Darwin Initiative: Half Year Report

(due 31 October 2007)

Project Ref. No.	14-056
Project Title	Cryo-conservation Centre of Excellence for Sub-Saharan Africa (CCESSA)
Country(ies)	UK, South Africa
UK Organisation	Royal Botanic Gardens Kew
Collaborator(s)	University of KwaZulu-Natal
Project Leader	Prof H.W. Pritchard
Report date	30th October 2007
Report No. (HYR 1/2/3/4)	HYR 3
Project website	http://www.sles.ukzn.ac.za/plantgermcons/

1. Outline progress over the last 6 months (April – September) against the agreed baseline timetable for the project (if your project has started less than 6 months ago, please report on the period since start up).

Progress has been maintained over the last six months. As we are coming towards the end of the grant period, the emphasis on screening has declined, although a few new recalcitrant seeded species have been identified. It has been established that the seeds of two species of *Strelitzia* can survive immersion in nitrogen slush, and so are probably amenable to cryopreservation. The difficulties associated with the excision of the cotyledons from seeds with large reserve-storing cotyledons continues to bedevil attempts at cryostorage of embryonic axes of these species (this problem was highlighted in the previous annual report). On the basis of the assumption (there are preliminary data to support this assumption) that the excision damage is mediated by reactive oxygen species, a multi-factorial experiment involving excision under anti-oxidant solutions, and subsequent treatment of cut surfaces with various anti-oxidant and other powders, was set up for *Trichilia dregeana* axes, which have proved to be particularly vulnerable. None of the treatments led to a curtailment of the problem, and the only axes in which shoot growth occurred were those where small pieces of cotyledon were left attached to the axes, irrespective of anti-oxidant treatment. We are in a position where we can 'cryopreserve' embryonic axes of seeds of a number of species, but almost without exception, those with large reserve-storing cotyledons and of tropical origin, suffer excision damage and do not produce shoots, even prior to partial drying and subsequent cooling. It is intended at some future date to study this phenomenon more deeply to identify the cause of damage and try to overcome this problem. We have, however, had success with some endospermous seeds where entire embryos can be removed from the seeds without requiring excision of cotyledons.

In order to obviate the excision damage problem effort has been put into the use of alternative explants as potential source material for germplasm cryopreservation. Apical bud and nodal segment tissue cultures for eight species have been established, and the protocols for plantlet regeneration from these have been developed. However, these explants have proved to be disappointing in terms of cryopreservation. In tissue culture the explants have very high water contents (in excess of those useful for cryopreservation) and when rapidly dried to the appropriate water contents, there is only limited (if any) survival. If explant size is reduced to reduce thermal mass for the rapid cooling cryopreservation approach, the explants do not develop further. A two-stage freezing approach is being attempted: slow cooling (1^{o} min^{-1}) to -40^{o}C to induce extra-cellular ice formation and freeze-induced dehydration, followed by rapid cooling in nitrogen slush.

In addition to the more empirical and direct approaches described above, more 'basic science' studies are being undertaken to better understand the phenomenon of seed recalcitrance, particularly with respect to the response to drying and freezing, and possible differences between tropical and temperate species. Included amongst these is the influence of cryoprotectants on cellular ultrastructure (using freeze-substitution) following cooling at different rates. Studies into the presence or absence of certain late embryogenic abundant proteins (LEAs) are also being initiated. Although similar studies have been carried out previously, a wider range of appropriate antibodies is now potentially available and the LEA data can be linked with accumulation of various sugars, and seeds of a range of desiccation and freezing response can be studied. In a 'flagship' study various biochemical responses of seeds of two amaryllid species that can be successfully cryopreserved, and two dicotyledonous woody species with seeds that are sensitive to cryo treatment, are being studied. The biochemical responses being measured include post-cryo vigour, production of reactive oxygen species and the activity of anti-oxidant enzymes, membrane damage (electrolyte leakage and ACC-oxidase activity), rate of protein synthesis, cytoskeleton integrity, and the profile of heat stable proteins.

2. Give details of any notable problems or unexpected developments that the project has encountered over the last 6 months. Explain what impact these could have on the project and whether the changes will affect the budget and timetable of project activities.

The unexpected damage associated with cotyledon excision, and the unsuitability of bud and nodal segment explants as suitable material for cryopreservation has necessitated a re-think of aspects of the project. The previous report indicated an intention to establish single-cell cultures as possible material for cryopreservation studies. Those based on coffee species are derived from somatic embryos which are subject to somaclonal variation and, because this project is ultimately aimed at biodiversity conservation, we are reluctant to go down that route. A distinct possibility though, and one that should be investigated thoroughly, is the use of the embryonic shoot meristems, as meristem cultures, and/or single cells and/or small groups of cells, Meristem cells are small and compact and may, particularly as single cells, behave more like materials such as sperm or yeast cells with respect to freezing response.

The initial agreement indicated that an output of the project would be a manual giving fairly detailed instructions for the cryopreservation of recalcitrant seeds. However, it is our view that such a manual is not appropriate at the moment. This is based on two factors, (i) because of the problems associated with cotyledon excision we are not yet in a position to provide detailed instructions, and (ii) there is the imminent publication of a book 'Plant Cryopreservation' edited by B. Reed (2008), in which the country partners are co-authors on a chapter on recalcitrant material; and the Project Leader a co-author on a 'recipe' chapter on orthodox seeds.

Have any of these issues been discussed with the Darwin Secretariat and if so, have changes been made to the original agreement?

Not yet, but, considering the interest in the potential of meristems, perhaps such discussions should be entered into.

Discussed with the DI Secretariat: **no/yes, in……. (month/yr)**

Changes to the project schedule/workplan: **no/yes, in…….(month/yr)**

3. Are there any other issues you wish to raise relating to the project or to Darwin's management, monitoring, or financial procedures?

If you were asked to provide a response to this year's annual report review with your next half year report, please attach your response to this document.

Please note: Any planned modifications to your project schedule/workplan or budget should not be discussed in this report but raised with the Darwin Secretariat directly.

Please send your **completed form email** to Eilidh Young, Darwin Initiative M&E Programme at Darwin-Projects@ectf-ed.org.uk . The report should be between 1-2 pages maximum. **Please state your project reference number in the header of your email message eg Subject: 14-075 Darwin Half Year Report**