Final Evaluation Report

Your Details	
Full Name	Maria de Lourdes Delgado Aceves
Project Title	Magueyes land: Sustainable strategy for the use and conservation of valuable natural resources of Mexico
Application ID	September 2020 to October 2021
Grant Amount	£6000
Email Address	bmlda108@gmail.com
Date of this Report	November 30, 2021

1. 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
Micropropagation of species for cultivation in communities.			■	
Permission procedures and links with the botanical gardens for the transfer of biological material.			■	
First workshop in the communities for the training and donation of plants.		■		Pandemic restriction period
Report of the material generated and development of in vitro plants.			■	
Realization of an educational pavilion at the University of Guadalajara.			■	
Second visit to the communities to monitor the cultivation and development			■	
Cryopreservation experiments.			■	
Evaluation of plants established in the field		■		Pandemic restriction period
Submission of scientific articles			■	
Participation in conferences for the dissemination of results -Participation in academic seminars that allow new generations to be involved in the conservation of various species.			■	

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

Workshop. It was difficult to plan the times to be able to carry out the visits to the community since the quarantine periods established by the government were different in each state.

Cryopreservation experiments. The labour work involved in cutting meristems from various agave species requires skill and time in the laboratory. The size and shape of the explants must be standardised to obtain homogeneous results; therefore, this stage was hard work and dedication. Some reagents were delayed in their delivery due to the pandemic.

Field. The transfer of the materials was carried out; however, the field evaluation of the plants was limited to a few days available in the community since due to pandemic protocols and healthy distance it was not possible to fully evaluate in the cultivation area. Counting and monitoring was carried out by online communication.

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

Research. New knowledge and protocols were generated for long-term conservation in the genus *Agave*, this project allowed publications that will be support for later studies.

Scientific dissemination. This project allowed to raise awareness and encourage the conservation of the species to future generations. The creation of new spaces, exhibition of specimens and linking of producers in educational centres (universities), favoured the implementation of new educational programs (Master in Agave) and the approach of ideas for the sustainable use in commercial species for the elaboration of distilled beverages.

Linking in communities. Finally, the continuous work with the communities in this project, generated greater interest and organisation in the study area. Currently, we are in constant communication to continue learning and knowing the way of working, way of life, habits and customs that help to understand and plan projects that have a greater impact on communities in the long term.

4. What do you consider to be the most significant achievement of this work?

5. Briefly describe the involvement of local communities and how they have benefitted from the project.

We believe that the information we provide, donation of plant material and constant communication with local people, encourages, generates interest and awareness of the natural resources that are around them. We have confirmed that the local people want to conserve and keep their wilderness areas in good condition, however, many of them are unaware of how to do it, even unaware of the wealth around them. The transfer of knowledge and involving the community in their own laboratory or research activities, provides tools and certainty to avoid and reduce the massive extraction of wild specimens.

6. Are there any plans to continue this work?

Yes, we consider that this project has great potential to improve the current production and conservation of agave species in our country. The work that has been done is a small part of many species that we wish to conserve. The interest and participation of students, teachers and local people, who have been involved in this 2nd Small Grant, has not allowed us to know that various activities can be carried out at the same time that they benefit local communities and the scientific community.

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

We have had the opportunity to participate in school seminars, symposia, interviews and publication of scientific articles, where we have shared the results and achievements generated in this project. This has helped us to generate links at a professional level that new projects arose in the future.

In addition, we have encouraged colleagues in the area to apply their projects to this great foundation. They have even asked us for information and advice to implement conservation projects. We are very happy to know that new projects have been accepted to continue the work of conservation of the species.

A mention to:

Leader: Mateo David León Durán
Conservation and soil restoration on forest fires degraded areas in the Tambillo Community Protected Area, Ecuador through native species reforestation

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

We consider that the time to meet the objectives was more complex due to the pandemic, however, the project achieved the objectives. We had to stop our work for 2 months due to the closure of public places, the time mentioned was the time we fell behind in meeting our objectives.

9. Budget: 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. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Technical support	420	420		was aimed at the biology student scholarship
Congresses, symposia and divulgation scientific	400	200	-200	some events were cancelled due to the pandemic, the rest of the money was used for workshops materials
Accommodation	400	400		
Food	500	500		
Gasoline	500	500		

Workshop materials	500	700	+200	some events were cancelled due to the pandemic, the rest of the money was used for workshops materials
Transference of biological material to community	100	100		
Laboratory equipment (scalpel, jars)	300	500	+200	The price of the equipment increased in the month it was purchased. We have received support of university to pay the rest.
Lab Reagents	600	600		
Liquid nitrogen	600	600		We have yet for future experiments
Tanks liquid nitrogen	780	780		
Administration cost	900	900		
TOTALS	**6000**	**6200**	**+200**	

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

The distance has been one of the disadvantages in order to better implement reforestation in the area. A future activity would be to establish a small laboratory, which is practical and accessible so that local people can be trained and can propagate their plants. It would be a sustainable and supportable practice for them, producing and marketing endemic agaves without the need to exploit protected natural areas. Thus, they will be able to be autonomous without depending on foreign companies for the production of traditional distilled beverages. This future project would be an economic support for many families that depend only on this cultural activity.

11. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

At all times. We are proud to partner with The Rufford foundation as it is internationally renowned. We have mentioned The Rufford Foundation in academic events, symposia, scientific articles, activities, school events, workshops. As mentioned above, information has been provided to interested colleagues for dissemination and support in their projects.

We have used the logo in different materials for workshops and virtual presentations.

12. Please provide a full list of all the members of your team and briefly what was their role in the project.

Liberato Portillo Martínez, PhD. (University of Guadalajara) (University of Guadalajara). He contributed to implement strategies for the workshops in the communities, gave talks to local producers and logistics within the university.

Raquel Folgado, PhD. (The Huntington Library, Art Collections, and Botanical Gardens). Contributed to the development and improvement of protocols for cryopreservation experiments. In addition, she gives talks and workshops to local people.

Ana Lilia Vigueras, PhD. (University of Guadalajara). It was valuable during the stage of linking with the communities, it also contributed to the review of works for publication. She provided ideas and troubleshooting during the project.

Felipe de Jesús Romo-Paz, PhD student. The participation in the executed techniques made the processes more efficient and successful. He contributed to the design of experiments and technical support in the laboratory.

Jorge Daniel Orozco Flores, student of biology. The support and perseverance in laboratory and field activities led to satisfactory results. The technical support and dedication contributed to the achievement of objectives in the planned time.

Ariosto Rafael Romero Guzmán, biologist. The support and shared knowledge were key to carrying out a propagation and adaptation in the field of the plants. In addition, the experience he has in botanical gardens and community work allowed that the work was carried out in an equable manner.

Juvenal Aragon Parada, biologist. The informative material that he produced for the workshops was highly accepted by the local people, we appreciate his dedication and effort in making this material.

13. Any other comments?

We are very grateful to The Rufford Foundation, their support in this project was essential to continue with the conservation of important endemic species in Mexico. We know that these are difficult times due to the pandemic that we are experiencing. However, we are comforted to know that our project was carried out with great enthusiasm and the objectives set could be met. The activities carried out will continue to be monitored and work will continue in conjunction with the communities. We still have many objectives to meet, which we are sure we will share with you for a future project. Thanks to Jane and her entire work team for the attention and facilities they gave us.

We remain open to future contributions and new goals to meet.

New Approaches for Micropropagation and Cryopreservation of Agave Peacockii, An Endangered Species

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Research Article

Keywords: Agavoideae, in vitro propagation, droplet-vitrification, vegetative growth

Posted Date: November 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1013233/v1

Abstract

More than 50% of Agave species are endemic to Mexico. Among them, *Agave peacockii* is listed within the list of threatened species that require special protection. In this work, we aimed at developing new supplementary strategies to achieve micropropagation and perform cryopreservation of *in vitro*-grown shoot-tips of *A. peacockii*. For multiplication, the addition of two cytokinins, 6-benzylaminopurine (26.6 µM) and kinetin (27.84 µM) to MS semisolid medium significantly favoured the morphogenetic response and produced the highest shoot generation (87.00±17.18) after 60 d of culture. This interaction was more effective than using the same growth regulators separately. Propagated and rooted plantlets were successfully acclimated with 100% survival and a normal morphological development during greenhouse performance. For cryopreservation, an optimized protocol following droplet-vitrification approach allowed obtaining 98% and 96% regrowth before and after cryopreservation, respectively. Shoot-tips were excised of *in vitro*-propagated plants, subjected to preculture on MS semisolid medium with 0.3 M sucrose for 1d, loaded in solution with 0.4 M sucrose and 1.6 M glycerol for 20 min, exposed to vitrification solution PVS2 for 15 min, and then, immersed in liquid nitrogen in droplets of PVS2 placed on aluminium foil strips. The vegetative growth of cryo-derived plants and of the *in vitro* propagated plants was compared under greenhouse culture conditions. No significant differences were detected in most assessed characteristics after 120 d of acclimatization. The results presented here constitute new viable biotechnological approaches for the *in vitro* propagation and long-term conservation of endangered *Agave* germplasm.

Key Message

Agave peacockii shoot micropropagation was induced combining 6-benzylaminopurine and 6-furfuryl-aminopurine. A droplet-vitrification protocol was optimized to cryopreserve shoot-tips. Greenhouse performance of *in vitro* and cryo-derived plants was similar.