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Morphogenetic effect of NAA (1-naphthalene acetic acid) on *in-vitro* Regeneration of *Aloe vera*

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ABSTRACT: The NAA (1-naphthalene acetic acid) is an important auxin, commonly utilize in plant tissue culture for micropropagation of various plant species. The degree and direction of regeneration response of explant, highly influences with the concentration of growth regulator. Therefore, in the present investigation, effects of different levels of NAA (0.5 to 4.0 mg/l) were evaluated for shoot proliferation, callus induction and root induction of *Aloe vera.* Various concentrations of NAA had shown differential effects on lateral shoot explant in MS (Murashige and Skoog) medium. The treatment of 2.5 mg/l induced highest number of shoots (3.10) per explant, followed by 3.0 mg/l NAA (2.90) with 80 per cent induction frequency. Maximum callus weight (1.93 g) was observed at 3.5 mg/l with 50 per cent frequency while, highest number of roots (5.0) was obtained at 1.5 mg/l with root induction frequency of 90 per cent. Thus, for best economical use, these identified levels of NAA can be utilized for getting maximum regeneration response of *Aloe* under *in vitro* condition.

Keywords: Aloe, Callus, Micropropagation, NAA, Root induction, Shoot proliferation.

INTRODUCTION

Aloe vera (L.) Burm. f. (Carter *et al.,* 2011) is a desert medicinal plant, immensely utilizes in the pharmaceutical, cosmetic and food industries. It is synonymously known as *Aloe barbadensis* and commonly called "Gwarpatha" or "Ghrit kumari" in Sanskrit (Raksha *et al.,* 2014; Ahmad *et al.,* 2020). Bioactive ingredients of *Aloe* gel known for, anti-inflammatory, anti-tumor, anti-ulcer, anti-cancer, anti-bacterial and anti-viral properties (Jayakrishna *et al.,* 2011). *Aloe vera* is a short stemmed, xerophytic, evergreen perennial shrub, grows up to height of 80-100 cm. Flowers are drooping produced in an inflorescence of 90-110 cm tall. Each flower is pendulous, with yellow tubular corolla length of 2-3 cm, hermaphrodite and possess male sterility. Traditionally, lateral shoots or suckers are used as planting material for cultivation of *Aloe vera* which is a slow and tedious method for its propagation (Bhandari *et al.* 2010). Due to insufficient availability of naturally developed shoots, large scale production of superior quality planting material of *Aloe* is only possible through *in vitro* micropropagation technique. The discovery and documentation of the role of plant hormones like auxins and cytokinins in plant 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 *in vitro* condition, as these two classes of hormones generally required for root and shoot regeneration (Choudhary *et al.,* 2011). The NAA (1-naphthalene acetic acid) is one of the important auxin, commonly utilize in plant tissue culture for micropropagation of various species. For *in vitro* propagation of *Aloe*, NAA concentrations/levels need to be carefully balanced and controlled for getting maximum regeneration response in view of reducing economic cost. Therefore, the present investigation was carried out to evaluate the morphogenetic effects of NAA on shoot proliferation, callus induction and root induction of *Aloe vera.*

MATERIALS AND METHODS

The present investigation was carried out at Sri Karan Narendra Agriculture University Jobner, India at Tissue Culture Laboratory under the department of Plant Breeding and Genetics, during the year 2020-2021. Lateral shoot explant of Jobner Aloe-1 genotype was used. Sizes of 3-4 cm length of lateral shoot explants were used for testing the morphogenetic effects of NAA in MS medium (Murashige and Skoog, 1962). Eight discrete concentrations/levels of NAA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0) were incorporated in the medium and responses over regeneration along with control (MS medium devoid of NAA) were observed. The experiment was laid out in Completely

Randomized Design (CRD) using ten replication of each treatment. Inoculated vessels kept in culture room which was maintained at standardized temperature (25 ± 2°C) with light intensity of 3000 lux for 14 hours followed by 10 hours dark in a day.

Cultures were observed periodically throughout the experiments and data on initiation of shoot, callus and root induction were recorded after 8 weeks of inoculation. Number of shoot and root per explant were counted and average was used for analysis. Shoot length and shoot width/diameter (at middle length of the shoot) were measured in centimeter (cm) using scale. Morphological data on root (length and thickness) were observed visually. Callus weight was measured in gram using electric balance while data on callus colour and texture were observed on visual basis. Morphogenetic response of explant (per cent) for induction of shoot, root and callus were calculated as:

Morphogenetic response (%) =

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

Data were analyzed by using XLSTAT software for means and standard error accordingly as described by Snedecor and Cochran (1972). Test of significance for treatment comparisons were done following Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984). Analysed data depicted in the table as Mean ± Standard Error (SE). Values followed by different letters (as per DMRT) in each column significantly differ at probability of 5 per cent.

RESULTS AND DISCUSSIONS

In the present investigation, effect of NAA (1-naphthalene acetic acid) was evaluated for micropropagation of *Aloe vera* using lateral shoot explant in MS medium. MS basal medium without supplementation of NAA (control) did not show regeneration response. However, differential levels of NAA revealed varied effects on shoot multiplication, callus induction and root induction (Table 1, 2 and 3).

Among all the studied levels of NAA (0.5-4.0 mg/l), treatment of 2.5 mg/l induced highest number of shoots (3.10) per explant, followed by 3.0 mg/l (2.90) with 80 per cent morphogenetic response [Table 1 and Fig. 2 (a)]. Shoots multiplied at these levels (2.5 and 3.0 mg/l) were significantly higher than other treatments levels. Days taken in shoot initiation were also found least (14.50 days) for the best performing level (2.5 mg/l) and was maximum (22.10 days) at lowest concentration of NAA (0.5 mg/l). Moreover, longest shoot length (3.91 cm) and shoot diameter (0.81 cm) were observed at 2.5 mg/l and 3.0 mg/l NAA. Graph represented in the Fig. 1 (a) indicated that progressive increment in shoot number was observed with increased concentration on NAA up to intermediate level (2.5 mg/l) and further raised levels declines the number of shoot induction per culture. It might be due to greater response for induction of root and callus at lower and higher concentration of NAA, respectively.

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

NAA (mg/l)	Days taken for shoot initiation	Morphogenetic response (%)	Number of shoots /explant	Shoot length (cm)	Shoot width (cm)
0.5	22.10±0.57^{a}	20	0.40±0.16^{e}	2.48+0.14^{d}	0.44±0.02^{f}
1.0	18.40 ±0.34^{b}	30	0.80±0.13^{de}	2.84+0.09^{cd}	0.53±0.02^{e}
1.5	17.10 ±0.23^{c}	40	1.10±0.10^{cd}	3.40+0.09^{b}	0.65±0.03^{d}
2.0	15.20±0.29^{de}	70	2.20±0.13^{b}	3.72+0.09^{a}	0.72±0.03^{bc}
2.5	14.50±0.17^{e}	80	3.10±0.10^{a}	3.91+0.08^{a}	0.76±0.01^{ab}
3.0	15.30±0.21^{de}	80	2.90±0.10^{a}	3.82+0.09^{a}	0.81±0.02^{a}
3.5	16.00±0.26^{d}	60	2.10±0.10^{b}	3.30+0.09^{b}	0.70±0.02^{cd}
4.0	16.20±0.25^{d}	50	1.30±0.15^{c}	2.90+0.11^{c}	0.58±0.02^{e}

Values in columns represent Mean ± SE

Values followed by different letters in each column are significantly different ($p<0.05$)

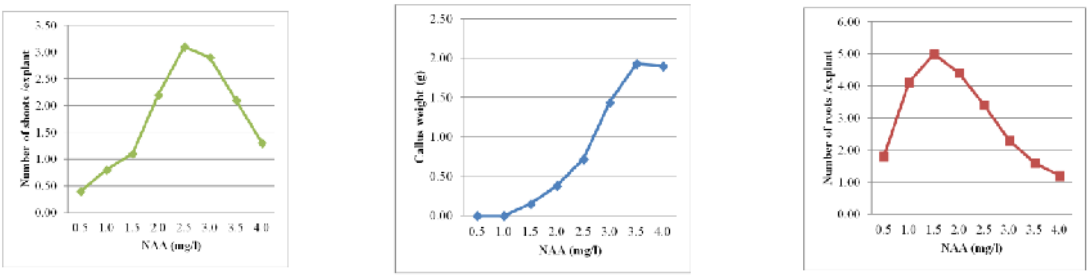
Callus induction at low concentrations (0.5 and 1.0 mg/l) was not reported while a trends of progressive increment in callus weight was observed on higher levels of NAA [Table 2 and Fig. 1 (b)]. Maximum callus weight (1.93 g) was recorded at 3.5 mg/l with 50 per cent frequency [Fig. 2 (b)]. Induced callus was characterized with yellow brown colour and semi compact textured at this level. Higher concentration of NAA, induced higher amount of callus in explant, probably due to the diversion of medium ingredients towards formation of unorganized cell mass rather than multiplying shoots and roots. The similar effects on callus induction of NAA at higher concentration levels were reported by Rathore *et al.,* (2011); Badar *et al.,* (2013) in *Aloe vera.* The enhanced callus induction response of NAA in combinations with other plant growth regulators were also reported by Kim and Lee, (2012); Kumari and Naseem, (2015); Wahab *et al.,* (2020).

All the treatments of NAA also showed root induction with discrete pattern having visual characteristics of short to long size and slightly thick to thicken root (Table 3). Highest number of roots per explant (5.0) was obtained at 1.5 mg/l with maximum root induction frequency of 90 per cent [Fig. 2 (c)]. In perspective of root induction, lower concentration of NAA revealed better results than medium to high levels [Fig. 1 (c)]. It may be because intermediate levels (near to 2.5 mg/l) were more prone to promote shoot multiplication, while higher concentration leads to enhanced callus induction in lateral shoot explants of *Aloe* (Ahmad *et al.,* 2020). In the same way, regeneration response of NAA for root induction was also reported by Lee *et al.,* (2011); Gupta *et al.,* (2014); Sahoo and Rout, (2014); Jakhar *et al.,* (2020) in *Aloe vera*.

Table 2: Effect of different concentrations of NAA on callus induction.

NAA (mg/l)	Days taken for callus initiation	Morphogenetic response (%)	Callus weight (g)	Callus colour	Callus texture
0.5	-	-	-	-	-
1.0	-	-	-	-	-
1.5	25.20±0.38[a]	10	0.15±0.01[e]	Light yellow	Friable
2.0	23.40±0.54[b]	20	0.38±0.01[d]	Light yellow	Friable
2.5	20.20±0.26[c]	30	0.72±0.05[c]	Light yellow	Semi compact
3.0	19.40±0.12[d]	40	1.44±0.04[b]	Yellow	Semi compact
3.5	18.90±0.48[e]	50	1.93±0.08[a]	Yellow brown	Semi compact
4.0	20.50±0.33[cf]	50	1.90±0.08[a]	Yellow brown	Semi compact

(-) = No response
Values in columns represent Mean ± SE
Values followed by different letters in each column are significantly different ($p<0.05$)



(a) (b) (c)

Fig. 1: Trends of responses of different concentrations of NAA for (a) shoot multiplication, (b) callus induction and (c) root induction

Table 3: Effect of different concentrations of NAA on root induction.

NAA (mg/l)	Days taken for root initiation	Morphogenetic response (%)	Number of roots /explant	Root morphology
0.5	20.10±0.28[a]	40	1.80±0.20[de]	Slightly thick and short
1.0	18.90±0.34[b]	70	4.10±0.23[b]	Slightly thick and medium long
1.5	17.20±0.21[c]	90	5.00±0.21[a]	Thick and long
2.0	16.10±0.20[d]	80	4.40±0.22[b]	Thick and long
2.5	15.40±0.25[de]	70	3.40±0.16[c]	Thick and short
3.0	15.80±0.15[de]	70	2.30±0.15[d]	Thick and short
3.5	16.20±0.24[d]	50	1.60±0.16[e]	Very thick and short
4.0	17.40±0.17[c]	40	1.20±0.13[e]	Very thick and short

Values in columns represent Mean ± SE
Values followed by different letters in each column are significantly different ($p<0.05$)

(a) Shoot proliferation (b) Callus induction (c) Root induction

Fig. 2. Response of NAA: (a) shoot multiplication at 2.5 mg/l, (b) subculture of callus induced at 3.5 mg/l, (c) Root induction at 1.5 mg/l

CONCLUSION

Based on our study it was found that, NAA in MS medium showed all rounder effects on lateral shoot explant under *in vitro* condition. It can be utilized for shoot proliferation at intermediate levels (2.0-3.0 mg/l), callus induction at higher levels (more than 3.0 mg/l) and root induction at lower levels (below 2.0 mg/l). Therefore, for best economical use, identified levels of NAA can be utilized for getting maximum regeneration response of *Aloe* under *in vitro* condition.

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Conflict of Interest. None.

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