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Plant species with extremely small populations (PSESP) in China: A seed and spore biology perspective

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A B S T R A C T

Approximately one fifth of the world's plants are at risk of extinction. Of these, a significant number exist as populations of few individuals, with limited distribution ranges and under enormous pressure due to habitat destruction. In China, these most-at-risk species are described as 'plant species with extremely small populations' (PSESP). Implementing conservation action for such listed species is urgent. Storing seeds is one of the main means of *ex situ* conservation for flowering plants. Spore storage could provide a simple and economical method for fern *ex situ* conservation. Seed and spore germination in nature is a critical step in species regeneration and thus *in situ* conservation. But what is known about the seed and spore biology (storage and germination) of at-risk species? We have used China's PSESP (the first group listing) as a case study to understand the gaps in knowledge on propagule biology of threatened plant species. We found that whilst germination information is available for 28 species (23% of PSESP), storage characteristics are only known for 8% of PSESP (10 species). Moreover, we estimate that 60% of the listed species may require cryopreservation for long-term storage. We conclude that comparative biology studies are urgently needed on the world's most threatened taxa so that conservation action can progress beyond species listing.

1. Introduction

China is one of the richest countries in the world in terms of plant diversity. Third only to Brazil and Columbia, China harbours over 30,000 plant species (Yang et al., 2005). However, at least 200 plant species have become extinct in the last 50 years and c.5000 more are currently threatened or on the brink of extinction, making China one of the highest priorities for global biodiversity conservation (Volis, 2016).

Among this rich diversity of plants in China, there are 120 wild plant species that were identified in 2012 as the first group for urgent protection nationally. These species have the following features: 1) estimated to have <5000 mature individuals in the wild; 2) distribution restricted to a limited range with a few locations; 3) recognition as national or regional endemic species of China; and 4) potential for economic development or scientific value. Reflecting these attributes, a descriptor is now used for this cluster of species: 'Plant Species with Extremely Small Populations (PSESP)' (Ma et al., 2013; Sun, 2013). As a result of a new policy framework, several national and regional-level conservation strategies and actions for conserving China's PSESP are being implemented. Such an approach is particularly important as a recent spatial distribution analysis of 33 species from the 120 PSESP list revealed that only 12 of these are considered to be well protected in the National Nature Reserves (Wang et al., 2016a,b). Importantly, significant progress has been made to increase the coverage of threatened species in China's botanic gardens. For example, Xishuangbanna Tropical Botanical Garden, Yunnan has an extensive collection of nationally red-listed species. Nonetheless, only around 60 PSESP (i.e., about half) have been propagated and cultivated *ex-situ* in China's botanic gardens (Sun, W—B., pers comm.).

The global need for botanical gardens to protect threatened species, e.g., trees, in dedicated conservation collections is

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recognised (Cavender et al., 2015). The broad aim of establishing and managing such living *ex situ* collections should be to maintain the greatest biodiversity at the greatest economic and logistic efficiency (Cibrian-Jaramillo et al., 2013). Yet the problem of limited genetic diversity of individual species in these collections is well known. Part of the solution is to establish a combined management strategy for the acquisition of living collections among botanic gardens and other organizations interested in plant conservation (Cibrian-Jaramillo et al., 2013). In the case of the cycad *Zamia decumbens*, collections can better conserve the genetic diversity of *in situ* populations as long as multiple accession are made over more than one year (Griffith et al., 2015).

Conservation efforts with the world's most threatened species is also hindered by gaps in fundamental biological information, e.g., on trees (Cavender et al., 2015) and on their seed biology (Pritchard et al., 2014). Two biological traits are of particular importance when considering the efficient and effective utilisation of seeds and fern spores in both the *ex situ* and *in situ* environment: desiccation tolerance and dormancy/germination. Storing seeds is one of the main means of *ex situ* conservation and involves drying as the first step in the preservation process. Tolerance of drying opens up the opportunity for reduced temperature storage, which is the main means of conserving millions of accessions of plant genetic resources (http://www.fao.org/agriculture/crops/core-themes/theme/seeds-pgr/sow/sow2/en/), and underpins the global market for seeds, which was valued at US$53.76 billion in 2014 (http://www.marketsandmarkets.com/Market-Reports/seed-market-126130457.html). Fern spores are a ready source of germplasm to aid re-establishment of waning fern populations (Pennisi, 2010). Since they tolerate high levels of desiccation (Ballesteros, 2010), dry storage of fern spores could provide simple and economical *ex situ* conservation of genetic diversity in a relatively small space (Pence, 2008; Ballesteros, 2010; Ibars and Estrelles, 2012). Germination timing, which is often strongly dependent on temperature (for dormancy loss and germination speed), is a key stage in species regeneration *in situ* and in the assessment of seed viability in *ex situ* collections (Smith et al., 2003; Suo et al., 2015).

Here we review what is known about the seed and fern spore biology (storage and germination) of at-risk species, using China's PSESP as a case study, and highlight some areas of future research that need addressing.

2. Materials and methods

The list of PSESP in Ma et al. (2013) and Sun (2013) was consulted and the plant name and authority checked against The Plant List (http://www.theplantlist.org/). Data searches were run on the 2008 Seed Information Database (http://data.kew.org/sid/), using the terms ‘seed storage’ and ‘seed germination.’ Individual species records from the published literature were summarised when available, particularly to bring the analysis up to date. Otherwise a seed biology perspective is given on either the genus or family basis, including some perceived scientific challenges.

3. Results and discussion

The taxonomic spread of the 120 PSESP extends to 76 genera from 33 families (Table 1) of mainly seed plants. Ferns (sensu lato, i.e. monilophytes) are represented by just one family and species. The species are not evenly spread among seed-bearing families with significant clustering in the Orchidaceae (37 species) and Cycadaceae (11 species). These two over-represented groups contribute 40% of PSESP species and should provide an urgent focus for future seed biology studies.

Of the 120 PSESP, there is readily available information on seed germination for 28 species (23% of PSESP), but storage characteristics are accessible for only 8% of PSESP (i.e., 10 species), for which germination protocols are also known. These 28 species are profiled below. Our interpretation of the published literature for the listed species, or close relatives, suggests that storage under international gene bank standards (i.e., drying to 15% RH, plus hermetic storage at −20 °C) may only be realistic for 40% of the PSESP (i.e. 48 species). The other 72 species (60%) may have to be cryopreserved for long-term storage, as spores, seeds or embryonic axes (Li and Pritchard, 2009; Walters et al., 2013).

3.1. Ferns in PSESP

There is only one fern species in PSESP for China, *Cystoathyrium chinense* Ching. This species was believed to be extinct in the wild but was recently rediscovered (Wei and Zhang, 2014). It is a critically endangered species endemic to China with no more than 40 extant individuals, closely related to North American taxa of the genus *Cystopteris* (Wei and Zhang, 2014).

To our knowledge there is no published information on the spore biology of *C. chinense*. However, fern spore storage and germination have been well studied (reviewed in Ballesteros, 2010; Suo et al., 2015) including related species, such as *Cystopteris fragilis* (e.g. Ballesteros et al., 2012). In terms of storage and conservation, spores of *C. fragilis* died at room conditions within 3 years, but initial germination percentage and rate was preserved after cryogenic storage at −80 and −196 °C. In general, fern spores appear to be tolerant to desiccation, showing increased longevity as storage temperature decreases (e.g. Quintanilla et al., 2002; Aragón and Pangua, 2004; Li et al., 2010; Ballesteros et al., 2011, 2012; Li and Shi, 2014). However faster than expected ageing of fern spores from some species at temperatures between −10 °C and −30 °C (Ballesteros, 2010, and references therein; Li et al., 2010; Ballesteros et al., 2012; Li and Shi, 2014) have led to the suggestion that fern spores may have a storage physiology similar to intermediate seeds (Pence, 2008; Ballesteros, 2010). Cryopreservation is feasible for many fern spores and may be necessary to maximize spore longevity for long-term *ex situ* conservation (Pence, 2008; Ballesteros, 2010; Li et al., 2010; Ballesteros et al., 2011, 2012; Li and Shi, 2014; Mikula et al., 2015).

Most fern spores germinate on diverse mineral culture medium at temperatures between 20 and 25 °C, however optimal light intensity and illumination time for spore germination are different among species (Suo et al., 2015). For *C. fragilis* spores, germination is successful in mineral culture medium, at 20 °C with a 12 h photoperiod (common fluorescent tubes, photon irradiance 25–50 μmol m^{-2} s^{-1} in the 400–700 nm region). Additionally, some spores may require specific metal ions, pH, or other specificities during the *in vitro* culture.

3.2. Over-represented groups in PSESP

There are two over-represented groups in PSESP for China constituting 40% of the species: cycads and orchids.

3.3. Cycads

Of the 26 gymnosperms (22% of species) listed, 11 species (9%) are from a single genus, *Cycas*, in the family Cycadaceae. Globally cycads (Cycadaceae, Stangeriaceae, Zamiaceae) are the most threatened group of plant species (Donaldson, 2003), with 196 out of 339 taxa (58%) recently listed by IUCN (IUCN, 2015). Cycads are also included in Appendix I or II of CITES (Rutherford et al., 2013).

There appears to be no published information on the seed biology of the 11 *Cycas* species on the China PSESP list, other than Jian et al. (2006) noting that all five populations of *Cycas fairylakea*