Darwin Initiative for the Survival of Species

Half Year Report (due 31 October each year)

Project Ref. No.	162/14/056
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
Report date	31st October 2006
Report No. (HYR 1/2/3/4)	HYR 2
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).

Good progress has been made over the last six months. Seeds of more than a further 20 species have been screened, the majority of them proving to be recalcitrant (this does not reflect the incidence of recalcitrance among the seeds of local species, simply that species were selected on the basis of indicators that suggested their seeds may be recalcitrant). Considerable effort has been expended on species of the Amaryllidaceae; the seeds of most of the species investigated proved to be recalcitrant, and excised embryonic axes from seeds of about half of these species could survive cryo treatment (if cryoprotectants are used), with survival greater than 50%. Success has also been achieved with two species of *Strychnos*. The amenability of seeds of members of the Amaryllidaceae and of the endospermous seeds of some *Strychnos* species suggest that the lack of large, reserve-storing cotyledons in these species might contribute to the minimization of damage associated with desiccation and cryo treatment.

Because of the difficulties being experienced with seeds of dicotyledonous woody species, effort is being put into the development of alternative explants (somatic embryos, apical and nodal buds) for species where excised axes do not survive cryo treatment. Currently, protocols for establishment of alternate explants are being developed for six species. It is felt that this aspect of the programme should not be extended to many additional species until it is clear that some success in cryopreservation of somatic embryos or buds can be achieved. A major success has been the ability to produce several buds from roots of *Ekebergia capensis* using the RITA container system

To gain insight into the damage associated with the processes of excision, partial dehydration and freezing associated with cryopreservation, studies on membrane permeability, production of reactive oxygen species and protein synthesis are being undertaken on excised embryos of some amaryllid species, two species of *Trichilia*, and on embryonic axes of *Ekebergia capensis*, a local species that seems to show variation in chilling sensitivity with provenance.

Another approach is being developed to assist in understanding the problems associated with cryopreservation – the use of cell suspension cultures. Although excised embryonic axes of most species are small, they are histologically complex, with a range of tissue types. It is possible that the different tissues are subjected to different degrees of stress, and respond differently to the 'insults' associated with cryopreservation. Cell suspension cultures provide

uniform material that can be investigated without the problems associated with complex embryonic axes. Suspension cultures of different *Coffea* species are being established. This genus has been chosen because there are differences in desiccation tolerance among species, and the technology to establish these cultures is locally available.

The problem of fungal contamination has largely been brought under control by the use of a combination of two systemic fungicides. This has resulted in bacterial proliferation, but this is also controllable. As a consequence of our ability to control (if not eliminate) fungal contamination, we have been able to considerably extend (by a factor of three or four) the short- to medium-term storage like span of seeds of a number of species. The limiting factor in hydrated storage is now the rate of germination of the seeds in storage, which can be reduced by low-temperature storage if the seeds are not chilling sensitive.

The B.Sc. (Hons) module on cryobiology that was introduced in 2005 was repeated in a much improved form in 2006, and we feel that this is now ready for students from elsewhere.

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.

As was mentioned in the previous annual report, and touched on above, we are experiencing more difficulties with tropical dicotyledonous species than originally anticipated. This may force us through the route of alternative explants for cryopreservation. Procedures to produce these explants are time-consuming and laborious and so the rate at which the suitability of cryopreservation for different species can be assessed will be reduced.

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

This matter has not yet been discussed with the Darwin Secretariat, but it may be a sensible step to take.

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?

No

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 <u>planned</u> modifications to your project schedule/workplan or budget should <u>not</u> be discussed in this report but raised with the Darwin Secretariat directly.

Please send your **completed form by 31 October each year per email** to Stefanie Halfmann, Darwin Initiative M&E Programme, stefanie.halfmann@ed.ac.uk . The report should be between 1-2 pages maximum. **<u>Please state your project reference number in the header of your email message.</u>**