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In vitro spore germination and gametophytic growth development of a critically endangered fern *Pteris tripartita* Sw.

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The effects of sucrose, pH and plant growth hormones on spore germination percentage and gametophyte growths of *Pteris tripartita* were studied. Various morphological structures of gametophytes were observed namely, filamentous, spatulate and heart stages in the MS culture medium with hormones. After 15 days, the spores of *P. tripartita* were sprouted in MS basal medium fortified with pH, sucrose and hormones. Maximum spore germination rates (84%) were observed in 70 g/L of sucrose and 79.33% in pH 5.7. On the other hand, the maximum gametophyte sizes were observed both in 40 and 50 g/l of sucrose on half strength MS medium. The maximum growth of gametophyte lengths (484.39 and 507.72 µm) and widths (846.58 and 1270.98 µm) were observed in both pH 5.7 and 6.7. Among three different hormones, the utmost number or percentage of spores were sprouted in GA_3. However, the *in vitro* cultures of spore having the capability to increase the spore germinated due to addition of adequate nutrition in the culture medium and also reduce the contamination as well as environmental factors.

Key words: *Pteris tripartita*, spore germination, sucrose, hormone, MS medium.

INTRODUCTION

Pteridophytes have alternation of generations, possessing distinct free-living diploid sporophyte and haploid gametophyte generations. The sporophyte forms are mainly used for various purposes such as ornamental, ethnobotanical, medicinal properties and mainly to maintain micro-ecological habitats (Martin et al., 2006). The free-living fern sporophytes are typically similar to vascular plants with laminate photosynthetic structures; on the other hand, fern gametophytes are very small and lack tissue organization only with a protonemal structure formed after germination of the spore (Fernandez et al., 2012). From conservation point of view, ferns are becoming endangered as the spores have difficulty to germinate under natural condition due to environmental factors. Fern spore germination and gametophyte development characteristics are much more helpful in taxonomic and phyletic studies (Raine et al., 1996; Chiou and Farrar, 1997; Chiou et al., 1998; Chandra et al., 2003). In spore culture, type of spore germination, development of the prothallial plate and the

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meristematic regions, form of the mature and old thallus, type, position and time of appearance of hairs when present and form of the sex organs (especially the antheridium) are proven valuables to the taxonomists. The earliest fern spore germination pattern was carried out in *Schizaea pusilla* (Britton and Taylor, 1901) and *S. bifida* (Thomas, 1902). Perez-Garcia and Mendoza-Ruiz (2004) indicated that gametophytes may be useful for taxonomic and phyletic studies at the family and generic levels as well as among species within the same genus. The genus *Pteris* L. (Pteridaceae) is estimated to comprise about 250 species and found in the tropics (Smith et al., 2006). Ferns from threatened ecosystems require special attention for their rescue and recovery. In the case of chlorophyllous fern spores, germination is faster than brown spores of other species. Mature brown spores have less viability than green spores but dry storage at 4 or -20°C is effective in many cases and cryopreservation is the only effective storage method for culture studies (Pence, 2000). The present investigation was focused on the effects of sucrose, pH and plant growth hormones on spore germination and their gametophyte growths of a critically endangered fern, *P. tripartita.*

MATERIALS AND METHODS

Collection and storage of spores

The matured sporophytes of *P. tripartita* were collected from matured fronds in Alagar hills, Madurai district and confirmed with the help of reference standards at Centre for Biodiversity and Biotechnology, St. Xavier's College, Palayamkottai, Tamil Nadu, India. The voucher specimen (XCH 25403) was numbered and deposited at St. Xavier's Herbarium. The fertile fronds were dried in shade condition for two days and spores were collected. The spores lose their viability if stored at room temperature. Therefore, collected spores were preserved at low temperature (4°C) for further studies. The spores were sown in culture medium within one month of their collection.

Spore germination

Before inoculation process, 5 mg of spores scooped from the storage bag and immersed in water for 2 h. Spores were sterilized with commercial bleach solution (NaClO, 0.5% v/v) with double distilled water for 10 min. All the spores were rinsed at least three times with sterile double distilled water and then they were centrifuged at 3,000 rpm for 3 min. After that, spores were collected in a sterile condition and cultured in 25 ml culture tubes (Borosil, India) containing 10 ml of Murashige and Skoog (1962) basal medium augmented with 3% (w/v) sucrose and 0.7% (w/v) agar and pH was adjusted to 5.7 with 0.1N NaOH and 0.1N HCl. Various strengths of sucrose concentrations (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 g/l), pH ranges (3.7, 4.7, 5.7, 6.7, 7.7) and plant growth hormones such as 6-benzylaminopurine (BAP), kinetin (KIN) and gibberellic acid (GA_3) (1, 2, 3, 4, 5 mg/l) were tested for their efficacy. All the cultures were maintained at 25°C under cool-white fluorescent light (40 µmol m^{-2} s^{-1}) with 16 h artificial photoperiod (Philips, India). After two weeks, hundred spores were scored per treatment to study the spore germination rate and 10 gametophytes were randomly selected and observed for the measurement of gametophytes growth.

Microscopical studies

The effects of sucrose, pH and hormones in spore germination after two weeks and gametophyte growth rates (length, width of gametophytes) were assessed after 45 days. Spore germination was scored after 14 days of inoculation and photographed in light microscope (Deep vision, India) equipped with an ocular micrometer. The morphological studies of the gametophytes were measured with the help of stereomicroscope (Nikon SMZ800, Japan) after 45 days of inoculation and photographed using stereomicroscope (Nikon Eclipse E200, Japan). The micromorphological characters such as meristematic zone, rhizoid formation, perine, obconic cell and shape of protonemal cell were also observed.

Statistical analysis

Results were represented as Mean±Standard error. Hundred spores were scored per plate for spore germination percentage and ten gametophytes were randomly selected and measured using microscopes. Spore germination percentage and its gametophyte growth values represent the average of three replicates. The analysis was carried out using SPSS 17.0 software (Chicago, USA) with one way ANOVA test with Duncan's multiple range test (DMRT) along with $p < 0.05$ as the limit of significance.

RESULTS AND DISCUSSION

Spore germination using tissue culture methods permits a spore population free from contamination by spores of other species as well as infection by bacteria, fungi, algae and mosses which usually constitute a major problem when growing under normal field conditions (Deberg, 1994). Thus, fern spore culture has been studied on *in vitro* condition to evaluate the effects of sucrose, pH and plant growth regulators on both spore sprouting rate and gametophytes growth. Moreover, these physical and chemical factors affect the processes involved in growth and development of prothallium (Fernandez et al., 1997a, b, 1999). Different morphological structures like filamentous, spatulate and heart shaped in gametophyte development of ferns were observed after 30-35 days (Delfin, 1998). However, gametophytic generation is essential in the fern life cycle and very little is known about its ecology and physiology (Greer and McCarthy, 1999; Johnson et al., 2000; Watkins et al., 2007a, b). In the present study, germination of each spore produced one gametophyte that evolved from one dimensional filamentous stage to two dimensional heart shaped stage. Germinated spores of *P. tripartita* also raised filamentous prothalli and later developed into heart shaped gametophytes.

Effect of sucrose on spore germination

According to Dyer (1979), sucrose is generally used as

Table 1. Effects of sucrose and pH on spore germination of *P. tripartita* Sw. in half strength MS medium after two weeks.

Treatment	Spore germinations (%)	Rhizoid formations (%)
Sucrose (g/L)		
0	36.33±0.88^{e}	-
10	45.00±1.15^{d}	-
20	50.00±1.52^{d}	-
30	60.66±3.38^{c}	-
40	70.33±1.45^{b}	1.33±0.33^{b}
50	68.33±2.02^{b}	4.33±0.88^{a}
60	59.66±1.45^{c}	1.66±0.33^{b}
70	84.00±1.73^{a}	2.33±0.88^{ab}
80	49.66±2.02^{d}	-
90	55.66±0.88^{c}	-
100	38.33±1.45^{e}	-
pH		
3.7	32.33±2.60^{d}	-
4.7	59.66±2.40^{c}	-
5.7	79.33±1.76^{a}	6.33±0.88^{a}
6.7	68.33±2.02^{b}	3.66±0.66^{b}
7.7	67.66±1.20^{b}	2.66±0.33^{b}

Data are shown as Mean±SE of triplicate values. Each replication consists of hundred spores. Means followed by the same letter within columns are not significantly different at P≤ 0.05 by Duncan's Multiple Range Test (DMRT).

carbon source to promote gametophyte growth of leptosporangiate ferns. Varied concentration of sucrose (0-100 g/L) was added with to ½ strength MS basal medium to study the effects of sucrose on germination of spore, since sucrose is an indispensable supplement of all plant tissue culture media to augment the carbon supply and the photosynthetic ability of the cultured tissue which is also influenced by low irradiance and gaseous exchange (Kozai, 1991; Capellades et al., 1991). Sucrose concentrations in the culture medium play a vital role in ferns tissue culture and are particularly involved in spore germination. Spores of *P. tripartita* were sprouted after 15 days of inoculation in MS basal culture medium. Maximum spore germination rate (84%) was observed with 2.33% of rhizoid formation in 70 g/L of sucrose (Table 1). Sheffield et al. (2001) and Wu et al. (2010) proved significant increase in spore germination and gametophyte growth of fern species by addition of sucrose in the medium. Furthermore, stimulation of *Platycerium bifurcatum* (Camloh, 1993) and *Osmunda regalis* (Fernandez et al., 1997b) gametophytes were increased by the addition of sucrose. Moderate spore germination rates (70.33 and 68.33%) were achieved in both 40 and 50 g/L of sucrose concentrations. Lowest spore germination rates (36.33 and 38.33%) with absence of rhizhoid formation have been noticed in both 10 and 100 g/L of sucrose. The present results directly overlap with previous outcome that the spore germination was lower with high amount of sucrose (Renner and Randi, 2004).

Effect of sucrose on gametophyte growth

The influence of sucrose on gametophyte growth was studied by the addition of sucrose (10-50 g/L) with ½ MS strength medium. Lowest gametophyte growths were observed in both concentrations 10 and 20 g/L of sucrose. At 30 g/L of sucrose, the gametophyte length was 445.51 µm with 1392.08 µm of width which showed moderate rhizoid length (309.96 µm) with 6.62 mean numbers of rhizoids. Furthermore, maximum growth rate of gametophyte lengths of 691.03 and 722.14 µm along with widths 1845.36 and 1950.91 µm were also observed in 40 and 50 g/L of sucrose on half strength MS medium which showed significant rhizoid lengths (376.62 and 436.62 µm) and rhizoid mean numbers (7.10 and 8.23). Among the five concentrations of sucrose (10-50 g/L), minimal growths of gametophytes were identified in both 10 and 20 g/L of sucrose. In 10 g/L of sucrose, 291.07 µm

Table 2. Effects of sucrose and pH on spore derived gametophytic growths of *Pteris tripartita* Sw. after 45 days of culture.

Treatment	Gametophyte length (µm)	Gametophyte width (µm)	Rhizoidal number	Rhizoidal length (µm)
Sucrose (g/L)				
10	291.07 ± 4.00^{c}	445.50 ± 7.77^{c}	-	-
20	321.07 ± 5.87^{c}	482.17 ± 10.60^{c}	2.63 ± 0.17^{c}	112.21 ± 6.75^{c}
30	445.51 ± 16.36^{b}	1392.08 ± 195.33^{b}	6.26 ± 0.66^{b}	309.96 ± 40.54^{b}
40	691.03 ± 12.81^{a}	1845.36 ± 15.43^{a}	7.10 ± 0.36^{b}	376.62 ± 16.44^{ab}
50	722.14 ± 7.28^{a}	1950.91 ± 25.83^{a}	8.23 ± 0.17^{a}	436.62 ± 19.52^{a}
pH				
3.7	158.87 ± 2.22^{c}	433.28 ± 6.93^{c}	-	-
4.7	426.62 ± 10.18^{b}	811.02 ± 18.98^{b}	-	-
5.7	484.39 ± 8.67^{a}	846.58 ± 23.33^{b}	32.13 ± 0.46^{a}	443.28 ± 15.02^{a}
6.7	507.72 ± 14.18^{a}	1270.98 ± 39.73^{a}	27.70 ± 0.86^{b}	451.06 ± 7.28^{a}
7.7	407.73 ± 8.01^{b}	853.24 ± 15.74^{b}	28.43 ± 0.98^{b}	394.39 ± 14.44^{b}

Data are shown as Mean±SE of three replications. Each replication consists of 10 gametophytes. Means followed by the same letter within columns are not significantly different at P≤ 0.05 by Duncan's multiple range test (DMRT).

of gametophyte length and 445.50 µm of width with the absence of rhizoids were observed. The gametophyte length (321.07 µm) and width (482.17 µm) with lowest growth of rhizoid length (112.21 µm) and rhizhoid mean numbers (2.63) were noticed in 20 g/L of sucrose (Table 2). Fernandez et al. (1999) observed the effect of sucrose on gametophyte development along with the presence of mineral salts in the culture medium. Moreover, the addition of sucrose to the medium not only increases gametophytic morphological growth and shoot number but also, increase embryo induction that ultimately shortening the period from gametophyte to sporophytes of both *Nephrolepis biserrata* and *Aleuritopteris argentea* (Ambrosio and De Melo, 2004; Huang et al., 2009). The results revealed that the length, width, rhizoid length and its numbers of *P. tripartita* could be increased by the addition of sucrose in ½ strength MS culture medium condition.

Effect of pH on spore germination

The influence of pH over the multiplication efficiency, establishment and quality of the propagated sporophytes of ferns as well as ornamental plants were well documented (Handreck, 1992; Koedam et al., 1992; Pevalek-Kozlina, 1996; Symonds et al., 2001; Ambrosio and De Melo, 2004). Maximum spore sprouting rate (79.33%) was achieved along with 6.33% of rhizoid formation in pH 5.7 among five different pH ranges (3.7-7.7). Subsequently, minimum spore germination rates (68.33 and 67.66%) were observed with 3.66 and 2.66% of rhizhoid formations at pH of 6.7 and 7.7, respectively (Table 1). In earlier studies, spores of *Drynaria fortunei* showed highest germination frequency occurred at the pH toward alkalinity (pH 7.7) (Raghavan, 1989; Chang et al., 2007). In both pH ranges 3.7 and 4.7, the spore germination rates were 32.33 and 59.66% with the absence of rhizoid formations, respectively. The present results concluded that increasing pH range in MS culture medium plays significant role on spore germination and its gametophyte growth.

Effect of pH on gametophyte growth

The influence of pH in culture medium significantly affects the gametophyte growths. Due to acidic conditions of MS culture medium, there were no rhizoidal formation in lowest pH ranges (3.7 and 4.7), but showed the lowest gametophyte lengths (158.87 and 426.62 µm) and widths (433.28 and 811.02 µm), respectively. The minimum growth of gametophyte was observed in pH 7.7 in which, 407.73 µm of length and 853.24 µm of width with 394.39 µm of rhizoidal length along with 28.43 mean numbers of rhizoid were also noticed. Moreover, maximum growth of 484.39 and 507.72 µm of gametophyte lengths and 846.58 and 1270.98 µm of widths of gametophytes were observed in both pH ranges of 5.7 and 6.7, correspondingly (Table 2). Earlier report also proved that pH 6.7 provided better culture condition for the gametophyte development of *Sphaeropteris lepifera* (Ma et al., 2010). In the pH ranges 5.7 and 6.7, mean numbers of rhizoid (32.13 and 27.70) were also noticed (Figure 1).

Effect of hormones on spore germination

Generally, spore germination capability increased while MS media was supplemented with hormones, sugar and casein hydrosylate (Renner and Randi, 2004; Mazumder

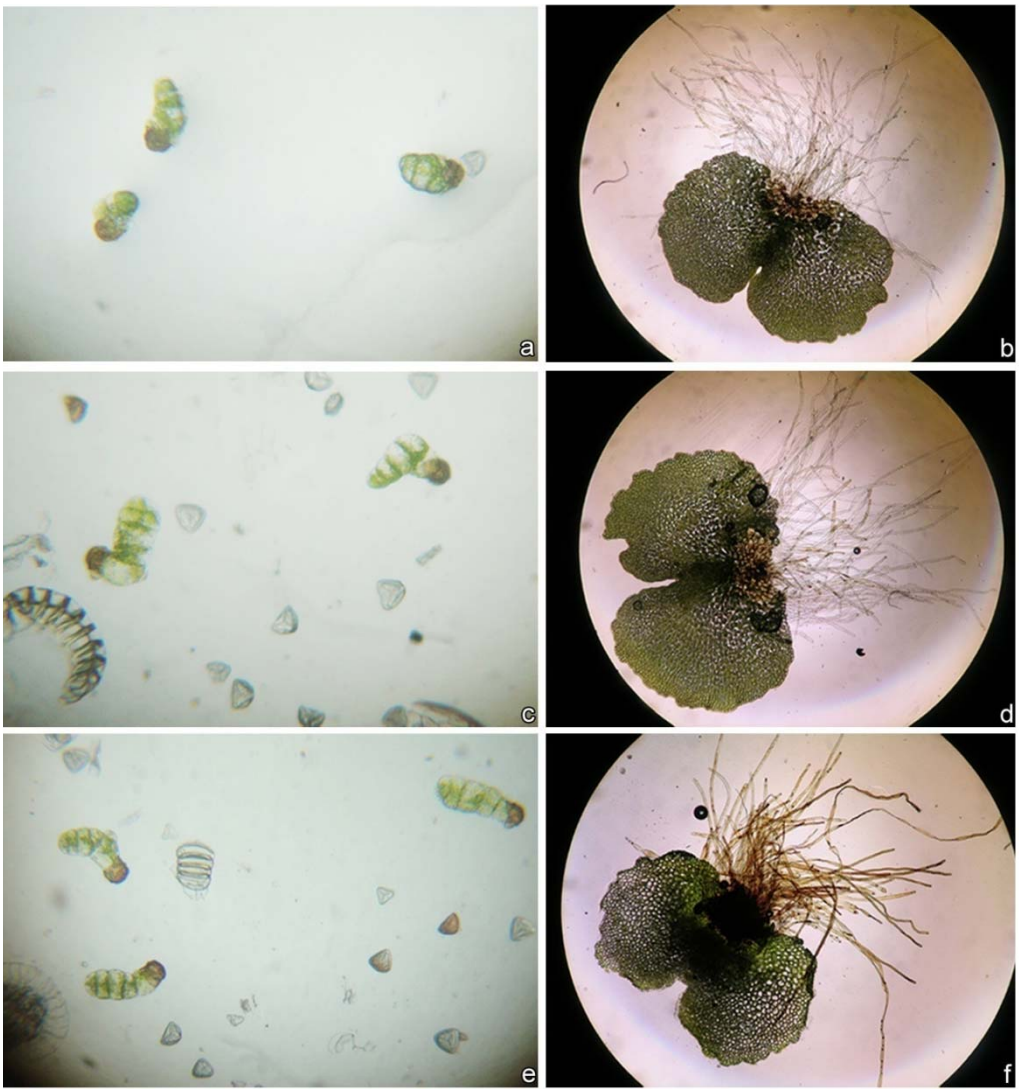

Figure 1. Effect of pH and sucrose on spore sprouting and gametophyte growth of *Pteris tripartita* Sw. (a) Germinated spores cultured in pH 5.7, (b) Heart shaped gametophyte in pH 5.7 after 45 days, (c) Germinated spores in pH 7.7 culture medium, (d) Heart shaped gametophyte formed in pH 7.7 after 45 days, (e) Spores grown in 30 g/L of sucrose, (f) Heart shaped gametophyte growth in 50 g/L of sucrose after 45 days.

et al., 2010). The influence of different hormones such as BAP, kinetin and GA_3 on spore germination and their gametophytes growth in various concentrations (1-5 mg/L) were studied. All hormonal concentrations significantly increased the percentage of spore germination. The cytokinin (BAP) showed spore sprouting percentages varying from 76 to 90.66% with the absence of rhizoids. The highest sprouting rate (90.66%) was achieved in 4 mg/l of BAP. The spore germination percentages were slightly reduced at 2 mg/L (80.66%), 3 mg/L (82.33%) and 5 mg/L (80.33%) of BAP (Table 3).

When compared to BAP, the Kinetin (KIN) showed minimum spore germination rate ranging from 63.33 to 70.66%. Maximum germination rate (70.66%) was obtained at 4 mg/L of KIN and other concentrations of KIN showed only least differences in their germination rates (1 mg, 63.33%; 2 mg, 63.66%; 3 mg, 64% and 5 mg, 65.33%). Among five concentrations of three hormones used in the study, the rhizoid was emerged only in GA_3 at 3 mg/L (1.66%), 4 mg/L (3.66%) and 5 mg/L (2%). Moreover, all concentrations of GA_3 appreciably increased the germination of *P. tripartita* spores (Figure 2). The similar results in germination rate with least difference were noticed at 2 mg/L (89%), 3 mg/L (90%) and 5 mg/L (91.33%), correspondingly. Among the three hormones, highest spore sprouting rate (95.33%) was observed in 4 mg/L of GA_3. Gibberellins have been used for breaking dormancy in numerous

Table 3. Effects of plant growth hormones on spore germination of *P. tripartita* Sw. after two weeks.

Hormone (mg/L)	Spore germination (%)	Rhizoid formation (%)
BAP		
1	76.00 ± 2.30^{b}	-
2	80.66 ± 5.60^{ab}	-
3	82.33 ± 2.02^{ab}	-
4	90.66 ± 1.45^{a}	-
5	80.33 ± 2.60^{ab}	-
KIN		
1	63.33 ± 3.17^{a}	-
2	63.66 ± 2.40^{a}	-
3	64.00 ± 2.08^{a}	-
4	70.66 ± 1.20^{a}	-
5	65.33 ± 2.02^{a}	-
GA_3		
1	83.00 ± 2.08^{c}	-
2	89.00 ± 1.15^{b}	-
3	90.00 ± 0.57^{ab}	1.66 ± 0.88^{ab}
4	95.33 ± 1.45^{a}	3.66 ± 0.88^{a}
5	91.33 ± 2.40^{ab}	2.00 ± 0.57^{ab}

Data are shown as mean±SE of triplicate values. Each replication consists of hundred spores. Means followed by the same letter within columns are not significantly different at P≤ 0.05 by Duncan's multiple range test (DMRT).

plant species and optimum concentrations accelerating germination depends on species (Sari et al., 1999). The solution containing GA_3 increased the spore germination of *Alsophila spinulosa* (Chen et al., 1991).

Effect of hormones on gametophyte growth

The high rate of gametophyte growth (516.60 µm length and 945.45 µm width) was achieved in 4 mg/L of BAP. In both 2 and 3 mg/L of BAP, moderate growth were observed, in which 354.40 µm of gametophyte length with 287.85 µm of width and 351.07 µm of length with 645.48 µm of width were recorded, respectively. As the BAP hormone concentration increases from 1 to 4 mg/L the growth rate was also increased but, at higher concentration (5 mg/L), the growth rate was rapidly reduced. There was no rhizoidal formation in the gametophytes at 1-3 mg/L of BAP culture condition and also at 5 mg/L of BAP only 324.40 µm of length and 224.41 µm with least number of rhizoid and their length were noticed. Among five concentrations of Kinetin, maximum rate of protonema length (593.27 µm), width (895.46 µm), rhizhoid mean number (3.33) and its length (125.53 µm) were recorded at 3 mg/L (Table 4; Figure 2). There were no rhizhoid formations in the other concentrations of Kinetin. The minimal lengths (335.52 and 307.74 µm) and widths (217.75 and 267.75 µm) of gametophyte growths were observed in both 4 and 5 mg/L of kinetin, respectively.

Rhizoids were noticed in all the concentrations of GA_3. The highest length of rhizhoid (787.69 µm) with maximum rate of gametophyte length (307.74 µm) and width (834.36 µm) were noticed in 1 mg/L of GA_3. The increased concentrations of GA_3 gradually reduced the growth of gametophyte. At both 2 and 3 mg/L of GA_3, moderate lengths (298.85 µm, 261.08 µm), widths (811.02 µm, 377.73 µm) with highest rhizoid numbers (12.30, 13.10) and its lengths (746.58 µm, 754.36 µm) were noticed. Lowest lengths (231.08 and 226.63 µm) and widths (474.39 and 488.83 µm) were noticed with 514.38 and 493.28 µm of rhizhoidal lengths at both 4 and 5 mg/L of GA_3. However, the lower concentration of GA_3 influenced overall morphological growth of gametophytes. Earlier studies also reported the influence of GA_3 on the gametophyte growth of *Lygodium japonicum* and *Blechnum spicant* (Swami and Raghavan, 1980; Fernandez et al., 1997c) and the plant growth regulators such as indol-3-acetic acid (IAA), BAP, gibberellins (GA_3 and GA_{4+7}) on the growth and sexual organ development in spore derived gametophytes of *Blechnum spicant* was also reported (Menendez et al., 2006). Chia and Raghavan (1982) found that GA_3 influenced the gametophyte growth of *Mohria caffrorum* than KIN hormone.

Even though Pteridaceae is a large family, influence of hormones, pH and carbohydrate source on their gametophyte growth are awfully limited. However, *in vitro* culture of *Pteris tripartita* spore summarizes that their spore germination is *Vittaria* type and followed by *Ceratopteris* type of prothallial development. The thalloid adult prothallus was cordate with broad wings, growing very fast and also with a distinct cushion. Rhizoids are nearly hyaline or pale brown that distributed in the lower surface of the cushion with thin cell walls. The adult prothallus is naked and further developments of gametangia are of the common leptosporangiate-type in which antheridia are formed from early development stages of the prothallus. The cap cell becomes loose and pushed off and finally releasing the spermatozoids. The neck of the archegonia is elongated and curving away from the apex of the prothallus (Nayar and Kaur, 1971). The present result agree with former observations on *Vittaria* type of spore germinations which was also reported in *Pteris vittata*, *P. multifida*, *P. wallichiana*, *P. cretica, P. ensiformis, P. inermis, P. fauriei, P. excelsa, P. finotii, Pleopeltis astrolepis*, *P. crassinervata*, *P. macrocarpa*, *P. polylepis, P. revoluta*, *Neocheiropteris palmatopedate, Neottopteris nidus*, *Blechnum appendiculatum*, *B. falciforme*, *B. gracile, B. occidentale, B. polypodioides*, *B. schiedeanum*, *B. serrulatum, B. caudatum* and nine species of *Callipteris* (Reyes Jaramillo

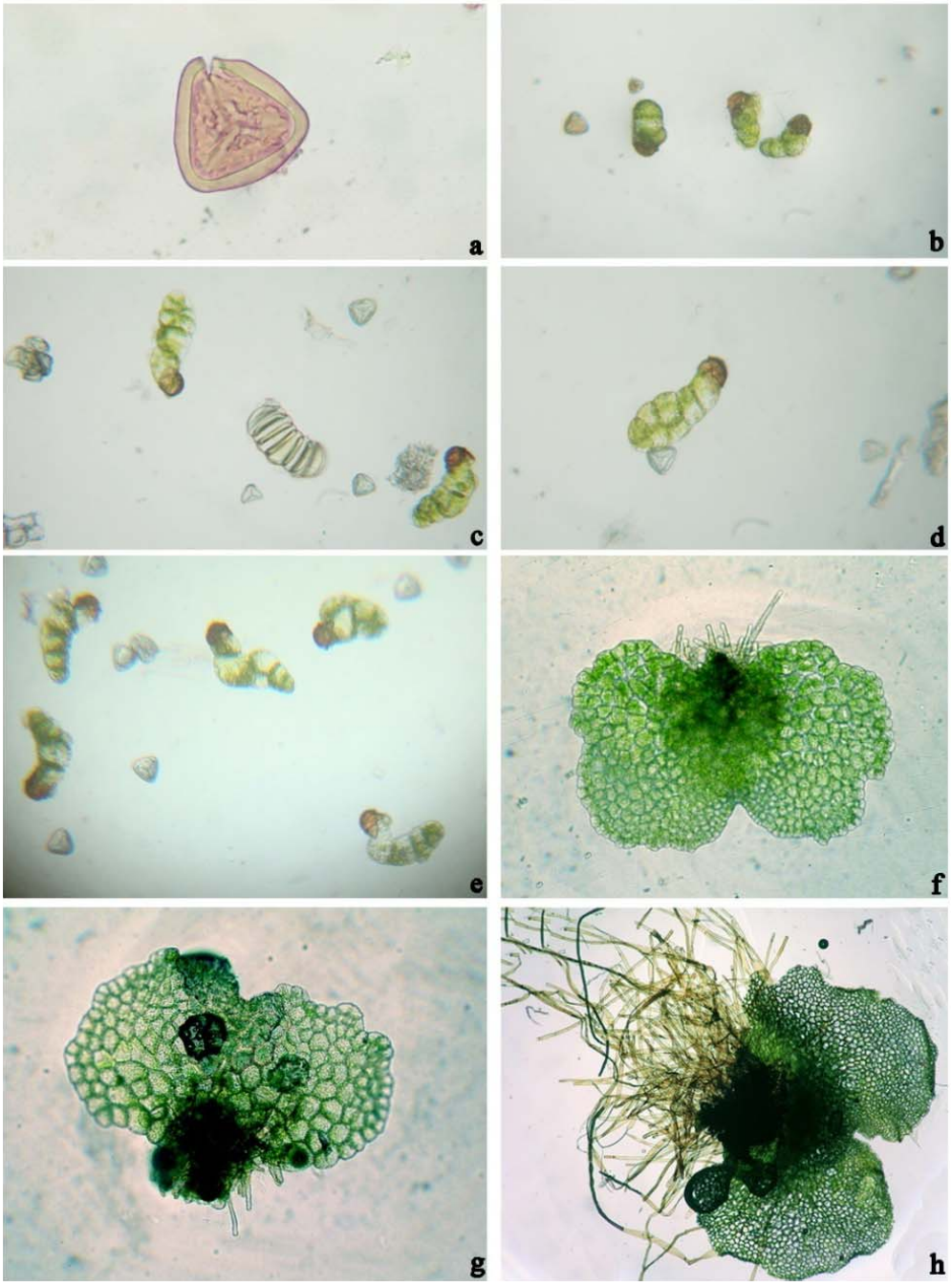

Figure 2. Effect of plant growth hormones on spore sprouting and gametophyte growth of *Pteris tripartita* Sw. (a) Spore, (b) Spore germination in 3 mg/L of KIN, (c) Germination in 3 mg/L of GA_3, (d) Protonemal development at 4 mg/L of BAP, (e) Protonemal cell at 4 mg/L of GA_3, (f) Gametophyte growth in 4 mg/L of BAP, (g) Heart shaped gametophyte at 3 mg/L of KIN (h) Gametophyte growth in 1 mg/L of GA_3.

et al. 2003; Pacheco and Riba, 2003; Zhang et al. 2008; Deng et al. 2009; Zhang, 2009; Mendoza-ruiz and Perez-garcia 2009; Tanco et al. 2009; Shen et al. 2009; Martinez 2010). *Ceratopteris* type of prothalli development was also seen in *Pteris vittata*, *P. multifida*, *P. wallichiana*, *P. finotii*, *P. excelsa*, *P. cretica*, *P. ensiformis*, *P. angusta*, *P. Mexicana* and *Neottopteris nidus* (Reyes Jaramillo et al., 2003; Zhang et al., 2008; Zhang, 2009; Martinez, 2010). However, growth hormones also considerably induced gametophyte morphological growths of *P. tripartita* on their length, width ratio, rhizhoidal and cell number.

Table 4. Effects of plant growth hormones on gametophytic growth of *P. tripartita* Sw. after 45 days.

Hormones (mg/L)	Gametophyte length (µm)	Gametophyte width (µm)	Rhizoidal number	Rhizoidal length (µm)	Gametophyte shape
BAP					
1	292.19 ± 11.60^{d}	246.63 ± 3.33^{d}	-	-	Heart
2	354.40 ± 4.00^{b}	287.85 ± 21.56^{c}	-	-	Heart
3	351.07 ± 7.78^{b}	645.48 ± 1.11^{b}	-	-	Heart
4	516.60 ± 3.33^{a}	945.45 ± 14.69^{a}	3.53 ± 0.88^{a}	108.87 ± 17.46^{a}	Heart
5	324.40 ± 2.22^{c}	224.41 ± 4.00^{d}	0.30 ± 0.30^{b}	8.88 ± 8.88^{b}	Heart
Kinetin					
1	176.64 ± 20.09^{d}	221.08 ± 1.11^{b}	-	-	Spatulate
2	261.08 ± 9.87^{c}	197.75 ± 13.51^{b}	-	-	Spatulate
3	593.27 ± 24.56^{a}	895.46 ± 64.58^{a}	3.33 ± 1.49^{a}	125.53 ± 59.00^{a}	Heart
4	335.52 ± 6.75^{b}	217.75 ± 2.93^{b}	-	-	Spatulate
5	307.74 ± 14.94^{bc}	267.75 ± 28.04^{b}	-	-	Spatulate
GA_3					
1	307.74 ± 4.00^{a}	834.36 ± 17.46^{a}	12.83 ± 1.27^{a}	787.69 ± 4.00^{a}	Heart
2	298.85 ± 7.28^{a}	811.02 ± 9.09^{a}	12.30 ± 0.40^{a}	746.58 ± 6.93^{a}	Heart
3	261.08 ± 6.75^{b}	377.73 ± 85.89^{b}	13.10 ± 0.56^{a}	754.36 ± 27.50^{a}	Heart
4	231.08 ± 1.11^{c}	474.39 ± 5.87^{b}	11.00 ± 0.55^{b}	514.38 ± 6.18^{b}	Heart
5	226.63 ± 3.33^{c}	488.83 ± 4.00^{b}	9.43 ± 0.24^{b}	493.28 ± 28^{b}	Heart

Data are shown as Mean±SE of three replications. Each replication consists of ten gametophytes. Means followed by the same letter within columns are not significantly different at P≤ 0.05 by Duncan's multiple range test (DMRT).

Conclusion

Spores of *P. tripartita* were successfully germinated with significant rates which further developed into normal young gametophytes. Various concentrations of sucrose, pH and plant growth hormones factors either enhance or inhibit the spore germination rate and their gametophyte development. The adult gametophytes of *P. tripartita* were spatulate and cordiform having wide wings without hairs.

Conflict of Interests

There is no conflict of interests.

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