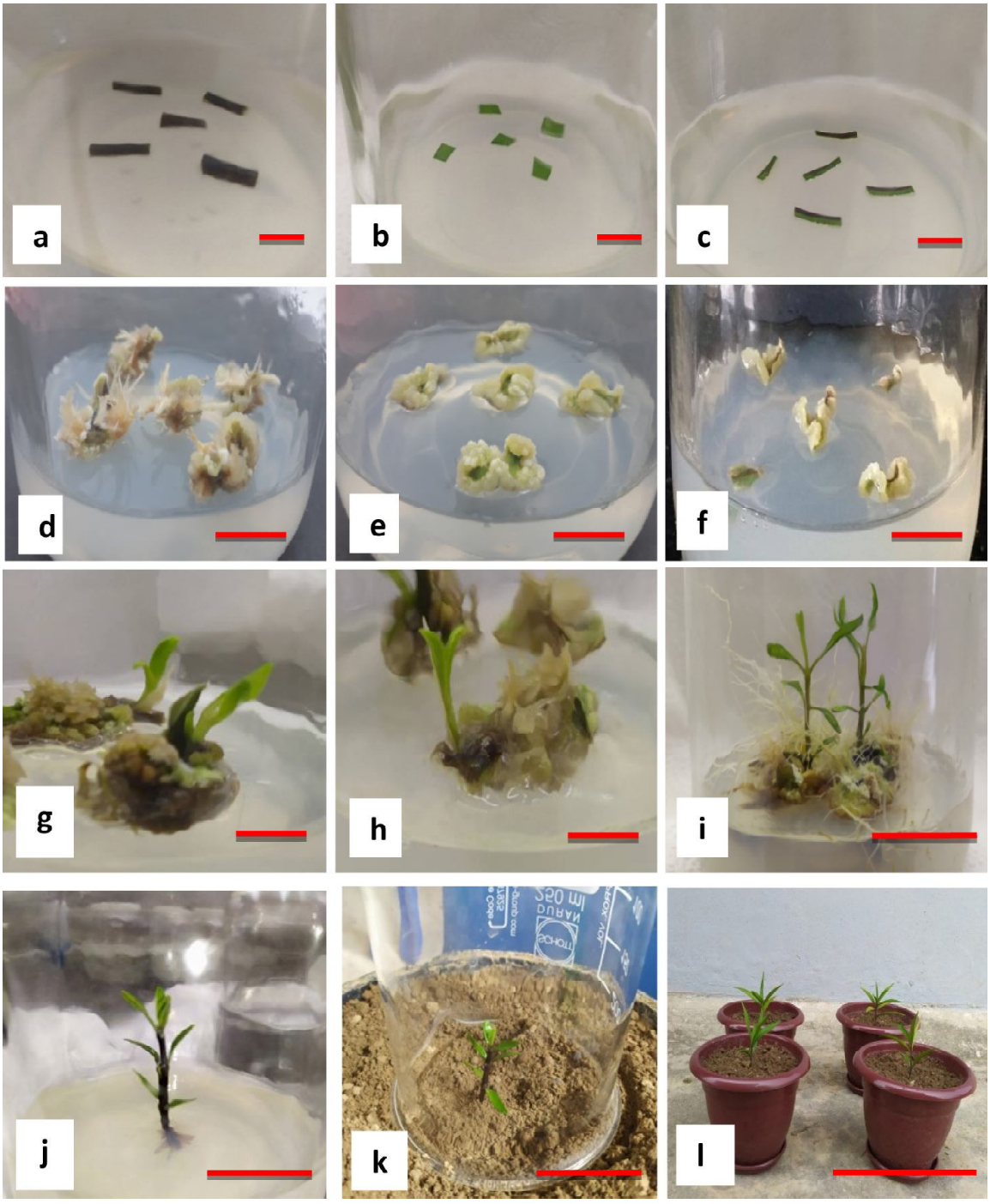


Fig. 1 : In vitro regeneration of Kalmegh explants on callus induction medium (MS + 0.5NAA + 2.0BA) at 1st day of culture, (b) Leaf explants on callus induction medium (MS + 0.5NAA + 2.0BA) at 1stday of culture, (c) Midrib explants on callus induction medium (MS + 0.5NAA + 2.0BA) at 1st day of culture, (d) 15 days old stem callus on callus induction media, (e) 15 days old leaf callus on callus induction media, (f) 15 days old midrib callus on callus induction media, g) shoot bud initiation from stem callus in shoot induction medium (½MS + 0.1NAA + 3.0BA) on 27 days of culture, (h) shoot bud initiation from leaf callus in shoot induction medium (½MS + 0.1NAA + 3.0BA) on 27 days of culture, (i) multiplication of shoot in shoot induction medium (MS + 0.1NAA + 3.0BA) on 6 weeks of culture, (j) initiation of root in MS + 2 IBA medium, (k) acclimatized plant in soil, (l) plants that survived in natural condition.*Scale bars* represent 0.5 cm (a, b & c), 1 cm (d, e, f, g & h), 2.5 cm (i, j & k), 1.0 cm (l).

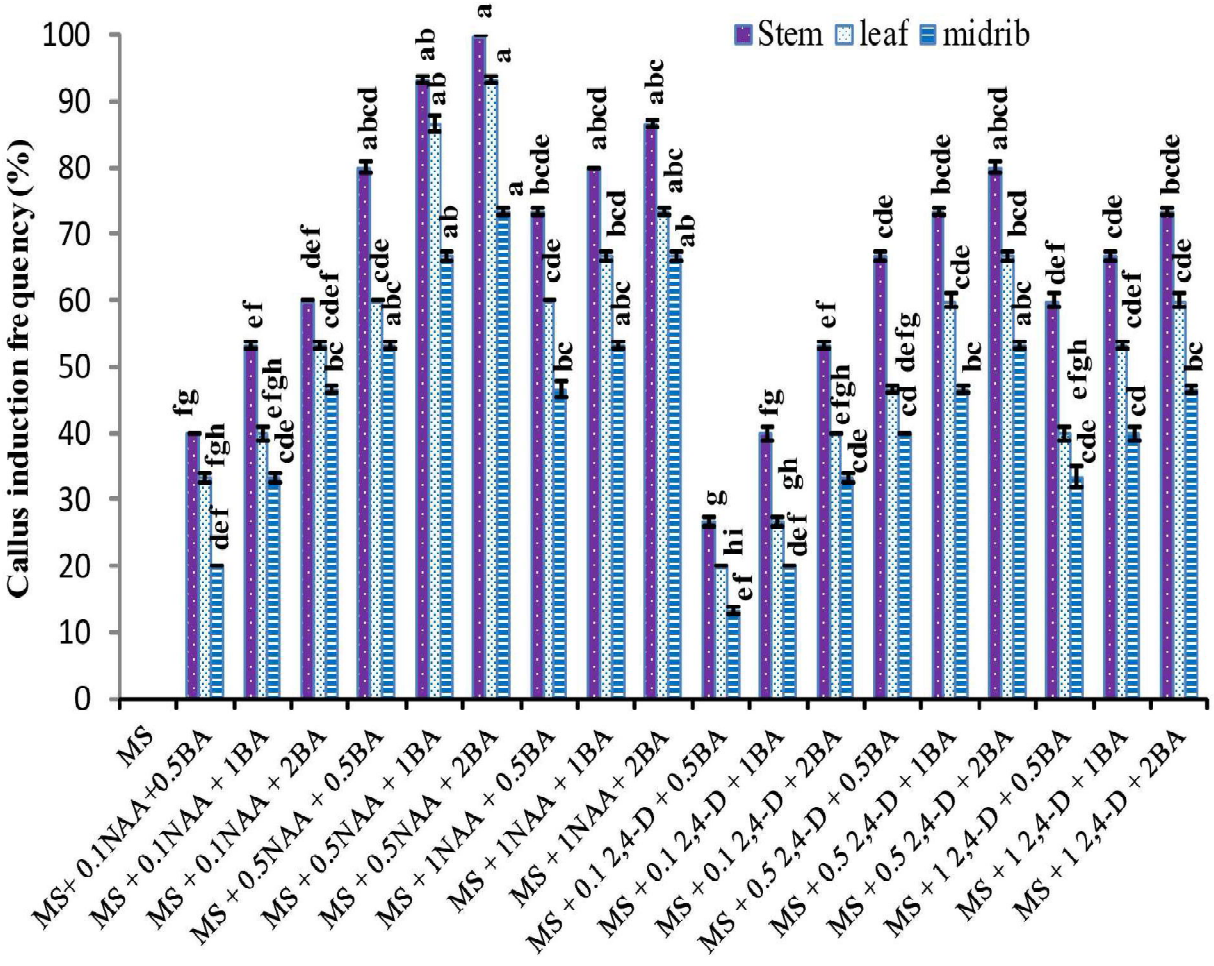


Fig. 2 : Callus initiation frequency from *In vivo* stem, leaf and midrib explants of *A. paniculata* on MS media fortified with various concentrations of BA, NAA and 2,4-D. Data consist of three replications and 5 explants were utilizedin each replication. Bars reflect the standard deviation of means. Values with different letters are significantly different at *P* value = .05 (DMRT).

Table 1 : Morphology of callus at 3rd week of culture periodbased on explant type

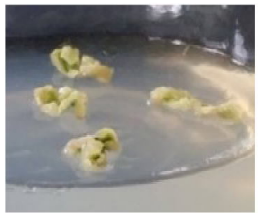
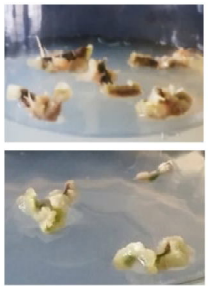
Stem explant	Leaf explant	Midrib explant
		
Compact, light green or brownish in colour	Compact to moderate compact and light green in color, sometimes friable and white	Fragile, Greenish white or whitish in color, very poor in size

Table 2 : Morphology of callus at 3rd week of culture period based on PGR concentration and combination

High Auxin + Low cytokinin (1.0 mg/L NAA + 0.5 mg/L BA; 1.0 mg/L 2,4-D + 0.5 mg/L BA)	Low Auxin + High cytokinin (0.5 mg/L NAA + 2.0 mg/L BA; 0.5 mg/L 2,4-D + 2.0 mg/L BA)	High Auxin + High cytokinin (1 mg/L NAA + 2 mg/L BA; 1 mg/L 2,4-D + 2 mg/L BA)	Low Auxin + Low Cytokinin (0.1 mg/L NAA + 0.5 mg/L BA; 0.1 mg/L 2,4-D + 0.5 mg/L BA)
			
Compact, whitish, brownish or brownish white in colour.	Compact, green, light green, greenish white or brownish in colour. Calli grew very fast.	Very compact, brownish, brownish white or white in colour.	Fragile, Brownish or whitish in colour.

buds was sub cultured on shoot regeneration media to obtain complete shoot buds. The percentage of shoot regeneration was varied with the difference of concentrations and combinations of growth hormones. Out of different combinations, ½ MS + 0.1 mg/L NAA + 3.0 mg/L BA was showed the highest 73.33% and 60% shoot regeneration frequency for leaf as well as stem explants respectively (Fig. 3). This result has proven that, ½ MS + 0.1 mg/L NAA + 3.0 mg/L BA is the best media combination for shoot initiation. Other findings of *in vitro* shoot regeneration of *A. paniculatas* (Bansi and Rout, 2013) also suggest that ½MS in a combination with 3.0 mg/L BA produce higher number of shoots. However, the better performance of stem explant in shoot regeneration is also supported by the findings of Roy (2014).

The strength of MS medium also influenced the shoot regeneration from stem and leaf callus. Full MS medium showed browning of the callus (Fig. 4c) and reduced shoot elongation process (Fig. 4b). These observations might be influenced by thehigh mineral concentrations, present in full strength MS media, excessive in amount for shoot morphogenesis (Purkayastha *et al.,* 2008; Kataky and Handique, 2011).

From the above data, it was found that the frequency of shoot regeneration from stem explant was higher than that of leaf explant (Fig. 3). To investigate the reason behind more shoot regeneration frequency of stem explants compared with leaf explant, anatomical study of stem and leaf segment of *A. paniculata* was carried out. Transverse section (T.S.) of stem possesses epidermis, collenchyma, chlorenchyma, sclerenchyma, phloem, xylem, pith cells (Fig. 5) whereas T.S. of leaf contains layer of epidermis, palisade parenchyma, spongy parenchyma (Fig. 5). Among them chlorenchyma, pith, palisade parenchyma, spongy parenchyma is parenchymatous cell. In microscopic observation, number of active parenchymatous cells was found more in stem than that of leaf (Fig. 5). And collenchyma cell was found in the stem which is absent in the leaf (Fig. 5).

Possibility of the highest shoot regeneration from the stem explant was due to presence of more active parenchymatous cells than leaf explant (Fig. 5). On the other hand, collenchyma cells serve the growing part of the plant like leaf and shoot which is absent in the leaf (Fig. 5). So, the highest shoot regeneration in the stem explant may be due to the presence of more active parenchymatous cells and collenchyma cells in the stem compared to the leaf explants (Abhilasha and Arpita, 2017).

Initiation of roots

For root initiation, well grown shoots were rescued and sub-cultured into MS media supplemented with different concentration of NAA and IBA (0, 0.5, 1.0

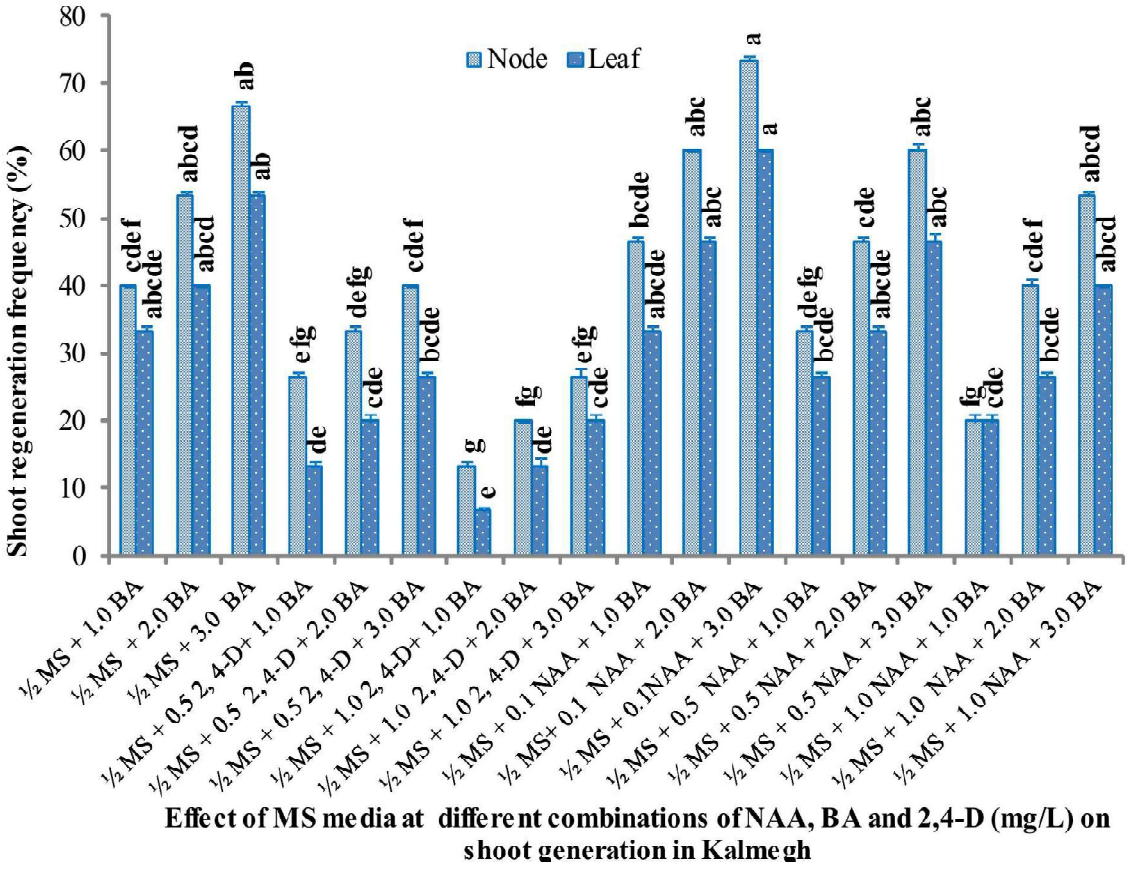


Fig. 3 : Shoot regeneration frequency at 6 weeks of culture of stem and leaf explant of *A. paniculata* on half strength MS media strengthen with various concentrations of BA, NAA and 2,4-D. Data consist of three replications and 5 explants were utilizedin each replication. Bars reflect the standard deviation of means. Values with different letters are significantly different at *P* value = .05 (DMRT).

and 2.0 mg/L). Among the fourteen tested media, the best result was obtained in half strength MS media containing 2.0 mg/L IBA. The maximum percentage of rooting (93.33%) was noticed in half MS medium fortified with 2.0 mg/L IBA whereas the minimum root formation frequency (33.33%) was found in full strength MS medium reinforced with 0.5 mg/L NAA (Table 3).Root development was noted within 7 days of transplanting, and plantlets established a well-developed root system by 15 days (Figure 1j). After that the plants were transferred to pot soil of acclimatization room for acclimatization (Figure 1k). The acclimatized plantlets were grown successfully in field condition.

Figure 4 (a) : Excessive rooting in the callus-on-callus induction medium contained higher concentration of NAA and 2,4-D (more than 1 mg/L), (b) no elongation of shoot in full strength MS medium, (c) browning of callus on full strength MS medium.*Scale bars* represent 1 cm (a, b, c).

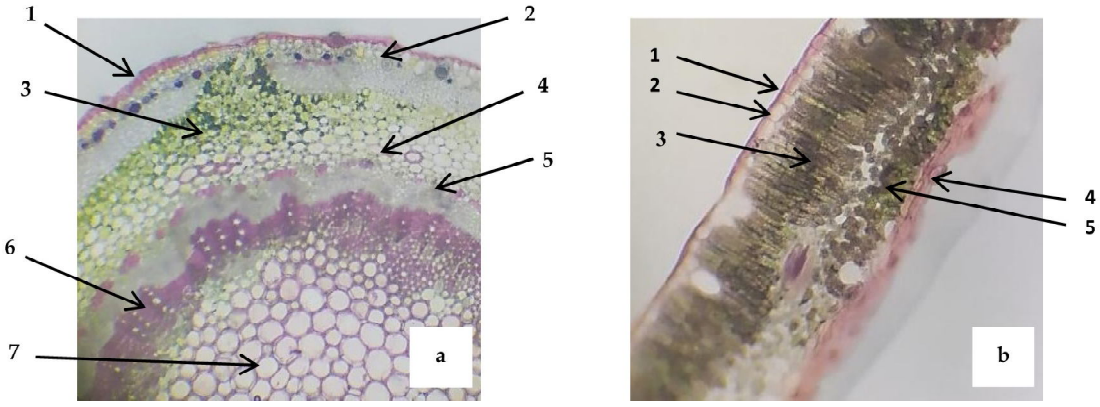


Figure 5(a) T.S. of Stem of *A. paniculata* Nees (× 40) where 1. Epidermis, 2. Collenchyma,3.Chlorenchyma, 4. Sclerenchyma, 5. Phloem, 6. Xylem and 7. Pith cells; (b), T.S. of Leaf of *A. paniculata*(× 40) where 1. Cuticle, 2. Lower epidermis, 3. Palisade parenchyma, 4. Upper epidermis and 5. Spongy parenchyma.

Table 3 : Root initiation frequency of A. *paniculata* on different strength of MS media reinforced with various concentrations of NAA& IBA.

Medium	Concentrations of growth hormone (mg/L)		Days of root initiation	Root formation frequency(%)
	NAA	IBA		
Full Strength MS Media	0	0	-	0^{f}
	0.5	0	12-15	33.33 ± 0.58^{e}
	1.0	0	10-12	40 ± 1^{e}
	2.0	0	8-10	53.33 ± 0.58^{cde}
	0	0.5	10-12	40 ± 1^{e}
	0	1.0	8-12	66.67 ± 0.58^{bcd}
	0	2.0	8-10	73.33 ± 0.58^{abc}
Half Strength MS Media	0	0	-	0^{f}
	0.5	0	10-15	46.67 ± 0.58^{de}
	1.0	0	8-12	53.33 ± 0.58^{cde}
	2.0	0	8-10	73.33 ± 0.58^{abc}
	0	0.5	10-12	73.33 ± 0.58^{abc}
	0	1.0	8-12	86.67 ± 0.58^{ab}
	0	2.0	7-10	93.33 ± 0.58^{a}

Data consist of three replications and 5 explants were used for each replication. The mean values were compared by DMRT. Mean ± SD followed by same letters are not significantly different at $P = 0.05$.

Growth hormone, NAA or IBA alone or in a combination provide positive effects on rooting *in vitro* for *A. paniculatas* (Bansi and Rout, 2013; Roy, 2014; Kataky and Handique, 2011). In this study, two auxins (NAA & IBA) were used for root initiation. Six weeks old shoots,obtained from the subsequent culture in callus initiation and shoot formation media, were used for root formation. However, the regenerated shoot did not produce any roots in control (full and half strength MS medium without hormones) even after six weeks of culture. This type of *in vitro* rooting behavior of *A. paniculatas* also reported by Kataky and Handique

(2011); Bansi and Rout (2013); Jindal *et al.* (2015) and Chandran *et al,* (2017). Root initiation was observed in full and half strength MS media supplemented with NAA or IBA after 7 days of inoculation. The highest (93.33%) rooting was found in half strength MS medium supplemented with 2.0 mg/L IBA whereas the lowest (33.33%) rooting was occurred in MS media with 0.5 mg/L NAA (Table 3). Similarly, Jindal *et al.* (2015); Chandran *et al.* (2017); Deshmukh *et al*. (2017); found maximum root formation in 2.0 mg/L IBA.

In this study, half strength MS media responded better to root initiation than full strength MS media which is in accordance with Chandran *et al*. (2017); Deshmukh *et al,* (2017); Roy (2014); Bansi and Rout (2013); Kataky and Handique (2011). This could be owing to half-strength MS media having lower osmotic strength and nutritional content than full-strength MS media. Half-strength MS media provided low osmotic potential for ease acclimation of plants and also created partial stress, causing plants to develop more roots and stimulate rhizogenesis.

Any medium with lower concentrations of auxin showed a poor response in root formation as compared to medium with higher concentrations of auxin and IBA was proved more responsive than NAA which is in accordance with Roy (2014) and Purkayastha *et al.* (2008) findings.

CONCLUSIONS

In conclusion, the present study demonstrates an easy, cost effective, rapid and highly efficient *in vitro* regeneration protocol of *A. paniculata*. Stem explants showed better response both for callus initiation and shoot regeneration. MS medium complemented with 0.5 mg/L NAA and 2.0 mg/L BA was found as proper medium forhigh frequency callus initiation.The medium ½MS medium enriched with 0.1 mg/L NAA and 3.0 mg/L BA resulted in maximum shoot regeneration. The best medium for root initiation of *A. paniculata* was MS medium enhanced with 0.2 mg/ L NAA. Commercial utilization of this protocol will allow the pharmaceutical industries to multiply vast quantities of this key medicinal plant species for developing novel drugs to fight against infectious disease with more safety.

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