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REVIEW

Fern spore germination in response to environmental factors

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Abstract **Fern spore germination gives rise to the rhizoid and protonemal cell through asymmetric cell division, and then develops into a gametophyte. Spore germination is also a representative single-cell model for the investigation of nuclear polar movement, asymmetrical cell division, polarity establishment and rhizoid tip-growth. These processes are affected by various environmental factors, such as light, gravity, phytohormones, metal ions, and temperature. Here, we present a catalog of spore germination in response to different environmental factors. They are as follows: (1) Representative modes of light affecting spore germination from different fern species include red light-stimulated and far red light-inhibited spore germination, far red light-uninhibited spore germination, blue light-inhibited spore germination, and spore germination in the dark. The optimal light intensity and illumination time for spore germination are different among various fern species. Light response upon spore germination is initiated from the cell mitosis that regulated by phytochromes (PHYs) and cryptochromes (CRYs). *Ac*PHY2, *Ac*CRY3 and/or *Ac*CRY4 are hypothesized to be involved in spore germination; (2) Gravity and calcium are crucial to early nuclear movement and polarity establishment of spores; (3) Gibberellin and antheridiogen can initiate and promote spore germination in many species, but abscisic acid, jasmonic acid, and ethylene pose only minor effects; (4) Spores can obtain the maximal germination rate in their favorable growth medium. Moreover, metal ions, pH, and spore density in the culture medium also affect spore germination; (5) Most fern spores germinate at 25°C, and an optimal CO_2 concentration is necessary for spore germination of certain fern plants. These provide valuable information for understanding fern spore germination in response to environmental factors.**

Keywords fern, spore, germination, environmental factors

Introduction

Ferns represent an ancient group of vascular plants, and the fern life cycle alternates between two distinct phases or generations: a sporophytic phase and a gametophytic phase. Fern spores generated from sporophytes can develop into gametophytes after germination. Gametophytes produce sperms and eggs to allow later fertilization and then develop into a new sporophytic generation. Following breaking of dormancy, the nucleus migration of fern spore occurs. Then rhizoids and protonemal cells are produced through the asymmetric cell division. The protonemal cell divides continuously and finally develops into a gametophyte. Obviously, spore germination is crucial to the alternation of fern generations. It is also a representative single-cell model for investigation of polarity establishment and growth processes, unlike the germination of plant pollen grains and seeds, which are two-/three-cellular and multicellular tip-growing systems, respectively (Chatterjee et al., 2000; Dai et al., 2006). Investigation of fern spore germination will provide more information for the comparison of the germination mechanisms of fern spores, pollen grains, and

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seeds. Importantly, it is vital for understanding the mechanisms of single-cellular light response, cell nucleus migration, cytoskeletal dynamics and polar growth (Dai et al., 2008). The spore germination of more than 200 fern species have been reported to be affected by various environmental factors including light, gravity, hormones (e.g., gibberellin (GA), antheridiogen (An), abscisic acid (ABA), jasmonic acid (JA), and ethylene), temperature, and nutrition. These fern species belong to 52 genera in 25 families, which represent diverse and somewhat disjointed features of spore germination in response to different exogenous and environmental factors (Fig. 1). Here, we present an extensive review as a catalog of existing data on environmental factors that affect fern spore germination.

The effect of light irradiation on spore germination

The quality, intensity, and duration of light are crucial to plant dormancy, germination, seedling development, reproduction, and shade avoidance (Kami et al., 2010). Plants perceive diverse light signals using specialized information-transducing photoreceptors, such as phytochrome (PHY), cryptochrome (CRY), and phototropin (PHOT). Fern spore germination is also affected by such a diversity of light conditions.

Spore germination is regulated by PHY and CRY

Fern spores from species with photosynthetic gametophytes typically germinate in the light, while the species with subterranean, nonphotosynthetic, mycorrhizal gametophytes germinate in the dark (e.g., fern species from Psilotaceae, Ophioglossaceae, as well as species from *Lycopodium* and *Tmesipteris*) (Whittier and Braggins, 1994; Whittier, 2006, 2008). It has been reported that the spore germination of two species, *Ophioglossum crotalophoroides* and *Lycopodium clavatum*, was induced in the dark and prevented by white light and red light, while initiated by far red light in a red/far red light reversible manner (Whittier, 2006, 2008). Similarly, spores of four species from *Psilotum* (*P. nudum* and *P. complanatum*) and *Tmesipteris* (*T. lanceolata* and *T. sigmatifolia*) also germinated in darkness (Whittier and Braggins, 1994).

Fern spore germination initiated from the cell mitosis is regulated by PHY and CRY (Furuya et al., 1997). PHY is an approximately 120 kDa biliprotein encoded by a large multigene family. PHY switches its protein conformation between P_r (red light absorbing form with a maximum absorption peak at 666 nm) and P_{fr} (far red light absorbing form with a maximum absorption peak at 730 nm), thus to turn on (red light) or off (far red light) the first asymmetric mitosis of spores (Raghavan, 1992). Experiments of partial (nuclear or cytoplasmic region) irradiation with a red/far red light microbeam on the darkness-imbibed spores from *Adiantum capillus-veneris* suggested that the PHY molecule was localized in the whole spores under darkness, but gradually migrated to the nuclear region under the irradiation with red light (Tsuboi et al., 2012). Some PHY genes have been identified in *Adiantum capillus-veneris*, including two typical PHY genes (*AcPHY1* and *AcPHY2*) (Nozue et al., 1998a), a photoreceptor gene (*AcPHY3*) (Nozue et al., 1998b), and a PHY-related sequence (*AcPHY4*) (Nozue et al., 1997). The expression levels of *AcPHY1* and *AcPHY2* were similar in the spores under red light. The transient expression of the PHY and glucuronidase (GUS) fusion gene implied that it was *Ac*PHY2, but not *Ac*PHY1, that migrated into the nuclear region upon spore germination (Tsuboi et al., 2012). In addition, *Ac*PHY3 is a PHY-nonphototropic hypocotyl 1 (NPH1) chimera photoreceptor. Its N terminus is similar to PHY chromophore binding domain and PHOT, and the C terminus is a full-length blue light receptor domain (Nozue et al., 1998b). As the photoreceptor of red/far red light and blue light, *Ac*PHY3 can mediate red light-induced phototropic and polarotropic responses and chloroplast movement in *Adiantum capillus-veneris* protonemal cells (Nozue et al., 1998b). However, the role of *Ac*PHY3 during fern spore germination is still unknown.

The P_r-induced first mitosis of spores is inhibited by blue light under the regulation of cell nucleus-located CRY (Furuya et al., 1997). CRYs function in the subsequent mitosis of protonemal cells which gives rise to protonema. In addition, the cell membrane-located PHY can inhibit the function of CRYs, resulting in the inhibition of protonema genesis. Five groups of *CRY* genes (*AcCRY1 – AcCRY5*) have been cloned in *Adiantum capillus-veneris* (Kanegae and Wada, 1998; Imaizumi et al., 2000; Suetsugu and Wada, 2003). All these *Ac*CRYs contain an N-terminal photolyase-homologous domain and a C-terminal extension (Kanegae and Wada, 1998). The C terminus of *Ac*CRY2, *Ac*CRY3, and *Ac*CRY4 contains a STAESSSS-related amino acid motif, which is proposed to be important for the direct interaction between PHYs and CRYs by phosphorylation during spore germination (Imaizumi et al., 2000). The expression of *CRY-GUS* fusion gene suggested that *Ac*CRY1, *Ac*CRY2, and *Ac*CRY5 were localized in the cytoplasm, while *Ac*CRY3 and *Ac*CRY4 showed clear nuclear localization. *Ac*CRY3 accumulated in the nucleus under darkness and red light, but not blue light, while the *Ac*CRY4 tended to accumulate in the nucleus region under any treatment conditions (Imaizumi et al., 2000). This implies that only *Ac*CRY3 and/or *Ac*CRY4 are involved in the inhibition of spore germination (Imaizumi et al., 2000). Moreover, *Ac*CRYs involved in the phototropism, apical swelling, and chloroplast orientational movement are proposed to be localized at the plasma membrane in protonema (Hayami et al., 1992).


Fern
Polypodiaceae
Campyloneurum
C. angustifolium An
C. phyllitidis An
Colysis
C. latiloba S-L
Drynaria
D. fortunei pH1
Lepisorus
L. thunbergianus An
Microgramma
M. heterophylla An
M. lindbergii Top-25
M. squamulosa Top-25
Microsorium
M. pteropus Top-20; T<15
Neocheiropteris
N. palmatopedata N1
Phlebodium
P. aureum An
Phymatosorus
P. scolopendria An
Platycerium
P. bifurcatum MS4; JA
Polypodium
P. aureum R; B; R-B; B-R; LD1
P. hirsutissimum Top-30
P. latipes Top-25
P. lepidopteris LI-h; LI-l
P. pellucidum An
P. pleopeltifolium Top-25
Pyrrosia
P. lingua S-L; MS1
P. piloselloides CO2a
Tectariaceae
Tectaria
T. heracleifolia Top-25; LI-w; LI-s
T. incisa Top-25; LI-w; LI-s
T. mexicana Top-25; LI-w; LI-s
T. transiens Top-25; LI-w; LI-s
Lomariopsidaceae
Nephrolepis
N. auriculata GA
Dryopteridaceae
Dryopteris
D. expansa S-H
D. filix-mas LD2
D. paleacea LD2; Ca2+; I2
D. varia S-L; MS2
Polystichum
P. mickelii Top-25
P. minutum LD2
Rumohra
R. adiantiformis Top-25; LI-h; LI-l
Onocleaceae
Matteuccia
M. struthiopteris ABA
Onoclea
O. sensibilis R; FR; B; D; Top-30; E; CO2a; CO2b; LD2; I1; N1
Blechnaceae
Blechnum
B. appendiculatum Top-25
B. brasiliense LI-h; LI-l
Woodwardia
W. radicans Top-25
Thelypteridaceae
Thelypteris
T. glandulosa Top-25
T. kunthii R; B; D; R-FR; R-B; B-R; R-FR-Rf; R-FR-Rs; LD1
T. serrata Top-25
Woodsiaceae
Athyrium
A. filix-femina S-H
A. multidentatum GA
Aspleniaceae
Asplenium
A. nidus R; R-FR; FR-R; FR>24h-R; LD1
A. ruta-muraria T<15
A. scolopendrium T<15
A. trichomanes T<15
Pteridaceae
Adiantopsis
A. radiata Top-25
Adiantum
A. capillus-veneris R; FR; B; D; FR-R; R-FR; R-B; B-R; R38h-B/FR-X
A. raddianum MS2
A. reniforme var. sinense MS1
Argyrochosma
A. formosa Top-25
Bommeria
B. ehrenbergiana An
B. hispida An
Ceratopteris
C. richardii Gr; E-im; E-m
C. thalictroides An; ID1; LI-h; LI-l; I2
Cheilanthes
C. farinosa R; D; R-FR; R-B; B-R; (R-)B-Rh/l; R-D3-5h-B/FR; (R-)B-Rh-B/FR; (R-)B-Rh-Bs
C. feei R; FR; B; G; W; D; Top-25; pH1; LI-h; LI-l
Hemionitis
H. subcordata Top-25
Pteris
P. cretica S-L; MS2
P. denticulata Top-30
P. tripartita Sw MS2
P. vittata R; D; R-FR; FR-R; R-B; B-R
P. wallichiana MS2
Dennstaedtiaceae
Dennstaedtia
D. globulifera Top-25
Pteridium
P. aquilinum An; GA; S-H; LD1; N1
P. aquilinum var. latiusculum GA; N2
P. revolutum N2
Lindsaeaceae
Sphenomeris
S. chinensis Top-30; GA;
Dicksoniaceae
Dicksonia
D. sellowiana LI-h; LI-l
Cyatheaceae
Alsophila
A. metteniana R; B; Y; W; D; Top-30; T<15; pH2; LI-h; LI-l
A. podophylla MS3
A. spinulosa GA
Cyathea
C. atrovirens pH2
C. delgadii LI-h; LI-l
Sphaeropteris
S. hainanensis GA
Cibotiaceae
Cibotium
C. barometz N1
Plagiogyriaceae
Plagiogyria
P. pectinata Top-25
Culcitaceae
Culcita
C. macrocarpa Top-20
Marsileaceae
Marsilea
M. vestita Top-25; T<15; pH3
Schizaeaceae
Schizaea
S. pusilla R; FR; B; G; W; D; FR-R; B-R; GA; GA4+7
S. dichotoma MS1
Anemiaceae
Anemia
A. mexicana pH2
A. phyllitidis GA; S-H; N2; N1
Mohria
M. caffrorum R; FR; B; D; R-FR; R-B; B-R; FR-B; B-FR; R(FR)-B6-72h; ABA
L. heterodoxum FR; W; D
Lygodiaceae
Lygodium
L. japonicum R; GA; N1
Gleicheniaceae
Gleichenella
G. pectinata LI-I; pH2; Top-25
Osmundaceae
Osmunda
O. japonica S-L; MS1; ID2
Psilotaceae
Psilotum
P. complanatum D
P. nudum D
Tmesipteris
T. sigmatifolia W; D
T. lanceolata W; D
Ophioglossaceae
Ophioglossum
O. crotalophoroides R; FR; W; D; R-FR; FR-R

The effects of red light and far red light on fern spore germination

The spore germination of some fern species was induced by red light (Table 1), such as *Onoclea sensibilis*, *Lygodium japonicum*, *Thelypteris kunthii*, *Pteris vittata*, and *Cheilanthes feei* (Nondorf et al., 2003). Longtime irradiation with red light was necessary for some fern spore germination. For example, 12 h illumination per day induced a high germination rate of *Alsophila metteniana* spores (Du et al., 2009a). Similarly, *Asplenium nidus* spores initiated rhizoids under 12 h illumination of red light. Spores from some species germinate under red light irradiation for several days. For example, the maximum germination rate of *Polypodium aureum* spores was obtained after 5-day continuous red light illumination. However, for some fern spores, several minutes or seconds were sufficient to promote a high percentage of germination, such as spores of *Cheilanthes farinosa* and *Onoclea sensibilis*. On the contrary, intermittent irradiation with red light does not work effectively for some spore germination. Under a 24 h continuous irradiation of red light, *Mohria caffrorum* spores showed a 79.1% germination rate, while the germination rate was reduced to 15.4% with the intermittent red light irradiation (5 min per 1 h, 5 min per 4 h, and 5 min per 8 h) within a 24 h time course (Reynolds and Raghavan, 1982). Similar regulation by red light has also been observed upon *Thelypteris kunthii* spore germination (Huckaby and Raghavan, 1981).

In addition, short time far red light irradiation significantly inhibited spore germination of *Adiantum capillus-veneris* (Furuya et al., 1997). However, far red light promoted the germination of spores from *Mohria caffrorum* and *Onoclea sensibilis* (Table 1). Intermittent illumination under far red light within 24 h was not conducive to *Mohria caffrorum* spore germination (Reynolds and Raghavan, 1982). In addition, far red light also promoted initiation of *Cheilanthes feei* spore germination, but all the spores stopped germinating at the two-cell protonemal stage (Nondorf et al., 2003). The reasons for lacking photoreversibility in those spores under far red light has been explained as follows: (1) spore germination was controlled by a low level of P_{fr}, which was produced by both red light and far red light; (2) once active P_{fr} was formed, it was rapidly used in the post-inductive phase, leading to the non-sensitity of spores to far red light; and (3) PHY was masked by some accessory pigments, which also promoted spore germination (Reynolds and Raghavan, 1982). However, further study is necessary to prove these hypotheses.

There is a significant "switching effect" of red/far red light on spore germination of some fern species (Table 2). Spore germination (e.g., *Adiantum capillus-veneris* and *Lycopodium clavatum*) promoted by short time red light illumination can be reversed by the irradiation of far red light in a red/far red light reversible manner (Furuya et al., 1997; Whittier, 2008). However, after 12 h irradiation with red light, the germination of *Mohria caffrorum* spores could not be reversed by 5 min or 1 h illumination of far red light (Reynolds and Raghavan, 1982). In addition, a brief exposure of red light (5 min) could not repromote the germination of far red light-inhibited *Thelypteris kunthii* spores. On the contrary, if the spores were exposed to red light for 10 to12 h, or to a repeated 8 h red light plus 5 min far red light twice following an extra 8 h red light, the inhibition of far red light could be nullified (Huckaby and Raghavan, 1981). This implies that

Figure 1 Phylogeny depicting relationships of fern species whose spore germination is affected by various environmental factors. The effects of environmental factor on spore germination were shown in red (promotion), green (inhibition) and blue (no influence). ABA: Abscisic acid; An: Antheridiogen; B: Blue light; B-R: Blue light followed by red light; CO_{2a}: CO_2 treatment concentration for the spores of *P. piloselloides* (219–3360 $cm^3 \cdot m^{-3}$) and *O. sensibilis* (0–2%); CO_{2b}: CO_2 treatment concentration for *O. sensibilis* spores (5%–15%); D: Darkness; E: Ethylene ($\geqslant 1\mu L \cdot L^{-1}$); E-im: The treatment of ethylene on the immature fern spores; E-m: The treatment of ethylene on the mature spores; FR: Far red light; $FR_{>24h}$-R: 24h far red light treatment followed by red light; FR(B)-B(FR): Far red light followed by blue light or blue light followed by far red light; FR-R: Far red light followed by red light; G: Green light; GA: Gibberellin acid; GA_{4+7}: A kind of gibberellin acid; Gr: Gravity; I_1: Ca^{2+}, Mg^{2+}, or Mn^{2+}; I_2: La^{3+} or Cd^{2+}; ID_1: Inoculation density, 50 spores$\cdot mL^{-1}$; ID_2: Inoculation density, 3000 spores/cm^2; JA: Jasmonic acid; LD_1: Long time of light irradiation ($\geqslant$24 h); LD_2: Short time irradiation ($<$24 h); LI-h: High light intensity; LI-l: Low light intensity; LI-s: Leaf shade; LI-w: White light; MS_1: Medium with lower concentration ($<$1/2MS: 1/4MS, 1/5MS, 1/10MS etc.); MS_2:1/2 MS; MS_3: MS or$\leqslant$1/2MS; MS_4: The liquid and solid medium; N_1: Other nutrition in the medium: such as, sulphite, oxybarbiturates, lipophilic solvents, and the leachates of *Eupatorium adenophorum*; N_2: Other nutrition in the medium: such as, thiamine, pyridoxine, glycine, glutamate, kinetin, and extract of *P. aquilinum* leave; pH_1: A wide pH range (3.7 to 9.7 or 4.5 to 8.5); pH_2: 3.7 to 6.7; pH_3: 7 to 8; R: Red light; R-B: Red light followed by blue light; R/FR-$B_{6\text{-}72h}$: Red or far red light followed by blue light for 6–72 h; R-$D_{3\text{-}5h}$-B/FR: Red light followed by blue or far red light after an intervening dark period (3–5 h); R-FR: Red light followed by far red light; R-FR-R_s: Red light followed by far red light, and then red light for short time (5 min); R-FR-R_l: Red light followed by far red light, and then red light for 8 h; R_{38h}-B/FR-X : 38 h red light treatment followed by blue or far red light, and then a series of brief red, far red, and blue light irradiation; (R-)B-$R_{h/l}$: (Red light-) Blue light followed by red light of high intensity (1–2 min) or long time (1h); (R-)B-R_h-B_s: (Red light-) Blue light followed by red light with high intensity (2 min), and then blue light for short time (1–2 min); (R-)B-R_h- B_l/FR: (Red light-)Blue light followed by red light with high intensity (2 min), and then blue light for long time (1–2 h) or far red light; S-L: Low concentration of sucrose in MS; S-H: High concentration of sucrose in MS; T < 15: The optimum germination temperature less than 15 °C; Top-20: The maximum germination rate occurred at 20 °C; Top-25: The optimum germination temperature is 25 °C; Top-30: The maximum germination rate occurred at 30 °C. W: White light; Y: Yellow light.

Table 1 Effects of different light source on fern spore germination

Environmental factors	Plant species	Treatment condition (concentration, time)	Effects on spore germination	References
Red light	*Adiantum capillus-veneris*	5 min (followed in darkness for 168 h)	P (58.3%)	Furuya et al., 1997
		38 h (followed in darkness for 38 h)	P (78.3%±2.6%)	
	Alsophila metteniana	12 h daily	P (65.66%±4.04%)	Du et al., 2009a
	Asplenium nidus	⩾72 h	P (initiated both rhizoid and protonema)	Raghavan, 1971
		12 h	Only formed rhizoids	
	Cheilanthes farinosa	30 s	P (80%)	Raghavan, 1973
	Cheilanthes feei	–	P (88%)	Nondorf et al., 2003
	Lycopodium clavatum	30 min daily	I (5.9%)	Whittier, 2008
	Lygodium japonicum	–	P	Sugai et al., 1977
	Mohria caffrorum	144 h or 12 h or 24 h	86.2%±2.7% or 42.3%±3.3% or 79.1%±3.9%	Reynolds and Raghavan., 1982
		5 min/h (during a 24 h period)	SI (77.6%±1.3%)	
		5 min/4 h (during a 24 h period)	I (39.4%±2.8%)	
		5 min/8 h (during a 24 h period)	I (15.4%±0.7%)	
	Onoclea sensibilis	5 min	P (63.2%±2.5%)	Towill et al., 1973
	Ophioglossum crotalophoroides	20 min daily	I (0%)	Whittier, 2006
	Polypodium aureum	1 d, 3 d, 5 d	33%, 61%, 79%	Spiess et al., 1977
	Pteris vittata	–	P	Sugai et al., 1967
	Thelypteris kunthii	8 h or 18 h	P (34.8% and 46.5%±2.05%)	Huckaby and Raghavan, 1981
		5 min/1 h for 18 h	I (37.2%±2.63%)	
		5 min/3 h for 18 h	I (32.9%±2.17%)	
		R 8 h, D 24 h	P (34.6%±4.45%)	
Far-red light	*Adiantum capillus-veneris*	5 min (followed in darkness for 168 h)	I (1.2%)	Furuya et al., 1997
	Cheilanthes feei	–	P	Nondorf et al., 2003
	Lycopodium clavatum	30 min daily	P (95.8%)	Whittier, 2008
	Lygodium heterodoxum	12 h daily	P (74%)	Pérez-García et al., 1994
	Mohria caffrorum	144 h	P (84.0%±3.1%)	Reynolds and Raghavan, 1982
		12 h	P	
		24 h	P (80.6%±1.2%)	
		5 min/h (during a 24 h period)	P (78.8%±1.9%)	
		5 min/4 h (during a 24 h period)	I (35.2%±3.3%)	
		5 min/8 h (during a 24 h period)	I (9.0%±2.6%)	
	Onoclea sensibilis	5 min	P (75.2%±3.1%)	Towill et al., 1973
	Ophioglossum crotalophoroides	20 min daily	P (25.3%)	Whittier, 2006

(*Continued*)

Environmental factors	Plant species	Treatment condition (concentration, time)	Effects on spore germination	References
Darkness	*Adiantum capillus-veneris*	168 h or 38 h	I (0%)	Furuya et al., 1997
	Alsophila metteniana	–	I (0%)	Du et al., 2009a
	Thelypteris kunthii	18 h or 24 h	I (0%)	Huckaby and Raghavan, 1981
	Cheilanthes farinosa	4 weeks	I (8%)	Raghavan, 1973
	Cheilanthes feei	–	I (7.7%)	Nondorf et al., 2003
	Lycopodium clavatum	24 h daily	P (98.7%)	Whittier, 2008
	Mohria caffrorum	144 h	I (0%)	Reynolds and Raghavan, 1982
	Onoclea sensibilis	6 d	I (12.0%±3.9%)	Towill et al., 1973
	Ophioglossum crotalophoroides	24 h daily	P (36.2%)	Whittier, 2006
	Psilotum complanatum *Psilotum nudum*	Several months (cultured on nutrient medium, PH = 5.4)	P (52.0% – 98.3%)	Whittier and Braggins, 1994
	Tmesipteris lanceolata, *Tmesipteris sigrnatifolia*		P	
	Tmesipteris elongata		I (0%)	
	Tmesipteris tannensis		I (< 0.1%)	
White light	*Adiantum reniforme* var. *sinense*	16 h daily	P (maximum germination rate, 50.1%)	Wu et al., 2010
	Alsophila metteniana	12 h daily	P (68.78%±1.81%)	Du et al., 2009a
	Cheilanthes feei	–	P (98%)	Nondorf et al., 2003
	Lycopodium clavatum	30 min daily or 12 h daily	I (2.2% or 0%)	Whittier, 2008
	Lygodium heterodoxum	12 h daily	P (90%)	Pérez-García et al., 1994
	Ophioglossum crotalophoroides	20 min daily	I (0%)	Whittier, 2006
Green light	*Cheilanthes feei*	–	P	Nondorf et al., 2003
Yellow light	*Alsophila metteniana*	12 h daily	P (63.74%±3.06%)	Du et al., 2009a
Blue light	*Adiantum capillus-veneris*	5 min (followed in darkness for 168 h)	I (0%)	Furuya et al., 1997
	Alsophila metteniana	12 h daily	I (7.51%±0.44%)	Du et al., 2009a
	Cheilanthes feei	–	P (68%)	Nondorf et al., 2003
	Mohria caffrorum	144 h	I (15.7%±3.5%)	Reynolds and Raghavan, 1982
		78 h	I (16%)	
	Onoclea sensibilis	5 min	P (51.7%±3.0%)	Towill et al., 1973
	Polypodium aureum	1 d, 3 d, 5 d	I (0%)	Spiess et al., 1977
	Thelypteris kunthii	24 h (followed in drakness for 24 h)	I (0%)	Huckaby and Raghavan, 1981

Abbreviation: I, inhibited; P, promoted; SI, slightly inhibited.

enough time of red light illumination can nullify far red light-induced inhibition of spore germination.

Spores germinated under darkness are induced by far red light, but inhibited by red light. Spores from *Psilotum* (*P. nudum* and *P. complanatum*) and *Tmesipteris* (*T. lanceolata* and *T. sigmatifolia*) germinated only in the darkness (Whittier and Braggins, 1994) (Table 1). Although *Tmesipteris lanceolata* spores could germinate in light, its gametophyte development never proceeded beyond the two-cell stage. Spores of *Ophioglossum crotalophoroides* and *Lycopodium clavatum* were able to complete germination under darkness and far red light, but were inhibited by red and white light (Whittier, 2006, 2008). In addition, over 24 h far red light treatment followed by 1 to 2 min red light promoted almost complete *Asplenium nidus* spore germination and rhizoids initiation. Similarly, spores of fern *Lygodium heterodoxum* also germinated under far red light (Pérez-García et al., 1994).

In addition, white light induced the spore germination of *Alsophila metteniana*, *Cheilanthes feei*, *Lygodium heterodoxum*, and *Adiantum reniforme* var. *sinense*, and the former two species also germinated well under yellow or green light conditions (Pérez-García et al., 1994; Nondorf et al., 2003; Du et al., 2009a; Wu et al., 2010) (Table 1).

Blue light inhibition of spore germination

Blue light significantly inhibits fern spore germination of some species (Table 1). For example, blue light could not initiate the spore germination of *Adiantum capillus-veneris* and *Polypodium aureum* (Furuya et al., 1997). For *Alsophila metteniana* spores (Du et al., 2009a), blue light exposure only induced a 7.51% germination rate, which was lower than that under other light conditions. However, for certain species, blue light didn't inhibit or only partially inhibit spore germination. An approximately 68% germination rate of *Cheilanthes feei* spores was obtained under blue light (Nondorf et al., 2003). Additionally, the germination rate of *Onoclea sensibilis* spores under 5 min irradiation with blue light was similar to those under other light sources.

The mechanism of photoreversibility germination between blue light and red/far red light remains unclear (Table 2). Previous physiological studies have reported that blue light could not inhibit the red light-induced germination of spores from *Cheilanthes farinosa, Polypodium aureum*, and *Mohria caffrorum* (Reynolds and Raghavan, 1982). For example, red light-promoted germination of *Cheilanthes farinosa* spores was reversed by a brief blue light, but the spores germinated again after a long time or high intensity of red light illumination. This is mainly due to the irradiation time of red light has reached the requirement for germination.

Light intensity and illumination time

The optimal light intensity for germination is different among various fern species. A relative low light intensity is more conducive to spore germination (Fig. 2). For example, when the light intensity increased from 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the germination rate of *Alsophila metteniana* spores was increased from 55.25% to 80.45%, whereas the germination rate was decreased to 60.44% when the light intensity continued to increase from 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Thus, 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was the optimal light intensity for *Alsophila metteniana* spore germination (Du et al., 2009a). Consistent with this, the germination rate of *Cheilanthes feei* spores also appeared to be increased under the light intensity from 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and then declined with the light intensity increasing from 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the maximal germination rate occurred at light intensity of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Nondorf et al., 2003). Germination rates of four species from *Tectaria* (*T. heracleifolia*, *T. incisa*, *T. mexicana*, and *T. transiens*) were also induced with the increase of light intensity. The germination rates were over 65% under the irradiation with white light, while less than 40% under the leaf shade conditions (Pérez-García et al., 2007). High light intensity inhibited the spore germination of *Rumohra adiantiformis*. The shortest germination time (8 days) was observed in a low light intensity (9% of nature light), whereas the germination time was extended to 23 days when the light intensity increased to 54% of natural light (Brum and Randi, 2002). In some cases, low light intensity seemed more efficient for spore germination of some species in Dicksoniaceae, such as *Dicksonia sellowiana*, *Cyathea delgadii*, and *Blechnum brasiliense* (Renner and Randi, 2004; Hiendlmeyer and Randi, 2007). Under 5%, 20%, 36%, and 50% of natural light irradiance, the spore germination rate of *Dicksonia sellowiana* was more than 85% over a 49-day incubation. Moreover, under 5% of natural light, the germination rate reached to 60% in a 21-day incubation (Renner and Randi, 2004). Similarly, under 5% natural light, the spore germination rate of *Cyathea delgadii* and *Blechnum brasiliense* was 83.5% and 84%, respectively (Hiendlmeyer and Randi, 2007). The highest spore germination rate of *Polypodium lepidopteris* was observed at 22% and 8% of natural light (Viviani and Randi, 2008). Similarly, *Gleichenella pectinata* spores also showed the highest germination percentage at 5% to 20% of sunlight irradiance (Santos et al., 2010).

The illumination time required for spore germination is diverse among different fern species (Fig. 2). Some fern spores need a long period of light irradiation for germination. For example, the spores of *Asplenium nidus* initiated rhizoids in 4 days under the irradiation with red light, and the maximal spore germination rate of *Polypodium aureum* was achieved in 5 days. In addition, after 4 days imbibition in darkness, the maximal spore germination rate of *Thelypteris kunthii* was detected under 7 days irradiation with white light or red light (Huckaby and Raghavan, 1981). However, for spores from some fern species, such as *Onoclea sensibilis*, *Dryopteris filix-mas, Dryopteris paleacea*, and *Polystichum minutum*,

Table 2 Effects of multi-light quality interaction on fern spore germination

Environmental factors	Plant species	Treatment condition (concentration, time)	Effects on spore germination	References
R-FR	*Adiantum capillus-veneris*	R 5 min-FR 5 min (followed in darkness for 168 h)	I (0.4%)	Furuya et al., 1997
		R 38 h-FR 10 min (followed in darkness for 38 h)	P (83.0%±2.2%)	
	Asplenium nidus	R 12 h-FR 48 h	I	Raghavan, 1971
	Cheilanthes farinosa	R 30 s-FR 30 s –1 h	N	Raghavan, 1973
	Lycopodium clavatum	R 30 min-FR 30 min daily	P (96.8%)	Whittier, 2008
	Mohria caffrorum	R 12 h-FR 5 min	N (41.1%±2.1%)	Reynolds and Raghavan, 1982
		R 12 h-FR 1 h	N (39.7%±4.4%)	
	Ophioglossum crotalophoroides	R 20 min-FR 20 min daily	P (28.4%)	Whittier, 2006
	Thelypteris kunthii	R 8 h-FR 5 min	I (0%)	Huckaby and Raghavan, 1981
FR-R	*Adiantum capillus-veneris*	FR 5 min-R 5 min (incubated in darkness for 168 h)	P (58.3%)	Furuya et al., 1997
	Asplenium nidus	FR 24–48 h-R 1–2 min	P	Raghavan, 1971
		FR > 72 h-R 0–10 min	P	
	Lycopodium clavatum	FR 30 min daily-R 30 min daily	I (6.4%)	Whittier, 2008
	Ophioglossum crotalophoroides	FR 20 min daily-R 20 min daily	I (0%)	Whittier, 2006
R-FR-R	*Adiantum capillus-veneris*	R 5 min-FR 5 min-R 5 min (followed in darkness for 168 h)	P (54.9%)	Furuya et al., 1997
		R 38 h-FR 10 min-R 5 min (followed in darkness for 38 h)	P (72.0%±2.6%)	
	Asplenium nidus	R 12 h-FR 48 h-R 10 min	P	Raghavan, 1971
	Thelypteris kunthii	R 8 h-FR 5 min-R 8 h	P (48.2%±4.91%)	Huckaby and Raghavan, 1981
		R 8 h-FR 5 min-R 5 min	I (6.8%±2.69%)	
FR-R-FR	*Adiantum capillus-veneris*	FR 5 min-R 5 min-FR 5 min (followed in darkness for 168 h)	I (0%)	Furuya et al., 1997
R-D-B	*Adiantum capillus-veneris*	R 5 min-B 5 min (followed in darkness for 168 h)	I (2.6%)	Furuya et al., 1997
		R 38 h-B 5 min (followed in darkness for 38 h)	P (87.3%±1.9%)	
	Cheilanthes farinosa	R 30 s-B 30 s	I (0%)	Raghavan, 1973
		R 30 s-D 3 h-B 30 s	SI (50%)	
		R 30 s-D 5 h-B 30 s	N	
	Mohria caffrorum	R 12 h-B 1 h	I (0.5%±1.6%)	Reynolds and Raghavan, 1982
		R 12 h-B 1–6 h	I	
		R 12 h-B 6–72 h	P	
	Polypodium aureum	R 1 d-B 1 d; R 1 d-B 3 d; R 1 d-B 5 d	I (11%, 12%, 15%)	Spiess et al., 1977
		R 3 d-B 1 d; R 3 d-B 3 d; R 3 d-B 5 d	SI (49%, 59%, 57%)	
		R 5 d-B 1 d; R 5 d-B 3 d; R 5 d-B 5 d	SI (64%, 66%, 76%)	
	Thelypteris kunthii	R 8 h-B 24 h	I (17.4%±1.09%)	Huckaby and Raghavan, 1981

(*Continued*)

Environmental factors	Plant species	Treatment condition (concentration, time)	Effects on spore germination	References
	Adiantum capillus-veneris	B 5 min-R 5 min (incubated in darkness for 168 h)	I (2.4%)	Furuya et al., 1997
	Cheilanthes farinosa	B 30 s-R 30 s	I	Raghavan, 1973
		B 30 s-R 1 h	P	
		B 30 s-R (high intensity) 1–2 min		
B-R	*Mohria caffrorum*	B 1 h-R 12 h	I (0.3%±1.1%)	Reynolds and Raghavan, 1982
	Polypodium aureum	B 1 d-R 1 d; B 3 d-R 1 d; B 5 d-R 1 d	P (53%; 63%; 63%)	Spiess et al, 1977
		B 1 d-R 3 d; B 3 d-R 3 d; B 5 d-R 3 d	P (68%; 71%; 63%)	
		B 1 d-R 5 d; B 3 d-R 5 d; B 5 d-R 5 d	P (52%; 68%; 65%)	
	Thelypteris kunthii	B 24 h-R 8 h	I (19.1%±1.47%)	Huckaby and Raghavan, 1981
	Mohria caffrorum	FR 12 h-B 1 h	I (0%)	Reynolds and Raghavan, 1982
FR-B		FR 12 h-B 1–6 h	I	
		FR 12 h-B 6–72 h	P	
B-FR	*Mohria caffrorum*	B 1 h, FR 12 h	I (0%)	Reynolds and Raghavan, 1982
R-B-R	*Adiantum capillus-veneris*	R 5 min-B 5 min-R 5 min (followed in darkness for 168 h)	I (5.4%)	Furuya et al., 1997
		R 38 h-B 5 min-R 5 min (followed in darkness for 38 h)	P (73.9%±3.1%)	
	Cheilanthes farinosa	R 30 s-B 30 s-R 1 h	P	Raghavan, 1973
		R 30 s-B 30 s-R (high intensity) 1–2 min	P	
	Adiantum capillus-veneris	R 38 h-FR 10 min-R 5 min-FR 10 min (followed in darkness for 38 h)	P (82.6%±2.2%)	Furuya et al., 1997
	Asplenium nidus	R 12 h-FR 48 h-R10 min-FR 8 h	I	Raghavan, 1971
R-FR-R-FR	*Thelypteris kunthii*	R 8 h-FR 5 min-R 8 h-FR 5 min	P (36.7%±1.83%)	Huckaby and Raghavan, 1981
		R 8 h-FR 5 min-R 5 min-FR 5 min	I (0%)	
		R 8 h-FR 5 min-R 8 h-FR 5 min-R 8vh	P (55.2%±4.83%)	
		R 8 h-FR 5 min-R 5 min-FR 5 min-R 5 min	I (5.2%±0.92%)	
D-R	*Asplenium nidus*	D 24–72 h-R 1–10 min	I	Raghavan, 1971
	Thelypteris kunthii	D 24 h-R 8 h	P (33.0%±3.04%)	Huckaby and Raghavan, 1981
B-R-D-FR	*Cheilanthes farinosa*	B 30 s-R (high intensity) 2 min-FR 1–2 min	I	Raghavan, 1973
		B 30 s-R (high intensity) 2 min-D 5 h-FR 1–2 min	N	
B-R-B	*Cheilanthes farinosa*	B 30 s-R (high intensity) 2 min-B 1–2 min	N	Raghavan, 1973
		B 30 s-R (high intensity) 2 min-B 1–2 h	I	
R-B-R-D-FR	*Cheilanthes farinosa*	R 30 s-B 30 s-R (high intensity) 2 min-FR 1–2 min	I	Raghavan, 1973
		R 30 s-B 30 s-R (high intensity) 2 min-D 5 h-FR 1–2 min	N	
R-B-R-B	*Cheilanthes farinosa*	R 30 s-B 30 s-R (high intensity) 2 min, B 1–2 min	N	Raghavan, 1973
		R 30 s-B 30 s-R (high intensity) 2 min-B 1–2 h	I	
	Adiantum capillus-veneris	R 38 h-B 5 min-R 5 min-B 5 min (incubated in darkness for 38 h)	P (67.9%±3.3%)	Furuya et al., 1997

Abbreviation: B, blue light; D, darkness; FR, Far-red light; I, inhibited; N, no effect; P, promoted; SI, slightly inhibited; R, red light.

short time red light irradiation (3 to 5 min) is enough for germination, In addition, treatment with red light for 24 h, *Pteridium aquilinum* spores required 3 to 5 days for germination, while the spores under red light for 12 h needed extra 3 to 4 days to germinate (Niu et al., 2002). The differences in the requirement of illumination probably attribute to the specific properties of fern species, the spore longevity and inoculation density of spores.

The effects of gravity and Ca^{2+} on spore germination

Gravity acting as an important ecological factor is required for the morphogenesis and generative development of plant normal growth and plays key roles in determining the spatial orientation of higher plants (Merkys et al., 1981). The polar development of *Ceratopteris richardii* spores is oriented by gravity. During the germination of *Ceratopteris richardii* spores, before the first mitosis took place, nuclear directional movement determined the asymmetry division of spore and the direction of rhizoid elongation. This period was called "polarity-determination window." Roux and his collaborators (Edwards and Roux, 1998; Chatterjee et al., 2000; Roux et al., 2003) have shown that gravity played a key role in nuclear migration. With the presence of gravity, the rhizoids of 94% spores grew in the direction of gravity. However, under the weightlessness condition, rhizoids exhibited random orientation and the cell nuclei also migrated to random positions (Edwards and Roux, 1998). It has been shown that the influence of light on spore polarity was less than gravity (Edwards and Roux, 1998). About 92% rhizoids grew downward (with respect to gravity) when spores were germinated in bidirectional light along the direction of gravity, or illuminated by uniform light from all directions. When the unilateral light was paralleled to the vector of gravity, 98% rhizoids grew downward. While the number of rhizoids growing downward was reduced to 81% with the light irradiation from the bottom of spores (Edwards and Roux, 1998).

As an essential element for plants, calcium is required for building various cellular structures such as cell wall and membrane, and it also act as a counter-cation for inorganic and organic anions in the vacuole. Cytosolic Ca^{2+} concentration is also an obligate intracellular messenger coordinating responses to numerous developmental cues and environmental challenges (White and Broadley, 2003). Calcium also plays key roles in the gravity response and polarity establishment during fern spore germination (Chatterjee et al., 2000). In *Ceratopteris richardii* spores, calcium current moved along the opposite direction of gravity (from the bottom to the top of the spores) (Chatterjee et al., 2000). Under nifedipine (a calcium-channel blocker) treatment, calcium transportation was disrupted, and the gravity-dependent spore polar development was also interrupted (Chatterjee et al., 2000). A recent study has shown that gravity perception in single-celled spores of the *Ceratopteris richardii* was mediated by mechanosensitive calcium channels and membrane calcium pumps (Salmi et al., 2011). In addition, two alternative models analyzing the role of calcium pumps and channels in the transcellular calcium current have been established. One hypothesis, which is favored, is that membrane calcium pumps and mechanosensitive calcium ion channels are uniformly distributed in the spore plasma membrane and are locally activated to generate the polar calcium current. The other hypothesis suggests that gravity must drive the redistribution of the calcium pumps and channels to the top and bottom of spores for the polar calcium current. Further work is required to identify the calcium channels and pumps involved in gravity-regulated polarity establishment of *Ceratopteris richardii* spores (Salmi et al., 2011). In addition, it has been found that Ca^{2+} concentration had a significant effect on *Dryopteris paleacea* spore germination with the optimal Ca^{2+}concentration of 0.1 mM (Scheuerlein et al., 1989).

The effects of phytohormones on fern spore germination

GA, ABA, JA, and ethylene play regulatory roles in plant growth and development. GA stimulates plant elongation, promotes flowering, and induces seed germination and dormancy release. ABA plays a key role in plant stress response and is also known as the main regulator of seed dormancy maintenance. JA inhibits the germination of non-dormant seeds but stimulates the germination of dormant seeds. JA also inhibits root growth, prevents tuber formation, and involves in the development of flower organs and regulation of senescence. In addition, ethylene is a gaseous hormone that activates fruit maturation, stimulates germination, accelerates senescence, inhibits elongation, promotes horizontal growth, and initiates adventitious roots and programmed cell death. Importantly, phytohormones exhibit extensive cross-talk with each other and with environmental and developmental signaling pathways (Wang and Irving, 2011). For fern spore germination, these hormones exhibited diverse regulation patterns.

GAs and An

The regulation mechanisms of GA on fern spore germination and seed germination are similar. The biosynthesis of endogenous GA in fern spores was suggested to be induced by red light via PHY system (Kagawa and Sugai, 1991). It has been shown that both GA_3 (4×10^{-7} M) and red light could induce the germination of *Lygodium japonicum* spores (Kagawa and Sugai, 1991). For most fern species, GA promoted their spore germination (Fig. 3), including spores of *Pteridium aquilinum* (Zhang and Niu, 1999), *Athyrium*

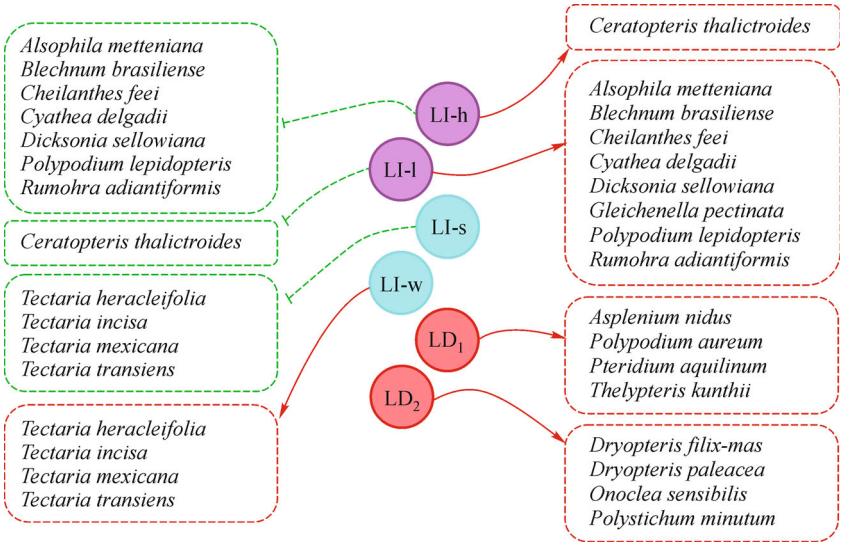

Figure 2 Effects of light intensity and illumination time on fern spore germination. The red solid lines represent 'promotion' and the 'inhibition' is shown in green dashed lines. LD_1: Long time of light irradiation ($\geqslant$24 h); LD_2: Short time irradiation ($<$24 h); LI-h: High light intensity; LI-l: Low light intensity; LI-s: Leaf shade; LI-w: White light.

multidentatum (Guo et al., 2007), *Nephrolepis auriculata* (Li et al., 1995). GA also functions in the spore maturation and dormancy breaking of Cyatheaceae fern species (Jiang et al., 2002). Moreover, the duration of GA treatment has an impact on spore germination. For example, after 12 h and 24 h of GA treatment, the germination rate of *Anemia phyllitidis* spores was 30% and 70%, respectively. Interestingly, different GA molecules have diverse effects on spore germination. The spore germination rate of *Schizaea pusilla* was increased under 0.1 mM GA_3, but not 0.1 mM GA_{4+7} (Guiragossian and Koning, 1986). However, spores of some fern species are not sensitive to GA. For example, GA_3 treatment didn't increase the spore germination rates of *Pteridium aquilinum* var. *latiusculum* (Zhai et al., 2007) and *Sphenomeris chinensis* (Ren et al., 2008).

An, secreted by mature fern gametophyte, is a small molecular and hydrophobic GA-like hormone. An can induce antheridium development (Chiou and Farrar, 1997), which has been suggested to occur in over 50 fern species of six genera (i.e., *Pteridium*, *Pteris*, *Anemia*, *Onoclea*, *Lygodium*, and *Ceratopteris*) (Chiou and Farrar, 1997). Various An purified from various ferns have been termed as Apt, Aps, Aan, Aon, Aly, Ace, and so on. Some of them were found to function in the initiation of spore germination in the dark (Chiou and Farrar, 1997) (Fig. 3). Interestingly, some An secreted by certain fern gametophytes only act on the spores generated from the same fern species. For example, various An from *Lepisorus thunbergianus*, *Phymatosorus scolopendria*, *Polypodium pellucidum*, *Campyloneurum angustifolium*, *Campyloneurum phyllitidis*, *Microgramma heterophylla*, and *Anemia phyllitidis* only induced the spore germination of the corresponding species (Chiou and Farrar, 1997). However, the An generated from *Phlebodium aureum* was able to promote spore germination of *Onoclea sensibilis* apart from its own spores in the dark. While Apt from *Pteridium aquilinum* did not have the species specificity and could induce many other fern spore germination in darkness (Chiou and Farrar, 1997).

ABA and JA

ABA has little effect on fern spore germination. The spore germination rates of *Mohria caffrorum* and *Matteuccia struthiopteris* were almost unaffected under ABA treatment (Fig. 3). Similarly, JA had no influence on the germination and primary rhizoid initiation of *Platycerium bifurcatum* spores (Fig. 3), although it significantly promoted rhizoid elongation and early gametophyte development (Camloh et al., 1996). This suggests that the pre-synthesis of mRNA, protein, and other storages in mature spores is adequate for spore germination, but the new mRNA synthesis stimulated by JA is necessary for the rhizoid elongation and early gametophyte development.

Ethylene

Ethylene can regulate the biosynthesis of RNA and protein, functioning in the plant growth and development (e.g. seed germination, plant mature and aging) (Li and Han, 2000). However, high concentration of ethylene can inhibit fern spore germination by affecting DNA synthesis, nucleus movement, and cell division. Ethylene completely inhibited spore germination of *Onoclea sensibilis* spores in the dark (Fig. 3). 1 μM of ethylene led to the decrease of germination rate of *Onoclea sensibilis* spores to 40%. However, this inhibition could be partly removed by light illumination and

CO_2 treatment. In addition, after absorbed into *Ceratopteris richardii*, (2-chloroethyl) phosphonic acid was degraded to produce ethylene, which promoted spore maturation, but reduced the germination rate of mature spores (Warne and Hickok, 1987) (Fig. 3).

The effects of growth medium and spore density on fern spore germination

In comparison with solid medium, liquid medium was more suitable for the early development of gametophytes of *Platycerium bifurcatum* (Camloh, 1993). The germination percentage was higher for *Anemia phyllitidis* spores in liquid medium (Douglas, 1994). However, it was more suitable for *Pteridium aquilinum* spores to germinate on solid medium than in liquid medium, and the difference was probably caused by the species-specific or ecotype-specific response to liquid medium among different fern species, as well as the interaction between medium composition and spore age (Sheffield et al., 2001). Medium composition is important for fern spore germination and gametophyte development (Table 3). For spores from certain fern species, such as *Schizaea dichotoma* (Cox et al., 2003), *Pyrrosia lingua* (Du et al., 2009b), *Osmunda japonica* (Yuan et al., 2002), and *Alsophila podophylla* (Zhang et al., 2007), contained all the required nutrient for early growth and had high germination rates when germinated in the medium with relative low concentration of mineral nutrient (e.g., 1/8 Knop's, 1/8 MS, 1/4 MS, or 1/10 MS). Similarly, 1/4 MS added with 15 $g \cdot L^{-1}$ sucrose was suitable for *Adiantum reniforme* var. *sinense* spore germination and early gametophyte development (Wu et al., 2010). Spore germination of *Pteris cretica*, *Pteris wallichiana* (Xu et al., 2005; Zhang et al., 2008b), *Pteris tripartita* Sw (Baskaran and Jeyachandran, 2012), and *Dryopteris varia* (Ouyang et al. 2008) were more efficient in the 1/2 MS when compared to that in full strength MS. The optimal media for the germination of *Adiantum raddianum* spores was 1/2 MS plus 3.0 $mg \cdot L^{-1}$ GA, 1.5 $g \cdot L^{-1}$ carbon, 30 $mg \cdot L^{-1}$ sucrose, and 6.5 $g \cdot L^{-1}$ agar (Tian et al., 2008).

Sucrose in the medium provides carbon source and osmolyte for spore germination. The size, coat, and reserve of fern spores among various species are different, which leads to diverse requirements for sucrose in medium. The spores of *Pteris cretica* (Xu et al., 2005), *Pyrrosia lingua*, *Dryopteris varia* (Ouyang et al., 2008; Du et al., 2009b), and *Osmunda japonica* (Yuan et al., 2002) germinated normally in the medium with sucrose content less than 2%. Similarly, the highest germination rate (86%) of *Colysis latiloba* spores was observed at 1% sucrose medium (Parajuli et al., 2013). However, the spore germination rates of some fern species (e.g., *Pteridium aquilinum*, *Athyrium filix-femina*, and *Dryopteris expansa*) cultured with 6.8% sucrose were higher than those with 1.7% sucrose in medium (Sheffield et al., 2001). For *Pteridium aquilinum* var. *latiusculum*, high contents of sucrose, thiamine, pyridoxine, glycine, glutamate, kinetin, and extracts of *Pteridium aquilinum* leaves promoted spore germination and the growth of prothallus (Bao et al., 2000). However, sulphites, oxybarbiturates, and lipophilic solvents inhibited spore germination of *Lygodium japonicum, Pteridium aquilinum*, *Anemia phyllitidis*, and *Onoclea sensibilis* (Bannon et al., 1991; Sahi and Singh, 1994). In addition, adding a certain does of *Eupatorium adenophorum* extracts (5% to 50%) into the culture media would result in a decrease of spore germination of *Macrothelypteris torresiana, Cibotium barometz, and Neocheiropteris palmatopedata* (Zhang et al., 2008a, 2008c, 2012).

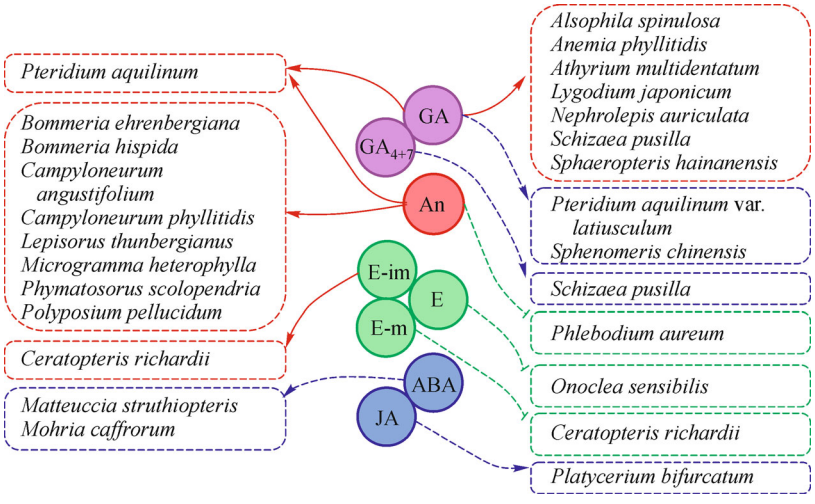

Figure 3 Effects of phytohormones on fern spore germination. The red solid lines represent 'promotion' and the 'inhibition' is shown in green dashed lines, whereas the blue dashed lines indicate no influence on the fern spore germination of current environmental factor. ABA: Abscisic acid; An: Antheridiogen; E: Ethylene ($\geqslant 1 \mu L \cdot L^{-1}$); E-im: The treatment of ethylene on the immature fern spores; E-m: The treatment of ethylene on the mature spore; GA: Gibberellin acid; GA_{4+7}: A kind of gibberellin acid; JA: Jasmonic acid.

Metal ions (e.g., Ca^{2+}, Mn^{2+}, and Mg^{2+}) in the medium function as the signaling molecules or chelating ions of enzymes (e.g., phosphatase, ATP synthase, and nucleotidase) during fern spore germination, which is essential for cell polarity and enzyme activity. It has been proved that these ions were necessary for the spore germination of *Onoclea sensibilis* and *Dryopteris paleacea* (Scheuerlein et al., 1989). However, Cd^{2+} and La^{3+} interfered with the activities of Ca^{2+} and other ions, leading to the inhibition of spore germination of *Ceratopteris thalictroides* (Gupta et al., 1992) and *Dryopteris paleacea* (Scheuerlein et al., 1989).

The pH of medium is also important for the germination of fern spores. The optimum pH value is varied from different fern species. Spores from *Drynaria fortunei* (Chang et al., 2007) and *Cheilanthes feei* (Nondorf et al., 2003) germinated on the medium with a relative wide range of pH from 3.7 to 9.7 and 4.5 to 8.5, respectively. However, some fern species only germinate in acidic or alkaline media. For example, the optimum pH for *Alsophila metteniana* spore germination was 4.7 (Du et al., 2009a). Acidic pH also promoted a relative higher germination rates of *Cyathea atrovirens* (pH 4.0 to 6.5), *Polypodium lepidopteris* Kunze (pH 4.0 to 6.7), *Ophioglossum palmatum* L. (Ophioglossaceae) (pH 4.0), and *Gleichenella pectinata* (pH 4.5 to 5.0) spores (Whittier and Moyroud, 1993; Viviani and Randi, 2008; Rechenmacher et al., 2010; Santos et al., 2010). *Anemia mexicana* and *Anemia phyllitidis* (Schizaeaceae) also germinated well at pH 5.0 to 6.5, but the germinability was very low in strong acidic conditions ($pH \leqslant 4.5$) (Nester and Coolbaugh, 1986). However, *Marsilea vestita* spore germinated in alkaline condition (pH 7 to 8).

In addition, the spore density affects the utilization of nutrient in medium for spore germination and gametophyte growth (Song et al., 2009) (Fig. 4). A high spore density would inhibit spore germination because of the competition for nutrient, physical impediment, biochemical inhibitors secreted by other spores, and a low density also causes a limitation of germination rate due to the individual biochemical basis of spores (Ashcroft and Sheffield, 2000).This suggests that an optimum spore density is crucial for fern spore germination. For example, 3000 spores/cm^2 was the optimal inoculation density for *Osmunda japonica* spores germination (Yuan et al., 2002). *Ceratopteris thalictroides* spores germinated completely with the inoculation density of 50 spores/mL medium (Guo et al., 2010). The germination rate of *Pteridium aquilinum* spores was 43% to 52% with the intermediate density ranging from 187 to 2114 spores/mm^2, and the highest germination rate (52%) occurred at 360 spores/mm^2 (Ashcroft and Sheffield, 2000).

The effects of temperature and CO_2 on fern spore germination

Temperature has a considerable influence throughout the plants development, such as plant morphology, flowering time, seed dormancy release, germination and vernalization. There is a considerable interaction between temperature and PHY-dependent light signaling pathways (Franklin, 2009). For fern spore germination, temperature also plays an important role through a similar regulation mechanism. Temperature affects the cell membrane permeability and enzyme activity during spore germination. High temperature seemed to affect the activity of PHY thus to regulate fern spore germination (Heschel et al., 2007). Some fern species can adapt to a wide range of temperature, and the optimum germination temperature is 25°C (Fig. 4), such as *Microgramma lindbergii*, *Microgramma squamulosa*, *Polypodium pleopeltifolium*, *Polypodium latipes, Adiantopsis radiata* (Ranal, 1999), *Argyrochosma formosa*, *Thelypteris glandulosa*, *Thelypteris serrata*, *Blechnum appendiculatum*, and *Dennstaedtia globulifera* (Juárez-Orozco et al., 2013). Similarly, spores of *Marsilea vestita*, *Woodwardia radicans* (Quintanilla et al., 2000), *Cheilanthes feei* (Nondorf et al., 2003), *Rumohra adiantiformis* (Brum and Randi, 2002), *Asplenium ruta-muraria* (Pangua et al., 1994), *Polypodium lepidopteris* (Viviani and Randi 2008), *Gleichenella pectinata* (Santos et al., 2010), *Plagiogyria pectinata*, *Polystichum mickelii*, and *Hemionitis subcordata* (Juárez-Orozco et al., 2013), as well as species of *Tectaria* (*T. heracleifolia*, *T. incisa*, *T. mexicana*, and *T. transiens*) reached the highest germination rates at 25 °C. While spores of *Culcita macrocarpa* (Quintanilla et al., 2000) and *Microsorium pteropus* (Xue et al., 2008) cultured at 20°C had an approximately 85% germination rate (Fig. 4). In addition, for the spores of fern *Sphenomeris chinensis* (Ren et al., 2008), a higher temperature (28°C) was better for germination (Fig. 4). Similarly, the spore germination rate of *Alsophila metteniana* was less than 46% when the temperature was lower than 15°C, and a higher germination rate (about 70%) was obtained under 20°C to 30°C (Du et al., 2009a). The maximum spore germination rates of *Onoclea sensibilis, Polypodium hirsutissimum*, and *Pteris denticulata* were observed at about 30°C (Ranal, 1999).

In addition, suitable concentration of CO_2 was necessary for *Pyrrosia piloselloides* spore germination (Fig. 4). Under 0.02% to 0.34% CO_2, spores had maximal germination rates (Ong et al., 1998). However, high CO_2 concentration inhibited *Onoclea sensibilis* spore germination (Fig. 4). When the CO_2 concentration increased from 2% to 10%, the spore germination rate of *Onoclea sensibilis* reduced from 95% to 25%.

Conclusions

Fern spore germination is a fine-tuned cellular process regulated by various complicated genes/proteins interactive networks. Meanwhile, the complex germination processes are also sensitive to various environmental factors (e.g., light,

Table 3 Effect of medium, sucrose and other environmental factors on the germination of fern spores

Environmental factors	Plant spices	Treatment condition (concentration, time)	Effects on spore germination (germination rate or time)	References
Mineral content in medium and type of Medium	*Adiantum raddianum*	Knop's or MS or 1/2 MS	42% or 32% or 48%	Tian et al., 2008
		Knop's, activated charcoal 0–1.5 $g \cdot L^{-1}$	42%–80%	
		Knop's, GA 0–3 $mg \cdot L^{-1}$	42%–80%	
	Adiantum reniforme var. *sinense*	1/4 MS, sucrose 0–60 $g \cdot L^{-1}$	Maximum germination 61.2%±16.2%	Wu et al., 2010
		1/2 MS, sucrose 0–60 $g \cdot L^{-1}$	Maximum germination 41.5%±7.9%	
		MS, sucrose 0–60 $g \cdot L^{-1}$	Maximum germination 29.8%±6.6%	
	Alsophila podophylla	1/2 MS or MS	60.2% or 58.7%	Zhang et al., 2007
		1/10 MS or 1/5 MS	67.5% or 66.9%	
	Anemia phyllitidis	Liquid medium	P	Douglas, 1994
	Dryopteris varia	Ms or 1/2 MS or 1/4 MS	33% or 38% or < 5%	Ouyang et al., 2008
	Osmunda japonica	1/2 MS or MS	50.3% or 80.2%	Yuan et al., 2002
		1/8 MS	95.3%	
	Platycerium bifurcatum	Solid media, 3 d or 6 d	53.5%±5.5% or 67.6%±6.0%	Camloh, 1993
		Liquid media, 3 d or 6 d	50.3%±3.6% or 68.8%±1.4%	
	Pteridium aquilinum	Solid media, 14 d	85.2%	Sheffield et al., 2001
		Liquid media, 14 d	60.0%	
	Pteris cretica	Knop's or MS	80.6% or 77.2%	Xu et al., 2005
		1/2 MS	82.3%	
		1/5 MS or 1/10 MS	64.7% or 62.5%	
	Pteris tripartita Sw	MS	70.66%±1.45%	Baskaran et al., 2012
		1/2 MS	80.00%±1.15%	
		1/4 MS or 1/8 MS	59.00%±1.15% or 56.00%±2.51%	
	Pteris wallichiana	MS	78.2%±2.1%	Zhang et al., 2008c
		1/2 MS	83.3%±2.3%	
		1/5 MS or 1/10 MS	66.7%±2.5% or 62.9%±0.9%	
	Pyrrosia lingua	MS	59.5%/9 d	Du et al., 2009b
		1/2 MS or 1/4 MS	82.3%/7d or 82.7%/7d	
		1/8 MS or Knop's	83.2%/6d or 84.1%/6 d	
	Schizaea dichotoma	1/4 MS	34%	Cox et al., 2003
		1/2 MS	12%	

(*Continued*)

Environmental factors	Plant spices	Treatment condition (concentration, time)	Effects on spore germination (germination rate or time)	References
	Athyrium filix-femina	Liquid medium, sucrose 0 M	87.2%	Sheffield et al., 2001
		Liquid medium, sucrose 0.05 M or 0.2 M	91.2% or 94.7%	
	Cibotium barometz	leachates of *Eupatorium adenophorum* (0%, 5%, 10%, 20%, 30%, 40%, 50%)	98% to 18%	Zhang et al., 2008b
	Colysis latiloba	0% Sucrose	15%	Parajuli et al., 2013
		1% Sucrose	86%	
		2%–5% Sucrose	75%–54%	
	Dryopteris expansa	Liquid medium, sucrose 0 M	57.8%	Sheffield et al., 2001
		Liquid medium, sucrose 0.05 M or 0.2 M	61.1% or 66.0%	
	Dryopteris varia	Sucrose 0% or 1% or 2%	37% or 39% or 38%	Ouyang et al., 2008
		Sucrose 3% or 4% or 5%	$<5\%$ or $<1\%$	
	Macrothelypteris torresiana	leachates of *Ageratina adenophora* (0%, 5%, 10%, 20%, 30%, 40%, 50%)	nearly 100% to 0%	Zhang et al., 2008a
	Neocheiropteris palmatopedata	leachates of *Ageratina adenophora* (0%, 5%, 10%, 20%, 30%, 40%, 50%)	nearly 100% to 0%	Zhang et al., 2012
Sucrose and other contents in culture medium	*Osmunda japonica*	1/8 MS sucrose 0%	34.8%	Yuan et al., 2002
		1/8 MS, sucrose 2% or 3%	90.2% or 95.1%	
		1/8 MS, sucrose 1% or 4% or 5%	80.1% or 83.3% or 64.5%	
	Pteridium aquilinum	Liquid medium, sucrose 0 M	30.4%	Sheffield et al., 2001
		Liquid medium, sucrose 0.05 M or 0.2 M	39.3% or 43.3%	
	Pteridium aquilinum var. *latiusculum*	Sucrose 50000 $mg \cdot L^{-1}$, Vitamin	8.0%	Bao et al., 2000
		Sucrose 50000 $mg \cdot L^{-1}$, Amino acid	9.8%	
		Sucrose 50000 $mg \cdot L^{-1}$, extract of *Pteridium aquilinum* leaves	20%	
		Sucrose 50000 $mg \cdot L^{-1}$, IAA, KN	6.8%	
		Sucrose 50000 $mg \cdot L^{-1}$, (did not add any growth-assisted substance)	6.7%	
	Pteris cretica	Sucrose 0% or 1% or 2%	82.3% or 83.0% or 82.6%	Xu et al., 2005
		Sucrose 3% or 4% or 5%	60.5% or 54.7% or 50.1%	
	Pyrrosia lingua	Sucrose 0% or 1% or 2%	82.4% or 81.3% or 80.6%	Du et al., 2009b
		Sucrose 3% or 4% or 5%	62.5% or 68.2% or 53.6%	

(*Continued*)

Environmental factors	Plant spices	Treatment condition (concentration, time)	Effects on spore germination (germination rate or time)	References
	Alsophila metteniana	3.7 or 5.7 or 6.7	65.6%–40%	Du et al., 2009a
		4.7	65.6%	
		⩾7.7	0%	
	Anemia mexicana	pH 5.0–6.5	Maximum germination	Nester and Coolbaugh, 1986
		pH⩽4.5	I	
	Anemia phyllitidis	pH 5.0–6.5	Maximum germination	
		pH⩽4.5	I	
	Cheilanthes feei	4.5 or 5.5 or 6.5 or 8.5	Nearly 95% or 75% or 55% or 50%	Nondorf et al., 2003
pH	*Cyathea atrovirens*	pH 4.0–6.5, 6 d–12 d	78%–93%	Rechenmacher et al., 2010
		pH 7.0, 6 d–12 d	40%–80%	
	Drynaria fortunei	7.7	63.3%	Chang et al., 2007
		9.7	0.8%	
		3.7	1%	
	Gleichenella pectinata	pH 4.0	0.8%±0.4%	Santos et al., 2010
		pH 4.5–5.0	1.2%±0.8%–2.2%±0.8%	
		pH 5.5–6.0	0.8%±0.4%–0.5%±0.5%	
	Marsilea vestita	pH 7 to 8	78%	Mahlberg and Yarus, 1977
	Ophioglossum palmatum	pH 3.4	14%	Whittier and Moyroud, 1993
		pH 4.0	55%	
		pH 4.6–6.2	48%–0%	
	Polypodium lepidopteris	pH 4.0–6.7	32%–38%	Viviani and Randi, 2008
Cd^{2+}	*Ceratopteris thalictroides*	0.1 $mg \cdot L^{-1}$ or 1.0 $mg \cdot L^{-1}$	N	Gupta et al., 1992
		2.5 $mg \cdot L^{-1}$or 5.0 $mg \cdot L^{-1}$	14% or 36%	
La^{3+}	*Dryopteris paleacea*	10^{-2} M or 10^{-4} M or 10^{-6} M or 10^{-8} M, R 1 min	2.0%±0.7% or 46.4%±2.2% or 58.0%±2.6% or 60.0%±2.4%	Scheuerlein et al., 1989
		0 M, R 1 min	60.2%±1.0%	
Ca^{2+}	*Dryopteris paleacea*	⩽10^{-7} M	I (5%)	Scheuerlein et al., 1989
		10^{-4} M	Maximum germination	
		⩾10^{-1} M	I	

Abbreviation: I, inhibited; N, no effect; P, promoted.

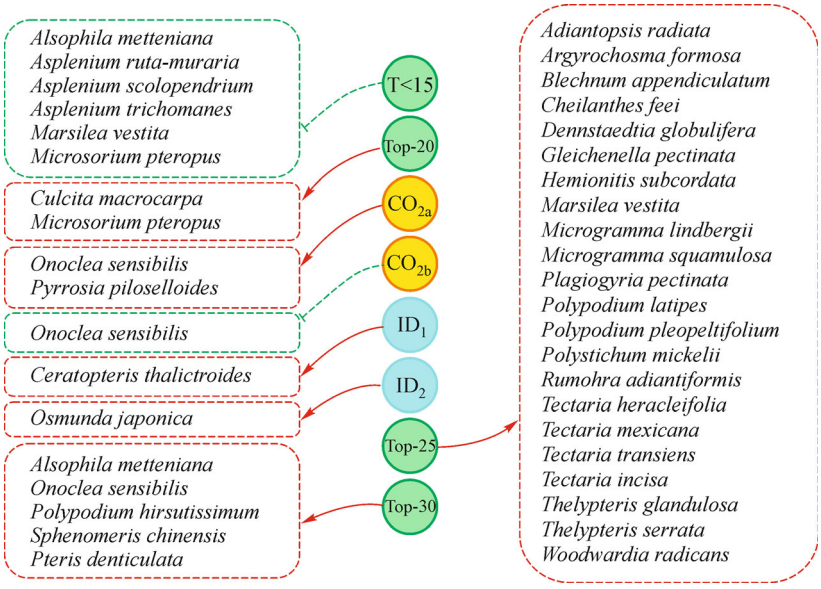

Figure 4 Effects of temperature and the other environmental factors on fern spore germination. The red solid lines represent 'promotion' and the 'inhibition' is shown in green dashed lines. CO_{2a}: CO_2 treatment concentration for the spores of *P. piloselloides* (219–3360 $cm^3 \cdot m^{-3}$) and *O. sensibilis* (0–2%); CO_{2b}: CO_2 treatment concentration for *O. sensibilis* spores (5%–15%); ID_1: Inoculation density, 50 spores$\cdot mL^{-1}$; ID_2: Inoculation density, 3000 spores$\cdot cm^{-2}$; T < 15: the optimal germination temperature less than 15 °C; Top-20: The maximal germination rate occurred at 20 °C; Top-25: The optimal germination temperature is 25 °C; Top-30: The maximal germination rate occurred at 30 °C.

gravity, Ca^{2+}, hormones, and temperature). Most of the aforementioned environment-responsive mechanisms in spore germination are deduced from physiologic and ecological investigations. The unrelated nature of diverse methods/conditions used to generate these data makes it very difficult to draw a broad conclusion across the evolutionarily divergent clade of ferns. In this review, we just give a simple catalog of existing information, which would be a valuable contribution for future studies on ferns. To date, although the molecular and metabolic mechanisms during fern spore germination are still lacking, the update of ESTs and cDNA database for *Ceratopteris*, as well as the refreshed technologies for plant genome sequencing and transcriptomic/proteomic analysis, provide a well opportunity to investigate the fern spore germination in response to environment factors. Using these modern molecular, biologic and "Omic" approaches, further investigations of the Ca^{2+} signaling, phytohormone regulation, light/gravity response upon fern spore germination would be novel hot topics in the future.

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Compliance with ethics guidelines

Jinwei Suo, Sixue Chen, Qi Zhao, Lei Shi, and Shaojun Dai declare that they have no conflict of interest. This manuscript is a review article and does not contain any studies with human or animal subjects performed by any of the authors.

References

Ashcroft C J, Sheffield E (2000). The effect of spore density on germination and development in *Pteridium*, monitored using a novel culture technique. Am Fern J, 90(3): 91–99

Bannon M E, Kordan H A, Sheffield E (1991). Effects of oxybarbiturates on fern spore germination and gametophyte development. Atla-Altern Lab Anim, 19(3): 308–315

Bao M, Wu X M, Ding L (2000). Effect of sucrose and the growth of auxiliary substances on spore artificial propagation of *Pteridium aquilinum*. J Qinghai Norm Univ, 3: 39–43 (Nat Sci Ed)

Baskaran X, Jeyachandran R (2012). *In vitro* spore germination and gametophyte growth assessment of a critically endangered fern: *Pteris tripartita* Sw. Pteridol Res, 1(1): 4–9

Brum F R, Randi A M (2002). High irradiance and temperature inhibit the germination of spores of the fern *Rumohra adiantiformis* (Forst.) Ching (Dryopteridaceae). Braz J Bot, 25(4): 391–396

Camloh M (1993). Spore germination and early gametophyte development of *Platycerium bifurcatum*. Am Fern J, 83(3): 79–85

Camloh M, Ravnikar M, Zel J (1996). Jasmonic acid promotes division of fern protoplasts, elongation of rhizoids and early development of gametophytes. Physiol Plant, 97(4): 659–664

Chang H C, Agrawal D C, Kuo C L, Wen J L, Chen C C, Tsay H S (2007). In vitro culture of *Drynaria fortunei*, a fern species source of Chinese medicine "Gu-Sui-Bu". In Vitro Cell Dev-Pl, 43(2): 133–139

Chatterjee A, Porterfield D M, Smith P S, Roux S J (2000). Gravity-directed calcium current in germinating spores of *Ceratopteris richardii*. Planta, 210(4): 607–610

Chiou W L, Farrar D R (1997). Antheridiogen production and response in *Polypodiaceae* species. Am J Bot, 84(5): 633–640

Cox J, Bhatia P, Ashwath N (2003). In vitro spore germination of the fern *Schizaea dichotoma*. Sci Hortic (Amsterdam), 97(3–4): 369–378

Dai S J, Gao J, Mu H F, Song Y Y (2008). Comparison of germination between fern spores and spermatophyte pollen. Chin Bull Bot, 25(2): 139–148

Dai S J, Li L, Chen T T, Chong K, Xue Y B, Wang T (2006). Proteomic analysis of *Oryza sativa* mature pollen reveal novel proteins associated with pollen germination and tube growth. Proteomics, 6 (8): 2504–2529

Douglas G E (1994). An investigation into the growth, development and ultrastructure of fern gametophytes in existing and novel culture systems. PhD Thesis, University of Manchester, UK

Du H H, Li Y, Li D, Dai S J, Jiang C D, Shi L (2009a). Effects of light, temperature and pH on spore germination and early gametophytic development of *Alsophila metteniana*. Biodiv Sci, 17(2): 182–187

Du J Z, Li L H, Li Y Q, Yin X H, Huang R S, Chen R (2009b). Effects of different factors on the germination and growth of *Pyrrosia lingua* spores. Guangxi Agric Sci, 40: 120–123

Edwards E S, Roux S J (1998). Influence of gravity and light on the developmental polarity of *Ceratopteris richardii* fern spores. Planta, 205(4): 553–560

Franklin K A (2009). Light and temperature signal crosstalk in plant development. Curr Opin Plant Biol, 12(1): 63–68

Furuya M, Kanno M, Okamoto H, Fukuda S, Wada M (1997). Control of mitosis by phytochrome and a blue-light receptor in fern spores. Plant Physiol, 113(3): 677–683

Guiragossian H A, Koning R E (1986). Induction of spore germination in *Schizaea pusilla* (Schizaeaceae). Am J Bot, 73(11): 1588–1594

Guo L L, Chen W B, Jiang N, Li X G, Dai X L (2010). The effect of culture density on spore germination and sex differentiation of *Ceratopteris thalictroides* (L.) Brongn. J Shanghai Norm Univ, 39 (2): 210–212 (Nat Sci)

Guo Q X, Shen Y X, Song X H, Zhao H T (2007). The effects of spores germination and planting rate of *Athyrium multidentatum*. Chin Agric Sci Bull, 23: 343–345

Gupta M, Devi S, Singh J (1992). Effects of long-term low-dose exposure to cadmium during the entire life cycle of *Ceratopteris thalictroides,* a water fern. Arch Environ Contam Toxicol, 23(2): 184–189

Hayami J, Kadota A, Wada M (1992). Intracellular dichroic orientation of the blue light-absorbing pigment and the blue-absorption band of the red-absorbing form of phytochrome responsible for phototropism of the fern *Adiantum* protonemata. Photochem Photobiol, 56(5): 661–666

Heschel M S, Selby J, Butler C, Whitelam G C, Sharrock R A, Donohue K (2007). A new role for phytochromes in temperature-dependent germination. New Phytol, 174(4): 735–741

Hiendlmeyer R, Randi A M (2007). Response of spores and young gametophytes of *Cyathea delgadii* Sternb. (Cyatheaceae) and *Blechnum brasiliense* Desv. (Blechnaceae) to different light levels. Acta Bot Bras, 21(4): 909–915

Huckaby C S, Raghavan V (1981). Photocontrol of spore germination in the fern *Thelypteris kunthii*. Physiol Plant, 51(1): 19–22

Imaizumi T, Kanegae T, Wada M (2000). Cryptochrome nucleocytoplasmic distribution and gene expression are regulated by light quality in the fern *Adiantum capillus-veneris*. Plant Cell, 12(1): 81–95

Jiang S J, Zeng X, Wang S P, Zhuang N S (2002). Study of tissue culture of *Sphaeropteris hainanensis*. Trop Agric Sci, 22: 9–12

Juárez-Orozco S, Orozco-Segovia A, Mendoza-Ruiz A, Pérez-García B (2013). Spore germination of eight homosporous ferns in a temperature gradient. S Afr J Bot, 87: 112–117

Kagawa T, Sugai M (1991). Involvement of gibberellic acid in phytochrome-mediated spore germination of the fern *Lygodium japonicum*. J Plant Physiol, 138(3): 299–303

Kami C, Lorrain S, Hornitschek P, Fankhauser C (2010). Chapter two-light-regulated plant growth and development. Curr Top Dev Biol, 91: 29–66

Kanegae T, Wada M (1998). Isolation and characterization of homologues of plant blue-light photoreceptor (cryptochrome) genes from the fern *Adiantum capillus-veneris*. Mol Gen Genet, 259(4): 345–353

Li J L, Yuan Y B, Cao Z X (1995). View of the cytology and biochemistry of sexual reproduction of algae and pteridophyta. Chin Bull Bot, 12(2): 1–8

Li M L, Han Y F (2000). Effect of ethylene on the growth and development of plants and inhibition of its biosynthesis by antisense RNA. Scient Silv Sin, 36(4): 77–84

Merkys A J, Laurinavichius R S, Rupainene O Y, Shvegzhdene D V, Yaroshius A V (1981). Gravity as an obligatory factor in normal higher plant growth and development. Adv Space Res, 1(14): 109–116

Nester J E, Coolbaugh R C (1986). Factors influencing spore germination and early gametophyte development in *Anemia mexicana* and *Anemia phyllitidis*. Plant Physiol, 82(1): 230–235

Niu J Y, Li S, Xu Z M, Qin S H, Lian R F (2002). Effect of illumination and exogenous substance on spore germination and seedling survival of *Pteridium aquilinum*. Acta Hortic Sin, 29(6): 584–586

Nondorf S L, Dooley M A, Palmieri M, Swatzell L J (2003). The effects of pH, temperature, light intensity, light quality, and moisture levels on spore germination in *Cheilanthes feei* of southeast Missouri. Am Fern J, 93(2): 56–69

Nozue K, Fukuda S, Kanegae T, Wada M (1998a). Isolation of a second phytochrome cDNA from *Adiantum capillus-veneris*. Plant Physiol, 118: 712

Nozue K, Kanegae T, Imaizumi T, Fukuda S, Okamoto H, Yeh K C, Lagarias J C, Wada M (1998b). A phytochrome from the fern *Adiantum* with features of the putative photoreceptor NPH1. Proc Natl Acad Sci USA, 95(26): 15826–15830

Nozue K, Kanegae T, Wada M (1997). A full length Ty3/gypsy-type retrotransposon in the fern *Adiantum*. J Plant Res, 110(4): 495–499

Ong B L, Koh C K K, Wee Y C (1998). Effects of CO_2 on growth and photosynthesis of *Pyrrosia piloselloides* (L.) Price gametophytes. Photosynthetica, 35(1): 21–27

Ouyang C J, Tang Y J, Wang R J (2008). Spore culture and gametophyte development of *Dryopteris varia* (L.) Ktunze. J Trop Subtrop Bot, 16 (4): 344–349

Pangua E, Lindsay S, Dyer A (1994). Spore germination and gametophyte development in three species of *Asplenium*. Ann Bot

(Lond), 73(6): 587–593

Parajuli J, Joshi S D, Nepal W W F (2013). *In vitro* morphogenesis of colysis latiloba (Ching.). Ching. Magnesium Res, 4: 1–6

Pérez-García B, Mendoza-Ruiz A, Sánchez-Coronado M E, Orozco-Segovia A (2007). Effect of light and temperature on germination of spores of four tropical fern species. Acta Oecol, 32(2): 172–179

Pérez-García B, Orozco-Segovia A, Riba R (1994). The effects of white fluorescent light, far-red light, darkness, and moisture on spore germination of *Lygodium heterodoxum* (Schizaeaceae). Am J Bot, 81 (11): 1367–1369

Quintanilla L G, Pajaron S, Pangua E, Amigo J (2000). Effect of temperature on germination in northernmost populations of *Culcita macrocarpa* and *Woodwardia radicans*. Plant Biol, 2(6): 612–617

Raghavan V (1992). Germination of fern spores. Am Sci, 80: 176–185

Ranal M A (1999). Effects of temperature on spore germination in some fern species from semideciduous mesophytic forest. Am Fern J, 89 (2): 149–158

Rechenmacher C, Schmitt J L, Droste A (2010). Spore germination and gametophyte development of *Cyathea atrovirens* (Langsd. & Fisch.) Domin (Cyatheaceae) under different pH conditions. Braz J Biol, 70 (4): 1155–1160

Ren B R, Xia B, Li W L, Wu J L, Zhao Y Y (2008). Investigation on spore germination of *Sphenomeris chinensis* (Lindsaeaceae). Acta Bot Yunnanica, 30(6): 713–717

Renner G D R, Randi A M (2004). Effects of sucrose and irradiance on germination and early gametophyte growth of the endangered tree fern *Dicksonia sellowiana* Hook (Dicksoniaceae). Acta Bot Bras, 18 (2): 375–380

Reynolds T L, Raghavan V (1982). Photoinduction of spore germination in a fern, *Mohria caffrorum*. Ann Bot (Lond), 49: 227–233

Roux S J, Chatterjee A, Hillier S, Cannon T (2003). Early development of fern gametophytes in microgravity. Adv Space Res, 31(1): 215–220

Sahi A N, Singh S K (1994). Effect of sulphite on spore germination and rhizoid development in the tropical fern *Lygodium japonicum* (Filicales: Lygodiaceae). Rev Biol Trop, 42(1–2): 53–57

Salmi M L, ul Haque A, Bushart T J, Stout S C, Roux S J, Porterfield D M (2011). Changes in gravity rapidly alter the magnitude and direction of a cellular calcium current. Planta, 233(5): 911–920

Santos E P G D, Lehmann D R M, Santos M, Randi A M (2010). Spore germination of *Gleichenella pectinata* (Willd.) Ching (Polypodiopsida-Gleicheniaceae) at different temperatures, levels of light and pH. Braz Arch Biol Techn, 53(6): 1309–1318

Scheuerlein R, Wayne R, Roux S J (1989). Calcium requirement of phytochrome-mediated fern-spore germination: No direct phytochrome-calcium interaction in the phytochrome-initiated transduction chain. Planta, 178(1): 25–30

Sheffield E, Douglas G E, Hearne S J, Huxham S, Wynn J M (2001). Enhancement of fern spore germination and gametophyte growth in artificial media. Am Fern J, 91(4): 179–186

Song Y Y, Gao J, Dai S J (2009). Sex differentiation in ferns response to environmental factors. Acta Ecol Sin, 29(9): 5030–5038

Suetsugu N, Wada M (2003). Cryptogam blue-light photoreceptors. Curr Opin Plant Biol, 6(1): 91–96

Tian X Y, Liu Y J, Wang S (2008). The fast establishment of micropropagation system on *Adiantum raddianum* using spore as explants. J Food Sci Biotech, 27(4): 103–106

Tsuboi H, Nakamura S, Schäfer E, Wada M (2012). Red Light-induced phytochrome relocation into the nucleus in *Adiantum capillus-veneris*. Mol Plant, 5(3): 611–618

Viviani D, Randi A M (2008). Effects of pH, temperature and light intensity on spore germination and growth analysis of young sporophytes of *Polypodium lepidopteris* (Pteridophyta, Polypodiaceae). Rodriguésia, 59(4): 751–761

Wang Y H, Irving H R (2011). Developing a model of plant hormone interactions. Plant Signal Behav, 6(4): 494–500

Warne T R, Hickok L G (1987). (2-Chloroethyl) phosphonic acid promotes germination of immature spores of *Ceratopteris richardii* Brongn. Plant Physiol, 83(4): 723–725

White P J, Broadley M R (2003). Calcium in plants. Ann Bot (Lond), 92 (4): 487–511

Whittier D P (2006). Red light inhibition of spore germination in *Ophioglossum crotalophoroides*. Am Fern J, 84(7): 1156–1158

Whittier D P (2008). Red light inhibition of spore germination in *Lycopodium clavatum*. Am Fern J, 98(4): 194–198

Whittier D P, Braggins J E (1994). Spore germination in the Psilotaceae. Can J Bot, 72(5): 688–692

Whittier D P, Moyroud R (1993). The promotion of spore germination and gametophyte development in *Ophioglossum palmatum* by low pH. Am Fern J, 83(2): 41–46

Wu H, Liu X Q, Ji H, Chen L Q (2010). Effects of light, macronutrients, and sucrose on germination and development of the endangered fern *Adiantum reniforme* var. *sinense* (Adiantaceae). Sci Hortic (Amsterdam), 125(3): 417–421

Xu Y, Shi L, Liu Y, Li D (2005). Studies on spore propagation of *Pteris cretica* 'Albo-lineata'. Acta Hortic Sin, 32(4): 658–662

Xue C L, Wang W J, Gao Y H (2008). Artificial culture of spore of *Microsorium pteropus*. Yunnan Agric Sci Tech, 5: 16–17

Yuan Y, Tian S N, Ye A H, Lu P L (2002). Studies on the rapid propagate of the *Osmunda japonica* Thund. Acta Hortic Sin, 29(3): 247–250

Zhai G Y, Bian K, Jia K G, Zhu L X (2007). Effect of GA_3 and MS medium ratio treatments on spore germination of wild Brake. China Veget, 8: 21–23

Zhang J W, Niu J Y (1999). The effects of culture ground substances, GA_3 and B, on spores germination and planting rate of *Pteridium aquilinum*. Acta Pratac Sin, 8(1): 62–68

Zhang K M, Liu J H, Cheng X, Zhang G F, Fang Y M, Zhang H J (2012). Effects of *Ageratina adenophora* on spore germination and gametophyte development of *Neocheiropteris palmatopedata*. Am Fern J, 102(3): 208–215

Zhang K M, Shi L, Jiang C D, Li Z Y (2008a). Inhibition of Ageratina adenophora on spore germination and gametophyte development of *Macrothelypteris torresiana*. J Integr Plant Biol, 50(5): 559–564

Zhang K M, Shi L, Jiang C D, Li Z Y (2008b). Allelopathic effects of *Eupatorium adenophorum* on spore germination and gametophyte development in *Cibotium barometz*. Acta Pratac Sin, 17(2): 19–25

Zhang K M, Shi L, Li D, Zhang X C (2008c). Development process and spore sterile culture of *Pteris wallichiana* Agardh. Acta Hortic Sin, 35(1): 94–98

Zhang Y L, Li Y, Ji M C, Li D, Shi L (2007). Spore sterile culture in *Alsophila podophylla* Hook. Plant Physiol Comm, 43(6): 1139–1140