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Research Article

OPTIMIZATION OF SHOOT MULTIPLICATION MEDIA FOR MICRO PROPAGATION OF *WITHANIA SOMNIFERA*: AN ENDANGERED MEDICINAL PLANT

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ABSTRACT

Effect of different types of plant growth regulators has been studied on *in vitro* multiplication of *Withania somnifera* using nodal explants. Maximum survival percentage 100 % of explants was observed using nodal explants were treated with 0.4 % Streptocyclin and 0.4 % Bavistin for 60 minutes followed by 3 minutes treatment with 0.1 % $HgCl_2$. MS medium supplemented with was found to be best for multiplication of nodal explants of *Withania somnifera* amongst seventeen media employed for multiplication. Maximum eight shoots/explants observed on MS basal medium supplemented with 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA after 45 days of culture. A maximum 77.8 % rooting was found on MS medium (half strength) supplemented with 0.5 mg l-1 NAA. Shoots with well developed roots were separated individually and transferred to plastic bag containing sand, soil and vermin-compost in ratio of 1:1:1 in the green house and was reported 100 % survival. The hardened plants were successfully transferred to field.

Keywords: Micro propagation, Multiplication, Rooting, *Withania somnifera*

INTRODUCTION

Withania somnifera, an herb is one of the common medicinal plants also known as ashwagandha in Sanskrit belongs to family Solanaceae. The plant has been used since Ayurvedic time to treat asthma, bronchitis, emaciation ulcers, insomnia and dementia. It is used as a liver tonic and astringent. Dry arid areas of Bangladesh, China, India and Pakistan are native of this plant. It can be found growing in the Africa and Mediterranean region. The plant possesses many pharmacological actions such as anti-arthritic, anti-rheumatic anti-inflammatory, abortifacient, adaptogenic, anti-stress, anti-tumour, immunomodulatory activities, anti-anxiety, anti-depression and aphrodisiac[1-3]. The medicinal properties of ashwagandha are due to major biochemical constituents such as alkaloids and steroids. Withanolides is main tropane alkaloids of the plant that posses anti-tumour activity. There are few reports available on *in vitro* culture of ashwagandha using different explants, such as from leaf[4-7], shoot tip[8-10], axillary buds[11-14], internodal[15,16], seed[17] and cotyledon[18,19]. For fulfilling ever increasing demand of pharmaceutical industry for the withanolides and to overcome problem of long gestation period between planting and harvesting of this plant there is great need to produce quality planting material of this plant throughout the year. Micropropagation is a feasible approach for the production of true –to-type planting material. Keeping in view all these points, the present investigation was conducted to study the effect of different plant growth regulators i.e. BAP, KIN and NAA alone and in combination on *in vitro* multiplication of *Withania somnifera* using nodal explants.

MATERIALS AND METHODS

Plant material

Young nodal explants were taken from field grown plant at Medicinal section of CCSHAU, Hisar, Haryana, India.

Media Preparation

The present study was conducted using MS^{20} basal medium fortified with various plant growth regulators. The pH of medium was adjusted to 5.8 using 0.1N HCL or 0.1N NaOH. Sucrose 30 g l^{-1} and agar 8 g l^{-1} was added in the medium and autoclaved at 121°C and 15 lbs $inch^{-2}$ pressure for 15 minutes.

Preparation and sterilization of Explants

Young, healthy nodal segments (2-3 cm) were excised from mother plant and washed with mild detergent for 8-10 minutes followed by rinsing under running tap water to remove detergent. The explants were then giving treatment with 0.4 % Bavistin + 0.4 % Streptocyclin for 60 minutes and followed by washing with double distilled water. Explants were then surface sterilized with 0.1 % $HgCl_2$ for 1-4 minutes in Laminar air flow and excess $HgCl_2$ was washed by 4 to 5 washing with double distilled sterilized water.

Culture conditions

The cultures vessels were incubated in growth room. The temperature of growth room was maintained at 25 ± 2°C and light intensity of 100 μEM^{-2} sec^{-1} (1000 lux) was provided using florescent tubes. Period of 16 h / 8 h light and dark was provided.

Shoot multiplication

Regenerated shoots were transferred to the MS medium supplemented with various concentrations of BAP (0.5, 1.0, 1.5, 2.0 mg l^{-1}), KIN (0.5, 1.0, 1.5, 2.0 mg l^{-1}) alone and in combination with NAA (0.5 mg l^{-1}) each for multiplication. The data were recorded for number of shoots/explant after 15, 30 and 45 days of inoculation after frequent sub culturing after 2 weeks.

In vitro rooting

The well elongated healthy shoots were separated aseptically and transferred individually to the half strength MS medium supplemented with various concentration of auxin NAA (0.5, 1.0, 1.5, 2.0 mg l^{-1}).

Acclimatization and Hardening

Rooted shoots were transferred in the pots containing different potting mixture of sand, soil and vermin-compost. Pots were kept in the Greenhouse for 4-6 weeks for hardening till the plant attained a height of approximately 15-20 cm. The hardened plants were successfully transferred in the field.

Table 1: Effect of MS Basal Medium Supplemented with Different Concentrations of Plant Growth Regulators on *In Vitro* Multiplication of *Withania somnifera*

S. No.	Media code	Average number of shoots/explants after		
		15 day	30 day	45 day
1	WSM0 (MS basal)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
2	WSM1 (MS + 0.5 mgl^{-1} BAP)	0.8 ± 0.13	1.7 ± 0.08	2.4 ± 0.09
3	WSM2 (MS + 1.0 mgl^{-1} BAP)	0.6 ± 0.11	2.2 ± 0.08	2.8 ± 0.11
4	WSM3 (MS + 1.5 mgl^{-1} BAP)	1.9 ± 0.27	2.5 ± 0.29	2.5 ± 0.12
5	WSM4 (MS + 2.0 mgl^{-1} BAP)	1.7 ± 0.08	2.2 ± 0.08	3.6 ± 0.10
6	WSM5 (MS + 0.5 mgl^{-1} KIN)	0.5 ± 0.13	0.5 ± 0.13	0.8 ± 0.13
7	WSM6 (MS + 1.0 mgl^{-1} KIN)	1.0 ± 0.10	1.7 ± 0.08	2.5 ± 0.12
8	WSM7 (MS + 1.5 mgl^{-1} KIN)	1.0 ± 0.00	2.4 ± 0.09	2.7 ± 0.62
9	WSM8 (MS + 2.0 mgl^{-1} KIN)	1.0 ± 0.00	2.4 ± 0.09	2.5 ± 0.12
10	WSM9 (MS + 0.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA)	2.1 ± 0.26	3.2 ± 0.56	5.6 ± 0.33
11	WSM10 (MS + 1.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA)	3.8 ± 0.20	4.6 ± 0.23	5.8 ± 0.27
12	WSM11 (MS + 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA)	6.7 ± 0.3	7.6 ± 0.54	8.0 ± 0.57
13	WSM12 (MS + 2.0 mgl^{-1} BAP + 0.5mgl^{-1} NAA)	5.8 ± 0.28	6.7 ± 0.3	7.2 ± 0.34
14	WSM13 (MS + 0.5 mgl^{-1} KIN + 0.5 mgl^{-1} NAA)	2.0 ± 0.22	4.5 ± 0.57	5.1 ± 0.82
15	WSM14 (MS + 1.0 mgl^{-1} KIN + 0.5 mgl^{-1} NAA)	1.6 ± 0.07	2.5 ± 0.29	4.5 ± 0.57
16	WSM15 (MS + 1.5 mgl^{-1} KIN + 0.5 mgl^{-1} NAA)	1.6 ± 0.07	3.2 ± 0.56	4.9 ± 0.63
17	WSM16 (MS + 2.0 mgl^{-1} KIN + 0.5 mgl^{-1} NAA)	2.7 ± 0.62	3.2 ± 0.56	3.2 ± 0.56

*Data is shown as mean of three replicates ± S.E.

Table 2: Effect of MS (Half Strength) Basal Medium Supplemented with Different Concentrations (0.5-2.0 mg l^{-1}) of Auxin (Naa) on Percentage Root Induction of Plantlets of *Withania somnifera*

S. No.	Media code	% root induction	No. of days required for rooting
1	WSR0 (½ MS Basal)	0.00 ± 0.00	-
2	WSR1 (½ MS + 0.5 mg l^{-1} NAA)	77.8 ± 0.08	8.1 ± 0.40
3	WSR2 (½ MS + 1.0 mg l^{-1} NAA)	55.6 ± 0.06	13.5 ± 0.38
4	WSR3 (½ MS + 1.5 mg l^{-1} NAA)	33.3 ± 0.03	15.3 ± 0.44
5	WSR4 (½ MS + 2.0 mg l^{-1} NAA)	22.2 ± 0.02	17.7 ± 0.44

*Data are shown as mean of three replicates ± S.E. - = no rooting

Table 3: Effect of Different Potting Mixture on Hardening of *In Vitro* Raised Plantlets of *Withania somnifera*

S. No.	Mixture Code	Potting mixture	% survival of rooted plantlets
1	WSPM1	Sand + FYM (1:1)	80
2	WSPM2	Sand + Soil + FYM (1:1:1)	80
3	WSPM3	Sand + Soil + vermin compost (1:1:1)	100

Figure 1: *In vitro* establishment, multiplication and rooting of *Withania somnifera*

(A) Regeneration of nodal explants on MS basal media, (B) Initiation of proliferation and multiple shoots from regenerated shoots on MS + 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA after 15 days (C) multiple shoots on MS + 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA after 45 days (D) *In vitro* rooting on MS + 0.5 mg l^{-1} NAA

RESULTS AND DISCUSSION

Effect of different duration of sterilization agents Bavistin, Streptocyclin and 0.1 % $HgCl_2$ studied on *in vitro* establishment of *Withania somnifera.* The explants were treated with 0.1 % $HgCl_2$ for different duration (1-4 minutes) and data were recorded for percent contamination and percent survival after 21 days of inoculation. A maximum 100 % survival and asepsis was observed when nodal explants treated with 0.4 % Streptocyclin and 0.4 % Bavistin for 60 minutes followed by 3 minutes treatment with 0.1 % $HgCl_2$. Long duration of $HgCl_2$ exceeding above 3 minutes treatment was resulted in browning and mortally of explants. Similar results were reported by[21] using 0.01 % $HgCl_2$ for 3-5 minutes in *Celastrus paniculatus*. Similarly, also found similar result in *Achyranthes aspera* medicinal plant using nodal explants[22]. In the present investigation effect of different types and concentrations of plant growth regulators Viz. BAP (0.5-2.0 mg l^{-1}), KIN (0.5-2.0 mg l^{-1}) alone and each in combination with (0.1 mg l^{-1}) NAA was employed for *in vitro* multiplication of nodal explants of *Withania somnifera* and data were recorded for number of shoots/ explants on fixed intervals of time (Table 1). Seventeen different media combinations (WSM_0 -WSM_{16}) employed for multiplication of nodal explants, MS medium supplemented with 1.5 mg l^{-1} BAP + 0.1 mg l^{-1} NAA was found to be best for multiplication. The maximum shoots/explant (8.0) was observed on medium WSM_{11} (MS + 1.5 mg l^{-1} BAP + 0.5 mgl^{-1} NAA) followed by medium WSM_{12} (MS + 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA) with 7.2 shoots/explants after 45 days (Table 1; Figure 1.C). Minimum shoots (0.8) were observed on WSM_5 medium MS basal medium supplemented with 0.5 mg l^{-1} KIN. It was observed that BAP was more effective than KIN for shoot multiplication (Figure 1 B, C). BAP was the most effective cytokinin for promoting shoot multiplication and proliferation from nodal segments. Results of present study are in close agreement with earlier reports for shoot multiplication in ashwagandha by several researchers[13, 18,23] suggesting BAP was most effective cytokinin. In the present study the lower concentration of cytokinin (BAP) in combination with lower auxin (NAA) was found to be superior over KIN alone and in combination with NAA for shoot proliferation of *Withania somnifera*. Result of present study in close agreement with[24] reported that BAP with NAA in low concentration produced direct shoots with a little callus development. While, 13 reported combination of 1.0 BAP and 1.0 KIN best for shoot multiplication in aswagandha using axillary explants. Hence the data presented here supports that BAP is better than KIN for *in vitro* multiplication of *Withania somnifera.* For root induction, the well developed shoots were separated aseptically and were transferred to the half strength MS media supplemented with various concentration of IAA (0.5-2.0 mg l^{-1}). The highest rooting (77.8 %) was observed within minimum 8.1 days on the WSR_1 medium supplemented with MS medium (half strength) medium supplemented with 0.5 mgl^{-1} NAA followed by medium WSR_2 (½ MS + 1.0 mg l-1 NAA) with 55.6 % rooting (Table 2). In present it was observed that on increasing concentration of NAA above 0.5 mgl-1 result in decrease rooting percentage. Low concentration of NAA was found more effective for root induction in *Withania somnifera* (Figure 1 D). Contrary, 85-90 % rooting using ms medium supplemented with 200 mgl^{-1} activated charcoal within 15-20 days[24]. Therefore, the data support that it is better to use lower concentration of auxins for *in vitro* root induction of *Withania somnifera*. Rooted shoots were separated individually and transferred to plastic bags containing different potting mixture of sand, soil and vermin-compost. A maximum 100 % survival was reported on potting mixture $WSPM_4$ (sand, soil and vermin-compost in ratio of 1:1:1). After that hardened plants transferred to pot or in field for commercial purpose (Table 3)

CONCLUSION

Amongst the different media employed MS media was found to be most suitable for growth and development of *Withania somnifera*. A high frequency of multiple shoots were obtained on MS media supplemented with 1.5 mg l^{-1} BAP supplemented with 0.5 mg l^{-1} NAA. In addition, the half strength MS medium supplemented with 0.5 mg l-1 NAA proved to be most effective for root induction. Thus in future this protocol will serve very useful for in vitro propagation of *Withania somnifera*.

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