Ferns represent an untapped biodiversity for improving crops for environmental stress tolerance

Ferns, which comprise c. 10,000 species and 250 genera, are the most conspicuous spore-bearing land plants. They have evolved remarkable adaptations to extreme environments, from tropical to cold temperate regions, from lowland to alpine zones and from xeric to aquatic conditions. Because the majority of living ferns result from a more recent diversification that is independent of angiosperm evolution, ferns represent a crucial, hitherto-unexplored genetic diversity that may be exploited for improving plants via gene-transfer technologies. The development of genome resources for select fern species, and the identification of functions for networks of genes, are crucial to achieve this.

Ferns represent an important early node in land plant evolution

Ferns are the second-largest group of vascular plants, comprising c. 10,000 living species. Molecular phylogenetic studies have revealed three monophyletic groups of early vascular plants: lycophytes; seed plants; and a clade including horsetails and ferns. Horsetails and ferns are the closest relatives of seed plants (Pryer et al., 2001).

Ferns attained remarkable levels of diversity and abundance from the Carboniferous period to the Jurassic period (from c. 300 to 150 million years ago (Ma)) (Skog, 2001). The rise of angiosperms during the Cretaceous period (c. 150 Ma) is often thought of as coincident with a dramatic decrease in the abundance of many seed-free vascular plant lineages, including ferns (Crane et al., 1995). Hence, ferns were thought to be 'evolutionary holdovers' from the late Palaeozoic and early Mesozoic eras. However, the first appearance of many modern fern genera in the early Tertiary era fossil record suggested that the majority of living ferns result from a more recent diversification (Smith, 1972; Lovis, 1977). Using both divergence time estimates for ferns and angiosperms, and molecular data, Schneider et al. (2004) showed that polypod ferns (> 80% of living fern species) diversified in the Cretaceous period, after angiosperms. This then implies that ferns evolved independently of angiosperms, but along with angiosperms in ecospaces created by the dominance of angiosperms (Schneider et al., 2004).

There are two examples of novel traits found in derived ferns, but not in basal ferns and angiosperms.

1. The polypod fern, *Adiantum*, has an unconventional photoreceptor, phytochrome 3 (PHY3). PHY3 may provide an advantage for photoreception under low-light canopy conditions (Kawai et al., 2003; Suetsugu et al., 2005).

2. Certain ferns in the Pteridales hyperaccumulate arsenic (Ma et al., 2001), while no angiosperm or basal fern is known to have this trait (Meharg, 2002; discussed further under the heading Heavy metal tolerance and hyperaccumulation).

Ferns are unique models for biological processes

While ferns, other lower plants and angiosperms share vital genes for the survival of green plants, certain biological features are enhanced in ferns. The evolutionary pressures on ferns have been somewhat different from those on angiosperms because of the lack of flowers, fruits, pollinators and seed-dispersal agents in ferns. This implies that angiosperms have evolved specific traits associated with flowering and pollination, which may be expected to be absent in ferns (and other nonflowering plants). However, most flowering plants have a lower dispersal capacity than ferns (Tryon, 1972). Because of their wider geographical distribution and unique habitats, ferns might have evolved novel environmental stress adaptations that may not be found in angiosperms.

In all plants, a multicellular haploid phase (the gametophyte) alternates with a diploid sporophyte phase. In flowering plants, the gametophyte is extremely reduced. In ferns, the gametophyte development is more distinct than in angiosperms. Biologists have recognized that ferns, in general, and *Ceratopteris richardii* (C-fern), in particular, are excellent models for studying gametophyte development and sexual reproduction in plants because their gametophytes are autotrophic, small and develop rapidly, and in C-fern can be crossed or manipulated to develop as males or hermaphrodites (Hickok et al., 1995; Banks, 1999). Spermiogenesis has been extensively studied in the C-fern and in *Marsilea vestita*, a water fern (Myles & Hepler, 1977; Pennell et al., 1986; Hart & Wolniak, 1998, 1999; Pennell et al., 1988; Vaughn et al., 1993; Hoffman et al., 1994; Hoffman & Vaughn, 1995).
Plants that grow in shaded conditions have adaptations that allow them to maximize the efficiency of photosynthesis and minimize their 'costs'. As ferns diverged under 'the shadow of angiosperms', they have remarkable shade adaptations. Typical shade plants are able to survive in habitats where the ambient light intensity is only a few per cent of direct sunlight. However, gametophytes of the fern *Tichomanes speciosum* thrive under low-light conditions (e.g. < 0.01% of full sunlight with optimum photosynthesis at 5–10 µmol m⁻² s⁻¹). Probably, this is the most shade tolerant of all plant species studied to date (Johnson *et al*., 2000). In *Teratophyllum rotundifoliatum*, a shade-tolerant tropical fern, novel anatomical and cytological features have been reported (Nasrulhaq-Boyce & Duckett, 1991).

Light affects various plant developmental processes. It is advantageous to study photomorphogenetic processes in lower plants because the entire photomorphogenetic process, from light reception to morphological change, often occurs within a single cell in the lower plants, avoiding the experimental difficulties associated with complex multicellular organs (Cooke *et al*., 1995). Three fern species – *A. capillus-veneris*, *C. richardii* and *Onoclea sensibilis* – have often been used as models for photobiological research (Cooke *et al*., 1995; Augustynowicz & Gabrys, 1999).

Polyploidy has played a significant role in the evolution of plants. While c. 50% of angiosperms are polyploids, polyploidy occurs more frequently in the Pteridophytes (Soltis & Soltis, 2000). The average haploid chromosome number for homosporous ferns is high (n = 57) but isozyme studies suggest that they behave like typical diploid plants (Hauffer & Soltis, 1986). This then led to the idea that either ferns have undergone repeated cycles of polyploidy and gene silencing or were initiated with high chromosome number. A study identifying the presence of defective genes in a gene family in a fern supports this idea (Pichersky *et al*., 1990).

**Ferns are adapted to extreme environments: an untapped biodiversity for plant biotechnology**

During the past few centuries, plant breeders have used the biodiversity available within plant families to improve traits in crop plants. These efforts were mostly confined to crossable species and occasionally, with wide crosses, to other genera. More recently, however, genetic transformation technologies have made it possible to improve crops by introducing genes from virtually any organism (Rathina-sabapathi, 2000). Many microbial genes have been used for the improvement of crops (Dunwell, 2000). However, microbial genes, while readily available and less likely to damage the host plant’s regulatory or metabolic machinery, also have some drawbacks for metabolic engineering. For example, the codon usage in a microbial gene may have to be optimized before high levels of expression can be achieved (e.g. Perlak *et al*., 1991). On the other hand, expression of fern genes in angiosperms will not be subject to codon usage problems (Wood *et al*., 1999). Plant biotechnologists have not yet used fern (and other lower plant) genes for improving angiosperm crops.

Ferns are adapted to extreme environments. Recent molecular phylogenetic analyses indicated that ferns have undergone recent adaptive radiations (Sanchez-Baracaldo, 2004). Harsh environments have led to the development of specific traits in organisms by convergent evolution. Ferns have been distributed widely and therefore might have undergone more extreme selection pressures than angiosperms. Hence, fern genetic diversity could be used to study the biological effects of certain genes, and these genes may be suitable for use in metabolic engineering to increase the stress tolerance of crops. Some examples of ferns with adaptations to extreme environments are illustrated below.

**Dessication tolerance**

Dessication tolerance is a rare adaptation among vascular plants. Several fern species of Actiniopteridaceae, Sinopteridaceae, Pteridaceae and Selaginellaceae have been documented to be distributed in rock outcrops in the tropics and to exhibit desiccation tolerance (Porembski & Barthlott, 2000).

**Salinity tolerance**

The genus *Acrostichum aureum*, the halophytic fern, grows associated with mangrove vegetation in the tropics. It accumulates the cyclitol d-1-O-methyl-muco-inositol (Medina *et al*., 1990), a cytoplasmic compatible solute, in response to increasing salinity.

**Heavy metal tolerance and hyperaccumulation**

The primitive fern, *Osmunda cinnamomea*, is tolerant of copper, cadmium and zinc (Francis & Petersen, 1983). *Athyrium yokoscense* accumulates lead in its tissues, particularly in roots (Nishizono *et al*., 1987), and the gametophytes of this species also exhibit lead tolerance and accumulation (Kamachi *et al*., 2005). Ma *et al*., (2001) discovered that the Chinese brake fern, *Pteris vittata*, could grow in arsenic-contaminated soils and accumulate large amounts of arsenic in its above-ground biomass (3280–20 000 ppm). Chinese brake fern was tolerant of and growing in soils containing as much as 1500 ppm arsenic, whereas most plants cannot survive 50 ppm arsenic. Following this report, others have also reported on the ability of this and other related ferns to hyperaccumulate arsenic. It was proposed that Chinese brake fern is an excellent model for using to study arsenic uptake, translocation, speciation, distribution and detoxification in plants (Ma *et al*., 2001; Singh *et al*., 2006).
High-altitude epiphytic adaptations

Many ferns are epiphytes adapted to forest ecosystems in high elevations. In a study on species richness of epiphytes in a forest ecosystem, pteridophytes showed a linear increase from the lowlands to the timberline (Kromer et al., 2005). The biology of epiphytes is not well understood.

Competitive ability and invasiveness

Certain ferns have high competitive abilities and hence they have the potential to be invasive weeds. The fern, Pityrogramma calomelanos, has been a nuisance in plantations (Wardlaw, 1962). The Old World climbing fern, Lygodium microphyllum, is an aggressive invasive weed in North and South America. Salvinia is an aquatic weed that threatens waterbodies of the world (Abbasi & Nipaney, 1986). Invasive nature is a poorly understood trait in biology (Bais et al., 2003) and ferns could be used to investigate the genetic basis of this trait.

Genome resources on ferns

Despite the importance of ferns in early plant evolution and in understanding novel biological structures, functions and adaptations, few community genomic resources are currently available. To date, no complete genomic sequence data are available for ferns (Jackson et al., 2006), and methods are not available for stable transformation of ferns. Being polyploids, special technical difficulties may be encountered when deciphering fern genome sequences. Genome sizes (Polito, 1980; Murray, 1985; Hanson & Leitch, 2002) of ferns are c. 20–70 times larger than that of Arabidopsis thaliana (1C = 157 Mb, Bennett et al., 2003). Expressed sequence tag (EST) collections will be cost-effective alternatives to whole-genome sequences and will aid us to annotate the genome sequence when obtained.

An EST sequence database represents a broad data set, with very few assumptions about the genes. A deep EST database, with large insert cDNAs, will therefore aid us, in a multitude of ways, to investigate the biology of the organism (Ohlrogge & Benning, 2000). Salmi et al. (2005) have reported the profile and analysis of gene expression changes, during early development, in germinating spores of the fern C. richardii. This work has identified genes likely to be critical for the germination and subsequent early development of diverse cells and tissues emerging from dormancy (Salmi et al., 2005). There are c. 5000 ESTs in the National Center of Biotechnology Information database from C. richardii germinating spores. An EST database from the prothallus of A. capillus-veneris has c. 10 000 ESTs (Yamauchi et al., 2005).

Large-scale EST sequence databases have now been established for many angiosperm species, generating a wealth of genomic resources (e.g. Newman et al., 1994; Sasaki et al., 1994; Cooke et al., 1996; Ronning et al., 2003; Ramirez et al., 2005). Recent EST analyses on lower plants – Acetabularia (Henry et al., 2004), cycad (Brenner et al., 2003), Physcomitrella (Rensing et al., 2005), Picea glauca (Pavy et al., 2005), Ginkgo (Brenner et al., 2005), Selaginella (Weng et al., 2005) and Tortula (Oliver et al., 2004) – have all uncovered novel transcripts not found in angiosperms and have provided tools to investigate land plant evolution.

In the present analysis, the Pteridophytes were specifically compared with angiosperms because most of the current molecular breeding efforts are focused on angiosperm crops. However, some gymnosperms are important forestry crops for wood pulp production (Tang & Newton, 2003) and their stress-tolerant characteristics can also be improved by the introduction of genes from the Pteridophytes.

We have initiated a functional genomics approach to identify genes implicated in arsenic metabolism in P. vittata, an extraordinary fern that hyperaccumulates arsenic (Rathinasabapathi et al., 2006a,b). Systematic and deep EST databases from ferns differing in stress-tolerance traits, and representing different phylogenetic nodes, will be a welcome community resource for understanding trait evolution in ferns and for engineering plants with improved stress tolerance for agriculture, forestry and phytoremediation applications.

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