



# Plant Archives

Journal homepage: <http://www.plantarchives.org>

DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.1.183>

## ORGANOGENESIS AND SOMATIC EMBRYOGENESIS FOR RAPID MULTIPLICATION AND REGENERATION OF *POLYGONATUM VERTICILLATUM* (L.) : AN ASHTAVARGA MEDICINAL HERB

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(Date of Receiving-21-01-2024; Date of Acceptance-24-03-2024)

### ABSTRACT

*Polygonatum* is a member of Ashtavarga group of medicinal plants that holds high medicinal value in Ayurveda. *P. verticillatum* (L.) All. is an immunity booster herb. Rhizomes being the medicinally important part of the plant, are harvested from the wild by uprooting the plant, pushing the species at higher threatening risks. Regeneration of this plant through seeds is a slow process as the seeds exhibit double dormancy. Therefore, the study is carried out for the rapid and mass propagation of this medicinal plant through *in vitro* methods. Leaves and rhizome buds were used as explant for culture establishment. Sterilised explants were inoculated in MS media complimented with various PGRs (mT, IAA, TDZ, NAA, IAA and GA<sub>3</sub>) and incubated at 25± 2°C with 16/8 hr photoperiod under white LED. Maximum callus (percentage) induction was found in MS medium complemented with TDZ (0.5 mg L<sup>-1</sup>) and 2, 4 -D (1.0 mg L<sup>-1</sup>). Maximum per cent (81.25 ± 3.6) shoot induction was achieved in MS fortified with mT (0.5 mg L<sup>-1</sup>) with maximum (23.67 ± 2.02) no. of shoots/explant. Maximum root (percent) induction (71.33 ± 8.3) was observed in MS medium supplemented with IBA (1.0 mg L<sup>-1</sup>) and maximum no. of roots/explants (10.33 ± 0.6) were observed in NAA (1.0 mg L<sup>-1</sup>) + Kn (0.5 mg L<sup>-1</sup>) fortified MS medium. Maximum per cent embryo induction (62.5 ± 7.2) was achieved in MS containing Kn (2.0 mg L<sup>-1</sup>) + NAA (1.0 mg L<sup>-1</sup>) followed by preparation of synthetic seeds in 2.5% sodium alginate and 2% CaCl<sub>2</sub>.

**Key words :** Organ culture, Plant Growth Regulators, Embryo induction, Artificial seeds.

### Introduction

Plants have been the foundation of traditional medicine since ancient times. In many parts of the world, plants are used as major sources of medicine. Since, Covid times, there is an upsurge in the demand of immunity boosting plant based drugs. Covid era clearly established the success of medicinal plants in healing, rejuvenation and rehabilitation. Today, these herbal products are increasingly being sought by the immune-compromised population throughout the globe. In India, a large number of plants are used as ethnomedicines. Many of these plants find use in different indigenous systems of medicines *viz.*, Siddha, Ayurveda etc. A considerable number of the population in the country uses a large

variety of plants as nutraceuticals. *Polygonatum verticillatum* (L.) is one such plant that has got huge nutraceutical value and is used for revitalization since ancient times.

*P. verticillatum* (Asparagaceae) belongs to the Astavarga group of revitalizing herbs that boosts cells' ability to rejuvenate. It grows in temperate Himalayas and is used widely in Unani and Ayurvedic systems of traditional medicine. It is known as Mahameda in Hindi and Solomon's seal in English. It has various ethnomedicinal uses, Bhutanese use it as tranquillizers, appetizers; Tibetan use it for curing kidney troubles whereas in India *Polygonatum* is used to treat digestive problems (Wangchuk *et al.*, 2008; Sharma *et al.*, 2011;

Ballabh and Chaurasia, 2009).

*P. verticillatum* is an indispensable component in the formulation of “Chyavanprash”, an age old health supplement. The plant is listed as a threatened species in various literatures (Kumari *et al.*, 2012). Its rhizome is illegally collected from the wild and is used for treating fatigue, rheumatism, wounds and boils etc. and are also used as emollient and vegetable (Ghayur, 2004; Wujisguleng *et al.*, 2012). Owing to overexploitation, the species needs immediate measures for conservation.

Conventional propagation approaches of *P. verticillatum*, via seeds or rhizomes, is a lengthy process requiring about 5 months' time for sprouting of rhizomes in the field (Lohani *et al.*, 2012). Hence, conventional method is not sufficient to fulfil the local and market demands. To address this, it is necessary to produce a rapid and efficient protocol for propagation of the plant which may eventually help to reduce the demand and supply gap besides facilitating *ex situ* conservation of the species. Therefore, the present study was conducted to develop a rapid and efficient protocol for organogenesis and somatic embryogenesis of *P. verticillatum* leading to formation of artificial seeds.

## Materials and Methods

### Plant material

Rhizomes of *Polygonatum verticillatum* (L.) All. were collected from Munsyari (30.07° N; 80.23° E) in Pithoragarh district of Uttarakhand during 2019-20. The plants were established from rhizomes and maintained at 25±2°C under white LED for 16/8 hr photoperiod in the glass house facility of the Dept. of Molecular Biology and Genetic Engineering, G. B. Pant University of Agriculture & Technology, Pantnagar. Sprouted buds from the rhizomes and young leaves were used for organogenesis and embryogenesis, respectively.

### Explant sterilization

Disinfection was done using 2% Tween 20 (Hi media) for 15 min. followed by 0.5% (w/v) treatment of Bavistin. Disinfection was done using 5 min. Treatment with 0.1% mercuric chloride (HgCl<sub>2</sub>) followed by 2-3 washing sterile water. Murashige and Skoog (MS) medium was supplemented with different concentration of plant growth regulators, added on with 3% (w/v) of sucrose and gelled with 0.7% (w/v) of agar. pH was adjusted to 5.8 ± 0.2 before autoclaving at 121°C at 15 psi for 15-20 min. Inoculated cultures were incubated at 25±2°C with 16/8 hr photoperiod under white LED at intensity 40 μmol m<sup>-2</sup>s<sup>-1</sup>. Sub-culturing was regularly done after 4 weeks and observations were recorded.

## In vitro organogenesis

### Shoot and root induction

Sterilized segments of sprouted rhizome buds were inoculated in MS medium complemented with different concentrations and combinations of plant growth regulators (thidiazuron (TDZ), 6-benzylaminopurine (BAP), kinetin (KN) (1.0, 2.0 mg L<sup>-1</sup>), gibberellic acid (GA<sub>3</sub>) (2.0 mg L<sup>-1</sup>), 2, 4-D (1.0 mg L<sup>-1</sup>), naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) (0.5, 1.0 mg L<sup>-1</sup>)) for the induction of shoots. For the rooting response, shoot clusters were transferred to full strength MS medium fortified with different conc. and combinations of plant hormones (auxins and cytokinins). Observations was noted for induction shoot and root induction (number and days to formation). Shoot and root induction (%) was calculated using the formulae:

$$\text{Induction (\%)} = \frac{\text{No. of explants producing shoots/roots}}{\text{Total no. of explants inoculated on culture media}} \times 100$$

### Callus induction

Sterilised leaves from pot grown plants were inoculated in MS medium complemented with different auxins and cytokinins (BAP, 2, 4-D, NAA, IAA, IBA, mT, Kn and TDZ) for callus induction. Callus induction percentage was calculated using the following formula:

$$\text{Callus Induction (\%)} = \frac{\text{No. of explants producing callus}}{\text{Total no. of explants on callus culture media}} \times 100$$

### Somatic embryogenesis

Induced calli (4 segments in each bottles) were transferred to full strength MS media containing different plant growth regulators namely casein hydrolysate (CH), IAA, IBA and Kn. Sub culturing was done after 4 weeks.

$$\text{Embryo induction (\%)} = \frac{\text{No. of explants producing somatic embryos}}{\text{Total no. of explants inoculated on culture media}} \times 100$$

### Histology

The embryos fixed in FAA solution (formalin, glacial acetic acid, 70% ethanol in the ratio of 5:5:90), dehydrated in graded ethanol followed by series of xylene and finally embedded in paraffin wax (Johansen, 1940). Microtome (Spencer “820”) was used to cut 8μm thick sections. The sections were stained with safranin and observed

under microscope (Olympus BX 51).

### Synthetic seeds preparation

Somatic embryos were suspended in autoclaved distilled water for 24 hours for aeration. Embryos were then encapsulated in 2.5% sodium alginate coated with calcium chloride (CaCl<sub>2</sub>) solution followed by 3-4 times washing of artificial seeds with liquid MS basal medium.

### Acclimatization of regenerated plantlets

Fully formed plantlets were taken for hardening. Plantlets were rinsed thoroughly and saplings were transferred to successively decreasing strength of nutrient media *i.e.* half strength with sucrose followed by ¼ MS liquid medium without sucrose and maintained in controlled conditions (25 ± 2°C, 16/8 hr photoperiod) for a week. Acclimatized plantlets were transferred to thermocol cups containing 2:1:1:1 mixture of soil: sand: vermiculite: vermicompost and kept at 25 ± 2°C and 70% humidity.

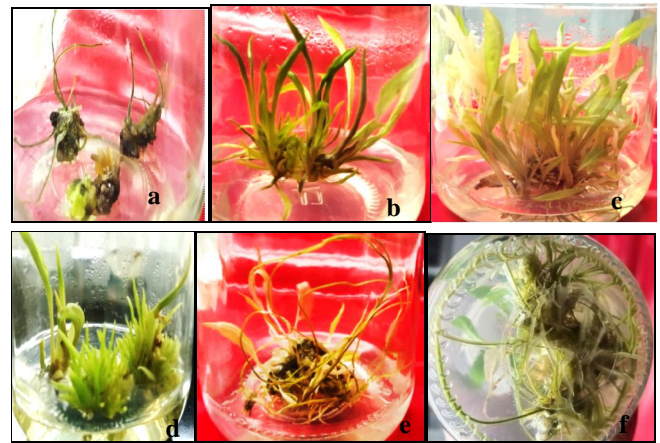
### Statistical analysis

All experimental data were expressed as Mean ± standard error (SE) of three replicates. The experimental data was subjected to analysis of variance (ANOVA) for completely randomized design (CRD). Significant difference was determined at  $p > 0.05$  by Duncan's Multiple Range Test (DMRT) using SPSS 26.0 version.

## Results and Discussion

### Shoot induction and proliferation

For shoot induction, *in vivo* raised sprouted rhizomes were used as explant in MS supplemented with different combinations and concentrations of cytokinins and auxins. In the present study, 0.5 mgL<sup>-1</sup> mT is found most effective for shoot morphogenesis of *P. verticillatum*. Of all the cytokinins used, metatopolin showed maximum shoot induction percentage (81.25±3.6%) with highest shoot proliferation (23.67±2.0) in minimum number of days (17.67±1.8) (Table 1, Fig. 1). TDZ (2.0 mg L<sup>-1</sup>) in combination with NAA (1.0 mg L<sup>-1</sup>) showed 68.75 ± 3.6 % shoot induction in 27.33±3.9 days with 14.33± 2.9 no. of shoots/explant. Although, cytokinins showed direct impact on shoot induction and proliferation, auxins (IAA and 2,4-D) did not show any response. Bisht *et al.* (2012), however, achieved direct shoot regeneration in *P. verticillatum* from stem explants on MS fortified with BAP and NAA (1mg/l + 0.5mg/l) with 8.60±0.58 shoots with an average length of 4.66±1.07cm. Studies done in *Syzygium cumini*, *Prunus* spp., *Daphne mezereum* also showed higher rate of shoot induction and proliferation with the application of mT (Naaz *et al.*, 2018; Monticelli *et al.*, 2015; Nowakowskam and Pacholczak, 2020). mT induced 100% organogenesis with considerable

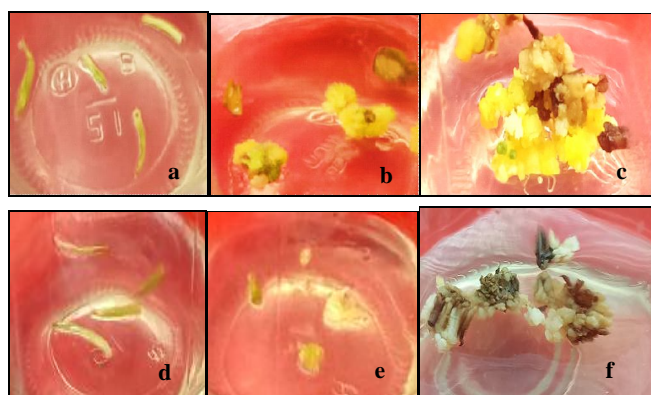


**Fig. 1 :** *In vitro* shoot and root induction in *P. verticillatum* from sprouted rhizome buds, (a–c) showing successive stages of shoot induction and proliferation; (d–f) successive stages of root induction and proliferation of *in vitro* generated shoots; (a) *in vivo* raised rhizome bud explants in fortified MS medium; (b) shoot induction in MS medium with 0.5 mgL<sup>-1</sup> mT (c) shoot proliferation stage after 25 days. (d) multiple shoots transferred to rooting media after 4 weeks; (e) rooting in MS media fortified with IBA at 1.0 mgL<sup>-1</sup>; (f) rooting after 30 days of subculturing.

improvement in leaf anatomy in *Santalum album* (Manokari *et al.*, 2021). mT being an aromatic cytokinin show more *in vivo* stability to cytokinin oxidases and dehydrogenases than isoprenoid cytokinins and hence promote good growth and development under *in vitro* system (Koc *et al.*, 2021). mT promotes highly efficient regeneration capacity with no reduction in root induction compared to other common cytokinins (Vrundha *et al.*, 2021). According to a study in *Pistacia vera* cultures, mT was found to be 6-fold more effective than BA (Abdouli *et al.*, 2022).

### Root induction and proliferation

For root induction, *in vitro* proliferated multiple shoots were cut into smaller clusters (5 to 10 shoots) and inoculated in full strength MS medium supplemented with 3% sucrose and different concentrations and combinations of auxins and cytokinins. Maximum root induction (%) was observed in MS+1.0mg L<sup>-1</sup>IBA in 28 days with 8.33 numbers of roots (Table 2). Of all the plant growth regulators, MS + IAA (1.0) + Kn (0.5) showed minimum days to rooting response (25.67 ± 1.20 days). Maximum (10.33) and minimum (4.33 ± 1.2) number of roots were observed in NAA (1.0) + Kn (0.5) fortified and IBA (1.0 mg L<sup>-1</sup>) + Kn (0.5 mg L<sup>-1</sup>) supplemented MS medium, respectively. IBA has polar cell to cell transfer potential and stimulates lateral roots making it a more efficient root proliferative auxin than IAA (Rashotte *et al.*, 2003; Khadr *et al.*, 2020).



**Fig. 2 :** *In vitro* callus induction from leaf explants (a-c) stages in callus induction in MS medium fortified with 0.5 mg L<sup>-1</sup> TDZ and 1.0 mg L<sup>-1</sup> 2,4 D yielding yellow coloured callus (d-f) stages in callus induction in MS medium fortified with 1.0 mg L<sup>-1</sup> SNP yielding pale white callus.

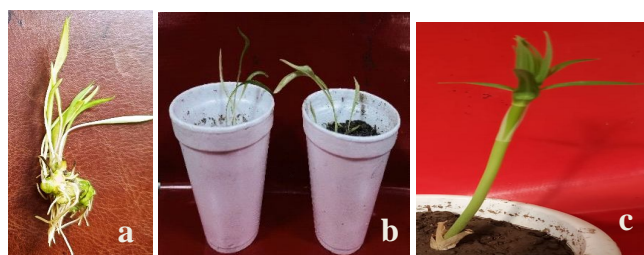


**Fig. 3 :** *In vitro* somatic embryogenesis of *P. verticillatum* through cultured calli. (a-c) successive stages of somatic embryos induction and further proliferation; (d) synthetic seeds by encapsulation of somatic embryos; (e) Histological observation of somatic embryogenesis from leaf explant showing globular somatic embryo stage; (f) Histological observation showing plumule development.

**Table 1 :** Effect of different concentrations and combinations of plant growth regulators on *in vitro* shoot induction.

Plant growth regulators (mg L <sup>-1</sup> )	Shoot induction (Days)	Shoot induction (%)	No. of shoots/explant
MS + BAP (1.0)	34.66 ± 2.0 <sup>d</sup>	41.67 ± 4.1 <sup>c</sup>	6.0 ± 1.1 <sup>bc</sup>
MS + KN (1.0)	33.0 ± 2.3 <sup>d</sup>	37.50 ± 7.2 <sup>c</sup>	1.0 ± 0.5 <sup>a</sup>
MS + TDZ (2.0) + NAA (1.0)	27.33 ± 3.9 <sup>de</sup>	68.75 ± 3.6 <sup>e</sup>	14.33 ± 2.9 <sup>e</sup>
MS + TDZ (1.5) + NAA (1.5)	28.0 ± 1.5 <sup>de</sup>	50.0 ± 7.2 <sup>cd</sup>	8.67 ± 0.88 <sup>d</sup>
MS + mT (0.5)	<b>17.67 ± 1.8<sup>b</sup></b>	<b>81.25 ± 3.6<sup>f</sup></b>	<b>23.67 ± 2.02<sup>f</sup></b>
MS + mT (1.0)	22.00 ± 3.21 <sup>b</sup>	60.41 ± 7.5 <sup>de</sup>	13.00 ± 2.5 <sup>de</sup>
MS + Kn (2.0) + IAA (1.0) + GA <sub>3</sub> (2.0)	20.67 ± 3.8 <sup>bc</sup>	52.91 ± 9.8 <sup>de</sup>	11.33 ± 1.7 <sup>de</sup>
MS + IAA (1.0)	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
MS + 2,4 D (1.0)	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
MS + NAA (1.0)	43.33 ± 2.6 <sup>e</sup>	18.75 ± 3.6 <sup>b</sup>	2.67 ± 0.8 <sup>a</sup>

Different letters following mean ± SE indicate significant difference among treatments (P<0.05). Values are expressed as Mean ± S.E. Kn, kinetin; 2,4-D, 2,4-dichlorophenoxyacetic acid; BAP, benzylaminopurine; NAA, naphthaleneacetic acid; TDZ, thidiazuron; mT, metatopolin; IAA, Indole acetic acid; GA<sub>3</sub>, Gibberellic acid.



**Fig. 4 :** (a-c) Hardened plants of *P. verticillatum* under glass house conditions (25 ± 2°C, 16/8hr photoperiod).

Cytokinins used alone did not show any rooting response. In a similar study on *P. odoratum*, 85% rooting response was observed at 3.0 mg L<sup>-1</sup> IBA supplemented MS medium (Kim *et al.*, 2014). Qadir *et al.* (2020), however, reported maximum root induction in *P. verticillatum* in 26 ± 0.55 days in MS medium fortified with 2 mg L<sup>-1</sup> IBA.

### Acclimatization

Regenerated plantlets were washed thoroughly with autoclaved distilled water and transferred to potting mixture of soil: sand: vermiculite: vermicompost (2:1:1:1) in thermocol cups in controlled conditions with 25 ± 2°C and 16/8 hr photoperiod provided with white fluorescent lamps. After one month, the plants were transferred to pots with sterilised soil.

### Callus induction from leaf explant

Leaf explants (1.5-2.0 cm) obtained from *in vivo* raised seedlings of *P. verticillatum* were used for callus induction on fortified MS medium with different conc. and combinations of plant growth hormones (Table 3, Fig. 2). MS medium fortified with TDZ (0.5 mg L<sup>-1</sup>) + 2, 4-D (1.0 mg L<sup>-1</sup>) yielded maximum callus induction (58.33 ± 8.33 %) in 20.33 ± 2.3 days followed by MS+SNP (1.0 mg L<sup>-1</sup>) giving creamish to brownish callus induction

**Table 2 :** Effect of different concentrations and combinations of plant growth regulator on *in vitro* root induction.

Plant growth regulators (mg L <sup>-1</sup> )	Root induction (Days)	Root induction (%)	No. of roots/ explants
MS basal medium	0 <sup>a</sup>	0000±000	0 <sup>a</sup>
MS + IBA (1.0)	28.0±1.5 <sup>c</sup>	<b>71.33± 8.3<sup>c</sup></b>	8.33±1.67 <sup>c</sup>
MS + IBA (1.0) + Kn (0.5)	43.33±2.0 <sup>d</sup>	32.33±6.3 <sup>a</sup>	4.33±1.2 <sup>b</sup>
MS + IAA (1.0) + Kn (0.5)	<b>25.67±1.2<sup>b</sup></b>	48.33±0.0 <sup>b</sup>	8.33±0.3 <sup>c</sup>
MS + NAA (1.0) + Kn (0.5)	27.66±1.4 <sup>b</sup>	66.67±6.2 <sup>c</sup>	<b>10.33±0.6<sup>d</sup></b>
Kn (0.5)	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
MS + 2,4 D (0.5) + Kn (0.5)	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
MS + IBA (0.5) + NAA (0.5)	31.33±2.3 <sup>c</sup>	41.67±0.1 <sup>b</sup>	7.33±2.0 <sup>c</sup>
MS + TDZ (0.5)	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
MS + mT (0.5)	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
MS + mT(1.0)	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>

Different letters following mean ± SE indicate significant difference among treatment (P<0.05). Values are expressed as Mean ± S.E. *Kn*- kinetin; *2,4-D*- 2,4-dichlorophenoxyacetic acid; *BAP*- benzylaminopurine; *NAA*- naphthaleneacetic acid; *TDZ*-thidiazuron; *mT*- metatopolin; *IAA*- Indole acetic acid; *IBA*- Indole-3-butyric acid.

**Table 3 :** Effect of different concentrations and combinations of plant growth regulators on *in vitro* callus induction.

Plant Growth Regulators (mg L <sup>-1</sup> )	Callus induction (Days)	Callus induction (%)
MS basal medium	0 <sup>a</sup>	0 <sup>a</sup>
MS + mT (0.5)	0 <sup>a</sup>	0 <sup>a</sup>
MS + mT (1.0)	0 <sup>a</sup>	0 <sup>a</sup>
MS + TDZ (0.5) + 2,4 D (1.0)	<b>20.33 ± 2.3<sup>c</sup></b>	<b>58.33 ± 8.3<sup>e</sup></b>
MS + TDZ (1.0) + 2,4 D (2.0)	32.67 ± 1.7 <sup>d</sup>	49.16 ± 4.1 <sup>e</sup>
MS + BA (1.0) + IAA (0.5)	0 <sup>a</sup>	0 <sup>a</sup>
MS + KN (1.0)	0 <sup>a</sup>	0 <sup>a</sup>
MS + BA (1.0) + 2,4 D (0.5)	30.66 ± 2.1 <sup>cd</sup>	23.33 ± 1.6 <sup>c</sup>
MS + BA (1.0) + NAA (0.5)	46.33 ± 3.7 <sup>e</sup>	30.00 ± 3.1 <sup>c</sup>
MS + BA (1.0) + NAA (1.0)	34.33 ± 3.7 <sup>d</sup>	16.67 ± 8.3 <sup>b</sup>
MS + SNP (0.5)	42 ± 3.5 <sup>e</sup>	40.33 ± 9.4 <sup>d</sup>
MS + SNP (1.0)	26 ± 2.3 <sup>b</sup>	51.67 ± 5.8 <sup>e</sup>
MS + SNP (2.0)	0 <sup>a</sup>	0 <sup>a</sup>
MS + KN (1.0) + IAA (0.5)	0 <sup>a</sup>	0 <sup>a</sup>
MS + KN (1.0) + IAA (1.0)	0 <sup>a</sup>	0 <sup>a</sup>

Different letters following mean ± SE indicate significant difference among treatments (P<0.05). Values are expressed as Mean ± S.E. *Kn*- kinetin; *2,4-D*-2,4-dichlorophenoxyacetic acid; *BA*- benzylaminopurine; *NAA*-naphthaleneacetic acid; *TDZ*- thidiazuron; *mT*- metatopolin; *IAA*- Indole acetic acid; *IBA*- Indole-3-butyric acid; *SNP*- *Sodium nitroprusside*.

(51.67±5.83%) in 26 ± 2.3 days, respectively. Synergistically, auxins and cytokinins induced effective callus formation. Differentiation of internal genetics trigger differential response in callusing, which is controlled by exogenous or endogenous level of plant growth hormones (Thorpe, 1983 and Patra *et al.*, 1998). MS+TDZ (1.0mg/L) +2,4-D(1.0mg/L) showed effective

callogenic effect in *P. verticillatum* from hypocotyl (70±5.00 %) and leaf explants (45%) of *in vitro* grown plants of *P. verticillatum* (Tiwari and Chaturvedi, 2018). Qadir *et al.* (2020) reported that *in vitro* sprouted seed explants pretreated with 1.0 mg/l GA<sub>3</sub> kept at -20° C for a month, produced 60% callus response on Kn (8.0 mg/l) and IAA (2.0 mg/l) fortified MS medium in 33±0.57 days (Park *et al.*, (2018), showed efficiency of 2,4-D at 0.5 mg·L<sup>-1</sup> for 87% production of callus in *P. stenophyllum* Maxim. In the present study, however, leaf explant from *in vivo* raised plants showed effective callusing (58%) in minimum number of days (20.33 ± 2.3) in MS medium supplemented with TDZ and 2,4-D.

### Somatic embryogenesis

Regenerated calli were further set for embryo induction in fortified MS medium with different combinations and concentrations of KN, NAA, IBA, IAA and casein hydrolysate (CH). Highest embryo induction percentage (62.5 ± 7.21%) was observed in minimum number of days (42.33 ± 1.76) on KN (2.0 mg L<sup>-1</sup>) + NAA (1.0 mg L<sup>-1</sup>) fortified MS medium (Table 3, Fig. 3). Kn (4.65 µM) along with NAA (0.54 µM) and BAP (4.44 µM) showed highest percentage of callus and somatic embryos induction in *Thymus hyemalis* (Nordine *et al.*, 2013). In another study, similar set of hormones, *viz.*, NAA (0.1 mg/L) and kinetin (0.5 mg/L) produced 4.83 viable embryos per culture in *Asparagus racemosus* (Choudhary *et al.*,

**Table 4 :** Effect of PGRs on *in vitro* embryo induction from leaf explants of *P. verticillatum*

Plant Growth Regulators (mg L <sup>-1</sup> )	Embryo induction (Days)	Embryo induction (%)
MS basal medium	0 <sup>a</sup>	0 <sup>a</sup>
MS + Kn (2.0)	0 <sup>a</sup>	0 <sup>a</sup>
MS + KN (2.0) + NAA (1.0)	42.33 ± 1.7 <sup>b</sup>	62.5 ± 7.2 <sup>c</sup>
MS + NAA (2.5) + BAP (0.5)	0 <sup>a</sup>	0 <sup>a</sup>
MS + NAA (3) + BAP (1)	0 <sup>a</sup>	0 <sup>a</sup>
MS + KN (2.0) + IAA (1.0)	0 <sup>a</sup>	0 <sup>a</sup>
MS + KN (1.5) + IAA (0.5) + CH (500)	59.33 ± 3.9 <sup>c</sup>	29.16 ± 11.0 <sup>b</sup>
MS + KN (2) + IAA (1) + CH (500)	84.0 ± 1.1 <sup>d</sup>	15.67 ± 4.2 <sup>ab</sup>

Different letters following mean ± SE indicate significant difference among treatment (P<0.05). Values are expressed as Mean ± S.E. *Kn*- kinetin; *BAP*- benzylaminopurine; *NAA*- naphthaleneacetic acid; *IAA*- Indole acetic acid; *CH*- Casein hydrolysate.

2019). During the present study, NAA, BAP, Kn, IAA used alone failed to show any response. CH (500 mg/l) along with KN and IAA induced significantly low number of somatic embryos (15.67 ± 4.2) in more number of days (84.0 ± 1.1). However, casein hydrolysate efficiently induced somatic embryos in *Eleusine coracana* (L.) Gaertn., *Abelmoschus esculentus* L. monech and *Setaria italica* L. (Venkatesan *et al.*, 2022; Daniel *et al.*, 2018; Sood and Prasad, 2020). The somatic embryos were confirmed by observational and histological studies carried out through Olympus BX 51 (Fig. 3 e-f). The synthetic seeds were achieved successfully in sodium alginate (2.5%) and CaCl<sub>2</sub> (2%) solution (Fig. 3 d). This is the first report of somatic embryo induction and artificial seed production in *P. verticillatum*.

### Conclusion

Seeds of *P. verticillatum* shows double dormancy and hence propagation through seeds is very slow. Underground rhizome being the important part of the plant, uprooted during harvesting posing a serious threat to natural population. Hence, in the present study, an efficient and rapid protocol for *in vitro* morphogenesis of *P. verticillatum* has been established through organogenesis, callogenesis and somatic embryogenesis. Somatic embryos were efficiently formed from the callus in IAA and Kn fortified MS medium. Embryos were confirmed by histological studies followed by encapsulation of embryos with sodium alginate to form artificial seeds. There is a need to bring out proper package of practice for establishing its cultivation through artificial seeds which

can eventually result in true to type plants.

### Acknowledgement

The authors would like to express their sincere thanks to G. B. Pant University of Agriculture & Technology, Pantnagar for providing necessary facilities to conduct this work. Council of Scientific and Industrial Research (CSIR), New Delhi is thankfully acknowledged for the financial support to carry out the work.

**Conflict of interest :** There is no conflict of interest.

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