Development of conservation biotechnologies in response to target 8 of the GSPC

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Target 8 of the Global Strategy on Plant Conservation (GSPC 2002) is to achieve *'60 per cent of threatened plant species in accessible ex situ collections'...,* giving clear recognition to the importance of *ex situ* conservation to support *in situ* initiatives. *Ex situ* conservation is now particularly urgent given the increased threat to plants *in situ* through climate change factors.

Target 8 also states the need for '*additional resources, technology development and transfer, especially for species with recalcitrant seeds*', thus identifying the importance of the development of new approaches to long-term *ex situ* conservation for these species. This paper will summarise the scope of the need for alternative conservation technologies, highlight recent research and propose directions for the future.

The seed of orthodox-seeded species can be routinely stored in seed banks using standard desiccation (5% Moisture Content) and freezing (-20^{o}C) protocols. However, not all species are amenable to these procedures and require the development of alternative conservation biotechnologies, particularly *in vitro* and cryopreservation approaches, before long-term *ex situ* storage can be achieved (Ashmore 1997; Pritchard 2004a). Conservation of these species is thus currently restricted to *in situ* approaches or field collections *ex situ*, making them particularly vulnerable to loss. Plant species for which conservation biotechnologies are needed include: Recalcitrant or desiccation sensitive (DS) seed, orchids and lower plants.

The numbers of DS species is unknown, but it may be up to 30% of flowering plants or >80,000 species. The seed of individual species can be screened for desiccation sensitivity (Pritchard *et al* 2004a & b). However, this approach will only be possible for a relatively small number of targeted species. Recent studies have investigated taxonomic and ecological aspects of DS species in order to better understand the distribution and prevalence of these (Dickie and Pritchard 2002; Tweddle *et al* 2003). Results indicate (i) the common occurrence and wide distribution across families of plants and (ii) the association of DS species with wetter habitats. Tweddle *et al* (2003) estimate that 48% of species in non-pioneer evergreen rainforest will have DS seed. Daws *et al* (2006) have recently reported a predictive model for likely DS species based on large seed mass and low seed coat ratio. Application of this model will allow better estimates of the numbers of DS species and may assist with prioritisation for *in situ* conservation planning. For example, protection of tropical rainforest regions is clearly a priority. It is also imperative that more generally applicable conservation biotechnologies be developed for DS species and other species with complex seed storage behaviour (e.g intermediate), but particularly for those under conservation threat or of socio-economic importance. Conservation biotechnologies are also vital for orchid seed storage and recovery and for lower plants. In addition seed storage at -20^{o}C may not be optimal for a number of essentially orthodox species such as *Carica papaya*, *Citrus* spp. or *Coffea* spp.

A partnership between the Millennium Seed Bank Project (MSBP), Royal Botanic Gardens Kew (RBG Kew) and the State of Queensland was established in 2004, known as the Seeds for Life project (SFL *www.greeningaustralia.org.au*). Collaborative research being undertaken under this partnership aims to improve our understanding of DS species and to develop conservation biotechnologies for targeted Queensland native species.

Queensland has a total of approximately 7811 native flowering plants (Henderson 2002), with around 1368 taxa (17.5%) listed as rare and threatened. Bioregions with the greatest numbers of threatened species are the wet tropics region in North Queensland (363 taxa) and the Southern coastal regions (230 taxa). Given that DS seeds are more likely to occur in wetter tropical regions (Tweedle *et al* 2003), this suggests that a significant number of taxa are both under threat *in situ* and unable to be stored in *ex situ* collections. As part of the Queensland project estimates have been made of the likely numbers of DS species in Queensland based on herbarium assessments of seed characteristics. This assessment has resulted in a preliminary listing of over 1800 species in over 100 families. Families containing the largest numbers of species (>50 each) in this preliminary list are Asclepiadaceae, Euphorbiaceae, Lauraceae, Myrtaceae, Proteaceae, Rubiaceae, Rutaceae and Sapindaceae. This list should prove useful in identifying areas rich in DS species and prioritisation for *in situ* conservation.

A list of targeted species for in depth research was generated. This includes genera containing rare and threatened species, of socio-economic value for horticulture and likely short-lived or DS seed, in order to address both targets 8 and 9 (*'70 per cent of the genetic diversity of crops and other major socio-economically valuable plant species conserved...')* of the GSPC (Ashmore and Drew 2005). Table 1 provides a listing of some of the genera identified by this process. Each of these genera contains species that are commercially cultivated (e.g. *Macadamia integrifolia*), crop wild relatives (CWRs) of commercially important species (e.g. *Macadamia* spp., *Citrus* spp., *Garcinia* spp.) or of local importance as bush foods (e.g. *Citrus australasica*, finger lime; *Davidsonia pruriens*, Davidson's plum). The Table also provides a summary of the percentage of species under threat in each genus as well as a listing of the rare and threatened species. It is also worth noting that the International Treaty on Plant Genetic Resources has identified 35 food crops of critical important to humanity and this includes citrus, breadfruit and coconut, all of which have non-orthodox seed (FAO 2005).

Some research outputs on the development of conservation biotechnologies in Australian native *Citrus* spp., Queensland orchids and *Carica* papaya (important crop species) are summarised below as examples. Work is on-going to for other species identified in Table 1

Genus	Common name	Category	Fraction(%) of Qld. species under threat	Rare and threatened species**
Alpinia	native ginger	CWR	1/5(20%)	*A. hylandii* (R)
Capparis	Australian caper	CWR	2/22(9%)	*C. humistrata* (E), *C. thozetiana* (V)
Citrus	wild limes	CWR/BF**	2/5(40%)	*C. garrawayi* (R), *C. inodora* (V)
Elaeocarpus	Quandong	BF***		*E. coorangooloo* (R), *E. johnsonii* (R), *E. stellaris* (R), *E. thelmae* (R)
Davidsonia	Davidson's plum	BF***	1/3(33%)	*D. johnsonii* (E)
Diploglottis	native tamarind	BF***	3/10(30%)	*D. campbellii* (E), *D. harpullioides* (R), *D. pedleyi* (R)
Garcinia	wild mangosteen	CWR	1/6(17%)	*Garcinia brassii* (R)
Macadamia	macadamia	CC/CWR	6/7(86%)	*M. claudiensis* (V), *M. grandis* (R), *M. integrifolia* (V), *M. jansenii* (E), *M. ternifolia* (V), *M. tetraphylla* (V)
Musa	wild banana	CWR/BF	2/3(66%)	*M. fitzalanii* (X), *M. jackeyi* (R)
Myristica	Australian nutmeg	CWR/BF	0/2(0%)	
Passiflora	wild passionfruit	CWR/BF	0/1(0%)	
Piper	wild pepper	CWR/BF	1/7(14%)	*P. mestonii* (R)
Syzygium	lilly pilly, rose apple	BF***	10/49(20%)	*S. aqueum* (R) *S. argyropedicum* (R) *S. buettnerianum* (R), *S. hodgkinsoniae* (V), *S. macilwraithianum* (R), *S. malaccense* (R), *S. moorei* (V), *S. pseudofastigiatum* (R), *S. rubrimolle* (R), *S. velarum* (V)

Information from Low (1991)* **Species conservation status sourced from Henderson (2002): **X E**x**tinct: **E** **E**ndangered: **R** **R**are**:** **V** **V**ulnerable. ***Species within this genus are cultivated as local crops.

Table 1 Some Queensland genera with likely short-lived seed (DS or complex seed storage behaviour) seed and of socio-economic importance as global Commercial Crops (CC), Crop Wild Relatives (CWR) or Bush Foods (BF). Numbers and percentages of species in each genus under conservation threat are given as well as listings of these species.*

Summary of research on Australian native *Citrus* spp.

Australia has the largest number of indigenous citrus species (6) of any country worldwide and these have greater genetic variability than many other members of the genus including cultivated species (cf. review Hamilton 2007). Two of these species (*C. inodora* and *C. garrawayi*) are threatened in the wild (Table 1).

Recent studies have reported cryopreservation of seeds of Australian native Citrus species is preferable to storage at -20oC (Hamilton et al 2005a; Hamilton, 2007). Optimal moisture content for seed storage is about 10% based on differential scanning calorimetry (DSC) studies (Hamilton 2007) which is consistent with the findings of Hor et al (2005) for seeds of cultivated citrus and confirms the need for alternative seed storage protocols for these species. A straightforward micropropagation system has also been developed, suitable for mass propagation and medium term storage of germplasm (Hamilton et al 2005b).

Summary of research on orchids

The Orchids Status Survey and Conservation Action Plan of the IUCN/SSC Orchid Specialist Group (Hágsater and Dumont 1996) recommends the use of aseptic propagation and seedbanking to support conservation. Queensland has >400 species, with and >100 of these under threat *in situ* (Henderson 2002).

In vitro germination and cryo-storage has successfully demonstrated in 5 Queensland orchid species including 3 threatened species and *in vitro* collections of a further 8 threatened species are being maintained. Storage of encapsulated protocorms has also been demonstrated and this provides a useful alternative for ease of handling for both cryopreservation and germination steps (Parisi pers. comm.)

Summary of research on *Carica papaya*

Papaya (Carica papaya) is an important crop in Queensland. On-going research includes (i) the introgression of Papaya Ringspot Virus (PRSV-P) resistance from CWRs of papaya (Vasconcellea spp.) into elite C. Papaya varieties and identification of the resistance gene(s) (Ashmore and Drew 2006), (ii) conservation of elite genotypes and CWRs using shoot tip cryopreservation (Ashmore et al In Press) and (iii) studies on seed biology and storage.

The major finding in terms of seed storage is that, papaya seed is essentially orthodox and can tolerate desiccation down to 5% moisture content(MC). However, Wood et al (2000) reported that desiccation–induced dormancy occurs in seed, which could be reversed by heat shock treatment. Results from Queensland confirm the view that papaya seed tolerates standard desiccation regimes but requires dormancy releasing agents to initiate germination after drying. In terms of tolerance to storage, desiccated seeds (5%MC) exposed to storage at 15oC, 4oC or -20oC were unable to germinate even in the presence of dormancy releasing agents. However, cryopreserved seed at 5%MC germinated at high levels (>87%) after 6 months. Thus storage in liquid nitrogen is preferred for long-term storage of papaya seed. Indeed, this result may prove to have application across a range of essentially orthodox seeded species, particularly those previously described as intermediate-seeded.

There is an urgent need to develop alternative long-term *ex situ* conservation biotechnologies for up to 30% of plant species which cannot currently be stored by standard seedbanking methods. Climate change threats to plants suggest that the adoption of mainly *in situ* conservation approaches for these species will be inadequate to avoid species loss. The following actions are proposed:

- Increased work to understand seed storage behaviour and traits associated with desiccation sensitivity for predictive use.

- Further work towards the development of general biotechnology approaches to *ex situ* conservation for DS seeds, orchids and lower plants
- Priority for research on species under conservation threat and/or of socio-economic value.
- Consideration of seed desiccation tolerance in prioritising for *in situ* conservation.
- Support for international networks/programs for research, capacity building, technology transfer and training in conservation biotechnologies for targeted species.

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