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- Short communication

HIGH FREQUENCY *IN VITRO* MULTIPLICATION OF *POLYGONATUM VERTICILLATUM* (L.) ALL. FROM RHIZOME BUD EXPLANTS

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Abstract

This work aims to develop a rapid protocol for the *in vitro* establishment of *Polygonatum verticillatum* (L.) All., an astounding medicinal herb of Astavarga group of plants. Successful *in vitro* establishment was achieved utilizing rhizome bud segments of approx. 0.5 to 1.0 cm size as explants. Sterilized explants were cultured on MS medium containing graded concentration of different plant growth regulators used either singly or in various combinations. Maximum (85 ± 10.0%) per cent shoot induction with maximum shoot length (6.50 ± 0.11cm) were recorded in TDZ (2.0 mg/l) + NAA(1.0 mg/l) + GA_3 (2.0 mg/l) supplemented MS medium. NAA (1.0 mg/l) + Kn (0.5 mg/l) and IBA (1.0 mg/l) supplemented full strength MS medium with 3% sugar showed highest 70 ± 5.0% rooting response. Half MS +1.5 % sucrose medium augmented with IBA (1.0 mg/l) showed the maximum average number of roots per explants (12.8 ± 1.07) with highest root length (6.12 ± 0.27 cm). After hardening plantlets were transferred to pots in glasshouse produced healthy plants with 56% survival rate.

*Polygonatum verticillatum*L. (All.) belong to Asparagaceae prominent medicinal herb of temperate Himalaya possesses a number of pharmacological properties such as antittussive, aphrodisiac, antipyretic, antiperiodic, appetizers, anti-inflammatory, anti-malarial, cardiotonic, diuretic, energizer, hypoglycemic, sedative, pain reliever etc (Khan *et al.* 2012, 2013). Being an important ingredient of Astavarga (group of eight astounding medicinal herbs), *P. verticillatum* is considerably utilized in Ayurvedic system of medicine. Wild stock of this herb is experiencing a steady decline due to its overexploitation from the wild. Agricultural practices for cultivation of this herb are also not well developed. Seeds of *P. verticillatum* show double dormancy making natural regeneration a lengthy process in nature. Moreover, rhizomes in nature are prone to various soil borne infections. Hence, it becomes imperative not only to conserve this species using *in vitro* approach but also to develop a simple and efficient methodology for fast multiplication of true to type planting material of *P. verticillatum*. Rhizome explants [collection site: Bhowali region (29.38^0N, 79.52^0E and 1654 m) Uttarakhand (India)] were sterilized with 2% detergent solution (Tween 20 for 10-15 min.) followed by bavistin [0.5% (w/v) for 15-20 min], aq. mercuric chloride [0.1 % (w/v) for 5 min.] and finally rinsed 4-5 times with double dist. water. Sterilized segments were then inoculated in shoot induction medium containing 60 ml of Murashige and Skoog medium (MS medium) fortified with different concentrations and combinations of plant growth regulators. *In vitro* regenerated microshoots (5-6 cm in length) were excised from the shoot cluster and advanced for root induction and elongation. ½ MS medium (medium containing MS salts diluted to the half) was supplemented with 1.5% (w/v) sucrose, whereas MS (full strength) was supplemented with 3% (w/v) sucrose. MS (full strength) medium without any plant growth regulators (PGRs) served as control. pH of the medium was adjusted to 5.8 ± 0.1 prior to autoclaving at 15 lbs at 121°C for 20 min. The cultures were maintained at 25 ± 1^0C for 16 hrs

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photoperiod using cool white fluorescent light (80 µmol $m^{-2}s^{-1}$) throughout the course of work and responses were examined at routine basis. Well rooted plantlets after washing with continuous flow of tap water were kept in plastic cups containing ¼ MS salt (without sucrose) and maintained in culture conditions (as mentioned earlier). Hardened plantlets were transferred in thermocol cups containing sterilized mixture of sand, soil and vermiculite in the ratio of 1:1:1. Plantlets were irrigated with dist. water on every alternate day. After 4 weeks, these acclimatized plants were planted to plastic pots containing garden soil and were kept in glass house for 2 months and were later transferred to field. Every treatment had five replicates having four explants each (5 replications x 4 explants = 20 explants). The data presented are mean± standard error. Effects of different treatments were quantified and the data were statistically analyzed using one-way ANOVA. The differences among treatments were significantly compared at $p < 0.05$.

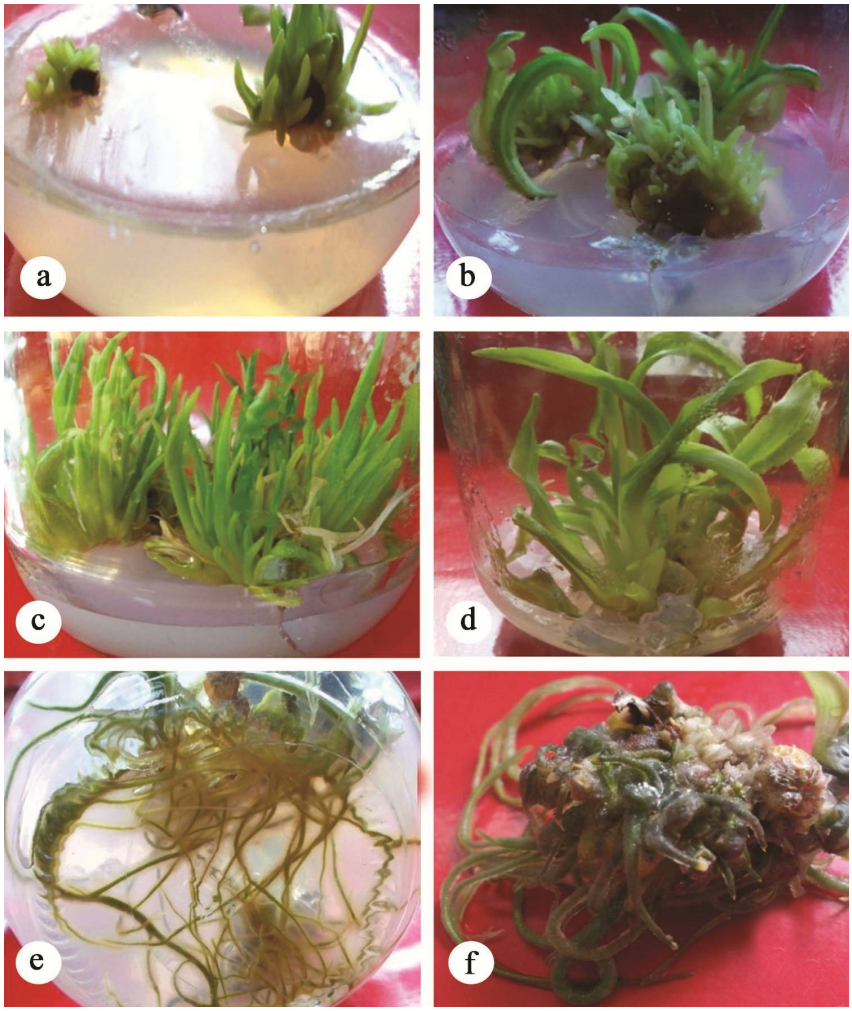

Fig. 1. *In vitro* establishment of *P. verticillatum* through bud segments from wildly grown rhizomes, (a-d) successive stages of shoot induction and proliferation (e) *in vitro* rooting (f) rooting in adventitious multi bud clusters (AMC).

Table 1. Effect of different concentrations and combinations of plant growth regulators on *in vitro* shoot induction and shoot proliferation from bud segments of wildly grown rhizomes of *P. verticillatum*

Plant growth regulators (mg/l)	Days to shoot induction	Shoot induction (%)	No of shoots/ explant	Shoot length (cm)	Shoot cluster weight (g)	Remarks
MS basal media	-	-	-	-	-	Only swelling of sprouts
MS+TDZ(1.0)	29±2.10^{cd}	45±5.00 ^{ab}	4.2±0.58 ^{b}	4.84±0.16 ^{c}	2.40±0.50 ^{b}	Shoot induction and multiplication
MS+BAP(1.0)	32±1.51 ^{d}	40±6.12 ^{ab}	4.2±0.37 ^{b}	4.36±0.44 ^{c}	2.16±0.15 ^{ab}	Shoot induction and multiplication
MS+KN(1.0)	32±1.21 ^{d}	40±6.12 ^{ab}	2.0±0.32 ^{a}	2.72±0.19 ^{a}	1.78±0.32 ^{ab}	Elongation of sprouts
MS+IAA(1.0)	-	-	-	-	-	Only swelling of sprouts
MS+ 2,4-D(1.0)	-	-	-	-	-	Only swelling of sprouts
MS+NAA(1.0)	32±1.90 ^{d}	30±9.35 ^{a}	2.0±0.32^{a}	2.98±0.30^{ab}	1.52±0.21 ^{a}	Elongation of sprouts
MS+IBA(1.0)	33±1.40 ^{d}	35±6.12 ^{a}	2.4±0.24 ^{ab}	2.56±0.35 ^{a}	1.98±0.20 ^{ab}	Elongation of sprouts
MS+KN(1.0)+IAA(0.5)	26±1.52 ^{bc}	55±9.35 ^{b}	7.4±1.08 ^{cd}	4.28±0.26 ^{c}	4.36±0.49 ^{d}	Shoot induction and multiplication
MS+KN(1.0)+IBA(0.5)	30±1.60 ^{cd}	45±5.0 ^{b}	2.4±0.24 ^{ab}	2.80±0.31^{a}	1.64±0.12 ^{ab}	Elongation of sprouts
MS+KN(1.0)+NAA(1.0)	23±1.21 ^{ab}	75±7.91 ^{c}	12.2±1.53 ^{e}	5.82±0.31 ^{d}	5.12±0.31 ^{de}	AMC formation
MS+BAP(1.0)+NAA(0.5)	29±1.12 ^{cd}	55±9.35 ^{b}	7.0±0.71 ^{c}	3.58±0.21 ^{b}	3.88±0.36 ^{cd}	Shoot induction and multiplication
MS+TDZ(2.0)+NAA(1.0)	22±1.60 ^{ab}	80±9.35 ^{c}	**19.4±1.63** ^{g}	6.10±0.20 ^{d}	**6.38±0.25** ^{f}	AMC formation
MS+TDZ(2.0)+IBA(1.0)	22±1.32 ^{ab}	65±6.12 ^{bc}	12.2±0.80 ^{e}	4.76±0.35 ^{c}	5.32±0.25 ^{e}	AMC formation
MS+TDZ(2.0)+2,4-D(1.0)	26±0.94 ^{bc}	60±6.12 ^{bc}	7.4±0.51 ^{cd}	4.50±0.34 ^{c}	4.54±0.32 ^{de}	AMC formation
MS+TDZ(2.0)+NAA(1.0)+ GA_3(2.0)	**19±1.62** ^{a}	**85±10.0** ^{c}	14.6±0.75 ^{f}	**6.50±0.11** ^{d}	5.56±0.24 ^{e}	AMC formation
MS+KN(2.0)+IAA(1.0)+G A_3(2.0)	24±1.53 ^{b}	60±6.12 ^{bc}	10.0±1.14 ^{d}	4.82±0.15 ^{c}	4.52±0.44 ^{de}	Shoot induction and multiplication
MS+BAP(2.0)+NAA(1.0)+ GA_3(2.0)	28±1.44 ^{c}	65±6.12 ^{bc}	9.4±0.51 ^{d}	4.12±0.16 ^{bc}	3.36±0.27 ^{c}	Shoot induction and multiplication

Different letters following mean±SE indicate significant difference among treatments ($p < 0.05$).

Propagation using axillary bud is a simple and safe method for steady production of true to type plants within a short period of time (Salvi *et al.* 2002). In the present study, axillary bud segments of rhizome inoculated in MS + TDZ (2.0 mg/l) + NAA (1.0 mg/l) + GA_3 (2.0 mg/l) showed minimum days(19 ± 1.62) to shoot induction with maximum per cent shoot induction (85±10.0%) and maximum shoot length (6.50 ± 0.11 cm) (Table 1, Fig. 1a-d). TDZ has proved as an effective PGR for shoot regeneration in other reports (Jones *et al.* 2007, Landi and Mezzetti 2007, Thomus 2007). The present finding doesn't conform to the earlier study in this plant, where MS + BAP (1.0 mg/l) + NAA (0.5 mg/l) was observed as the most effective medium for shoot multiplication giving 8.60 ± 0.58 number of shoots with on an average 4.66 ± 1.07 cm. shoot

Table 2. Effect of different concentrations and combinations of plant growth regulators on *in vitro* root induction and root length in microshoots of *P. verticillatum*.

Medium	Plant growth regulators (mg/l)	Days to root Induction	Rooting response (%)	Avg. no. of roots/explants	Root length (cm)
MS+3 % sucrose	MS basal media	-	-	-	-
MS+3 % sucrose	IAA (1.0)	24±1.59 ^{ab}	60±6.12 ^{b}	11.2±1.59 ^{c}	4.58±0.24 ^{c}
MS+3 % sucrose	IBA (1.0)	21±1.46 ^{a}	**70±5.00** ^{b}	12.4±1.17 ^{c}	5.82±0.56 ^{d}
MS+3 % sucrose	2, 4-D (1.0)	28±1.16 ^{bc}	40±6.12 ^{ab}	7.8±1.07 ^{ab}	2.64±0.17 ^{a}
MS+3 % sucrose	NAA (1.0)	25±0.84 ^{b}	55±9.35 ^{b}	9.8±1.16 $_{bc}$	3.54±0.30 ^{b}
MS+3 % sucrose	IAA (1.0)+ Kn (0.5)	24±2.03 ^{ab}	65±6.12 ^{b}	11.4±0.75 ^{c}	5.08±0.16 ^{cd}
MS+3 % sucrose	IBA (1.0)+ Kn (0.5)	28±1.87 ^{bc}	45±9.35 ^{ab}	6.2±0.80 ^{ab}	3.68±0.17 ^{bc}
MS+3 % sucrose	2, 4-D (1.0)+ Kn (0.5)	-	-	-	-
MS+3 % sucrose	IBA (1.0)+TDZ (0.5)	30±0.86 ^{c}	35±6.12 ^{a}	5.6±1.08 ^{a}	2.58±0.43 ^{a}
MS+3 % sucrose	IAA (1.0)+TDZ (0.5)	-	-	-	-
MS+3 % sucrose	NAA (1.0)+KN (0.5)	23±1.11 ^{ab}	**70±5.00** ^{b}	11.0±0.55 ^{bc}	5.04±0.46 ^{cd}
MS+3 % sucrose	IBA (0.5)+IAA (0.5)	26±1,07 ^{b}	60±6.12 ^{b}	8.6±0.68 ^{b}	3.62±0.35 ^{bc}
MS+3 % sucrose	IBA (0.5)+NAA (0.5)	27±0.84 ^{bc}	50±11.18 ^{ab}	7.0± 0.71 ^{ab}	2.58±0.60 ^{a}
MS+3 % sucrose	IBA (0.5)+2, 4-D (0.5)	-	-	-	-
1/2 MS+1.5 % sucrose	IAA (1.0)	23±1.69 ^{ab}	60±6.12 ^{b}	11.6±1.17 ^{c}	4.94±0.29 ^{c}
1/2 MS+1.5 % sucrose	IBA (1.0)	**21±0.86** ^{a}	65±6.12 ^{b}	**12.8±1.07** ^{c}	**6.12±0.27** ^{d}
1/2 MS+1.5 % sucrose	2, 4-D (1.0)	28±2.01 ^{bc}	45±9.35 ^{ab}	7.6±0.68 ^{ab}	4.06±0.18 ^{bc}
1/2 MS+1.5 % sucrose	NAA (1.0)	25±1.50 ^{b}	60±6.12 ^{b}	10.0±0.89 ^{bc}	4.44±0.19 ^{c}

Different letters following mean ± SE indicate significant difference among treatments ($p < 0.05$).

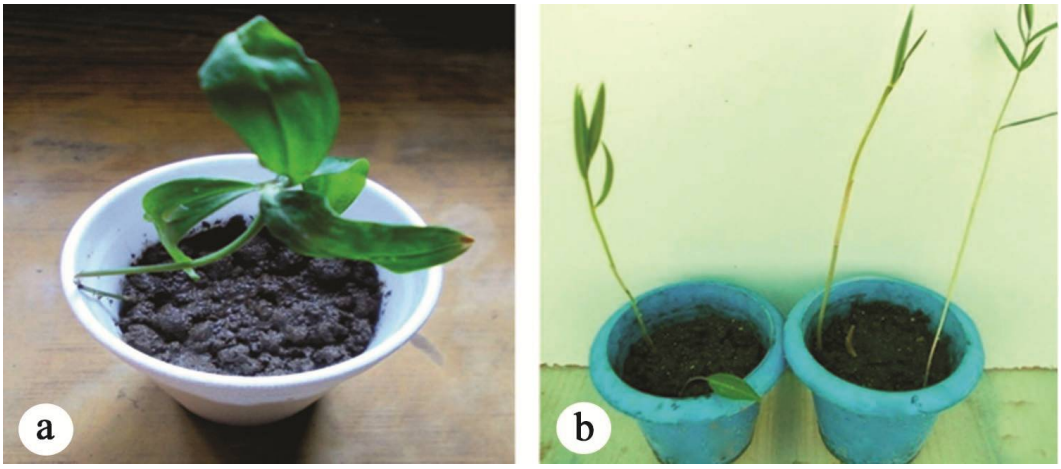

Fig. 2. *In vitro* raised plants of *P. verticillatum* (a) acclimatization in thermocol cups (b) complete plants after 2 weeks of growth in the glass house.

length (Bisht *et al.* 2012). In the present study, few PGR combinations were found suitable for shoot induction and multiplication, others are for adventitious multi-bud cluster (AMC) formations and still others for elongation of sprouts (Table 1). In AMC, swellings of bases occurred, from which bud induction took place. Among tested root induction medium, highest number of roots (12.8 ± 1.07) with maximum root length (6.12 ± 0.27cm) was observed in minimum number of days (21 ± 0.86 days) in half strength MS + IBA (1.0 mg/l) (Table 2, Fig.1e-f). Maximum per cent rooting response (70 ± 5.00%) was achieved in MS + IBA (1.0 mg/l) and MS + NAA (1.0 mg/l) + Kn (0.5 mg/l). The present finding is in contradiction with the earlier report in this plant, where NAA (0.5 mg/l) in half strength MS medium was most effective in producing maximum number of roots (4.79 ± 0.69) with maximum root length (1.00 ± 0.30 cm) (Bisht *et al.* 2012). In another study on *P. verticillatum*, 1.93 ± 0.34 cm long roots regenerated from shoots when inoculated on MS medium supplemented with 2.0 mg/l IBA within 26 ± 0.55 days (Qadir *et al.* 2020). Compared to these two studies, in the present study, more number of roots with higher average root length is formed in significantly lesser number of days. IBA treatment stimulates active division of the cambial cells, resulting in the formation of a functional root system. Complete plantlets regenerated were hardened in ¼ MS salts (liquid medium) and later on transferred for acclimatization in thermocol cups containing sterilized mixture of sand, soil and vermiculite in the ratio of 1 : 1 : 1 (Fig. 2a). Pots containing plantlets were enclosed with transparent polythene bags to ensure humidity. After 4 weeks, these acclimatized plants were transferred to plastic pots containing garden soil and maintained in glass house (Fig. 2b). Plants raised showed 56% survival rate. Present investigation gives a simple, fast, efficient and reproducible protocol for the rapid multiplication of *P. verticillatum.* Through this protocol, it becomes possible to obtain approx. 30 to 40 plantlets from single explant after three subculture cycles of multiplication. Plants thus generated are true-to type and are healthier as these were raised from rhizome buds cultured under aseptic conditions.

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