The Rufford Small Grants Foundation

Final Report

Congratulations on the completion of your project that was supported by The Rufford Small Grants Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. The Final Report must be sent in **word format** and not PDF format or any other format. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. Please note that the information may be edited for clarity. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to jane@rufford.org.

Thank you for your help.

Josh Cole, Grants Director

Grant Recipient Details	
Your name	Nicolas Niemenak
Project title	Implementation, monitoring and conservation of *Prunus africana* seeds in Cameroon
RSG reference	70.05.09
Reporting period	September 2009 – December 2010
Amount of grant	£6000
Your email address	niemenak@yahoo.com
Date of this report	January 25, 2011

1. Please indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Search for suitable conditions for seeds germination after six months conservation at 4 °C			Yes	
Establishment of seed garden and monitoring of seedling growth		Yes		We faced seedlings attack by Psyllids in our seeds garden
Callus and somatic embryos induction		Yes		Callus was induced from young leaves. Search to induce competent embryonic cells are ongoing.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled (if relevant).

- We faced seedlings attack by Psyllids in our seeds garden. By testing different insecticides, we found one which was able to control the insect.
- The seeds availability time is too short during the year.

3. Briefly describe the three most important outcomes of your project.

Prunus africana (*Rosaceae*) is endemic to the montane forests of tropical Africa in altitudes between 1200 and 3000 m. The interest of *P. africana* is laid in the curative virtue of its bark extracts used in the manufacture of over 19 drugs sold in 23 European and U.S. companies for the treatment of benign prostate cancer. According to the literature, the seeds of *Prunus africana* do not germinate after 6 months of conservation. In this study, we observed a gradual increase in germination after 6 months of storage at 4°C. The effects of temperature, light and different plant growth stimulators on germination were evaluated. GA3 (10 µM, 100 µM) and $NaNO_3$ (0.5 mM, 1mM, 1.5 mM, 5 mM) were tested. It emerges from this that alternation between cold (4°C) and room temperature (ca. 25 °C) is favorable for seed germination of *P. africana*. The maximum rate of germination was 100 % and was observed with 1 mM $NaNO_3$. GA3 at 100 µM, associated with an alternate temperature, is also critical for seeds germination. By 15 days after incubation up to 65 % of seeds germinated under this condition. Incubation at 4°C (in the night) and room temperature (in the daytime) during our experiments mimic the natural condition where *Prunus* grows and highlights the importance of ecological condition on seed management.

Histological study allowed identifying storage compounds like proteins and starch. Toluidine blue staining highlighted the presence of unstained bodies largely distributed in the seed. Isolation and characterisation of this compound will be a breakthrough of identifying chemicals involved in prostate treatment. If it is chosen to be enrolled in this disease treatment, alternative use of seeds instead of bark will help managing the conservation of *P. africana*, In fact in rural areas where *P. africana* grows, farmers say, seeds have a medicinal property as well.

Callus was initiated from young leaf fragments cultivated in DKW (Driver and Kuniyuki Woody plant medium) basal salts at different strength. The growth regulator 2, 4-D was added in each medium at 2 mg l^{-1}. For each medium 100 explants leaves were cultivated (five explants per petri dish). Callogenesis rate was 22.5; 47.25 % and 68.75 % for explants derived from DKW/4, DKW and DKW/2 respectively. Calli are yellowish or greenish-brown and compact.

4. Briefly describe the involvement of local communities and how they have benefitted from the project (if relevant).

The local communities will be involved in the project next year after seedlings transfer to their plantation. We contacted some Chiefs (who also use *Prunus* bark for diseases treatment) in the rural area (Haut-Nkam department, West region) who received positively our idea of giving them *Prunus* seedling to be used as hedge trees.

5. Are there any plans to continue this work?

- Analysis of the influence of UV light on germination capacity
- Seed germination study after 12, 18- and 24-months conservation at 4°C
- Tissue culture
- Continuing monitoring seedling development until their transfer to farmer.

6. How do you plan to share the results of your work with others?

- Publication in peer review journal like *Seed Science Research.*
- Attending national and international conference.

7. Timescale: Over what period was the RSG used? How does this compare to the anticipated or actual length of the project?

We started using the RSG fund in October 2009 until December 2010. We are trying as much as we can to save money for seeds collection (which is the cornerstone of the programme) for future experiments.

8. Budget: Please provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used.

Item	**Budgeted Amount 1 £ = 850 FCFA**	**Actual Amount**	**Difference**
Laboratories supplies and consumables	**3000.0**	**3600.5882**	**- 600.5882**
Freezer (-20 °C)		735.294	
Protected Outlet for the freezer		14.705	
Vortex Mixer (Stuart SAZ)		411.764	
Balance (Kern 512)		588.235	
pH meter (Hanna PH 211)		588.235	
UV Lamp 8 Watts		352.941	

Stativ for UV Lamp		35.294	
Hydrogene Peroxide		5.882	
Carboy.PTIO salt (10 mg)		117.647	
Gelrite (1 kg)		235.294	
L-Glutamine (500 g)		117.647	
PVPP (500 g)		235.294	
Glucose anhydrous (500 g)		88.823	
Sodium Nitroprusside (250 g)		73.529	
Administration	**200**	**191.176**	**- 8.823**
12 months Internet connections in the Lab		141.176	
Keyboard (1)		5.882	
Computer screen (1)		31.25	
Mouses (2)		12.5	
DVD-R (20)		3.125	
Construct nursery	**500**	**500**	**0**
Nursery equipment, germplasm collection and facilities	**500**	**145.882**	**+ 354.117**
Sprayer (2 L)		4.705	
Sprayer AGRO (16 L)		47.058	
Plastic bags 40 x 40 x 150 (100)		17.647	
Sawdust		11.764	
Gross plant 480EC, Polyvalent insecticide		17.647	
HYDROX SUPER insecticide		23.529	
Actara 25 WG insecticide		11.764	
Polyhix insecticide		11.764	
Expedition in Bamenda for *Prunus* seeds collection	**300**	**235.294**	**+ 64.705**
4. Communication and publications	**700**	**294.117**	**+ 405.882**
16th Annual Cameroon Bioscience conference registration fees (held in Yaounde)		29.411	
17th Annual Cameroon Bioscience conference transport, registration, accommodation fees (held in Bagangté, Cameroon)		264.705	
Transport of plant materials from nursery to planting and training of farmers on nursery	**500**	-	**+500**
Project management	**300**	**300**	**0**
Total*	**6000.0**	**5267.0572**	**732.942**

*The main difference was due to the fact that we have to buy new balance and pH meter (the old one was defective) in order to carry out experiments. The money we still have is for plant transfer in the field since the plants are not ready for transplantation and for seeds collection for further experiments.

9. Looking ahead, what do you feel are the important next steps?

- Seeds ultra-structure analysis under electronic microscope.
- Gas Chromatography and HPLC-MS analysis of seed components in collaboration with foreign laboratories in Europe or America.
- Rooted cutting - Tissue culture in Bioreactors

10. Did you use the RSGF logo in any materials produced in relation to this project? Did the RSGF receive any publicity during the course of your work?

We acknowledge RSGF in our article published in Cameroon Biosciences Proceedings.

11. Any other comments?

Scientifics production from the project

Nzweundji J.G., Niemenak N., Dongfagsiteli T.N., Tiya N., Noka H.T.D, Omokolo N.D. 2010. Variation de certains paramètres biochimiques au cours de la callogenèse chez *Prunus africana*. *Biosciences Proceedings, Vol. 16: 46 – 50*

Nzweundji J.G., Niemenak N. Mengoum T., Noumi C, Dongfagsiteli T.N., Omokolo N.D. Influence of temperature, light and growth stimulators on germination and growth of *Prunus Africana*. Abstract, 17th Annual Conference, Cameroon Bioscience Society, Université des Montagnes, November 30 – December 04, 2010, Bagangté, Cameroon.

Master students trained and upcoming students

Didier Noka Huisken Takouo. 2010. Induction de la callogenèse chez *Prunus africana* : influence de la force ionique du milieu de culture. Master thesis, DIPES II, Higher Teacher Training College, University of Yaounde I. 67 p.

Nadege Tiya. 2010. Variations des sucres solubles et des acides aminés au cours de la callogenèse chez Prunus africana. Master thesis, DIPES II, Higher Teacher Training College, University of Yaounde I. 50 p

Christelle Noumi. Vegetative propagation of *Prunus africana*: microcuttings and identification of biochemecals markers. Master thesis in preparation, 2011, DIPES II, Higher Teacher Training College, University of Yaounde I.

Theophile Mengoum. Influence of temperature, light and growth stimulators on germination and growth of *Prunus africana*. Master thesis in preparation, 2011, DIPES II, Higher Teacher Training College, University of Yaounde I.