Propagation of the endangered fern *Adiantum reniforme* var. *sinense* for conservation

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Introduction

Adiantum reniforme var. *sinense* was first discovered in China in 1978 (Lin 1980). The discovery of this Tertiary relict attracted immediate attention from botanists due to its intercontinental distribution, with *A. reniforme* being found in the Azores and *A. reniforme* var. *asarifolium* in south-central Africa. The discovery has great scientific significance in the study of fern phylogeny and phytogeography (Fu & Jin 1992).

Adiantum reniforme var. *sinense* occurs only in one small area in the Three Gorge Area (TGRA), Wanzhou, Chongqing (Fig. 1), where it is restricted to a narrow strip along the Yangtze River valley (approximately 150 km2), between 120 and 480 m above sea level (Shi et al. 2005). This fern was listed as endangered in the Chinese Red Data Book shortly after its discovery, due to its sparse distribution, weak reproductivity, limited population number and small population size (Fu & Jin 1992). Despite its recent 'scientific' discovery, local residents of the TGRA have a long tradition of using *A. reniforme* var. *sinense* in herbal medicine to treat fever, carbuncles and inflammation, dropsy, humidness and jaundice (Qin & Xing 1990). Following increased accessibility due to dam-related infrastructure, populations of *A. reniforme* var. *sinense* have been severely depleted by illegal harvests, with many now of the brink of extinction. Furthermore, the Three-Gorge Reservoir will directly submerge the individuals distributed below the proposed water level (alt. 175m), and climate changes caused by the huge reservoir will affect the future survival of this species. Population surveys, ex-situ conservation in botanic gardens (Xu et al. 1987; Lin 1989; Xie 1993; Xu et al. 1998), and some translocation and reinforcement programmes have been undertaken to rescue populations doomed to submergence in 2009 (Shi et al. 2005).

Adiantum reniforme var. *sinense* is a perennial homosporous herbaceous fern and has caespitose, round-reniform leaves with oblong or short-linear sori on the margins. Leaves are 2-6 cm wide, with a deep sinus, and the dark chestnut stipes are 3-14 cm long and 0.5-1.5 mm in diameter. Fronds unfold in early spring, sori occur in July, and spores mature gradually in August and September. Both sexual and asexual reproduction has been observed in the wild (Lin 1989). *A. reniforme* var. *sinense* grows on moist and exposed thin soils on rocks and in rocky crevices, and occasionally with grasses on warm and humid sites. Due to its ornamental value, it has also attracted plant breeders, gardeners and horticulturalists' attention. Studies of its propagation techniques will help to relieve the wild collection pressures and to produce material for reintroduction, reinforcement, habitat restoration and floral markets.

Asexual propagation

Asexual propagation does not involve exchange of genetic material, so it almost always produces plants that are identical to a single parent. We used division method for the asexual propagation of *Adiantum reniforme* var. *sinense*. It is the easiest way of producing mature plants but only a limited numbers of individuals. Carefully dig the plant, loosening the roots and lifting the plant from the soil. Split apart the main clump with two spades or forks. The segments are replanted and let them grow under shading shed or in the open. To investigate the best season for division propagation of *A. reniforme* var. *sinense*, we conducted divisions of *A. reniforme* var. *sinense* in three different seasons: spring (March), summer (June) and autumn (October). The result showed that spring (March) was the best season for division in Wuhan and the segments should planted under a shading shed (Table 1).

Table 1 The effect of season on division propagation: survival percentage and growth status

Season of propogation	Condition of propagation	Percentage of survival (%)	Growth status
Spring (March)	Planted on seedbed under shading shed	100	Good
Spring (March)	Planted in pots under shading shed	100	Good
Spring (March)	Planted on seedbed in the open	0	-
Summer (June)	Planted on seedbed under shading shed	40	bad
Autumn (October)	Planted on seedbed under shading shed	60	bad

Sexual propagation

Sexual propagation of plants involves the exchange of genetic material between parents to produce a new generation. It is often the cheapest and easiest method of producing large numbers of plants. Fronds that have produced spores were collected and stored in an envelope until used. Germinating fern spores requires more time and care than germinating the seeds of seed plant. It always takes from 3 to 6 months to grow ferns from spores, because growing ferns from spores involves two different generations of ferns. Spores first produce a sexual plant called a gametophyte which is very small and has none of the usual plant parts and resembles a moss-like growth. The gametophyte reproduces sexually and forms sporophytes which have visible roots, stems and leaves. During the first phase of growing ferns, sterile conditions are critical.

Spore propagation *in vitro*

The optimal medium of spore germination was suggest as 1/2MS medium supplemented 5.0mg/l GA3, 3.0% sucrose and 0.7% agar(pH 6.0), and the spore germination percentages were 90-100%; Suitable gametophyte increment medium was obtained on MS medium supplemented 2.0mg/l NAA; Optimal sporophyte generation medium was on river sand as medium of partial tissue culture.

Spore propagation in pots

The compost used for sowing consists of two parts of peat mixed with one part of garden loam and one part of fine sand. The constituents are thoroughly mixed and are thoroughly steam-sterilized in order to kill any

animal eggs and the spores of algae bryophytes and pathogenic fungi. Clay pots of diameter 10-30cm are used and sterilized by putting in the boiling water for 10 min. Prior to sowing, the compost is thoroughly moistened by the passage of water up through the pots by capillarity (Lin 1989). The spores are sown directly from the packet in which they were collected. The clay pot that covered with a piece of glass was then put in a tray filled with water and cultured at 25±1°C under fluorescent light at an intensity of 2000-2500 lx 12h per day. Spores begun to germinate at 20 days after sowing and the first gametophyte was seen at week 6.

Effect of spore storage time on the formation of gametophyte and sporophyte

Interest in the conservation of pteridophyte spores has become evident in recent decades, because they are easy to obtain, can be stored in large quantities, and can germinate rapidly in simple media (Aragon and Pangua 2004). Little is known about the effect of spore storage time on the formation of gametophyte and sporophyte. We found that the spores of *A. reniforme* var. *sinense* were non-chlorophyllous and could remain viable in ambient storage for at least five years, but different storage time (spore age) has a significant effect on formation time of both gametophytes and sporophytes (Table 2): with that 7 days ambient storage resulting in gametophyte formation in 29 days, prolonged five year ambient storage resulting in sporophyte formation in 38 days, whereas 7 days ambient storage resulted in sporophyte formation in 96 days; prolonged four months storage resulted in sporophyte formation in 105 days.

Table 2 The effect of different spore storage period on gametophyte and sporophyte formation and fungus contamination

Spore age	Formation of gametophyte (days)	Formation of sporophyte (day)	Contaminated by fungi
One week	29.3aA	96.0a	2.50aA
Four months	32.5aAB	105.0b	4.50bAB
Five years	38.0bB	-	10.00B
F	5.78*	9.00*	67.88**

Note: The capital and small letters represent significance level of P<0.01 and P<0.05 respectively. Level of significance: *P<0.05; **P<0.01; -: No sporophyte formation because of fungus contamination

Effect of density of gametophyte and culture conditions on the formation of sporophyte

About twenty-day-old gametophytes were used in this experiment. The different density of gametophyte , culture conditions and their interactions had significant effects on the formation of sporophyte. The culture condition (controlled temperature and light: 25±1°C and 2000-2500 lux for 12 hrs/day) in sand media resulted in a shortest sporophyte formation time, 87 days after spores were inoculated. The sand medium is more favorable than peat soil for sporophyte formation and high density of gametophytes enhanced the sporophyte formation .

Table 3 The effect of different culture environment on the sporophyte formation

		Days of sporophyte formation from gametophyte	F
Culture method			567.23**
	Temperature: 25±1°C; illumination: 2000-	29.9	

	2500lx;12h Temperature: 10-27°C; illumination: natural	46.8	
Medium			27. 37**
	Sand	36.2	
	Peat soil	40.2	
Density (individual/cm^2)			75. 83**
	1	44.3aA	
	2	37.1bB	
	3	33.7cB	
Interaction effect			
	culture method* medium		3. 811*
	culture method * density		6. 024**
	medium * density		3. 610**
	culture method* medium * density		. 463

Note: The capital and small letters represent significance level of P<0.01 and P<0.05 respectively.□Level of significance: *P<0.05; **P<0.01.

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