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Investigation on effect of different growth regulators on shoot induction, multiplication and length of shoots in case of *Plantagoafra L.*micropropagation

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ABSTRACT:

The modern world is attracted towards our ancient Ayurveda and natural products for therapeutic purpose. Even most of the drugs are designed by isolating and analyzing the natural constituents from medicinal plants like various secondary metabolites of such plants are extracted and analyzed for their structure and reactive groups which can be utilized for treating range of diseases. Thus, endangered medicinal plant species must be conserved and grown at mass scale for extracting best from them. In thirst of it, endangered *Plantago* species micro-propagation has been investigated and presented in this article. Within the present research article, different findings regarding shoot induction and multiplication from different explants of *Plantagoafra L.* and effect of different growth regulators and plant hormones over it is focused. The different explants were preferred from which significant result in form of shoot induction and multiplication was obtained in case of cotyledon and hypocotyl explants. The maximum shoot regenerated from cotyledon explants was 24 in MS medium contained with 0.85 µM IBA and 0.80 TDZ. The shoots were rooted in different rooting medium out of which best shot was obtained in medium contained with 3.8 µM NAA. The maximum length of the shoot was 1.25 cm after 2 weeks of incubation. The plantlets were transferred to soil pots of green house for hardening.

KEYWORDS: Medicinal plants, micro propagation, endangered plant species, metabolites

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INTRODUCTION:

The requirement of resonators which having capability to get mounted on surface of *Plantagoafra L*. is also called as Black psyllium. It belongs to family of Plantaginaceae which is an herb having stem less leaves with alternate assortment. It has been very popular all around the globe due to its various medicinal properties such as detoxification of digestive tract, soothing effect on gastrointestinal wall and lubricant for very high constipation like situations. Other than that it is helpful in treating chronic kidney diseases, urethritis and bladder diseases[1]. The seed mucilage is known to have peristaltic effects on gut wall and believed to lower down blood cholesterol level when incorporated in regular diet. According to former investigations, it has been experimented that it actively lowers the serum LDL-cholesterol with less significant effect on HDL cholesterol and serum triglycerides. It has prominent effect on intestinal wall and diminishes uptake of glucose from blood and in such a way beneficial in hyperglycemia or type-2 diabetes. It is indigenous species of North Africa, Asia and western Mediterranean region which is cultivated almost all over the globe[2]. The World Health Organization has documented literature on seeds of different species of Plantago. The Biochemical composition of seeds revealed that there is 12-15 % mucilaginous polysaccharides such as galacturonic acid, xylose, arabinose and ramose are commonly found. Other than that around 10 % lipids with sterols and unsaturated fatty acids are also present[3].

The plant tissue culture techniques can be applied for mass scale cultivation of economically and medicinally important plants. Another thing which can be achieved by in-vitro propagation is to preserve endangered species. There is inadequate availability of literature of Plantago tissue culture[4,5]. Thus, the present investigation was intended to standardize culture conditions for organogenesis from different explants. The phytochemical composition of plant extract was experimented to identify secondary metabolites which were aucubuin, geniposidic acid, verbascoside, isoverbascoside, phenyl-ethanoside, glucosides, sorbitol and others[6,7,8,9].

MATERIALS AND METHODS:

In current paper an attempt made for standardization of shoot induction and proliferation from four different explants of *P. afra* plant like cotyledon, hypocotyl, seedling tips and young leaves. The plant was 2 months old and grown at botanical garden of DMAPR, ICAR research institute, Boriavi. It is followed by rooting of microshoots and majority of plantlets were developed via callus induction from different explants.

Explant source: Plant parts of an elite plant of *P. afra L*. grown at botanical garden of Directorate of Medicinal and Aromatic Plant Research (DMAPR), Boriavi, Anand, Gujarat

EXPLANT STERILIZATION:

Apart from collection of plant parts like young leaves, seeds of same plant were collected and treated further. Seeds were sterilized by 30% bleach for 15 minutes to remove the dust particles it was followed by treatment of 2% (v/v) detergent solution (Teepol, Qualigen, India) for 15 minutes and washed in running tap water. The further disinfectant 1% Bavistin fungicide was used for 15 minutes followed by washing with running tap water. Repeated washing with double distilled water for 4 to 5 times was done prior to inoculation. Following sterilization, seeds were socked in to the water. The healthy seeds were sitting to the bottom of the container and were collected for further use. Health seeds were inoculated in MS nutrient media with 3% carbohydrate and 1% agar within petriplates sealed by paraffin tap. The germination time after which seedlings are collected for inoculation was two weeks.

INOCULATION OF EXPLANTS WITHIN MS MEDIA CONTAINING DIFFERENT GROWTH REGULATORS:

The medium for shoot multiplication used was contained with 2.0 µM of BAP and 1.30 µM of NAA, 1.75 µM BAP and 1.30 µM NAA, 1.25 µM BAP and 1.0 µM IBA, 1.25 µM BAP and 0.08 µM IBA, 1.25 µM TDZ ad 1.00 µM IBA, 1.25 µM along with 0.08 µM IBA, 1.75 µM kinetin along with 1.00 µM and 0.08 µM IBA containing MS medium. The control was taken as plain MS medium without any cytokine or growth regulators.

The pH of medium was adjusted in range of 5.8- 6.0 by 0.1 N NaOH and 1 N HCl. It was followed by incorporating agar in to the medium which transferred in autoclave for sterilization for 30 minutes. All experiments were governed under complete sterile condition. Hand gloves, all glass wares and tools used for inoculation were surface sterilized under UV light of laminar air flow hood. The callus induction was followed by shoot induction from the calli. Shoots were inoculated within rooting medium contained with different contents of NAA viz 0.05 µM, 1.0 to 6.0 µM. Rooted plantlets were trans-located to pots with soil having coconut water and other nutrients required for plant growth called as hardening[10].

RESULTS AND DISCUSSION:

In the thirst of the presented study different trials and errors was done for standardization of culture conditions in terms of concentration and explants. Callus growth was observed in each tube having all kind of media composition as well as all four explants within 2 weeks.

SHOOT REGENERATION AND MULTIPLICATION:

Average number of shoots regenerated from each explant within 35 days was 0.35 to 12.90 for cotyledons, 1.15 to 23.82 in case of hypocotyl, 0.09 to 8.17 for seeding tips and 1.64 to 7.89 for young leaves. Amongst all trials the highest number of shoots were regenerated within MS media possessing 1.25 µM TDZ in combination with 0.08 µM IBA in case of hypocotyl explant. BAP in both the combination with NAA and IBA revealed positive effect on callus and shoot induction in all explants in which cotyledon was 10.10 and was better than hypocotyl. The combination of kinetin with IBA was the least significant. Here effect of concentration of growth regulators can directly revealed in case of TDZ, 1.25 µM TDZ with 1.00 µM IBA gave significant regeneration whereas 1.25 µM TDZ with 0.08 µM IBA gave less regeneration in most of the explants and thus can be considered as inhibitor for shot multiplication as shown in Table1.

There was no shoot induction was observed in case of control for cotyledons whereas all other explants revealed little callus and shoot induction in MS medium. Thus, from table.1, it can be observed that hypocotyl was the best explant for more number of shoot regeneration per explant and concentration of growth regulators significantly affect the shoot multiplication in which TDZ and BAP in combination of IBA gave very good regeneration in concentration dependent manner.

Table 1: Mean values of shoot regeneration from four different explants of *P. afra L.* upon 6 weeks of culture
1 Each one is the mean of 3 replicates with 10 number of explants

Plant growth regulators (mg/l)		**Average number of shoots per replication**			
		Cotyledon	**Hypocotyl**	**Seedling tips**	**Young leaves**
BAP (µM)	NAA (µM)				
2.00	1.30	10.10[1] b[2]	9.45 bc	3.78 c	6.95 c
1.75	1.30	0.35 a	9.66 c	4.75 b	4.77 d
BAP (µM)	IBA (µM)				
1.25	1.00	0.44 c	6.28 b	2.15 d	1.64 d
1.25	0.08	2.15 d	2.17 c	4.29 c	2.28 c
TDZ (µM)	IBA (µM)				
1.25	1.00	12.90 c	22.90 a	8.17 c	7.89 a
1.25	0.08	3.50 c	23.82 a	7.68 d	1.99 d
Kinetin (µM)	IBA (µM)				
1.75	1.00	1.80 a	1.15 d	1.72 d	1.85 d
1.75	0.08	2.58 cd	2.60 cd	0.09 c	2.40 c
Control (MS medium)		0.00 d	2.05 c	1.85 c	2.17 d

2 Column contains the digits followed by letters which denotes significance difference at 0.01 level according toDMR test

Table 2: Effects of different growth regulators on shoot length of *P. afra L.*

	No. of explants revealed shoot induction (%)	Length of shoots (cm)
BAP and NAA (µM)		
2.00 and 1.30	100	2.20 a
1.75 and 1.30	100	4.50 c
BAP and IBA(µM)		
1.25 and 1.00	87	5.25 b
1.25 and 0.08	87	4.00 c
TDZ and IBA(µM)		
1.25 and 1.00	76	3.72 ab
1.25 and 0.08	65	2.43 c
Kinetin and IBA(µM)		
1.75 and 1.00	70	2.89 d
1.75 and 0.08	60	2.76 c

1 Each one is the mean of 3 replicates with 10 number of explants
2 Column contains the digits followed by letters which denotes significance difference at 0.01level according to DMR test

Other parameters of investigation were average length of shoots and effect of growth regulators over it. After 35 days of incubation, the shootswere measured for their length by Veneercaliper. The shoot length measured in case of TDZ 1.25 µM and IBA 1.00 µM was 3.72 cm whereas BAP 2.00 µM in combination with 1.30 µM NAA was 2.20 cm. Table 2 revealed that reduction in BAP which was at concentration of 1.75 µM gave shoot length up to 4.5 cm. The highest shoot length was obtained for 1.25 µM BAP in combination with 1.00 µM IBA.

ROOTING OF SHOOTS:

Shoots were aseptically transferred in the tubes containing rooting media which contained with different contents of growth regulator NAA. Root regenerating frequency of each medium is presented in Table 3. NAA concentration significantly enhance rooting within media having 5.00 µM NAA which was 88.67, which was maximum. Further increase in content down fall the frequency of rooting. Other parameters were measured side by side were average number of roots which was concentration dependent and gave highest count in case of NAA content of 4.0µM. It has been observed that the same content of NAA gave highest length of the root which was 3.37 cm as shown in Table 3.

Table 3. Effects of concentrations of NAA on *In vitro* rooting of *P. afra*

NAA (µM)	Root regenerating frequency of each inoculated shoot (%)	Average number of roots regerated from shoot	Average length of root(cm)
0.05	25.55[1] ab[2]	0.75 c	0.87 b
1.00	54.66 c	1.45 d	1.38 b
2.00	35.74 ab	3.25 b	2.55 a
3.00	80.34 bc	3.87 d	3.36 bc
4.00	78.42 a	5.40 a	3.37 bc
5.00	88.67 bc	3.33 b	2.75 a
6.00	57.33 b	2.35 c	1.82 b

1 Each one is the mean of 3 replicates with 10 number of explants

2 Column contains the digits followed by letters which denotes significance difference at 0.01 level according to DMR test

The findings of presented study were comparable with other species of Plantago. Similar work was done in 2001 upon *Plantago ovata* in which they used Casein hydrolysate and coconut water as additives in MS media fortified with NAA and NBA[11,12]. They got significant amount of callus induction and additives also revealed the role in augmentation of somatic embryogenesis. Similar findings were successfully recorded, in which it was documented soot induction from cotyledon and hypocotyl explants in case of *P. lanceolata* by inoculating them within MS media having different contents of BAP and IBA[13].

CONCLUSION

It has been concluded that out of four, hypocotyl explant revealed highest number of shoot regeneration capacity. The growth regulators TDZ in combination with IBA was proved to be the best for shoot induction from callus in case of *P. afra L.* Frequency of shoot regeneration and length of shoots was significantly dependent on concentration of growth regulators such as BAP and IBA of 1.25 µM and 1.00 µM has got the most positive effect on shoot length which was 5.25 cm. Along with shoot proliferation, rooting within different rooting medium was also investigated which revealed that 3.00 µM NAA was optimum for root prolifration whereas 4.00µM NAA was ideal for higher number of roots per explant as well as average length of roots which was 5.40 and 3.37 cm respectively.

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