Darwin Initiative Annual Report (Year 1)

Cryo-conservation Centre of Excellence for Sub-Saharan Africa (CCESSA)

Profs. Patricia Berjak, Norman Pammenter

(Univ. KwaZulu-Natal, RSA)

and Hugh W. Pritchard (Kew, UK)

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Darwin Initiative Annual Report

1. Darwin Project Information

Project Ref. Number	**14-056**
Project Title	Cryo-conservation Centre of Excellence for Sub-Saharan Africa (CCESSA)
Country(ies)	UK, South Africa
UK Contractor	Royal Botanic Gardens Kew
Partner Organisation(s)	University of KwaZulu-Natal
Darwin Grant Value	£168,852
Start/End dates	April 2005 – March 2008
Reporting period (1 Apr 2005 to 31 Mar 2006) and annual report number (1,2,3..)	1 April 2005 to 31 March 2006, annual report 1
Project website	African Centre for Germplasm Conservation (http://www.ukzn.ac.za/Biology/AfricanGermplasmConservation451.aspx) has a link to the DI.
Author(s), date	Berjak, Pammenter, Pritchard; 26th April 2006

2. Project Background

- The project is located predominantly in the KwaZulu-Natal Province of South Africa. It aims to address the problem that biodiversity conservation of plant species producing recalcitrant seeds is not possible by conventional seeds storage, as recalcitrant seeds are desiccation-sensitive.

3. Project Purpose and Outputs

- The purpose of the project is to develop a centre of excellence for the cryo-conservation of germplasm of species producing recalcitrant seeds. The outputs from this centre will be the development and optimisation of technologies to permit *ex situ* preservation of such species; the training of students in cryo-biology and cryo-conservation; and, ultimately, the establishment of a cryo-bank for the storage of appropriate germplasm, and making this available to users.
- Neither the outputs nor proposed operational plan has been modified over the last year.

4. Progress

- Prior to the initiation of this Darwin project, the project partners (Berjak and Pammenter) had been involved in research into recalcitrant seeds for some 20 years and had elucidated the basic physiology of these seeds. Milestones include the recognition that the on-going metabolism and lack of metabolic switch–off and ultrastructural de-differentiation underlie the desiccation sensitivity of these seeds; the development of the technique of flash- (ultra-rapid) drying to enable drying, without loss of viability, to water contents that may permit non-lethal freezing; and the use of ultra-rapid (non-equilibrium)

freezing to reduce the likelihood of lethal ice crystal formation during cryo procedures. The objective of the present project is to use the information and understanding accumulated over the last 20 years to develop and optimise generic technologies permitting cryopreservation of germplasm of species producing recalcitrant seeds.

- Good progress has been made in most aspects of the project. The storage behaviour of seeds from 30 southern African species has been identified, and nine of these have been subjected to cryopreservation protocols (with three successes). This is somewhat in excess of that anticipated in the logframe, due partly to the popularity of the programme with post-graduate students. The B.Sc. (Hons) level cryobiology module has been implemented, and will be revised according to experience and student assessment. The development of a database that links collection site to South African weather, soils and geology is nearly complete

- This project is more than an empirical approach to improving success in cryopreservation. It includes basic studies on the phenomena of desiccation sensitivity vs tolerance, and cellular responses to freezing using different freezing techniques. This is to enable optimisation of cryo-protocols to be made on a sound knowledge base, rather than simply empirically. At the research level achievements include: the initiation of studies on the responses of the cyto- and nucleo-skeletons to drying of both recalcitrant and orthodox seeds; the production of reactive oxygen species resulting from cotyledon excision, drying and freezing; and DNA extraction so as to enable assessment of genetic consequences of cryopreservation protocols (because the presence of cotyledonary reserves impairs DNA extraction, this work has centred on members of the Amaryllidaceae). Studies on the localisation and size of inter-cellular ice crystals following different freezing protocols, and the effect of cryo-protectants on this, are also underway. A problem associated with working with recalcitrant seeds is the ubiquitous fungal contamination, some of which is internal, in addition to surface contaminants. A series of systemic fungicides, as well as the potential for biocontrol using *Trichoderma* spp, is being investigated to deal with this problem. From a training perspective, the B.Sc.(Hons) level cryobiology module has been initiated and run, and post-graduate training in cryopreservation methodology has been provided to three visiting students/researchers from Africa (A. Ajayi, Nigeria; M. Quain, Ghana and H.P. Msanga, Tanzania). In terms of assessment and monitoring of the project, achievement of successful cryopreservation is the yard-stick. (At this stage it is important to point out that by 'success' we mean the ability of germplasm subjected to cryopreservation to establish independent plants, rather than simply tissue greening or expansion that many researchers use.) Assessment of the training aspect is by the numbers of students trained at various levels (six week training course, post-graduate degrees.

- One thing that has become apparent is that the success rate in the cryopreservation of tropical species is considerably less than that of temperate species. The reason for this is unknown; to elucidate this, future studies will incorporate a temperate species known to be amenable to cryopreservation in parallel with the test tropical species to assess at what stages of the procedures failure occurs. Most of the cryopreservation work by the South African partners prior to the initiation of the Darwin project was carried out on three species of the Meliaceae; *Trichilia dregeana, T. emetica* and *Ekekbergia capensis* (this was not intentional, simply that seeds in sufficient quantities were readily available from these species). Although excised embryonic axes could produce a root subsequent to cryopreservation, axes of all three species failed to produce shoots. This is probably a consequence of physical damage associated with the excision of the cotyledons required to produce an explant of suitable size for cryopreservation. However, it has been shown that if cryopreserved axes of *Ekebergia capensis* are incubated on a medium containing benzyl adenine (a synthetic cytokinin), multiple shoot production occurs. Although this does introduce an additional step in cryopreservation procedures, the problem can be overcome. Additionally, encapsulating excised axes in alginate beads prior to cryopreservation is showing promise.

- The design of the project has not been modified substantially.

Work plan for the next reporting period:

- *o* A further at least 30 species will be screened to establish the storage category of their seeds.
- *o* At least nine of those found to be recalcitrant will be subjected to cryopreservation protocols.
- *o* Some temperate species (where available) whose response to cryo procedures has been assessed (by other workers) by tissue greening or expansion, will be subject to our cryopreservation and assessment protocols.
- *o* In some cases it may not be possible to cryopreserve zygotic embryonic axes. Alternative explants (buds, somatic embryos) will be generated and subjected to cryo- protocols.
- *o* The potential for the production of 'synthetic seeds' (alginate encapsulation of the explant prior or subsequent to freezing) will be further investigated.
- *o* The effect of cryopreservation on the vigour and stress tolerance of recovered plantlets will be assessed.
- *o* The B.Sc. (Hons) level cryobiology course will be refined.
- *o* Basic scientific studies will be pursued. These include work on the cyto- and nucleo-skeletons, the production and potential control of reactive oxygen species, development of molecular techniques to assess potential effects of cryopreservation on genetic fidelity, and studies on the size and location of ice crystals formed in response to different freezing techniques.
- *o* Many species of the family Amaryllidaceae are heavily utilised in traditional practices and are endangered. Also the 'germination' process of this family (initial protrusion of a cotyledonary body which encloses the embryonic axis) permits simple excision of the axis, and, importantly, permits DNA analysis of the same seed before and after cryopreservation. For these reasons considerable attention will be paid to this family.

5. Actions taken in response to previous reviews (if applicable)

- N/A

6. Partnerships

- Collaboration between the UK and host country partners is excellent. Dr Wood visited RSA from 10 – 15 July 2005 to discuss management arrangements with the UK, to review facilities, discuss target species and student numbers / arrangements. The resignation of the initial UK project leader (Dr C. Wood) has had no adverse effect as the new leader (Prof H. Pritchard) is fully conversant with the project. Prof Pritchard met with Prof Berjak in Accra, Ghana on 26 March 2006 to discuss progress on the CCESSA project just prior to the DIRECTS meeting in Kumasi, Ghana (27-31 March 2006).
- Promoting partnerships was a major theme of the recent DIRECTS - Millennium Seed Bank Project conference in Ghana (27-31 March 2006). The work of the CCESSA project was given prominence in two keynote presentations, reaching an audience of nearly 50 scientists and technologists from 16 African countries, including representatives of IUCN, OECD and IPGRI (see list of attendees in Appendix 1). The presentations were on: 'African tree seed science' by Patricia Berjak; and 'AU Africa's Consolidated Plan for Science and Technology: the role of seed conservation science' by Patricia Berjak and Hugh W. Pritchard (this latter presentation is included in as Appendix 2).

7. Impact and Sustainability

- Within the professional botanical community in South Africa, the project has a high profile. We have initiated contact with the Co-ordinator of the Biosciences Initiative of the Science Desk of the New Partnership for Africa's Development, but are awaiting a response. Post-graduate students passing through the programme will have acquired expertise in cryopreservation; as this project is directly aimed at biodiversity conservation, it will concomitantly increase awareness of an interest in biodiversity amongst its graduates. The exit strategy outlined in the original application still stands.

8. Outputs, Outcomes and Dissemination

- Outputs, particularly in terms of scientific achievements and training, are in line with, or even exceed those in the 'Project Implementation Timetable' and 'Project Outputs Schedule'. If there is a lag it is in the publicising of the project.
- Dissemination has been mainly at the level of scientific meetings and congresses. Berjak presented the programme and the Darwin project at the seed section of the South African Association of Botanists, which was attended by conservation biologists, as well as seed scientists (local dissemination). She presented two keynote addresses at the Darwin DIRECTS meeting in Ghana (dissemination amongst African seed biologists) and addressed the TWAS - Academy of Sciences for the Developing World annual meeting (international dissemination). Contact with the Science Desk of NEPAD has been initiated.
- Quantification of project outputs: Note that the number of post-graduate students in training in Table 1 (Output 4C) is less than that given in Annex 1. This is because a number of students joined the project in January or February 2006 and have not been included in Table 1.

Table 1. Project Outputs (According to Standard Output Measures)

Code No.	Description	Year 1 Total	Year 2 Total	Year 3 Total	Year 4 Total	TOTAL
2	M.Sc. degree	1				
3	B.Sc.(Hons) degree with seed-associated project	8				
4C	Post-graduate students in training, 2005	5				
4D	Training weeks per student	40				
6A	Visiting scientist from Africa given cryopreservation experience	3				
6B	Average training weeks per visitor	4				
8	One visit by UK project leader	1				
11A	Papers published in peer reviewed	1				

	journals	
11B	Papers submitted to peer-reviewed journals	2
15B	Release to UKZN in-house publication	1
15C	Release in Kew publications	2

Table 2: Publications

Type * (e.g. journals, manual, CDs)	Detail (title, author, year)	Publishers (name, city)	Available from (e.g. contact address, website)	Cost £
Journal	Perán R, Berjak P, Pammenter NW and Kioko JI (2006). Cryopreservation, encapsulation and promotion of shoot production of embryonic axes of a recalcitrant species *Ekebergia capensis* Sparrm.	CryoLetters 27, 5-16	Country partners	nil

9. Project Expenditure

10. Monitoring, Evaluation and Lessons

- The purposes of this project are basically two-fold – to develop technologies for the cryo-conservation of the germplasm of species producing recalcitrant seeds, and to build capacity in sub-Saharan Africa in this field. The outputs can almost be quantified in terms of the number of species successfully cryopreserved, and the number of people undergoing training. In the first year of this project the training was confined to B.Sc. (Hons) students at the country partners' university (University of KwaZulu-Natal) who enrolled for the cryobiology module. When students from elsewhere in Africa take this module, the effectiveness of the training will have to be evaluated. The model of Reed *et al.* (2004, *CryoLetters* **24**, 341-352) may be useful in this context.
- Based on discussions at the DIRECTS – MSBP workshop (Ghana, 27-31 March 2006) there are clearly substantial challenges with respect to science and technology take-up across Africa. It is the intention of the project management team to be more proactive in promoting S&T on cryo and seed conservation science in general. Preliminary discussions (26 March and 1 April 2006) have been held in Accra with Dr Karl Harmsen, DG of the United Nations University Institute of Natural Resources for Africa (UNU-INRA), about running a short course in seed conservation science theory and practice for about 12 Africans in Durban in January 2007. This is subject to extra funding, of course.

11. OPTIONAL: Outstanding achievements of your project during the reporting period (300-400 words maximum)

■ **I agree for ECTF and the Darwin Secretariat to publish the content of this section**

In this section you have the chance to let us know about outstanding achievements of your project over the year that you consider worth highlighting to ECTF and the Darwin Secretariat. This could relate to achievements already mentioned in this report, on which you would like to expand further, or achievements that were in addition to the ones planned and deserve particular attention e.g. in terms of best practice. The idea is to use this section for various promotion and dissemination purposes, including e.g. publication in the Defra Annual Report, Darwin promotion material, or on the Darwin website. As we will not be able to ask projects on an individual basis for their consent to publish the content of this section, please note the above agreement clause.

In the first year, this DI project has enabled Profs. P. Berjak, N. Pammenter (RSA) and Hugh W. Pritchard (UK) to establish a Cryoconservation Centre of Excellence for Sub-Sahara Africa (CCESSA) in KwaZulu Natal (RSA). This centre has already provided capacity building to 17 African students and visitors and produced one peer-review paper. Such advances strongly support the call made in the AU Africa's Science and Technology Consolidated Plan of Action for centres of excellence in conservation science with state-of-the art research facilities.

Annex 1 Report of progress and achievements against Logical Framework for Financial Year: 2005/2006

Project summary	Measurable Indicators	Progress and Achievements April 2005-Mar 2006	Actions required/planned for next period
Goal*:* To draw on expertise relevant to biodiversity from within the United Kingdom to work with local partners in countries rich in biodiversity but poor in resources to achieve • The conservation of biological diversity, • The sustainable use of its components, and • The fair and equitable sharing of the benefits arising out of the utilisation of genetic resources			
Purpose *(insert original project purpose statement)*	*(insert original purpose level indicators)*	*(report impacts and achievements resulting from the project against purpose indicators – if any)*	*(report any lessons learned resulting from the project & highlight key actions planning for next period)*
The establishment of a Centre of Excellence for cryo-banking for sub-Saharan Africa.	Number or requests for research and training placements at CCESSA from African and other students	Considerable interest shown at the recent Darwin DIRECTS programme meeting, Kumasi, Ghana	The project, and particularly the training aspect, require higher profile advertising in Africa
The development and embedding of 'generic' technologies for *ex situ* collection, storage and utilisation of plant species producing recalcitrant seeds.	Inward investment (grants) in CCESSA from national and international agencies. Techniques and technologies applied to non-target species by other groups.	Grant received from International Plant Genetic Resources Institute; too early for other groups to use techniques developed in this programme.	

Outputs			
(insert original outputs – one per line)	*(insert original output level indicators)*	*(report completed activities and outcomes that contribute toward outputs and indicators)*	*(report any lessons learned resulting from the project & highlight key actions planning for next period)*
Recalcitrant-seeded species in cryo-storage (conserved) and utilisable through propagation and 'extension' activities	Facility up and running and handling > 15 difficult to store (conventionally) species in 3 years, with 5 species reaching the nursery stage ex vitro.	Using the '100 seed test' developed by MSB, 30 'likely' species have been screened and confirmed to be recalcitrant. Of these, nine have been subjected to cryo-preservation. Greater than 60% survival was recorded in three species, 30 – 60% in a further two species, and failure in four species.	Work on geophytes, particularly of the family Amaryllidaceae, must be extended
Staff and students (particularly from Africa) trained in cryo-biology (both on 6 week honours and post-graduate courses).	Over 3 years, > 10 post-docs and / or graduate students (MSc to Post-doc) given specialised training (6 training weeks per year) and / or research project guidance (continuous, throughout project).	B.Sc. (Hons) module on cryobiology implemented. Currently one post-doctoral, three Ph.D. and 14 M.Sc. students are registered through the programme.	Honours module requires some modification before it is suitable for external students. Need to recruit post-graduate students from the rest of Africa.
Cryo-preservation technologies refined, through research and made available.	(Y3) Cryo-preservation modules released as hardcopy / electronically, following review of market need; (Y2) 4 publications submitted to ISI-accredited journals.		

Long term ex situ species conservation strategies developed and implemented.	> 45 species collected and evaluated for desiccation tolerance over 3 years; any conventionally bankable species conserved in the Millennium Seed Bank.	Good progress being made with evaluation of species.	As an increasing proportion of species are evaluated, attention will shift from screening to refinement of cryo protocols

Note: Please do NOT expand rows to include activities since their completion and outcomes should be reported under the column on progress and achievements at output and purpose levels.

LOGICAL FRAMEWORK

19. Please enter the details of your project onto the matrix using the note at Annex B of the Guidance Note. This should not have substantially changed from the Logical Framework submitted with your Stage 1 application. Please highlight any changes.

Project summary	Measurable Indicators	Means of verification	Important Assumptions
Goal: To draw on expertise relevant to biodiversity from within the United Kingdom to work with local partners in countries rich in biodiversity but poor in resources to achieve • the conservation of biological diversity, • the sustainable use of its components, and • the fair and equitable sharing of benefits arising out of the utilisation of genetic resources			
Purpose The establishment of a Centre of Excellence for cryo-banking for sub-Saharan Africa. The development and embedding of 'generic technologies' for ex situ collection, storage and utilisation of plant species producing recalcitrant seeds.	Number of requests for research and training placements at CCESSA from African students and from elsewhere. Inward investment (grants) in CCESSA from national and international agencies. Techniques / technologies applied to non-target species by other groups.	Univ. KZN Annual Report; Independent audit reports, e.g. by IPGRI; NRF Annual Report; RBG Kew / MSBP Annual Report. Peer-review papers and other forms of scientific articles / reports.	Institutional support is sustained, resources are not limiting to delivery, and partnerships continue. New protocols are seen as a valuable component of CBD-related conservation action; students / staff apply knowledge routinely on return to their institutes.
Outputs Recalcitrant-seeded species in cryo-storage (conserved) and utilisable through propagation and 'extension' activities.	Facility up and running and handling > 15 difficult to store (conventionally) species in 3 years, with 5 species reaching the nursery stage ex vitro.	University KZN records; database entries, and greenhouse and/or field evaluations of performance of plants established from cryo-preserved explants.	Protocols developed are effective and serve as 'exemplars' for other stakeholders. Sufficient material can be made available to sustainable utilisation projects.
Staff and students (particularly from Africa) trained in cryo-biology (both on 6 week honours and post-graduate courses).	Over 3 years, > 10 post-docs and / or graduate students (MSc to Post-doc) given specialised training (6 training weeks per year) and / or research project guidance (continuous, throughout project).	UKZN Science Faculty handbook; Review of successfully completed student theses.	Wide interest by staff / students across Africa for training. Theses available for consultation.
Cryo-preservation technologies refined, through research and	(Y3) Cryo-preservation modules released as hardcopy / electronically, following review of market	Review IPGRI list of publications / Kew - MSBP web site; Consult reprints/	Optimisation of methods is possible.

made available.	need; (Y2) 4 publications submitted to ISI-accredited journals.	preprints of publications submitted and review journal contents pages.	Information as presented meets stringent publication requirements.
Long term ex situ species conservation strategies developed and implemented.	> 45 species collected and evaluated for desiccation tolerance over 3 years; any conventionally bankable species conserved in the Millennium Seed Bank.	Data entered into project data base and, once verified, into the Seed Information Data base on the WWW.	Data standards are to international standard and information is used by appropriate agencies e.g. IUCN, IPGRI.

APPENDIX 1: LIST OF PARTICIPANTS/INVITEES (DIRECTS –MSBP WORKSHOP – GHANA)

No.	*NAME*	*ROLE*			*COUNTRY*
	INVITEES				
1	*Dr. Oscar Eyog-Matig*	*SAFORGEN co-ordinator*			***Benin***
2	*Mr. Dah-Dovonon Z. Jean*	*Researcher*			***Benin***
3	*Masego Kruger*	*PhD student*			***Botswana***
4	*Mr. Oble Neya*	*PhD student*			***Burkina Faso***
5	*Ms. Edith Daboue*	*Researcher*			***Burkina Faso***
6	*Sibidou Sina*	*PhD student*			***Burkina Faso***
7	*Dr. Edmond Kouablan KOFFI*	*Research Officer*			***Cote d' Ivoire***
8	*Anatole K N. Guessan*				***Cote d' Ivoire***
9	*Mr. Guy M. Gnahoua*	*Research Officer*			***Cote d' Ivoire***
10	*Abeje Eshete Wassie*	*Project Coordinator*			***Ethiopia***
11	*Mr. Desterio Nyamongo*	*Research Officer*			***Kenya***
12	*Mrs. Lolona Ramamonjisoa*	*Head of Production Department*			***Madagascar***
13	*Dr. Guy Rakotondranony*	*Research Officer*			***Madagascar***
14	*Mr. Maganiso Namoto*	*Ag. Seed Centre Manager*			***Malawi***
15	*Mr. Chanyenga Tembo F.*	*P.F.R.0. Seed*			***Malawi***
16	*Mr. Sidi Sanogo*	*Research Scientist*			***Mali***

17	*Mr. Mahamane Larwanou*	*Research Scientist*			***Niger***
18	*Dr. Julius Olaoye Faluyi*	*Leader*			***Nigeria***
19	*Dr. S. Adesola Ajayi*	*Senior Lecturer*			***Nigeria***
20	*Dr. Y.A.O. Olaniran*	*Coordinator*			***Nigeria***
21	*Sershen Naidoo*	*PhD Student*			***South Africa***
22	*Patricia Berjak*	*Researcher*			***South Africa***
23	*Mr. Ludovick Uronu O.N.*	*Technical Unit Manager*			***Tanzania***
24	*Heriel P. Msanga*	*Chief Executive*			***Tanzania***
25	*Mr. Boundjouw Sama*	*Manager*			***Togo***
26	*Stephen Khaukha*	*Manager National Tree Seed Centre*			***Uganda***
27	*Prof. Hugh Pritchard*	*Head of Research; Leader of DIRECTS and CCESSA Projects*			***United Kingdom***
28	*Dr. Moctar Sacande*	*International co-ordinator; DIRECTS Project Manager*			***United Kingdom***
29	*Dr. Paul Smith*	*Head of Department & MSB Project Leader*			***United Kingdom***
	FORIG INVITEES				
30	*Mrs. Theresa Peprah*	*Research Scientist*			***Ghana***

31	*Mr. Joseph Mireku Asomaning*	*Research Scientist*			***Ghana***
32	*Dr. J.R. Cobbinah,*	*Director*			***Ghana***
33	*Dr. J. Ofori, Deputy Director*	*C.R.S.*			***Ghana***
34	*Dr. Victor Agyemang*	*Research Scientist*			***Ghana***
35	*Mrs. Augustina Gyimah*	*P.R.S.*			***Ghana***
36	*Dr. Daniel A. Ofori*	*Senior Research Scientist*			***Ghana***
37	*Dr. Dominic Blay*	*Senior Research Scientist*			***Ghana***
38	*Dr. Ernest Foli*	*Senior Research Scientist*			***Ghana***
39	*Mrs. Mary Apetorgbor*	*Research Scientist*			***Ghana***
40	*Mrs. Sraku-Lartey*	*Research Scientist*			***Ghana***
41	*Ms. Jacqueline J. Twintoh*	*Technician*			***Ghana***
42	*Mr. Addo-Danso Shalom*	*Technician*			***Ghana***
	KNUST				
43	*Dr. Mrs. Sakyiwaa Olympio*	*Senior Lecturer*			***Ghana***
44	*Dr. Kyere Boateng, FRNR, KNUST*	*Senior Lecturer*			***Ghana***
45	*Dr. Steve Amisah, FRNR, KNUST*	*Senior Lecturer, Dean*			***Ghana***
46	*Dr. Bennet Lartey, University College of Winneba, Winneba.*	*Chief Research Scientist*			***Ghana***
47	*Dr. L. Aboagye*	*Senior Research Scientist*			***Ghana***
48	*Mr. Samuel Kofi*	*Project*			***Ghana***

	*Nyame (**IUCN**), Accra*	*Coordinator*			
49	*Mr. Kester K. Mensah*	*Protocol*			***Ghana***

APPENDIX 2: Powerpoint presentation on –

'Africa's Science & Technology Plan of Action: how does the practice of seed conservation science fit in?' Profs. Patricia Berjak and Hugh W. Pritchard

APPENDIX 3: Peer-reviewed publication from Year 1.

Perán R, Berjak P, Pammenter NW and Kioko JI (2006). Cryopreservation, encapsulation and promotion of shoot production of embryonic axes of a recalcitrant species *Ekebergia capensis* Sparrm. *CryoLetters* **27**, 5-16.