RESEARCH ARTICLE

SEASONAL PROFILE OF SOIL SPORE BANK OF FERNS IN A SEMI-NATURAL FOREST OF HOOGHLY DISTRICT, WEST BENGAL, INDIA AND ITS IMPLICATION IN CONSERVATION

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Received-30.12.2015, Revised-11.01.2016

Abstract: The vertical structures of live and total fern spore banks were studied during summer, rainy, and winter seasons in a semi-natural forest situated at Mankundu region (22.885877, 88.391903 and 22.848333, 88.342603) of Hooghly District, West Bengal, India. A reservoir of vertically distributed live fern spore bank (LFSB) is established in the region. However, not all the spores present in soil samples could retain their viability for germination to establish gametophytic generation and subsequently sporophyte formation. The best reservoirs are 0-5 cm soil depth in summer and rainy seasons; while, 5-10 cm in winter. The sporophytic plants developed from gametophytes through *in vitro* soil culturing have adapted successfully in natural environment, and fulfilled the objective for establishing fern conservation through natural soil spore bank study.

Keywords: Mankundu, Spore germination, Prothallial development, Sporophytic generation, *Ex situ* conservation

INTRODUCTION

Natural spore bank of ferns is a biotic component having potentiality for *in situ* conservation and regeneration processes of the fern community (Dyer 1992, 1994; Dyer and Lindsay 1996; Simabukuro *et al.* 1998, 1999; Ranal 2003; Ramirez-Trezo *et al.* 2004). The bank can be enriched each year by adding new spores in the soil or can also be worn out by spore depletion from the soil due to predation, loss of spore viability, anthropogenic activities, among others. The major advantages of such conservation technique are easy way for soil collection throughout the year and cost effective culturing method in suitable growing conditions. The collected soil samples can be stored for several years and used later on for raising sporophytic plants through germination of viable spores present in the soil. Besides conservation aspects, the soil spore bank has significant role in the natural life cycle of a fern by maximizing the scope for spore germination time and minimizing the risk for extinction of population, if any. Even it can enhance or modify the fern breeding system as a soil spore bank has chance to contain more than one type of spores mostly. The objective of the work is to establish the natural soil spore bank of ferns in West Bengal. Such endeavour is vital for fern conservation in India (Gupta *et al.* 2014) and from available literatures it seems that such reports are lacking. Present investigation is an attempt to explore soil spore bank of ferns seasonally in a semi-natural forest of Hooghly district of West Bengal, India.

MATERIAL AND METHODS

Sampling

Sampling was carried out in two sites (I and II) of a semi-natural forest situated at Mankundu region (22.885877, 88.391903 and 22.848333, 88.342603) of Hooghly District, West Bengal, India (Fig. 1a). Sampling was done in three seasons namely summer, rainy, and winter by soil coring method up to the depth of 25 cm from surface at regular interval of 5 cm. Physical and chemical analyses of soil revealed sandy soil with pH ranging from 6.82 to 8.15 and per cent of total organic matter (OM) from 2.76 to 4.82. Three replicates from each sample were made to obtain result up to the level of specificity. Precautions were taken to minimize contamination of the soil by airborne spores at all the stages. The soil collected from each depth was analyzed in two ways for getting total fern spore count (TFSC) and live fern spore count (LFSC) in the soil by palynological and soil culturing techniques, respectively.

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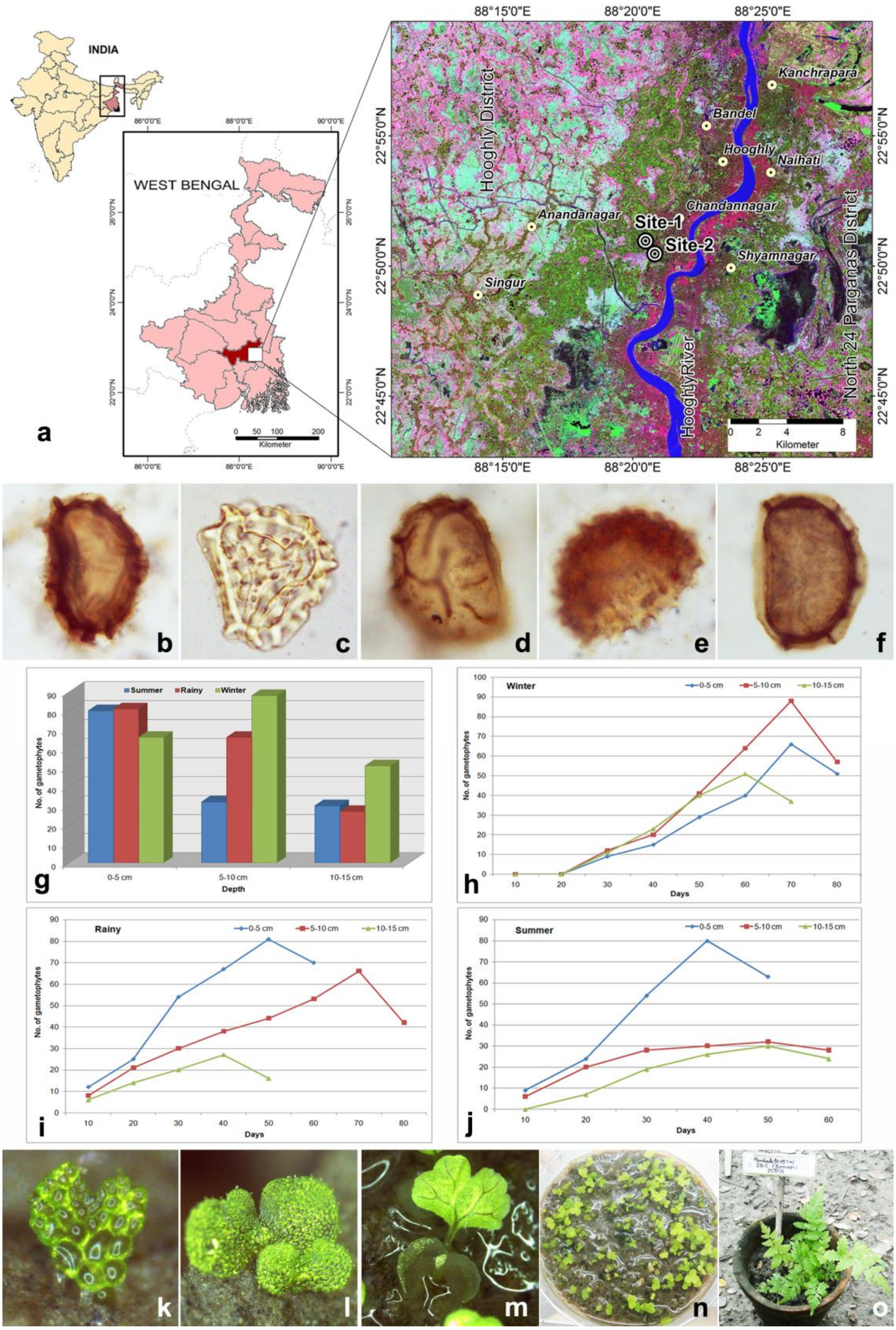

Figure Legends

Fig. 1 (a) Map of West Bengal depicting study area (*marked in black and white circles*); **(1b-f)** recovered spores in soil samples: **(b)** *Diplazium esculentum*, **(c)** *Pteris vittata*, **(d)** *Christella dentata*, **(e)** *Tectaria* sp. **(f)** *Pyrossia lanceolata*; **(1g)** depth wise seasonal variation in LFSB profile; **(1h-j)** number of gametophytes developed at different soil depths in three seasons; **(1k-o)** stages of gametophytic (*in vitro*) and sporophytic (*ex situ*) development from laboratory to field conditions.

Table 1. TFSC profile from different soil depths in studied area

Study area	Type of spores	Frequency distribution of spores (%) (depth wise in cm)				
		0-5	5-10	10-15	15-20	20-25
Site-I	*Pteris vittata*	20	-	-	-	-
	Diplazium esculentum	10	44.48	23.08	-	-
	Pyrossia lanceolata	5	11.12	38.46	80	-
	Lygodium flexuosum	20	-	-	-	-
	Polypodium sp.	5	-	-	-	-
	Christella dentata	25	16.68	30.77	20	100
	Asplenium sp.	5	27.8	-	-	-
	Dryopteris sp.	5	-	-	-	-
	Tectaria sp.	5	-	-	-	-
	Chilanthes sp.	-	-	7.69	-	-
Site-II	*Pyrossia lanceolata*	87.5	57.14	-	-	-
	Christella dentata	12.5	42.86	100	-	-

Table 2. Depth wise seasonal variations in LFSB profile

Seasons	No. of gametophytes scored at different depths (in cm)		
	0-5	5-10	10-15
Summer	80	32	30
Rainy	81	66	27
Winter	66	88	51
χ^2 value of heterogeneity	11.34	140.50	68.77
Probability level at 2 DF	< 0.01	<0.001	<0.001

Palynological Technique

Aliquots of 10 g of each sample were treated for two hours with hydrochloric acid (HCl) to remove the carbonate present in the soil samples. HCl treated samples were washed thoroughly by distilled water by discarding the supernatant by centrifugation at 3000 rpm for 10 minutes. The residue was kept for six days in hydrofluoric acid for removal of silica. The hydrofluoric acid was eliminated by dilution in water (1:2::acid:water) and then centrifuged (10 minutes at 3000 rpm each time) till the acid was removed from the sample. Then the samples were treated with KOH to remove the clay particles. To eradicate base from the samples completely, further centrifugation was done with distilled water for 10 minutes at 3000 rpm. Acetolysis technique (Erdtman 1952, 1960) was applied to the precipitated sample for recovery of pteridophytic spores from the soil. Finally, permanent slides from acetolysed samples were prepared by using polyvinyl alcohol and Canada balsam and observed under Leitz Laborlux S compound microscope.

Soil Culturing Technique

Soil culture was done by breaking up aggregation of soil particles, removing stones, roots, invertebrates, if present. Each sub sample was mixed thoroughly to make a homogeneous sample. Approximately, 10 cm^3 of soil from each core was placed on top of 3 cm^3 of sterile sand (in each of 3 replicates) in 5 cm diameter sterile Petri plates for culturing. About 8-10 ml of water was added to the Petri plates. Wet sand acts as a reservoir preventing small samples of soil from flooding or desiccation. The Petri plates were placed in gametophyte culture room (8-10 weeks; temperature 15-17°C; light 1800-2000 lx; relative humidity around 65-70 %) of Pteridology-Palaeobotany Section of Botany Department, University of Kalyani to promote germination and gametophyte development of pteridophytic spores present in soil samples. At regular interval the Petri dishes containing the cultured soils were observed under Leica S8 APO StereoZoom Microscope.

RESULT

The fern vegetation of two explored sites are recorded by the occurrence of *Adiantum caudatum, A. philippense, Ampelopteris prolifera, Christella dentata, Diplazium esculentum*, along with rich epiphytic flora (mostly growing on mango trees) namely, *Asplenium* sp., *Drynaria quercifolia, Microsorium punctatum, Pyrossia lanceolata.*

Palynological analysis reveals total fern spore assemblage at site I by the recovery of spores of *Pteris vittata, Diplazium esculentum, Pyrossia lanceolata, Lygodium flexuosum, Polypodium* sp., *Christella dentata, Asplenium* sp., *Dryopteris* sp., *Tectaria* sp., and *Chilanthes* sp. The fern spore assemblage at site II encompasses *Christella dentata*, and *Pyrossia lanceolata* only (Table 1; Figs. 1b-f).

LFSB of sampled soils is established through soil culture. Among the total fern spore assemblages, spores of *Christella dentata* only remain viable. A similar trend of germination of live spores in cultured is observed at both the sites. However, χ^2 test of heterogeneity reveals considerable seasonal variability in the prothallial frequency emerging from soil samples at different depths (0-5 cm to 10-15 cm) (Table 2; Fig. 1g). Cultured soils from remaining two depths (15-20 cm and 20-25 cm) have given negative results for prothallial development. Spore germination time is relatively lower in winter than summer and rainy seasons. The prothallial development started after 30 days in winter; while, it was only after 10 days in other two seasons. Time taken for prothallial maturation and subsequent initiation of sporophytic generation varied markedly as evinced from gradual decrease in number of emerging prothallia after 60 (10-15 cm), and 70 (0-5 cm; 5-10 cm) days in winter; 40 (10-15 cm), 60 (0-5 cm), and 70 (5-10 cm) days in rainy; 40 (0-5 cm), and 50 (5-10 cm; 10-15 cm) days in summer (Figs. 1h-j). The growing sporophytic plants are relocated from laboratory to field condition (Figs. 1k-o) for the purpose of *ex situ* conservation by adapting themselves in natural environment.

DISCUSSION

The pteridoflora in the studied area is covered by about 10 taxa of ferns though all the taxa (*Adiantum caudatum*, *A. philippense*, *Ampelopteris prolifera*, and *Microsorium punctatum*) represented in TFSC profile in soil. On the contrary, species namely, *Polypodium*, *Dryopteris*, *Tectaria*, and *Chilanthes* are not present in the studied flora but represented well in soil samples which may be the consequence of their presence in distant or nearby areas.

LFSB profile reveals that all spores present in the soil samples are not viable. For spore viability the vertical length of soil profile is recorded up to 15 cm, below which, the spores could not retain their viability. Gametophyte development has demonstrated seasonal variations at different depths of soils. Soil depth of 0-5 cm is found as best reservoir in summer and rainy; while, 5-10 cm depth is significant in winter.

CONCLUSION

The present study clearly established a reservoir of vertically distributed LFSB in the soil of Mankundu region of Hooghly district, West Bengal for conserving the fern flora. The emerging sporophytes from prothalli growing in *in vitro* conditions are brought to natural habitat with the objective for *ex situ* conservation. The sporophytic plants survived and enriched fern community, and highlight the significance of the work.

ACKNOWLEDGEMENT

The financial grant is aided by UGC-MRP (F. No. 41-489/2012 (SR) dated 16th July, 2012) and DST-PURSE programme of University of Kalyani. The authors gratefully acknowledge the kind help of former Prof. Manju Banerjee of Botany Department, University of Calcutta and Prof. Animesh Kumar Datta of Botany Department, University of Kalyani. The authors are also grateful to Mr. Debabrata Mondal, Department of Geography, Raiganj University for his kind help.

REFERENCES

Dyer, A.F. (1992). Natural soil spore banks—can they be used to retrieve lost ferns? *In: Proceedings of the Kew conservation conference*, September 1991.

Dyer, A.F. (1994). Natural soil spore banks—can they be used to retrieve lost ferns? *Biodiversity and Conservation*, **3:** 160–175.

Dyer, A.F. and Lindsay, S. (1992). Soil spore banks of temperate ferns. *American Fern Journal*, **82:** 89–122.

Simabukuro, E.A.; Esteves, L.M. and Felippe, G.M. (1998). Analysis of a fern spore bank in Southeast, Brazil. *Hoehnea*, **25:** 45–57.

Simabukuro, E.A.; Begovacz, A.; Esteves, L.M. and Felippe, G.M. (1999). Fern spore bank at Pedregulho (Itirapina, Sao Paulo, Brazil). *Revista Brasileira de Biologia*, **59:** 131–139.

Ranal, M.A. (2003). Soil spore bank of ferns in a gallery forest of the Ecological Station of Pangg, Uberlandia, MG, Brazil. *American Fern Journal*, **93:** 97–115.

Ramirez-Trezo, M.D.R.; Perez-Garcia, B. and Orozco-Segovia, A. (2004). Analysis of fern spore banks from the soil of three vegetation types in the central region of Mexico. *American Fern Journal*, **91:** 682–688.

Gupta, S.; Hore, M. and Biswas, S. (2014). An overview of the study of soil spore bank of ferns: need for suitable exploitation in India. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, **84:** 779–798.

Erdtman, G. (1952). *Pollen Morphology and Plant Taxonomy: Angiosperms*. Almqvist and Wiksell, Stockholm.

Erdtman, G. (1960). The acetolysis method, revised description. *Svensk Botanisk Tidskrskrift*, **54:** 561–564.