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Histological and SEM studies on somatic embryogenesis in rhizome- derived callus of *Panax assamicus.* Ban.

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Abstract
Somatic embryogenesis is an important technique of plant biotechnology in medicinally important plants. Ginseng which is the common name referred to species of the genus *Panax* (Araliaceae), has been valued as a potentiating oriental herbal medicine. Somatic embryogenesis of *Panax assamicus* Ban. was initiated from approximately four-five years old rhizome explant through callogenesis. Highest callus proliferation was observed in MS medium supplemented with 5 mg/L 2,4-D. Eight weeks old callus on being transferred to MS medium supplemented with 5mg/L 2,4-D and 0.5mg/ L BAP in combination showed best result in embryogenic calli which eventually formed somatic embryos. Induction of somatic embryos occurred after 3 months of sub culture in the same medium. Highest germination of somatic embryos occurred in 1/2MS medium incorporated with 1.5mg/L gibberellic acid. To confirm the appearance of the embryo-like structures in greater detail, the structures were observed microscopically by scanning electron microscopy and through histological sections. Complete normal plantlets were regenerated within 4 to 5 months after the induction of somatic embryos Plantlets were acclimatized successfully to *ex vitro* conditions with the survivability of 70% in the green house.

Keywords: *Panax assamicus, Somatic embryogenesis, 2,4-D · dichlorophenoxyacetic acid, medicinal plant, Conservation*

1. Introduction
"Panax" is derived from the Greek words "*pan*" (all) "*akos*" (cure), meaning "cure-all". This alone tells us a lot about this herb: no single herb can be considered a panacea but ginseng comes close to it [1]. *Panax* root has been used in Oriental medicine since ancient times. The crude root extracts are known to have tonic, stimulatory and adaptogenic properties [2] owing to the presence of a wide range of saponins and sapogenins [3]. The root of this plant is characterized by the presence of triterpene saponins and other phenolic compounds which are known for their enormous medicinal properties [4]. Different ginseng species may be used slightly differently in traditional medicines, all species contain ginsenosides as an active components and most of the pharmacological activity of ginseng can be attributed to these compounds [5]. Materia Medica of Divine Plowman written in China about 2,000 years ago records ginseng as the highest quality herb. Ginseng plant has been used widely in Asia, Europe and America [1]. In India roots are used as stimulant, carminative tonic and expectorant [6]. *P. pseudoginseng*, *P. vietnamensis* and *P. japonicus* have been used as a folk medicine among the local people in the Himalayan regions, Vietnam and the middle parts of China [7]. Recently, ginseng has become a popular tonic and health food complement in Western countries too. Therefore, the demand for the plant has increased dramatically worldwide.
Ginseng has got a lengthy field production cycle of up to 5-7 years from seeds [8]. Because of its long-term growth, the plant is very expensive. The seeds of most *Panax* species are known to exhibit morpho-physiological dormancy, a type of seed dormancy where warm-cold sequence of seasonal temperature changes are needed to complete the germination process. Thus, in *Panax* species the warm and cold stratification may take up to 18 to 22 months to fully germinate [9]. This type of seed dormancy has been reported in *P. quinquefolius* [9], *P.* ginseng [10] and *P.* stipuleanatus [11]. Because of long dormancy period and over exploitation of the plant, seed availability are limited, hence propagation via somatic embryogenesis was carried out.
Somatic embryogenesis is a process whereby embryos develop from somatic cells or tissues in tissue culture system, thereby achieving maturity and subsequently germinate to form normal plantlets [12]. Furthermore somatic embryogenesis is defined as a process in which a bipolar

Structure resembling a zygotic embryo develops from a non-zygotic cell without vascular connection with the original tissue [13]. Propagation methods of ginseng by plant tissue culture and particularly by somatic embryogenesis have been investigated [14]. There have been several reports on plant regeneration of ginseng via somatic embryogenesis from the root, leaf, petiole, seed, zygotic embryos, flower buds as the explants [15-25] but no reports from the rhizome. For the Indian species, experiments had been carried out with *P. Psuedoginseng* and *P. sikkimensis*, where *P. pseudoginseng* calli were highly recalcitrant and failed to respond towards any organogenetic pattern so far [26]. Callus line was induced from root explants of *P. sikkimensis* Ban for screening of anthocynin [27], no tissue culture work had been carried out from this species including P.assamicus Ban.

The objective of this study was to establish the optimal conditions for (i) callus induction (ii) induction of somatic embryos (iii) plantlet regeneration from somatic embryo and (iv) establishment of hardened plantlets of *Panax assamicus* Ban. a highly medicinal plant of Meghalaya, India. The study was successfully carried out through somatic embryogenesis from rhizome derived callus. The protocol established in the present study is simple and cost effective. This protocol can be effectively used for rapid and mass propagation of this highly medicinal plant without disturbing its natural habitat. Furthermore, the derived somatic embryos can be subjected for variation studies and for subsequent genetic manipulation and control of specific secondary metabolite pathway like ginsenoside. The somatic embryos can also be a good source of material for genetic transformation and somatic hybridization.

2. Materials and methods

2.1 Plant material

Four-five years-old *in vivo* rhizome of *Panax assamicus* Ban. Collected from Upper Shillong East Khasi Hills Meghalaya, India, was used throughout the study. Thoroughly washed rhizome was surfaced sterilized in ethanol (70%, v/v) for 5 min, then in 10% sodium hypochlorite (4 % available chlorine, Himedia) plus few drops of tween-20 (Himedia) for 10 min, followed by mercuric chloride (0.1% w/v) for 2 min. Rinsed four to five times with sterile distilled water.

2.2 Induction of callus

Sterilized rhizome was cut transversely into 0.5-1.0 mm thick sections that serve as initial explants for callus induction. The explant was cultured on medium prepared by dissolving Schenk and Hildebrandt (SH) [28] and Murashige and Skoog (MS)[29] medium supplemented with different concentrations (0.5, 2.5, 5.0, 7.5, 10.0 mg/l) of 2,4-dichlorophenoxy acetic acid (2,4-D) with 3% sucrose and 8g/L of agar (Himedia) . The pH was adjusted to 5.8, and growth regulator was added to the medium before autoclaving (20 min, 121ºC, 1.06 kg/cm^2). Each treatment consisted of ten replicates and the experiment was repeated three times. All the cultures were kept at 2 ±2 ºC in the dark for 8 weeks in the same medium, till the callus proliferated into good amount. The percentage of callusing was calculated as follows.

$$percentage\ of\ callusing = \frac{No.of\ rhizome - derivedcalli}{Total\ No.of\ cultured\ rhizome}\ x\ 100$$

2.3 Somatic embryos induction

0.5g of callus clump was transferred onto MS and 1/2MS basal medium supplemented with various concentrations (0.25,0.5,1.0,2.5,5.0,7.5,10.0 mg/l) of 6-benzyladenine (BAP) with 5.0 mg/l of 2,4-D and a combination of kinetin (KN) (0.25,0.5,1.0,2.5,5.0,7.5,10.0 mg/l) with 5.0mg/l 2,4-D to check the response on the induction of callus into somatic embryos. Ten callus clumps (0.5g) were considered for each treatment and the experiment was repeated three times. The cultures were kept in the same culture condition as described earlier. Embryogenic potential of the callus was first observed after 10 weeks of innoculation where the compact, opaque callus of *P. assamicus* turned into a white loose and fragile callus. The frequency of somatic embryogenesis induction and the mean number of embryos per explants (0.5g of callus) was recorded after 3 months. Somatic embryos were used for further proliferation by repeated subcultures onto fresh medium of same composition. The frequency of somatic embryogenesis was calculated as the percentage of explants producing SEs. Both the frequency of embryo formation and the mean number of SEs were combined together as index of embryo forming capacity (EFC) that was calculated as follows:

EFC= [(mean no.of SEs per explant) X (% of explants forming SEs)] ÷100

2.4 Effect of different sucrose concentrations on embryogenic callus induction

Different concentrations of sucrose (2, 3, 6 and 9%), were used to examine the response of calli into embryogenic callus induction. 0.5g of the callus was transferred to MS basal medium supplemented with 5mg/L 2, 4-D and 0.5 mg/L BAP that showed the best response of somatic embryogenesis, 8 g/L agar was added with the different sucrose concentrations. The experiment consisted of ten callus clumps (0.5g) for each treatment, repeated three times. All cultures were maintained in the same culture condition as described earlier, subculture was done after 10weeks. This study mainly focused on the frequency of somatic embryogenesis and the readings were taken after 4 months of embryos initiation.

2.5 Germination of somatic embryos and plantlet regeneration

The matured embryos were transferred to a germinating medium, 1/2MS and MS medium supplemented (after autoclaving) with gibberellic acid (GA_3) at 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/L. GA_3 was filter sterilized and added into the medium that had cooled to about 50–60 ºC. The cultured tubes were subjected to 25±2 ºC, 14/10 h photoperiod by cool white fluorescent tubes at an intensity of 60.2 µmol m^{-2} sec^{-1}. Well developed plantlets with both roots and shoots, were regenerated within 4 to 5 months after sub culturing the germinated somatic embryos in the same medium, at an interval of 6 months. Each experiment was repeated three times.

2.6 Histological observation and scanning electron microscopy studies

SEs were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 3 h at 4 ºC, dehydrated through a graded ethanol series for 10 min each and embedded in saturated paraffin wax (Himedia), at 60 ºC. Sections of 7µm were cut using a rotary microtome (LeicaRM2125RT). Paraffin wax was removed by xylene prior to rehydration of the tissues in a graded ethanol series, it was then stained with 0.05% toluidine blue and mounted in DPX. The sections were observed and

photographed using a Leica light Microscope.
Scanning electron microscopy (SEM) was used for morphological observation of somatic embryos formation. The embryos were fixed as described earlier, washed in phosphate buffer for 1h, fixed in 1% osmium tetroxide for 1h and dehydrated through an increasing series of acetone. Dehydrated tissues were dried in a HCP-2 (Hitachi) critical point-drier. The dried samples were positioned horizontally to brass stubs with double-sticky tape, coated with gold in a JFC-1100 (JEOL) ion sputter Coater and observed using a JEOL, JSM-6360 SEM at 20 kV.

2.7 Ex vitro plant establishment
Well-developed plantlets were taken out, washed under running tap water to remove agar from the roots, and transferred to the pot containing sterilized mixture of black garden soil, compost and leaf litter in the ratio of 2:1:1. The plantlets were maintained under greenhouse condition with a temperature of 25 ± 2 ºC. The plantlets were irrigated twice a day. Plantlets acclimatized successfully to *ex vitro* conditions with the survivability of 70%.

2.8 Statistical analysis
Statistical calculations were carried out according to the analysis of variance (ANOVA), and the results were examined according to tukey's test. In all the cases, values represented means of ten replicates per treatment, repeated three times.

3. Results and discussion
3.1 Induction of callus
This is the first report of somatic embryogenesis from the rhizome in *P. assamicus*, via callus. We investigate the influence of MS and SH culture media with 2,4-D (0.5-10.0mg/l), on the induction of callus from four-five years old *in vivo* rhizome (fig 1a) as the explants. Though callogenesis occurred in both media supplemented with 2,4-D (0.5-10.0mg/l) but the response was significantly different at MS medium supplemented with 5.0mg/l 2,4-D, that resulted in the higher rate (94.44%) of callus induction (table 1). Generally 2,4-D was claimed to be one of the most effective auxins in the induction and growth of callus[30]. Similarly, in this study white coloured, fragile callus formation was observed in MS medium supplemented with 2,4-D (0.5-10.0mg/l) compared to control where no callogenesis developed. Initiation of callogenesis occurred in SH medium, the growth was not sustained followed by browning of the callus and the explants. The frequency of callus induction was obtained in MS medium supplemented with 2,4-D (fig 1b), present study showed that higher concentration of 2,4-D showed inhibitory effect for callogenesis, Similar findings was reported by Reddy and[31] in *Gardenia latifolia*. First initiation of callus was observed after two weeks of inoculation which showed to be earlier when compared to *P.notoginseng* as reported by [32] which took more than 12 weeks for callogenesis.

3.2 Induction of somatic embryos
0.5g/ test tube of the white coloured, fragile calli (fig 1c) was cultured in MS and ½MS medium with 5.0mg/l 2,4-D and different concentrations (0.25-10.0mg/l) of cytokinins, BAP and KIN for somatic embryo induction. Somatic embryogenesis was observed both in MS and ½Ms medium supplemented with different concentrations of BAP and KIN. No somatic embryogenesis occurred in the medium containing 2,4-D alone (table not shown). Similarly study was reported by [33]. In our present study, callus cultured on MS medium supplanted with 5.0mg/l of 2,4-D and 0.5mg/l of BAP resulted in higher conversion of (63.33%) to embryogenic calli (table 2). Whereas the combined effect of 2,4-D (5.0mg/l) and all the different concentrations (0.25-10.0mg/l) of KIN in both MS and 1/2MS resulted in lower conversion percentage of callus to embryogenic calli (table3).

Table 1: Effect of 2,4-D on callus induction from the rhizome segment (0.5-1.0 mm)

Basal medium	PGR (mg/l) 2,4-D	Callus induction (%)*
	0	0
MS	0.5	44.44 ± 0.12bc
	2.5	72.22 ± 0.11ab
	5.0	94.44 ± 0.05a
	7.5	50.00 ± 0.12abd
	10.0	38.89 ± 0.12bd
SH	0.5	22.22 ± 0.10cd
	2.5	44.44 ± 0.12bd
	5.0	27.77 ± 0.10bd
	7.5	11.11 ± 0.07cd
	10.0	05.55 ± 0.05cd

Somatic embryogenesis study on *P.ginseng* and P. *vietnamensis* by [14] and [34] respectively revealed that benzoselenienyl-3 acetic acid (BSAA) and indoleacetic acid (IAA) were useful in induction of somatic embryos. In accordance with the finding of [35], our present study showed that BAP gave the best response for embryogenesis frequency and number of somatic embryos per explants. In plants, auxins and cytokinins are the main regulators of cell division and differentiation. The influences of exogenous auxin/cytokinin on the induction of somatic embryogenesis, particularly 2,4-D, are well known[36]. Highest frequency of embryogenesis was obtained in *Eucalyptus camaldulensis* in the combination of BAP and NAA [37]. Unlike other species of *Panax*, somatic embryogenesis in *P. assamicus* was obtained through a combination of the auxin (2,4-D) and the cytokinin (BAP) at a lower concentrations (fig 2e), similar results to our finding was also seen in the study of *in vitro* somatic embryogenesis in *Acacia Arabica* [38].

Table 2: Effect of BAP in MS and ½ MS on the induction of somatic embryos from the derived callus (0.5g)

Basal medium	PGRs (mg/L)		Somatic embryogenesis %*	Mean no. of SEs/ explant*
	2,4-D	BAP		
MS		0.0	0.00	0.00
		0.25	30.00± 0.08abc	11± 0.86bd
		0.5	63.33 ± 0.08a	11.2 ± 0.92bd
		1.0	46.66 ± 0.09ab	12.2 ± 1.1bc
	5.0	2.5	30.00 ± 0.08abc	23.10 ± 1.2a
		5.0	16.66 ± 0.06bc	15.5 ± 0.68c
		7.5	10.00 ± 0.05cd	12.1 ± 1.04bc
		10.0	3.33 ± 0.03cd	12.1 ± 1.04d
½ MS		0.25	13.33 ± 0.06bd	7.4 ± 0.68bd
		0.5	23.33 ± 0.07bc	12.6 ± 0.63bc
	5.0	1.0	26.67 ± 0.08bc	16.1 ± 1.2c
		2.5	46.66 ± 0.09ab	18.5 ± 0.52c
		5.0	30.00 ± 0.08ac	24.9 ± 1.04a
		7.5	16.67 ± 0.06bc	14.2 ± 0.8b
		10.0	6.67 ± 0.04c	9.2 ± 0.95bd

* Different letters within each column represent significant difference at $P \leq 0.05$ by Tukey–test

Table 3: Effect of KN in MS and ½ MS on the induction of somatic embryos from the derived callus (0.5g)

Basal medium	PGRs (mg/L)		Somatic embryogenesis %*	Mean no. of SEs/ explant*
	2,4-D	KN		
MS		0.0	0.00	0.00
		0.25	10.00 ± 0.0ab	8.0 ± 0.86bc
		0.5	20.00 ± 0.07ab	9.2 ± 0.92bc
	5.0	1.0	43.33 ± 0.09a	15.5 ± 0.68af
		2.5	33.66 ± 0.08ab	10.1 ± 1.04bf
		5.0	26.67 ± 0.08ab	5.8 ± 0.57cd
		7.5	16.67 ± 0.06ab	4.1 ± 0.37de
		10.0	6.67 ± 0.04b	2.1 ± 0.64e
1/2MS		0.25	6.67 ± 0.04b	11.1 ± 0.73bf
		0.5	16.16 ± 0.06ab	12.8 ± 0.8f
	5.0	1.0	33.33 ± 0.08ab	16.6 ± 0.6a
		2.5	23.33 ± 0.07ab	13.6 ± 0.81ae
		5.0	13.33 ± 0.06ab	10.5 ± 0.65be
		7.5	10.00 ± 0.05b	8.3 ± 0.55bcf
		10.0	3.33 ± 0.03b	6.0 ± 0.55df

* Different letters within each column represent significant difference at $P \leq 0.05$ by Tukey–test

3.3 Effect of different sucrose concentrations on embryogenic callus induction

To check the response of callus for somatic embryogenesis in different concentrations of sucrose, MS medium was supplemented with optimal concentrations of 2, 4-D and BAP at 5.0 mg/L and 0.5 mg/L respectively, with 8g/L agar. Sucrose concentrations were adjusted to 2, 3, 6 or 9% before autoclaving. The treatments containing different sucrose concentrations presented significant effect on the induction of somatic embryogenesis. Among the different concentration of sucrose used, 30 g/l of sucrose performed well followed by 60 and 20, and 90 that showed the least (fig 5).

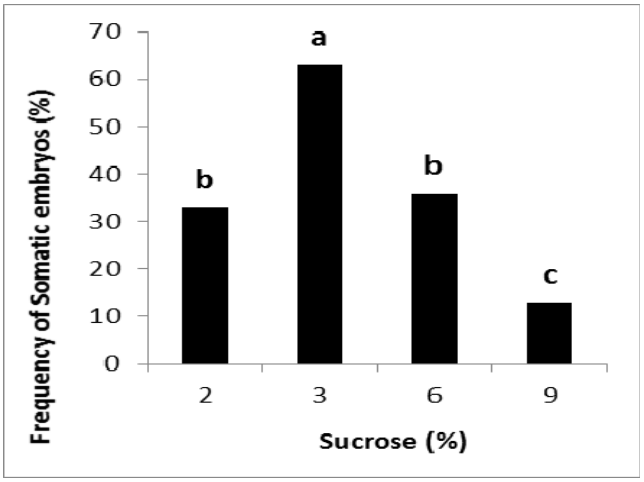

Fig 5: Effect of different concentrations of sucrose on the induction of embryogenic calli. *Columns* denoted by *different letters* represent significant difference at $P \leq 0.05$ according to Tukey test.

Most *in vitro* studies using carbohydrate have indicated that sucrose is the best carbon source for optimal cell growth [39] concluded that specific carbohydrates had differential effects on *in vitro* morphogenesis and the osmotic potential provided by these sugars support embryogenesis. In the present study, differences were also found for somatic embryogenesis capacity when using sucrose at various concentrations. The resent study showed that the frequency of embryogenesis (63.33%) cultured in medium with 3.0% added sucrose was highest among the different treatment, and the differences were significant. However with the increasing sucrose concentration, somatic embryogenesis gradually decreased (fig 5), this is in accordance with the study in *Picea abies* [40], *P. rubens* [41]. Qualitative variations in SE development with different concentrations of sucrose have been observed in *Eucalyptus camaldulensis* [37]. Some of these embryos were observed under a light microscope showing the different stages of development (Fig 2f). [42] reported increasing sucrose concentration in culture medium enhanced secondary embryogenesis as well. Differences were also found for somatic embryogenesis capacity when using sucrose at different concentrations.

3.4 Germination of somatic embryos and plantlet regeneration

When somatic embryos were cultured on ½ MS medium containing 3% sucrose supplemented with GA_3, germination occurred after 4-5 months of culture. Highest response was seen in GA_3 (1.5mg/L) with 31.67% of somatic embryos germinated on the media (table 4). Shoot was formed at the apex of somatic embryo and the root was developed to form a complete normal plantlet (Fig 2g), the development took place side by side after 3 months of transferring into germination medium. Present study showed the use of GA_3 to induce plantlet development which was also reported by [43] in grapevines. [44] Demonstrated that GA_3 inhibits somatic embryogenesis, but [45] showed that GA_3 promotes somatic embryogenesis. According to [46] study on *P.ginseng* showed that IBA was more effective in root initiation and proliferation. But in accordance with the earlier reports of [47] our observations showed that GA_3 played a very important role in germination of somatic embryos. Complete plantlets were obtained in ½ MS supplemented with GA_3 (1.5mg/l) (table4).

Table 4: Effect of different concentrations of GA_3 on regeneration of somatic embryos in ½ MS.

Plant growth regulator	Mean number of regenerated plantlets*
0.0	0.0
0.2	08.33±0.31b
0.5	11.67±0.31bc
1.0	28.33±0.31ac
1.5	31.67±0.6a
2.0	26.67±0.5ac
2.5	11.67±0.31bc
3.0	06.67±0.33b

* Different letters within the column represent significant difference at $P \leq 0.05$ by Tukey–test

3.5 Ex vitro plant establishment

After 7 months when plantlets reached in size of 4–5 cm, they were removed from culture vessel and plantlets were transferred to the pots containing sterilized mixture of garden soil, compost and leaf litter in the ratio of 2:1:1, and covered with a plastic sheet for acclimation. The plantlets were maintained under greenhouse condition with a temperature of 25±2 ºC. The plantlets were irrigated twice a day and 70% of the plantlets survived in *ex vitro* condition (fig2 h).

3.6 Histological observation and scanning electron microscopy studies

The ontogeny of the SEs was further analyzed by histological sections which showed successive stages of SEs. According to the results obtained from histological study, the embryogenic calli (fig1c) composed of cells with dense cytoplasm and a nucleus, indicating the presence of meristematic zones that could lead to the development of somatic embryos (fig3 k). The embryos attached to the callus by the suspensor were observed (fig3 i). Globular embryos and the elongated cotyledonary staged embryos with a distinctly differentiated protodermis further differentiated showing a group of actively dividing cells, the more advanced stage of somatic embryo development was observed with the shoot apical meristem (Fig3 j). Somatic embryos were observed to be initiated directly from the epidermal layer of the explant tissue. No apparent vascular connections were observed between SEs and maternal tissue in the process. Globular embryos with a distinctly differentiated protodermis further differentiated and developed through the early cotyledonary stage. Attempt has been made to develop the early ontogeny stages of somatic embryo differentiation from different plants, i.e *Vigna* species[48,49,50,51], *Phaseolus*[52,53], Glycine[54,55,56], Arachis *hypogaea*[57,58], *Cajanus cajan* [59] and *Macrotyloma uniflorum*[60], *Paris polyphylla* Sm [61]. Our approach was similar to previously reported procedures in which somatic embryo development through a division of spherical or elongated cells. Different stages of embryos appeared on the surface of callus.

To confirm the appearance of embryo-like structures in greater detail, we observed the structure microscopically by scanning electron microscopy (SEM). SEM study showed that embryos were developed in the surface of callus, and are clustered together in in a common mass. These structures revealed typical early stage of globular shape (Fig4 l) and mid stage of cotyledonary embryo (Fig4 m). The other forms of embryos (torpedo, heart shaped) were in less number compared to globular embryos. Embryogenic callus have been used for scanning electron microscopic study in a number of plant systems [62, 63]. In our present study we noticed that 2,4-D help in inducing embryogenic calli, and together with BAP and KIN it resulted in induction and proliferation of embryos. The findings agreed with the results obtained from the other plant species, where 2,4-D influences embryo induction [64, 65].

4. Conclusion

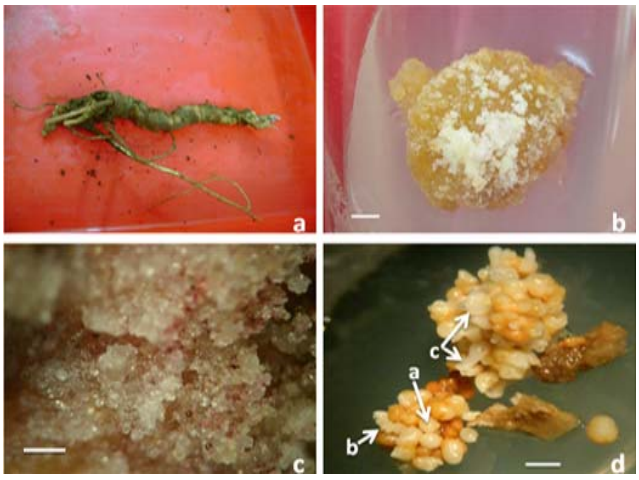

Fig 1.

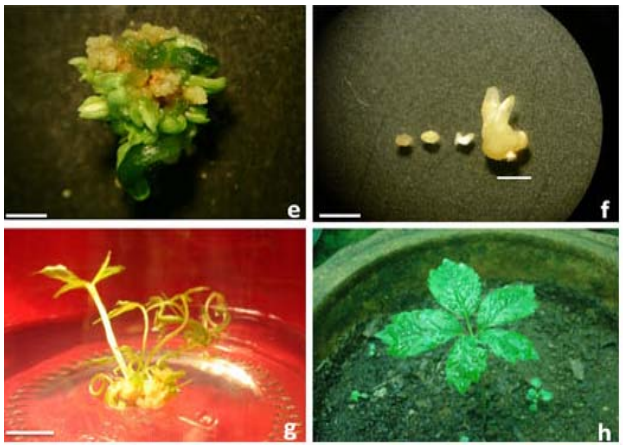

Fig 2.

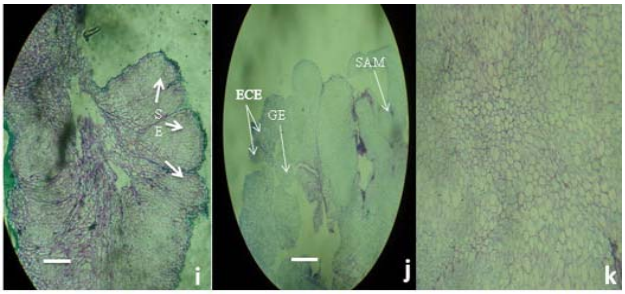

Fig 3

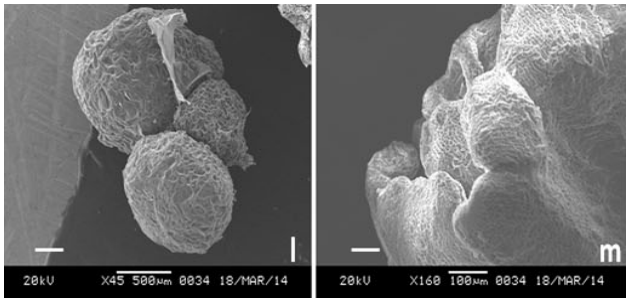

Fig 4

Fig.1 (a) Rhizome, 4-5 years old (b) callus induced from the rhizome explant with 5mg/l 2,4-D after 4 weeks of culture (c) embryogenic callus obtained after 8 weeks of subculture in MS medium supplemented with 5mg/L 2,4-D and 0.5mg/L BAP (d) Different developmental stages of embryos:(GE) globular embryos (HE) heart- shaped embryos (CE) cotyledonary embryos. Bar b,c ─1mm; d ─0.5mm.

Fig.2 (e) Clusters of germinating embryos in ½ MS supplemented with GA_3 (f) Different stages of embryo arranged singly (g) regenerated plantlets in basal ½ MS medium (h) first year hardened plant. Bar e, f─0.5mm; g ─5mm.

fig.3 (i)The embryogenic cells were possibly elongated densely cytoplasmic cells with prominent nuclei located on the callus surface (**j**) Different developmental stages of embryos (GE) globular embryo (ECE) elongated cotyledonary stage embryo (SAM) shoot apical meristem. Bar ─0.05mm

fig.4 (k) SEM view of globular (**l**) and the cotyledonary staged embryos. Bar ─0.1mm.

In conclusion, for the first time we have successfully established an efficient and reliable plant regeneration system via somatic embryogenesis for *P.assamicus* supported by histological and electron microscopy study. No noticeable

morphological abnormalities were observed in tissue cultured plants growing in the polyhouse. There is a lack of systematic effort for the cultivation of *P. assamicus*. Therefore, the protocol described above is simple, cost effective can contribute to the establishment of a large scale tissue culture production with specific reference to germplasm conservation, commercial cultivation and genetic improvement studies, as well as for ginsenoside production. Today ginseng is one of the most important medicinal herb and widely used medicine as a carminative tonic and expectorant, for blood pressure, cancer etc. We expect that *P. assamicus,* will serve well for a large-scale production for ginsenoside and other related compounds.

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