Cryo-conservation Centre of Excellence for sub-Sahara Africa (CCESSA) 14-056

Final Report: Volume 1

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Darwin project information

Project Reference	14-056
Project Title	Cryo-conservation Centre of Excellence for Sub-Saharan Africa (CCESSA)
Host country(ies)	South Africa
UK Contract Holder Institution	Royal Botanic Gardens Kew
UK Partner Institution(s)	Royal Botanic Gardens Kew
Host Country Partner Institution(s)	University of KwaZulu-Natal
Darwin Grant Value	£168,852
Start/End dates of Project	April 2005 – March 2008
Project Leader Name	H.W. Pritchard
Project Website	www://ukzn.ac.za/plantgermcons
Report Author(s) and date	P. Berjak, N.W. Pammenter, H.W. Pritchard; 26th June 2008

Darwin Initiative – Final Report

*(To be completed with reference to the Reporting Guidance Notes for Project Leaders (http://darwin.defra.gov.uk/resources/reporting/) - it is expected that this report will be a **maximum** of 20 pages in length, excluding annexes)*

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1 Project Background

The project was located predominantly in the KwaZulu-Natal Province of South Africa. It aimed 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. The immediate objective was to develop the technology to cryopreserve seeds, embryonic axes or other suitable tissue of these species. This has been achieved with a number of species (mostly non-graminoid monocotyledonous). A recent achievement has been the successful cryopreservation of meristems of a woody species, an approach that shows considerable potential where cryopreservation of embryonic axes has not been possible

2 Project support to the Convention on Biological Diversity (CBD)

The project was specifically aimed at contributing to the CBD 2010 Biodiversity Target through Target 8 of the Global Strategy for Plant Conservation (60% of threatened plant species to be in accessible *ex situ* collections and technology development for species with recalcitrant seeds). The project has developed the technology for the successful cryopreservation of tissues of some plant species producing recalcitrant (unstorable) seeds (see below for details). As many of the species producing recalcitrant seeds are forest species, the programme has indirectly contributed to the Forest Biodiversity theme. It has also had an impact on the Technology Transfer and Co-operation cross-cutting issue through training and co-operation with seed scientists from three African countries.

3 Project Partnerships

Collaboration between the UK and host country partners is excellent, and has been from the beginning of the project. The partnership was established on the basis of common interest and problems, and all parties were involved in project conceptualisation and planning. Day-to-day management has been largely the responsibility of the host country partners. A particular strength of the partnership has been that the partners were well

acquainted with each other and each others' work before the commencement of the project. Thus the project was a natural extension of a pre-existing relationship and was in no way 'forced'.

4 Project Achievements

4.1 Impact: achievement of positive impact on biodiversity, sustainable use or equitable sharing of biodiversity benefits

The impacts of this project have been both scientific/technical, and social/political. At the scientific/technical level we have demonstrated that it is possible to cryoconserve tissue of recalcitrant-seeded species, but that the amenability to cryopreservation varies among species. Cryopreservation of embryonic axes of some species is feasible, but is not possible for dicotyledonous species with fleshy cotyledons (due to unavoidable excision damage), and meristems are likely to be the most suitable material. The project contributes directly to the CBD 2010 Target 3.1 in that it enhances our ability to conserve genetic diversity. Similarly it contributes to the potential for achieving Target (viii) of the Global Strategy for Plant Conservation (60% of threatened species in *ex situ* collections). Strategic Objective 3.1 of the South African National Biodiversity Strategic Action Plan notes the importance of gene banks in conserving biodiversity, and the 3rd National Report mentions the co-operation between the South African National Biodiversity Institute and the Millennium Seed Bank in storing seed of some South African species. However, these are orthodox seeds; what the present project has done is to enhance the possibility of cryo-storage of tissues of recalcitrant seeded species, and so extend the range of species that can be stored.

The social/political impacts of the project were not initially envisaged, but being DI grant-holders permitted us to take the opportunities to raise awareness among decision makers of the contribution seed storage can make towards biodiversity conservation, and the difficulties associated with recalcitrant seeds. Examples of these impacts include the following.

(i) Two presentations were made at the DIRECTS meeting, Kumasi, Ghana (2006).

(ii) A presentation and display were delivered at the African Union(AU)/ New Partnership for African Development (NEPAD) meeting of Heads of State and Ministers of Science and Technology meeting, Addis Ababa (2007).

(iii) Berjak (host country project co-leader) attended the Economic Commission for Africa: Science with Africa meeting where she made a presentation and displayed a number of posters highlighting the DI project (2008)

(iv) Berjak has become involved with the New Partnership for African Development (NEPAD) Southern African Network for Biosciences (SANBio). She sits on a task force aimed at enhancing the capabilities for conservation of plant genetic resources, including cryopreservation of tissues of recalcitrant-seeded species.

(v) CCESSA, with mention of the Darwin Initiative, is covered in the publication Profiles of Institutions for Scientific Exchange and Training in the South, 4th edition (2007), published by TWAS, the Academy of Sciences for the Developing World.

(vi) We have signed a Memorandum of Understanding with the National Zoological Gardens of South Africa Wildlife Biological Resource Centre (NZG/wBRC). The wBRC cryo-stores certain animal tissues, and the realisation that many of the problems of cryoconservation are common to plants and animals has led to this MoU. The ultimate objective is to establish a national cryo-bank encompassing the entire spectrum of biological organisms.

(vii) Negotiations are currently underway for the establishment of a Biodiversity Park to the north of Durban. We are proposing (a proposal that has been well received) that a seed banking facility be included in the biodiversity conservation activities of the park.

Although these impacts were not presented in the original proposal, the DI was highlighted and in fact made many of these activities possible.

4.2 Outcomes: achievement of the project purpose and outcomes

The project has, to a large extent, achieved its purpose and outcomes. Although not formally constituted, the project has significantly contributed towards the establishment of the partnership as a centre of excellence for cryo-conservation of recalcitrant seeded species. The project is also beginning to have social/political impact. It has not attracted students from the rest of Africa, probably partly because of funding issues. Similarly, a 'recipe' style manual has not been produced. This has been because it proved more difficult than anticipated to cryopreserve excised embryonic axes, and no success was achieved with nodal explants. As such, the project has not reached the stage at which such a manual can be produced. However, the current thinking of the project team on the cryopreservation of desiccation-sensitive and -tolerant seeds appeared as chapters of a book edited by Reed (2008) – see list of publications.

4.3 Outputs (and activities)

To some extent the outputs have been achieved. The seeds of 69 species have been screened, and 51 of these proved to be recalcitrant, and of these, excised axes of 28 have been successfully cryopreserved (see attached list). The reason that only six of these species have as yet been banked is one of seasonal availability of seeds.

A problem that was encountered was the unexpected difficulty posed by seeds with fleshy cotyledons, which would not produce shoots, even immediately after excision without the manipulations required fro cryopreservation. This problem was addressed by using explants alternative to excised axes (see section 4.5 for details).

The B.Sc. (Hons)-level cryobiology module has been developed and refined and in total 16 students have taken the module

4.4 Project standard measures and publications

See Annexures 4 and 5, respectively, for details.

4.5 Technical and Scientific achievements and co-operation

The host country staff complement of the project consists of two co-leaders (part-time), one full-time and one part-time assistant and a variable number of Masters and Doctoral students.

When the project was initiated, some success had already been had with excised embryonic axes of temperate recalcitrant seeded species, using the techniques of very rapid ('flash') drying to water contents that were not lethal but would permit very rapid cooling in liquid nitrogen, without ice crystal damage, thereby achieving successful cryopreservation. The basic assumption was that it would require only modifications to the protocol to similarly cryopreserve axes from tropical and subtropical recalcitrant seeded species.

It became apparent early in the project that this assumption was incorrect; it was found for several species, particularly those with fleshy cotyledons, that although germination, in terms of radicle growth, after cryopreservation, was observed, no shoot growth occurred. Even immediately after excision, prior to treatment for cryopreservation, excised axes would not produce shoots. Treatment of such cryopreserved axes in tissue culture with benzyl adenine did lead to adventitious bud formation, but this approach requires further investigation because of considerable variability, even within the same species, in the response to this growth regulator. It was shown that the physical damage associated with

the excision of the cotyledons led to prolonged production of reactive oxygen species (ROS) which probably severely damaged the apical meristem. Shoot production will occur if part of the cotyledon is left attached to the axis, but this provides an explant that is too large for rapid drying and cooling, and furthermore the cotyledon remnants rupture on rapid cooling, releasing reactive oxygen species.

In an attempt to overcome this problem of ROS production a major multi-factorial experiment involving excision in anti-oxidant solutions followed by short-term drying and treatment of the sites of excision with antioxidant powders was performed, but no shoot production occurred in any treatment. However, current work using a glycerol pre-treatment appears very promising (see below).

The second approach to the problem of shoot meristem damage is to use explants other than excised embryonic axes. This approach is also the only feasible one in the case of species that produce seeds with embryonic axes that are too large for rapid drying and cooling. Consequently, considerable effort has been expended on the production and culturing of alternative explants, particularly nodal segments and shoot meristem tips. Cryopreservation of nodal segments has proved to be unsuccessful, largely because the water content of these explants is very high, and drying to suitable water contents is generally lethal. An additional problem is that explants that are small enough for cryopreservation are too small to develop *in vitro*. A range of cryoprotectants and cooling rates has been tried, but without success.

Recently success has been achieved with meristem culture cultures of *Trichilia emetica* and *T. dregeana*, species which are very susceptible to damage on excision of the cotyledons. Although the process of meristem culture is laborious, this is considered to be a major breakthrough as no other explant proved suitable for cryopreservation. We are sanguine that this approach will be successful with other species susceptible to excision damage.

Success has been achieved with the cryopreservation of excised axes of a number of species of the Amaryllidaceae. Seeds of this family ‘germinate’ by initially protruding the cotyledonary body, with the embryonic axis enclosed in this structure. Axes can thus be excised with a small portion of the cotyledonary body, thereby avoiding the excision damage associated with removing fleshy cotyledons.

A major problem in the past has been the high incidence of fungal contamination of (especially tropical) recalcitrant seeds, with this contamination often being internal as well as surface. However, judicious use of a number of fungicide ‘cocktails’, combining systemic and topical fungicides, largely has resolved this problem.

Although this project, perforce, has a certain amount of empiricism in it, it also includes basic studies on the phenomena of desiccation sensitivity vs tolerance, and cellular responses to freezing using different freezing techniques. An aspect receiving some attention is the biochemical response of axes to rates of drying and freezing, to assess the extent that these can ‘explain’ the observed physiological response(vigour and viability). The biochemical parameters being investigated include ROS production, anti-oxidant activity, protein synthesis, membrane integrity, lipid peroxidation and cyto- and nuclear skeleton integrity. We have found glycerol to be the most successful cryoprotectant with most species, and so studies are underway on the influence of glycerol on a range of biochemical activities and ultrastructural status.

Also of interest is the variation between seeds of the same species of different provenances. Seeds of *Ekebergia capensis* from a latitudinal gradient from warm temperate to tropical show differences in chilling sensitivity, and molecular studies show that these are genetically distinct populations, verging on sub-species. In a related project the influence of hardening of explants to various stresses, on response to cryopreservation is being assessed

Preliminary molecular studies are being undertaken to assess the potential for genetic ‘selection’ by the cryopreservation procedures. It is proving difficult to get clean DNA from

the cotyledons of many species, and so the work has been confined to members of the Amaryllidaceae. Results are pending on the acquisition of suitable primers

Studies on growth rates and stress sensitivity of plants derived from explants retrieved from cryopreservation have been undertaken. Many of the steps in the cryotreatment, especially treatment with fungicides, partial drying and cooling to cryogenic temperatures, are themselves potentially injurious, and it has been found that plants produced by embryonic axes retrieved from cryogenic storage are initially of reduced vigour. Whilst not a limiting factor in the development of cryopreservation protocols, this low-vigour phenomenon could impose some constraints on the re-establishment and re-introduction phases of the cryoconservation procedures.

The outputs of these studies are subject to peer review when submitted for publication. To date seven papers have been published and several more will appear over the next two years. The DI will be acknowledged in these papers.

4.6 Capacity building

The capacity building component of this project lies mainly in training and human resource development. Several (approaching 20) post graduate students have been or are involved in projects associated with this DI project. Additionally, a number of undergraduates have been given work experience during the vacations. One of our Masters graduates is about to take up a position as a cryo-biologist at the wBRC.

4.7 Sustainability and Legacy

The achievements most likely to endure are the development of the technology for cryopreservation of tissues of recalcitrant seeded species. It is hoped that the social/political impact will be just that – it will have a long-lasting effect on policy decision makers.

The staff (one full-time, two part-time) are on short term contracts and are aware of the limited duration of the project. We will try to raise funds to retain them, as the project will continue after the end of DI support. The UK and host country partners will remain in contact as there is the common interest in seed science.

A potential problem of a project of this nature is sustainability and succession. The ultimate aim of the programme is the establishment of a cryo-bank. This, however, this is not the provenance of a university, and this is part of the reason that we have established an MoU with the Wildlife Biological Resource Centre. This Centre is part of a parastatal organization (National Zoological Gardens) and is the ideal body to curate such a gene bank.

5 Lessons learned, dissemination and communication

Perhaps one lesson from the experience of this project is not to let it get too big. Although there were two co-leaders in the host country, they were part-time and at times it was rather demanding looking after so many post-graduate students.

Information relating to project achievements has been through the scientific literature, but additionally there has been considerable exposure to parastatal organisations (see section 4.1). Dissemination will certainly continue through papers in the scientific literature, and less formally through the NEPAD/SANBio connection

5.1 Darwin identity

The Darwin Initiative was publicised through acknowledgements in published papers (and will continue to be so). Exposure was also given to the DI on posters presented at both national and international meetings.

In our instance the Darwin Initiative was a major component of an on-going programme, and has certainly increased the achievements and outputs of the host country.

Locally, the DI is well known amongst conservation biologists

6 Monitoring and evaluation

No major changes were made to the project design and the logframe was not altered.

The logframe indicators were developed during project design and have been useful in terms of ensuring that activities were in line with the initial purpose and objectives of the project. In the case of this project, monitoring and evaluation was confined to half-yearly and annual reports.

The National Research Foundation of South Africa operates a system by which it rates academic researchers. During the course of the project Berjak was awarded an A rating. Pammenter has re-applied for a rating and the outcome is still pending

6.1 Actions taken in response to annual report reviews

The only serious criticism in any of the reports concerned the dissemination of the project and its outcomes. Advantage has been taken of a number of opportunities to publicise the project (see points i to v in section 4.1). Particularly, the involvement with the SANBio task force on conservation of plant genetic resources will increase the exposure of the project in southern Africa.

7 Finance and administration

7.1 Project expenditure

Darwin CCESSA Totals for Project to Date – Final

Current Year's Costs	**Grant**	**Claimed**	**Unclaimed**	**Variance**
Staff costs				
Rent, rates, heating, lighting, cleaning				
Postage, telephone, stationery				
Travel and subsistence				
Printing				
Conferences, seminars etc				
Capital items				
Others (please specify)				
TOTAL				

Variance for 'travel and subsistence' and for 'conferences and seminars' are related. There was a wish to make regular visits to the project team in country (increased expense), which was counterbalanced by lower costs for conference attendance. In the latter case however, promotion of CCESSA at meetings was not hindered as all opportunities were taken to use funds from other projects. As result CCESSA had an excellent presence at conferences (***see list of posters and conferences attended in Volume 2***).

7.2 Additional funds or in-kind contributions secured

Bioversity International (formerly IPGRI): USD 3,000 (not handled by project team in UK).

7.3 Value of DI funding

The Darwin Initiative grant has permitted much more rapid progress with the project, particularly with respect to salaries, as this has created available time. The funds for laboratory consumables have made an important contribution to the cost of running the laboratory. The DI has also given some status to the grant holders, which has facilitated the activities considered that have led to social/political impacts.

Annex 1 Report of progress and achievements against final project logframe for the life of the project

Project summary	Measurable Indicators	Progress and Achievements April 2007 - March 2008	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 constrained 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		(report on any contribution towards positive impact on biodiversity or positive changes in the conditions of human communities associated with biodiversity eg steps towards sustainable use or equitable sharing of costs or benefits)	(do not fill not applicable)
Purpose The establishment of a Centre of Excellence for cryo-banking for sub-Saharan Africa.	Number of requests for research and training placements at CCESSA from African students and from elsewhere.	Presentations at AU/NEPAD Heads of State Meeting, Addis Ababa, Presentations at ECA Science in Africa meeting, Addis Ababa Establishment of an MOU with NZG/wBRC that will lead to a national germplasm cryobank Involvement with the NEPAD SANBio task force on enhancement gene banking capabilities	(Highlight key actions planned for next period)
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 / 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.	

Output 1. 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*.	Six species (five belonging to the family Amaryllidaceae) showing non-orthodox behaviour are in long-term cryo-storage. All of these have been successfully retrieved from storage and grown up in the nursery
Activity 1.1 Screen seeds of a range of species to determine desiccation response and storage characteristics		(report completed or progress on activities that contribute toward achieving this output), and what will be carried out in the next period Seeds of 69 southern African species have been screened. Of these, 51 proved to be recalcitrant and four intermediate
Activity 1.2 Develop cryo-preservation protocols for excised embryonic axes of recalcitrant seeds		It was discovered that axes of dicotyledonous woody species suffered physical damage associated with the excision process, and could not produce shoots: this is a consequence of free radical mediated oxidative damage at the site where the cotyledons were excised. However, success was achieved with some monocotyledonous herbaceous species, particularly of the family Amaryllidaceae.
Activity 1.3 Develop cryo-preservation protocols for alternative explants, particularly nodal explants and meristems of plants grown *in vitro*		Despite a considerable amount of effort, nodal explants proved to be unsuitable material for cryopreservation. This was probably because water contents were too high for successful cryo-preservation, but further drying induced desiccation damage. Recently success has been achieved with meristems of a dicotyledonous species. This is considered to be a major breakthrough as all other attempts at cryopreservation failed, because of the damage to shoots
Output 2. 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).	Training aspect has been good with respect to local students, but there has been little activity regarding students from the rest of Africa. Considering that 'generic' cryo-preservation protocols have not yet been established, this has not been a bad thing. Further more, our involvement with the NEPAD/SANBio initiative should lead to a increased interest from the rest of Africa
Activity 2.1. Establish, run and assess B.Sc. (Hons) Cryobiology module		Module up and running; minor details have been improved upon

Activity 2.2. Attract post-graduate students		Have attracted a number of local students and a few from the rest of Africa, but at the moment cannot accept more until the current cohort has graduated (within the next few months)
Output 3. 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	Difficulties have been experienced with dicotyledonous, non-endospermous seeds making a recipe-style manual inappropriate at this stage. Nonetheless 2 chapters were published in a new plant cryopreservation book by Reed (2008) – ***see publications in Volume 2***. If the preliminary success achieved with meristem cultures can be achieved across a wide range of species, then such a manual becomes a possibility. Course outline and reading matter for the Cryobiology Honours level course can be made available on the web. Seven papers have been published in peer-reviewed international journals and a further one is in review. Fourteen posters have been displayed at a range of conferences, including the AU/NEPAD Heads of State and Ministers of Science and Technology meeting (2007), and the Economic Commission for Africa/Science with Africa meeting (2008), both in Addis Ababa.
Activity 3.1 Attempt to reduce damage associated with cotyledon excision		Data suggest that this damage is free-radical mediated. A range of anti-oxidant treatments and excision techniques were attempted, but without success.
Activity 3.2 Establish other explants as potentially cryopreserveable material		See Activity 1.3 above: nodal explants unsuccessful; success with meristems of a dicot species
Output 4 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.	69 species collected and evaluated. Bankable species not conserved in the Millennium Seed Bank, largely because of small seed numbers available.
Activity 4.1 See Activities 1.2, 1.3, 3.1 and 3.2		

Annex 2 Project's final logframe, including criteria and indicators

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 constrained 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 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 (Bioversity International) 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 actions; students / staff apply knowledge routinely on return to their institutes.
Output 1. 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*.	Univ KZN records; database entries and greenhouse and / or field evaluations of performance of plants established from cryopreserved explants	Protocols developed are effective and serve as 'exemplars' for other stakeholders. Sufficient material can be made available to sustainable utilisation projects
Output 2. Staff and students (particularly from Africa) trained in cryo-biology (both on 6 week honours and post-	Over 3 years, > 10 post-docs and / or graduate students (MSc to Post-doc) given specialised training (6 training weeks per year) and / or	Univ KZN Science Faculty handbook;	Wide interest by staff / students across Africa for training. Theses available for consultation

graduate courses).	research project guidance (continuous, throughout project).	Review of successfully completed student theses	
Output 3. 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	Review of IPGRI (Bioversity International) list of publications / Kew MSBP web site; Consult reprints/ preprints of publications submitted	Optimisation of methods is possible Information as presented meets stringent publication requirements
Output 4 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 database and, once verified, into the Seed Information Database on the www.	Data standards are to international standard and information used by appropriate agencies, e.g. IUCN, IPGRI (Bioversity International)

Annex 3 Project contribution to Articles under the CBD

Project Contribution to Articles under the Convention on Biological Diversity

Article No./Title	Project %	Article Description
6. General Measures for Conservation & Sustainable Use		Develop national strategies that integrate conservation and sustainable use.
7. Identification and Monitoring		Identify and monitor components of biological diversity, particularly those requiring urgent conservation; identify processes and activities that have adverse effects; maintain and organise relevant data.
8. In-situ Conservation		Establish systems of protected areas with guidelines for selection and management; regulate biological resources, promote protection of habitats; manage areas adjacent to protected areas; restore degraded ecosystems and recovery of threatened species; control risks associated with organisms modified by biotechnology; control spread of alien species; ensure compatibility between sustainable use of resources and their conservation; protect traditional lifestyles and knowledge on biological resources.
9. Ex-situ Conservation	50	Adopt ex-situ measures to conserve and research components of biological diversity, preferably in country of origin; facilitate recovery of threatened species; regulate and manage collection of biological resources.
10. Sustainable Use of Components of Biological Diversity		Integrate conservation and sustainable use in national decisions; protect sustainable customary uses; support local populations to implement remedial actions; encourage co-operation between governments and the private sector.
11. Incentive Measures		Establish economically and socially sound incentives to conserve and promote sustainable use of biological diversity.
12. Research and Training	40	Establish programmes for scientific and technical education in identification, conservation and sustainable use of biodiversity components; promote research contributing to the conservation and sustainable use of biological diversity, particularly in developing countries (in accordance with SBSTTA recommendations).
13. Public Education and Awareness		Promote understanding of the importance of measures to conserve biological diversity and propagate these measures through the media; cooperate with other states and organisations in developing awareness programmes.
14. Impact Assessment and Minimizing Adverse Impacts		Introduce EIAs of appropriate projects and allow public participation; take into account environmental consequences of policies; exchange information on impacts beyond State boundaries and work to reduce hazards; promote emergency responses to hazards; examine mechanisms for re-dress of international damage.
15. Access to Genetic Resources		Whilst governments control access to their genetic resources they should also facilitate access of environmentally sound uses on mutually agreed terms; scientific research based on a country's genetic resources should ensure sharing in a fair and equitable way of results and benefits.

Article No./Title	Project %	Article Description
16. Access to and Transfer of Technology		Countries shall ensure access to technologies relevant to conservation and sustainable use of biodiversity under fair and most favourable terms to the source countries (subject to patents and intellectual property rights) and ensure the private sector facilitates such assess and joint development of technologies.
17. Exchange of Information		Countries shall facilitate information exchange and repatriation including technical scientific and socio-economic research, information on training and surveying programmes and local knowledge
19. Bio-safety Protocol		Countries shall take legislative, administrative or policy measures to provide for the effective participation in biotechnological research activities and to ensure all practicable measures to promote and advance priority access on a fair and equitable basis, especially where they provide the genetic resources for such research.
Other Contribution	10	Smaller contributions (eg of 5%) or less should be summed and included here.
Total %	100%	Check % = total 100

Annex 4 Standard Measures

Code	Description	Totals (plus additional detail as required)
Training Measures		
1a	Number of people to submit PhD thesis	
1b	Number of PhD qualifications obtained	2 (South African and Tanzanian)
2	Number of Masters qualifications obtained	4 (South African)
3	Number of other qualifications obtained	16 (all SA). B.Sc. (Hons) degree with seed-associated project
4a	Number of undergraduate students receiving training	9 (all SA)
4b	Number of training weeks provided to undergraduate students	6
4c	Number of postgraduate students receiving training (not 1-3 above)	14 (11 SA, 2 Indian, 1 Spanish); post-docs, and M.Sc. and Ph.D. currently in training
4d	Number of training weeks for postgraduate students	40
5	Number of people receiving other forms of long-term (>1yr) training not leading to formal qualification(ie not categories 1-4 above)	
6a	Number of people receiving other forms of short-term education/training (ie not categories 1-5 above)	2; Visiting scientists from Africa given experience
6b	Number of training weeks not leading to formal qualification	8
7	Number of types of training materials produced for use by host country(s)	
Research Measures		
8	Number of weeks spent by UK project staff on project work in host country(s)	3 +
9	Number of species/habitat management plans (or action plans) produced for Governments, public authorities or other implementing agencies in the host country (s)	
10	Number of formal documents produced to assist work related to species identification, classification and recording.	
11a	Number of papers published or accepted for publication in peer reviewed journals	7 plus 1 in review
11b	Number of papers published or accepted for publication elsewhere	1
12a	Number of computer-based databases established (containing species/generic information) and handed over to host country	

Code	Description	Totals (plus additional detail as required)
12b	Number of computer-based databases enhanced (containing species/genetic information) and handed over to host country	
13a	Number of species reference collections established and handed over to host country(s)	
13b	Number of species reference collections enhanced and handed over to host country(s)	
Dissemination Measures		
14a	Number of conferences/seminars/workshops organised to present/disseminate findings from Darwin project work	1
14b	Number of conferences/seminars/ workshops attended at which findings from Darwin project work will be presented/ disseminated.	5
15a	Number of national press releases or publicity articles in host country(s)	1
15b	Number of local press releases or publicity articles in host country(s)	
15c	Number of national press releases or publicity articles in UK	2 (Samara magazine)
15d	Number of local press releases or publicity articles in UK	
16a	Number of issues of newsletters produced in the host country(s)	
16b	Estimated circulation of each newsletter in the host country(s)	
16c	Estimated circulation of each newsletter in the UK	
17a	Number of dissemination networks established	
17b	Number of dissemination networks enhanced or extended	
18a	Number of national TV programmes/features in host country(s)	1
18b	Number of national TV programme/features in the UK	
18c	Number of local TV programme/features in host country	
18d	Number of local TV programme features in the UK	
19a	Number of national radio interviews/features in host country(s)	1
19b	Number of national radio interviews/features in the UK	
19c	Number of local radio interviews/features in host country (s)	

Code	Description	Totals (plus additional detail as required)
19d	Number of local radio interviews/features in the UK	
Physical Measures		
20	Estimated value (£s) of physical assets handed over to host country(s)	5500 (liquid nitrogen storage tanks)
21	Number of permanent educational/training/research facilities or organisation established	
22	Number of permanent field plots established	
23	Value of additional resources raised for project	£ 87,500
Other Measures used by the project and not currently including in DI standard measures		

Annex 5 Publications

Type * (eg journals, manual, CDs)	Detail (title, author, year)	Publishers (name, city)	Available from (eg contact address, website)	Cost £
Journal	Berjak P (2006) Unifying perspectives of some mechanisms basic to desiccation tolerance across life forms	Seed Science Research 16, 1-15	Host country	nil
Journal	Sershen, Pammenter, NW and Berjak P (2008) Post-harvest behaviour and short- to medium-term storage of recalcitrant seeds and encapsulated embryonic axes of selected amaryllid species	Seed Science and Technology 36, 133-147	Host country	nil
Journal	Sershen, Pammenter NW, Berjak P and Wesley-Smith J (2007) Cryopreservation of embryonic axes of selected amaryllid species	CryoLetters 28, 387-399	Host country	nil
Journal	Berjak P and Pammenter NW (2007) From *Avicennia* to *Zizania*: seed recalcitrance in perspective	Annals of Botany 101, 1-16	Host country	nil
Journal	Berjak P (2006)The challenge of	Journal of	Host country	nil

	recalcitrant germplasm cryopreservation (Guest editorial)	Horticultural Science and Biotechnology 81, 781-782		
Journal	Peran R, Berjak P, Pammenter NW and Kioko J (2006) Cryopreservation, encapsulation and promotion of shoot production of embryonic axes of a recalcitrant species *Ekebergia capensis* Sparrm.	CryoLetters 27, 5-16	Host country	nil
Journal	Sershen, Berjak P and Pammenter NW (2008) Desiccation sensitivity of excised embryonic axes of selected amaryllid species	Seed Science Research 18, 1-11	Host country	nil
Journal	Hajari E, Berjak P, Pammenter NW and Watt MP. Strategies for the micropropagation of *Ekebergia capensis* Sparrm.	Submitted to Journal of Horticultural Science and Biotechnology	Host Country	nil
Article in popular journal	Berjak P (2008) Storing the hitherto unstorable: cryopreservation of plant genetic resources	Quest, in press	Host country	nil

Annex 6 Darwin Contacts

Ref No	14-056
Project Title	Cryo-conservation Centre of Excellence for Sub-Saharan Africa (CCESSA)
UK Leader Details	
Name	Hugh W. Pritchard
Role within Darwin Project	Project Leader
Address	Seed Conservation Dept., Royal Botanic Gardens Kew, Wakehurst Place, Ardingly, West Sussex RH17 6TN.
Phone	
Fax	
Email	
Partner 1	
Name	Patricia Berjak
Organisation	University of KwaZulu-Natal
Role within Darwin Project	Host country project co-leader
Address	School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville Campus, Private Bag X54001 Durban, 4000 South Africa
Fax	
Email	
Partner 2 (if relevant)	
Name	N.W. Pammenter
Organisation	University of KwaZulu-Natal
Role within Darwin Project	Host country project co-leader
Address	School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville Campus, Private Bag X54001 Durban, 4000 South Africa
Fax	
Email	

APPENDIX 1: Course outline

B.Sc. (Hons): CRYOBIOLOGY

Main areas of research

1. Natural resistance to freezing: hibernation, cold acclimation (e.g. commercially relevant crops)
2. Short or long term conservation of genetics resources
    a. Genetic 'catalogue' or reference
    b. Breeding purposes
3. Pharmaceutical Industry: Stability of freeze-dried products (e.g. antibiotics)
4. Medicine
    a. Storage of blood, organs and embryos
    b. Preservation of cell lines *in vitro*
    c. Cryosurgery in the treatment of e.g. cancer

Course Objectives

1. To develop understanding of how biological cells, tissues, organs respond to freezing temperatures

2. To understand how the cellular environment, and the cooling and warming conditions, can be manipulated to obtain the desired result
    a. Desired Result?
        i. reliable preservation of genetic repertoire (heterogeneous material)
        ii. preservation of 'sufficient' material for later use (e.g. homogeneous / clonal material)
        iii. damage-free preservation (e.g. medicine)
    b. Manipulation
        i. Controlling whether ice forms or not
        ii. Where it forms, and final size attained
            1. Intracellular viscosity: dilute, syrup/rubber, vitreous state
            2. Cooling rate: 'fast' *vs.* 'slow' cooling. (*Relative to what?*)
            3. Sample Properties
                a. size
                b. water content
                c. geometry
                d. developmental status
                e. main type of storage reserve
                f. vacuolation
                g. etc.

3. Assessment of cryopreservation success or failure

4. Applying the lessons learned in one system to another
    a. Empirical *vs.* systematic approaches

SYLLABUS

- Biophysical properties of water and aqueous solutions
    - The unique properties of water
    - Freezing point depression
    - Vitrification
    - Phase diagrams

- Membrane composition and permeability
    - Diffusion in different cell types

- Cooling rates, nucleation and ice crystal distribution
    - The relative meaning of slow, intermediate and fast freezing
    - Supercooling, homogeneous *vs.* heterogeneous nucleation
    - Propagation of ice through cells and tissues; sub-cellular compartments

- Mechanisms of freezing damage in respect of cooling rates
    - Slow-cooling damage; solution effects
    - Intracellular mechanical freezing damage
    - Chilling damage; Phase transitions

- Principles of cryoprotection
    - Freezing sensitivity / hardiness of various cells and tissues
    - Penetrating *vs.* non-penetrating cryoprotectants
    - Loading / off-loading
    - Toxicity

- Recovery from cryostorage
    - Dependence of thawing rates on previous thermal history
    - *In vitro* techniques
    - Assessment of survival
    - Hardening and dissemination of germplasm

- Surviving low temperature in nature
    - Permafrost
    - Chilling injury by snow / frost

PRACTICALS

- Measuring freezing point depression of various cryoprotectants solutions
- Cryo-microscopy:
    - Observing ice propagation in living tissues
    - SEM observation of ice crystal 'ghosts' in freeze-dried material cooled at different rates
- Cryopreservation of plant material at different cooling rates, and assessment of *in vitro* survival after recovery

APPENDIX 2: STUDENTS TRAINED AND VISITORS SUPERVISED IN CRYOBIOLOGY

Post-graduate students: graduated

Post-doctoral	Rosa Peran (completed Sept 2006)
Ph.D.	Claire Whitaker, 2007
	H.P. Msanga, 2008
M.Sc.	Elliosha Hajari, 2005
	Nokuthula Myeza, 2005
	Sershen Naidoo, 2006
	Viloshanie Reddy, 2006
	Sharon Eggers, 2008
	Meagan Goveia, 2008

Post-graduate students: current

Post-doctoral	Boby Varghese (started Oct 2006)
	Dalia Matthews Varghese (started Oct 2006)
Ph.D.	Elliosha Hajari
	Sershen Naidoo
M.Sc.	Deshnidevi Govender
	Havendren Chetty
	Melissa Timothy
	Wynston Woodenberg
	Varsha Premsager
	Ashley Subbiah
	Prabashni Naidoo
	Vishal Bahruth

Visitors

Dr Adesola Ajayi, Obafemi Awolowo University, Ile-Ife, Nigeria; 2005, 3 months

Marion Quain, CSIR, Kumasi, Ghana; 2007, 4 months

Clifford Nxumalo, Director, National Zoological Gardens; 2008, 1 day

Dr Luke Mumba, Director, NEPAD/SANBio; 2008, 2 days

SANBio Task Force; 2008, 2 days