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Effect of Indole-3-acetic acid (IAA) on *in-vitro* Regeneration of Medicinal Plant-Gwarpatha (*Aloe vera*)

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ABSTRACT: The discovery and documentation of the role of plant growth regulators in tissue culture served as major thrust for the crop improvement. For getting maximum response, utilization of suitable growth regulator at appropriate concentration needs to be carefully balanced and controlled. Therefore, the present investigation was carried out to optimize the levels of Indole-3-acetic acid (IAA) for micropropagation of Gwarpatha [*Aloe vera* (L.) Burm. f.]. Lateral shoot explant was used for inoculation with incorporation of different concentrations of IAA (0.5 – 4.0 mg/L) in Murashige and Skoog (MS) medium. Diverse regeneration responses were observed for shoot multiplication, root induction and for callus induction at varied levels of IAA. Highest number of shoot per explant (2.90) was recorded in the treatment of 3.0 mg/L with 70 % induction frequency while, maximum number of root (4.10) was induced at 2.0 mg/L. Moreover, highest callus weight (1.24 g) was reported at 4.0 mg/L with yellow brown colour and semi-compact texture. These identified levels of IAA, further can be utilized for efficient micropropagation of *Aloe vera* in view of reducing economic cost.

Keywords: *Aloe vera*, Callus, Gwarpatha, Indole-3-acetic acid, Micropropagation, IAA, Root induction, Shoot proliferation.

INTRODUCTION

Gwarpatha [*Aloe vera* (L.) Burm. f.] is a succulent perennial plant, have valuable medicinal properties (Carter *et al.*, 2011). Worldwide it is utilizes in the formulation of traditional medicine and in food and cosmetics industries due to over abundance of biological activities of some of its primary and secondary metabolites. Bioactive ingredients of *Aloe* gel decreases total cholesterol level, triglyceride and blood sugar levels in diabetic patients and remarkably increases HDL (good cholesterol) level and helpful for curing gastrointestinal disorders and coronary heart diseases (Tiwari and Upadhayay, 2018). *Aloe* gel obtained from leaves contains 98-99 % water while dry matters of gel have lipids (4-5 %), protein (8.92 %), soluble sugars, polysaccharides, dietary fiber (35.5 %), ash (23.6 %), minerals and vitamins. Most common minerals of gel are Ca (16 %), K (4.06 %), Na (3.66 %) and Mg (1.22 %). *Aloe* gel is rich source of vitamins such as vitamin C (127.6 mg/100 g), E (0.25 mg/100 g), A, B, B12, choline and folic acid (Sanchez-Machado *et al.*, 2017). Acemannan (carbohydrate), a major fraction of *Aloe* gel, has been extensively investigated and proven to stimulate wound healing and anti-cancer activity *in vivo* through activation of immune responses (Chantarawaratit, 2014; Sanchez-Machado *et al.*, 2017). In conventional cultivation method of *Aloe vera*, lateral shoots or suckers utilizes as planting material. A single mother plant produces up to 5 lateral shoots per year which is insufficient for large scale propagation. Slow growth of shoots, also creates shortage of planting material to fulfill the demand of growing pharmaceutical industries. (Bhandari *et al.* 2010; Ahmad *et al.*, 2021). Hence, utilization of plant tissue culture techniques for micropropagation is a pre-requisite for large scale multiplication of *Aloe*. The discovery and documentation of the role of plant growth regulators (PGRs) like auxins and cytokinins in tissue culture served as major thrust for the crop improvement. Auxins, cytokinins, and auxin-cytokinin interactions are usually considered to be the most important for regulating growth of plant under control condition, as these two classes of hormones generally required for root and shoot regeneration (Choudhary *et al.*, 2011; Ahmad *et al.*, 2020). Root induction and callus proliferation is a dominating characteristic for most of the auxins (Adelberg and Naylor-adelberg 2012). However, regeneration response of auxins highly dependent on the concentrations of growth regulators and genotypes of plant from which explant collected. Indole-3-acetic acid (IAA) is one of the important auxin, utilizes in plant tissue culture. For *in vitro* propagation of *Aloe*, IAA concentrations/levels need to be carefully balanced and controlled for getting highest regeneration response in view of enhanced profitability. Therefore, the present investigation was carried out to see the effects of IAA on micropropagation of *Aloe* under *in-vitro* condition.

MATERIALS AND METHODS

To test the morphogenetic effect of Indole-3-acetic acid (IAA), lateral shoot explant of genotype "Jobner Aloe-1" was used. Eight treatments were made by using the different concentrations of IAA *viz.* 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/L in the MS medium (Murashige and Skoog, 1962). Culture medium without incorporation of IAA was used as control. Ten replications of each treatment were used to lay out the experiment following CRD (Completely Randomized Design). All inoculated cultures were placed in culture room and aseptic condition was maintained at 25 ± 2°C temperature. Frequent light was given to the cultures (14 hours light and 10 hours dark) with intensity of 3000 lux.

Cultures were observed periodically throughout the investigation and data were recorded after 6 weeks of inoculation. Number of shoot per explant and number of root per explant were counted and mean data was used for analysis. Shoot length (cm) and shoot width/diameter (at middle length of the shoot) in centimeter were measured using scale. Data on morphological aspects of root (length and thickness) were recorded on visual basis. Callus weight was measured in gram using electric balance while data on callus colour and texture were observed visually. Percent morphogenetic response for shoot, root and callus induction were calculated as:

Morphogenetic response (%) =

$$\frac{\text{No. of explants response}}{\text{Total no. of inoculated explants}} \times 100$$

Software XLSTAT was used for data analysis for means and standard error. Test of significance for treatment comparisons were done by following the DMRT (Duncan's Multiple Range Test) (Gomez and Gomez, 1984). In the tables, data were represented as Mean ± Standard Error (SE). Values followed by same letters in each column differ non-significantly at 5 per cent probability.

RESULTS AND DISCUSSIONS

Perusal to Table 1, 2 and 3, IAA (Indole-3-acetic acid) showed diverse morphogenetic effects on lateral shoot explants of *Aloe* in MS medium. The degree and direction of regeneration for shoots, roots and callus induction were fluctuated with different levels, however, MS medium devoid of IAA (control), did not show any morphogenetic response on explant. Days taken in shoot initiation and number of shoots per explant ranged between 15.50 to 31.10 days and 0.30 to 2.90, respectively. Highest number of shoot (2.90) was induced at the concentration of 3.0 mg/L IAA with 70 per cent frequency in 16.50 days [Table 1 and Fig. 2 (a)]. At this level, shoot multiplied per explant were significantly higher than rest of the treatments. Similarly effect on shoot multiplication of IAA added singly or in combination with other plant growth regulators was reported by Chaudhuri and Mukundan (2001); Rizwan *et al.*, 2014; Sahoo and Rout, 2014. However, shooting efficiency reported by them was much higher which might be due to the differences in type of explant, genotype, shape and capacity of container used for culture and culture room environment. The present study revealed that number of shoot per explant gradually increases with increased levels of IAA up to 3.0 mg/L, and then higher levels declined the efficacy of shoot multiplication may be due to initiation of callus [(Fig. 1 (a)]. However, continuous growth in shoot length and width were observed with increasing levels of IAA and maximum shoot length (4.63 cm) and width (0.93 cm) were obtained at highest level of IAA (4.0 mg/L).

Similarly, all the levels of IAA showed root induction with 50 – 80 per cent induction frequency. Days taken for root initiation gradually declined with the enhanced levels of IAA (21.92 to 15.96). Highest number of root (4.10) per explant was induced at 2.0 mg/L with 80 per cent morphogenetic response [Table 2 and Fig. 2 (b)]. In the same way, effects of IAA on root induction were also reported by various researchers in different genotypes (Lee *et al.*, 2011; Rizwan *et al.*, 2014; Kumar *et al.*, 2017). The trend of root induction represented in the Fig. 1 (b), indicates that increased levels of IAA gradually stimulates root formation up to 2.0 mg/L and further raised levels more inclined the ingredients of culture medium for the formation of shoots and callus, rather than root induction, progressively. Similarly, length of root was also increases initially and reduced on higher levels, while thickness of root was continuously increased with raised levels.

Table 1: Effect of different concentrations of IAA on shoot proliferation.

IAA (mg/L)	Days taken for shoot initiation	Morphogenetic response (%)	Number of shoots /explant	Shoot length (cm)	Shoot width (cm)
0.5	31.10±0.35^{a}	20	0.30±0.15^{e}	2.94±0.22^{e}	0.47±0.02^{g}
1.0	27.50 ±0.27^{b}	30	0.80±0.13^{de}	3.21±0.20^{de}	0.58±0.02^{f}
1.5	22.80±0.39^{c}	30	0.90±0.10^{d}	3.52±0.12^{cd}	0.64±0.03^{ef}
2.0	20.10±0.28^{d}	40	1.40±0.22^{c}	3.85±0.09^{bc}	0.69±0.03^{de}
2.5	17.80±0.25^{e}	60	2.30±0.15^{b}	3.99±0.09^{bc}	0.75±0.04^{cd}
3.0	16.50±0.31^{f}	70	2.90±0.23^{a}	4.15±0.06^{ab}	0.80±0.03^{bc}
3.5	16.10±0.28^{f}	70	2.70±0.15^{ab}	4.23±0.17^{ab}	0.86±0.02^{ab}
4.0	15.50±0.31^{f}	60	2.60±0.22^{ab}	4.63±0.26^{a}	0.93±0.02^{a}

Values represented as Mean ± SE (Standard Error)
Values followed by different letters in each column differ significantly as per DMRT

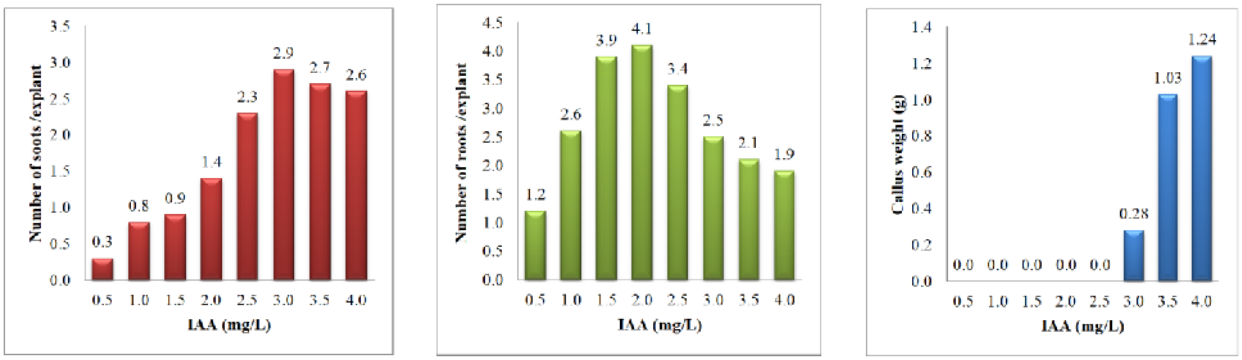
Slight amount of callus induction was observed at higher levels (3.0 to 4.0 mg/L) with increased callus weight [Fig. 1 (c)] and callus induction frequency. Maximum weight of callus (1.24 g) was reported at highest level of IAA (4.0 mg/L) with yellow brown colour and semi-compact texture [Table 3 and Fig. 2 (c)]. Efficacy of IAA for callus induction was reported low for the current studied treatments which may increase upon higher concentration levels. Similarly, efficacy of IAA for callus induction was also reported many researchers by Saggoo and Kaur, 2010; Kumari and Naseem, 2015 in *Aloe vera* and their findings were in accordance to the present results.

Table 2: Effect of different concentrations of IAA on root induction.

IAA (mg/L)	Days taken for root initiation	Morphogenetic response (%)	Number of roots /explant	Root morphology
0.5	21.92±0.34^{a}	50	1.20±0.13^{e}	Very thin and short
1.0	19.15±0.16^{b}	60	2.60±0.16^{c}	Very thin and short
1.5	18.12±0.41^{c}	80	3.90±0.18^{ab}	Thin and long
2.0	17.50±0.66^{d}	80	4.10±0.18^{a}	Thin and long
2.5	16.95±0.55^{ef}	70	3.40±0.31^{b}	Thin and long
3.0	16.58±0.36^{ef}	60	2.50±0.17^{c}	Slightly thick and long
3.5	16.34±0.22^{ef}	60	2.10±0.18^{cd}	Slightly thick and short
4.0	15.96±0.65^{f}	60	1.90±0.18^{d}	Slightly thick and short

Table 3: Effect of different concentrations of IAA on callus induction.

IAA (mg/L)	Days taken for callus initiation	Morphogenetic response (%)	Callus weight (g)	Callus colour	Callus texture
0.5	—	—	—	—	—
1.0	—	—	—	—	—
1.5	—	—	—	—	—
2.0	—	—	—	—	—
2.5	—	—	—	—	—
3.0	23.20±0.34^{a}	10	0.28±0.03^{c}	Yellow	Friable
3.5	20.65±0.52^{b}	30	1.03±0.08^{b}	Yellow brown	Semi compact
4.0	19.28±0.22^{c}	30	1.24±0.11^{a}	Yellow brown	Semi compact



(a) Shoot multiplication **(b)** Root induction **(c)** Callus induction

Fig. 1. Trends of responses of different concentrations of IAA on shoot, root and callus induction.

(a) Shoot multiplication at 3.0 mg/L **(b)** Root induction at 2.0 mg/L **(c)** Callus induction at 4.0 mg/L

Fig. 2. Regeneration responses of different concentrations of IAA on shoot, root and callus induction.

CONCLUSION

Indole-3-acetic acid (IAA) plays multidimensional role on regeneration of *Aloe vera* under *in vitro* condition. However, different concentrations showed variable responses for shoot multiplication, root induction and callus proliferation in lateral shoot explant. The best performing levels for shoot multiplication and root induction were obtained at 3.0 mg/L and 2.0 mg/L, respectively. Moreover, maximum callus was obtained at the concentration of 4.0 mg/L. These identified treatment levels of IAA further can be utilized for better economic use in the propagation of *Aloe vera* under *in vitro* condition.

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Conflict of Interest. None.

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