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OPTIMIZATION OF CONCENTRATIONS OF PLANT GROWTH REGULATORS FOR *In vitro* MULTIPLE SHOOT FORMATION AND EFFICIENT ROOT INDUCTION IN *Phyla nodiflora* L. (*Lippia nodiflora* L.) AN IMPORTANT MEDICINAL PLANT

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ABSTRACT

In the present study nodal and shoot tip explants were cultured in MS medium supplemented with different concentrations of BAP and KN separately, which were fortified with three different concentrations of NAA. It was noted that when nodal explants was inoculated in MS+ 2.0 mg/l BAP and 1.5 mg/l NAA, the percentage of response for shoot bud induction was 88.84 and the number of shoots per explants in this medium was 9.64. These were the highest percentage of response and the number shoots in this medium. In the same culture condition the percentage response for shoot tip explants was 82.26, while the number of shoots buds per explants was 7.25, respectively. When nodal explants were inoculated in MS+ 2.0 mg/l KN + 1.5 mg/l NAA, the percentage response for shoot bud induction was 75.25 and umber of shoots per explants in this medium was 6.39, respectively. In the same medium, when the shoot tip explant was inoculated, the percentage of response for shoot bud initiation was 70.36 and number of shoots per explant was 4.75 respectively. It was further noted that although the above explants, also responded to other concentrations of BAP and KN, along with NAA in MS basal medium but there was discrepancy in both the percentage response as well as the number of shoot buds initiated per explants. Above *in vitro* raised and well grown plantlets were used for rooting. For this both ½ strength as well ¼ strength MS basal medium were supplemented with seven different concentrations of IAA and IBA separately. Shoots were excised and inoculated in the aforesaid rooting medium. Here again it was noted that the explants said rooting medium. Here again it was noted that the explants inoculated in ½ MS + 1.0 mg/l IBA revealed maximum percentage (73.54) after 36th days of inoculation. The number of roots per explants was 34. ½ MS + 1.0 mg/l IAA, induced roots and the percentage of response was 66.36, while the number of roots per explants was 2.6 only. It may be concluded that for shoot bud induction, nodal explant and MS + 2.0 mg/l BAP + 1.5 mg/l IBA was most promising culture condition. Similarly, for root induction ½ MS + 1.0 mg/l IBA was the best medium for *Phyla nodiflora.*

KEYWORDS: Nodal Explants, Shoot Bud Explants, Axillary Shoots, Regeneration, *Phyla nodiflora*, *In vitro*

ABBREVIATION: MS – Murashige & Skoog medium, BAP- 6- Benzyl amino purine, KN- Kinetin, NAA- Naphthalene acetic acid, IAA- Indole acetic acid.

In spite of herbal treatments for curing different ailments from the ancient times, even today large numbers of medicinal plants are being harvested from their wild habitat. Because there is no control over their harvesting, so the agents of traders and Vaidaya, do harvest them mercilessly and due to this several species have become extinct or are on the verge of extinction. Plant tissue culture techniques are said to be more suitable alternative to help in this alarming problems. Establishment of cell, tissue and organ culture and regeneration of plantlets under *in vitro* conditions has opened up new avenues in the areas of plant biotechnology (Dagla, 2012). Micropropagation is the process of vegetative growth and multiplication from viable and regenerative cells in aseptic and favorable condition on suitable culture medium using various plant tissue culture techniques (Zhou and Wu, 2006). Because through *in vitro* propagation large numbers of identical plants can be produced within a limited space and time, which can be used as planting materials, this technique is being used in the Micropropagation of different medicinal plants in and outside of our country. *Phyla nodiflora* L. (*Lippia nodiflora* L) of family *Verbenaceae* is found growing in different habitat under wild conditions. Plants, due to abundance of branching system form a mat where it grows. Stem reveals a runner habit with scanty roots near the nodes.

Rural people use these plants for the treatment of different diseases. The paste of stem and leaves are placed on head to cure Jaundice. This plant contains different secondary metabolites which have been isolated and identified by different workers (Ravikant *et al*., 2000). Modern researches have confirmed that the secondary metabolites are being used as anti-cancer, anti-tumor, anti-fungal and cytotoxic agents (Nishino *et al*., 1988). Tissue culture and micropropagation of plants have been done by Jullani *et al*., (1999), *Lippia junelliana*., Rout *et al*., (2000), Gupta *et al*., (2001), *Lippia alba*., Been and Martin (2003) in *Ceropegia candelabrum*., Bhavisha and Jasrai (2003), Arumugan *et al*., (2003), Teli and Tinko (2004), Lee (2005), Aziz *et*

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al., (2006), Zhou and Wu (2006), Arya *et al*., (2008), Kayo (2008), Balaraju *et al*., (2008), Sasikumar *et al*., (2009), Singh *et al*., (2009), Sidhu (2010), Ahmad *et al*., (2011), Bakrudeen *et al*., (2011), Parven and Shahzad (2011), Priya and Ravindra (2011), Arumugan and Gopinath (2012), Dagla (2012), Garg and Malik (2012), Thiyagrajan and Venkatachalam (2012), Vidya (2012), Balaji and Ebnezer (2013), Daksh *et al*., (2014), Cristiano (2015), and Kavita and Gopal (2018). Keeping the importance of this plant, present study was carried out for *in vitro* micropropagation of *Phyla nodiflora* (*Lippia nodiflora* L.).

MATERIALS AND METHODS

Preparation of Culture Medium

MS basal medium was used for the present work. Above basal medium was supplemented with 3% sucrose (w/v), different concentrations of BAP and KN separately. Similarly, different concentrations of NAA were also added to them. The pH of the medium was adjusted to 5.8, with either 1 N NaOH or 1N HCl, before gelling with 0.8% (w/v) melted agar. 40 ml of above medium was dispensed into 150 ml conical flasks and were plugged with suitable cotton plugs, which were wrapped with Aluminum foil to prevent soaking with water vapour during sterilization at 15 lb pressure for 25 minutes. These cultures were allowed to cool and preserved at low temperature. Inoculation was done after two days and contaminated culture flasks were discarded after autoclaving.

Preparation of Explants

Healthy branches of *Phyla nodiflora* were collected for the campus and brought to the laboratory immediately. Above branches were taken in 2 L conical flask and mouth was covered with muslin cloth with the help of rubber band.

The flask was placed under running tap water for 40 min, so that it was washed thoroughly. Above plant materials were soaked in 5% liquid detergent for 5 min, and then washed under running tap water of 10 min so that the remains of the detergents were completely washed away. They were then treated with 0.1% systemic fungicide (Bavistin) for 10 min and then rinsed with sterile distilled water thrice. For further surface sterilization the *explants* were treated with 0.1% solution of mercuric chloride for 1.5 to 2 min. Above explants were taken in fresh culture flask and immediately washed thoroughly with sterilized distilled water 3-4 times, so that even the traces of the chemical was removed completely. Above sterilization was done in front of running laminar air flow chamber. Above plant material was preserved in pre-sterilized and moisten muslin cloth in the aseptic condition of laminar air flow chamber.

Inoculation

From the above plant materials, nodal segments and shoot tip *explants* were cut and directly inoculated in the culture medium containing flasks. These flasks were incubated in the culture room, where the temperature light and humidity were maintained artificially. The temperature was adjusted at 26 ± 1^0C, moisture 66-72% and light period 16/8 h (Light and Dark) with cool white fluorescent tubes. Observation was made on an alternate day and flasks showing contamination were discarded after autoclaving. Shoots regenerated were noted and percentage response, number of shoots and shoot length were measured after certain periods. They were subcultured and well grown plantlets were inoculated in rooting medium. Here again the percentage of response for rooting was calculated. The number and length were noted. All the experiments were done in triplicate and mean of the data have been tabulated in table 1 and 2.

Based on the above the conclusion for best plant growth regulator and its concentrations were noted.

RESULTS AND DISCUSSION

In the present work, four different concentrations of BAP and KN were supplemented separately in MS medium. Further three different concentrations of NAA were supplemented in the same medium separately. The mean of the data was taken and placed in the table 1. From the table it may be noted that highest percentages of response for axillary shoot generation was among the nodal explants inoculated in MS + 2.0 mg/l BAP + 1.5 mg/l NAA that was 88.74. This was followed in MS + 2.0 mg/l BAP + 2.0 mg/l NAA that was 78.34.

Similarly, MS + 2.0 mg/l BAP + 1.0 mg/l NAA induced shoot buds that was 74.45. MS + 2.0 mg/l KN + 1.5 mg/l NAA induced shoot buds which was 76.25%, where as MS + 2.0 mg/l KN + 2.0 mg/l NAA induced shoot buds in nodal explants that was 63.84% only. It may further be noted form the table that MS + 2.0 mg/l BAP + 1.5 mg/l NAA, induced shoot buds that was in 82.26% in shoot tip explants, which was followed by 71.86% in MS + 2.0 mg/l BAP + 2.0 mg/l NAA. Percentage response for shoot bud induction was 76.25 in

nodal explants when cultured in MS + 2.0 mg/l KN + 1.5 mg/l NAA followed by 63.84% in MS + 2.0 mg/l KN + 2.0 mg/l NAA. The percentage response for shoot bud induction on nodal explants in MS+ 2.0 mg/l BAP + 1.0 mg/l NAA was 74.45, while in MS + 2.0 mg/l BAP + 1.0 mg/l NAA was 52.38 in shoot tip explants. In the similar concentration of KN + NAA the percentage of shoot bud induction in nodal explants was 76.25% and 63.84, while in shoot tip explants it was 70.36 and 57.28 respectively.

Number of shoots per explant and its length were also studied. Form the table it may be noted that maximum shoot number was 9.64 among the nodal explants cultured in MS + 2.0 mg/l BAP + 1.5 mg/l NAA, followed by 6.44 on the nodal explants cultured on MS+ 2.0 mg/l BAP + 2.0 mg/l NAA. On the similar concentration of KN & NA, the number of shoots was 6.34 and 5.72 respectively on the nodal explants. While number of shoots in shoot tip explants was 7.25 and 6.88 in similar concentrations of BAP and NAA. In the similar concentration of KN + NAA the number shoots on shoot tip explants it was 5.75 and 3.62 respectively. Similarly, maximum length of the induced shoot was 4.62 followed by 3.54 can noted on plantlets induced form nodal explants in MS+ 2.0 mg/l BAP + 1.5 mg/l NAA and MS + 2.0 mg/l BAP + 2.0 mg/l NAA respectively.

Table 1: Effect of different concentrations and combination on shoot regeneration on tow explants of *Phyla nodiflora,* supplemented in MS medium after 56 days of inoculation

Plant Growth Regulators (mg/l)		Percentage of response (%)		Induction Shoot		Shoot length (cm)	
		Nodal explants	Shoot tip	Nodal	Shoot tip	Nodal	Shoot tip
BAP	NAA						
1.0	1.0	62.74	42.64	5.56	5.18	2.36	2.18
1.5		66.24	48.54	6.34	5.66	2.86	2.54
2.0		74.45	52.38	7.84	7.36	3.18	2.75
2.5		59.64	41.28	4.72	4.48	2.38	2.46
1.0	1.5	64.32	51.46	6.72	6.36	2.86	2.68
1.5		76.56	63.88	7.28	6.84	3.28	2.88
2.0		88.84	82.26	9.64	7.25	4.62	3.4
2.5		61.66	48.76	5.42	5.12	2.72	2.16
1.0	2.0	60.28	55.28	5.38	5.12	2.66	2.46
1.5		71.45	68.45	6.44	6.24	2.82	2.62
2.0		78.34	71.86	7.86	6.88	3.54	3.12
2.5		57.64	46.15	4.24	4.72	2.38	2.28
KN	NAA						
1.0	1.0	66.54	65.46	3.24	3.48	2.68	2.12
1.5		68.72	67.18	4.58	4.32	2.88	2.74
2.0		74.36	68.56	5.44	4.26	3.36	3.24
2.5		64.28	56.18	2.84	3.28	2.24	1.80
1.0	1.5	68.54	61.24	4.64	4.18	2.12	1.88
1.5		70.48	66.44	5.48	4.36	2.72	2.62
2.0		76.25	70.36	6.34	5.75	3.86	3.70
2.5		62.36	50.48	5.18	4.12	2.54	2.18
1.0	2.0	46.74	44.66	3.84	2.54	1.46	1.28
1.5		54.38	51.42	4.36	2.24	1.86	1.42
2.0		63.84	57.28	5.72	3.62	2.18	1.78
2.5		51.22	42.16	3.58	2.45	1.64	1.22

Well developed plantlets were excised from the bunch of plantlets and inoculated in rooting medium. The mean of the data obtained was placed in the table 2. Here both ½ strength as well as ¼ strength MS medium were supplemented with 7 different concentrations of IBA and IAA separately. Form the table it was found that maximum percentage of response 68.82 for rooting was in ½ MS + 1.0 mg/l IAA, and maximum length of the

roots was 3.28 cm. Similarly, the highest percentage of response for rooting was 74.54 and maximum length of the roots 5.66 cm in ½ MS + 1.0 mg/l IBA after 42 days of inoculation. The maximum percentage of response 64.58 and length of the roots were 3.72 cm in ¼ MS+ 1.0 mg/l IAA, while at the similar concentration of IBA percentage response for rooting was 68.54 and the number of the roots 6.4, while the length was 5.28 cm respectively.

It was further noted that at both the lowest concentrations or at the highest concentrations of both the growth regulator had no promising effects on root induction. Minimum percentage of response, lowest number of roots and lesser length were fond at 0.2 mg/l of both the hormones in either ½ or ¼ strength of MS medium.

Table 2: Effect of different concentrations of IAA and IBA supplemented in ½ and ¼ strength of MS medium on root induction on excised plantlets of *Phyla nodiflora,* raised through tissue culture after 42 days of inoculation

Growth Regulators (mg/l)		Percentage response for root induction	Number of roots/plantlet	Length of roots (cm)
½ MS	IAA			
	0.2	46.52	2.46	1.66
	0.4	55.78	2.88	1.84
	0.6	61.36	3.54	2.36
	0.8	63.24	3.86	2.74
	1.0	68.82	4.62	3.28
	1.2	62.44	3.28	2.78
	1.4	41.16	2.74	2.24
½ MS	IBA			
	0.2	52.34	2.74	1.88
	0.4	63.78	3.18	2.54
	0.6	66.86	3.76	2.72
	0.8	69.24	4.24	3.84
	1.0	74.54	6.56	5.44
	1.2	65.18	4.28	3.72
	1.4	52.64	3.34	3.18
¼ MS	IAA			
	0.2	38.28	2.58	1.84
	0.4	46.34	3.16	2.26
	0.6	54.68	3.74	2.78
	0.8	59.44	4.36	3.54
	1.0	64.58	5.88	4.68
	1.2	50.76	4.66	3.72
	1.4	42.24	3.76	2.88
¼ MS	IBA			
	0.2	50.38	2.86	2.26
	0.4	55.24	3.18	2.82
	0.6	63.56	3.76	3.42
	0.8	64.86	4.54	4.64
	1.0	68.54	6.44	6.36
	1.2	60.32	5.28	5.28
	1.4	51.44	4.36	3.85

DISCUSSION

From the table 1, where the percentage response for shoot bud induction, the number of shoots per explants and the length of the shoots are mentioned it appeared that shoot bud induction, in both the *explants* was found at all the concentrations of BAP + NAA and

KN+ NAA, but the quantum of response was different in the similar concentrations of BAP+ NAA and KN+ NAA. This was also true for the number of shoot buds and their lengths. Tissue culture studies for *in vitro* multiple shoots induction in medicinal plants have been done by Gupta *et al*., (2001) in *Lippia alba*., Pan *et al*., (2003) in *Artemisia* and *Echinops* spp. Findings of these workers are in agreement with the findings of the present work. Maximum number of shoots on nodal explants of *Phyla nodiflora* than the shoot tip explants have been reported by Ahmad *et al*., (2010). The present findings corroborate with the findings of the above workers. Bhavisha and Jasrai (2010) reported that maximum number of roots were induced in plantlets of *Curculigo orchioides* raised through tissue culture in lowest. 1.0 mg/l concentrations of NAA. Whereas Sasikumar *et al*., (2009) reported that thick and long roots were induced in plantlets of *Baliospermum montanum* in MS + 1.0 mg/l IBA and 0.5 mg/l IAA. All these findings support the findings of the present works.

CONCLUSION

For multiple shoots induction in *Phyla nodiflora* (*Lippia nodiflora*) the best explants is the nodal segments. Similarly, among the plant growth regulator MS + 2.0 mg/l BAP + 1.5 mg/l NAA is the most promising culture condition for maximum multiple shoot buds induction. For rooting in the plantlets of *Phyla nodiflora* raised through tissue culture half strength MS + 1.0 mg/l IBA was most promising condition in the present work.

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