New insights in conservation of *Sophora toromiro* (Phil.) Skottsb., emblematic species of the South Pacific

Jaime Espejo and Francisco Rodriguez

This paper presents advances in a conservation and reintroduction program of Sophora toromiro *(Phil.) Skottsb. A* status quo *of specimens growing in Chile is compiled and propagation of germplasm is performed by inter-specific grafts (scion and rootstock are different species). To date, over 300 grafts are ready for the establishment of a multipurpose Clonal Seed Orchard (CSO) with a germplasm collection based on three lines known as the National Botanic Garden of Viña del Mar (JBV), Goteborg (Got) and Titze (Tit). Additionally, the first controlled pollination seeds were obtained in order to understand basic aspects of reproductive biology. Activities and a work program to ensure gradual recovery of* S. toromiro *in Rapa Nui are identified. This is the second documented experience of rescuing a species listed as extinct in the wild by a traditional but effective technique in the field of horticulture, the first being made with Molokai koki'o (*Kokia cookei *O. Deg).*

En este documento se entregan avances de un programa de conservación y reintroducción de Sophora toromiro *(Phil.) Skottsb. Se registra el estado de ejemplares creciendo en Chile y se realiza una masificación de germoplasma mediante injertos inter específicos (injerto y patrón son especies diferentes). A la fecha se cuentan con más de 300 ejemplares para el establecimiento de un huerto semillero clonal (multipropósito), con una colección de germoplasma basado en tres líneas conocidas que son: el Jardín Botánico Nacional de Viña del Mar (JBV); Goteborg (Got) y Titze (Tit). Adicionalmente, se obtuvieron las primeras semillas de polinizaciones controladas, con el fin de entender los aspectos básicos de su biología reproductiva. Se identifican actividades y programas de trabajos para asegurar la recuperación gradual de* S. toromiro *en Rapa Nui. Esta es la segunda experiencia documentada de rescate de una especie catalogada como extinta en su hábitat natural, mediante una técnica no tradicional pero eficaz en el ámbito de la horticultura, siendo la primera la que fue llevada a cabo por Molokai koki'o (*Kokia cookei *O. Deg).*

Introduction

Flora growing on Oceanic islands is of particular interest because of their endemic status and evolutionary aspects of the genera present on these islands. These features have led to the designation of a hotspot in the South Pacific by Myers et al. (2000). Easter Island, or Rapa Nui, is an island belonging to the Chilean State since 1888, and is located about 3,765km west of continental Chile. The fragility (insularity) of its ecosystems and the human intervention experienced throughout the past 500 years gives us a meadow-like landscape, dominated by grasses and sedges. The flora of Rapa Nui is made up of 49% of introduced species, whose origin dates from the initial colonization by ethnic Rapanui and also increased in the mid-nineteenth century to the present (Ibáñez et al. 2001; Moreira-Muñoz 2007; Zizka 1991). The same authors note that only 7.7% of the species are endemic.

The flora of Rapa Nui has lost 25 species. Among these is the *Sophora toromiro* (Phil.) Skottsb., a small tree that is distinctive for its high wood density and was used by people as an energy source as well as raw material for the manufacture of ritual artifacts (Oyarzun, 1921; Palmer 1870; Pinart 1877) and the tablets known as *kohau motu mo rongorongo* (Englert 1988).

The last native specimen of this species grew on the slopes of Rano Kao and was cut down after a seed collection made by Heyerdahl in 1956. Today, a small population of re-introduced material is cultivated on the island by Sonia Haoa (Mata Ki te Rangi Foundation). This paper presents a new procedure to increase the success of reintroduction of this species.

Background

I) **Ex situ germplasm in Chile.** Maunder et al. (1999) reports five different lines of *S. toromiro* growing in Chile by the time of the study. Currently, only three lines can be found: The National Botanical Garden of Viña del Mar (JBV), Titze (Tit), and Goteborg (Got). Only the first line is documented with regard to the

Jaime Espejo | Programa de Postgrado. Facultad de Ciencias Forestales, Universidad de Concepción, Victoria 631, Casilla 160-C. Correo 3. Concepción, Chile
Francisco Rodriguez | Gerencia Tecnología Silvícola. Forestal Mininco S.A. Avenida Alemania # 751. Casilla 399. Los Angeles, Chile

year of harvesting and the identity of the collector. The same authors determined by molecular analysis (RAPD, ISSR) a greater genetic variability for these copies when compared with plants growing in Europe and Australia (Maunder et al. 2000).

II) **Experience using grafts for conservation purposes.** There are few experiments reported in the literature on the use of grafting as a means of conservation for extinct plants in their natural habitat, with the exception of *Kokia cookei* O. Deg. This tree became extinct by human action in Moloka'i (Hawai'i), and has been recovered using *Kokia drynarioides* (Seem) Lewt. as rootstock. The limitation to using grafts is that these do not show blooming events (Mehroff 1996). Specific inter grafts appear as an effective horticultural tool and their use is only justified for species which have gone extinct in the wild (Guerrant 1996).

III) **Clonal Seed Orchard (CSO)**. The direct benefit of forest orchards is the production of seeds, a product of high economic value. The management and success of the CSO lies in the knowledge of the reproductive biology of the species, as appropriate horticultural practices play a key role in productivity (Sedgley & Griffin 1989). The CSO design requirement reflects the type of seed. Usually, when producing open-pollinated seed available as grafts or ramets, the design is random and arranged in blocks. In the case of controlled pollination, the designs are prepared to maximize the productivity of the crosses. Thus, in the last generation of forest seed orchards, grafts are found in linear arrangements or in blocks (Espejo et al. 2001). Regarding the choice of site, the orchard should preferably be located close to a water source in the case of intensive management (fertilization and irrigation). Also, a good quality soil must be selected. Soils that are rich in organic content are necessary for the smooth development of the grafts and also to ensure good seed production.

The soils of the island are a volcanic series and at least thirteen soils of Rapa Nui are cataloged according to studies by the Development Corporation (CORFO). Most have a process of "base wash" given the percolation which is favored by the recorded annual rainfall on Rapa Nui (Rivera 2003).

Establishment and Maintenance Proposals

The preservation of specimens in Chile is guaranteed by the Botanical Garden of Viña del Mar and private collections. However, the big challenge is still the reintroduction of *S. toromiro* to Rapa Nui. In 1994, the Toromiro Management Group (TMG) initiated actions and main lines of research for the reintroduction of this species, however, they did not consider a grafting mode for both the formation of a breeding arboretum as germplasm or CSO with seed production objectives at the operational level.

Based on these proposals and progress in various fields of forestry, Forestal Mininco S.A. (CMPC holding) revised the proposals of TMG and is currently developing activities and actions (Table 1) to ensure the establishment of *S.toromiro* on Rapa Nui in the medium term.

Grafts

For rootstock selection, we used a species related to *S. toromiro*, in this case *Sophora cassioides* (Phil.) Sparre. The species has a wide distribution (easy access to germplasm) in south-central Chile and so it is adapted to different soil conditions. It grows between 5-900m above sea level, but its habitats are wet ravines and deep soils near streams, rivers or lakes that can be found in areas with rocky outcrops. There are other species growing on Oceanic islands such as *Sophora fernandeziana* (Phil.) Skottsb. and *Sophora masafuerana* (Phil.) Skottsb., however, they were discarded because of low numbers of specimens and

Table 1. Activities and actions of *S. toromiro* conservation.

Activity	Time (horizon)	Advances	Observation
Propagation by grafting	Short term	Over 300 grafts. Mean set 70% survive	Lines used Jardin Botánico Nacional Viña del Mar (JBV); Goteborg (Got) and Titze (Tit)
Reproductive biology: (pollen management, stigma receptivity etc…)	Medium term (3 years)	150 seeds by self pollination (68% of germination)	Pollination in grafts of JBV and Got
Establishment Clonal Seed Orchard/arboretum	Medium term	Ongoing (2012)	Two sites: Easter Island and a continental back up
Orthopedics* and induction (Paclobutrazol)	Medium term (3 – 5 years)		

*crown management

Table 2. Summary of grafts by year.

Season	N	Provenance ("line")	Set (%)	Observation
2007	301	Tit	79.8	
2008	36	JBV	58.4	
2008	36	Got	62.5	
2010	104	JBV	52.9	Mini grafting

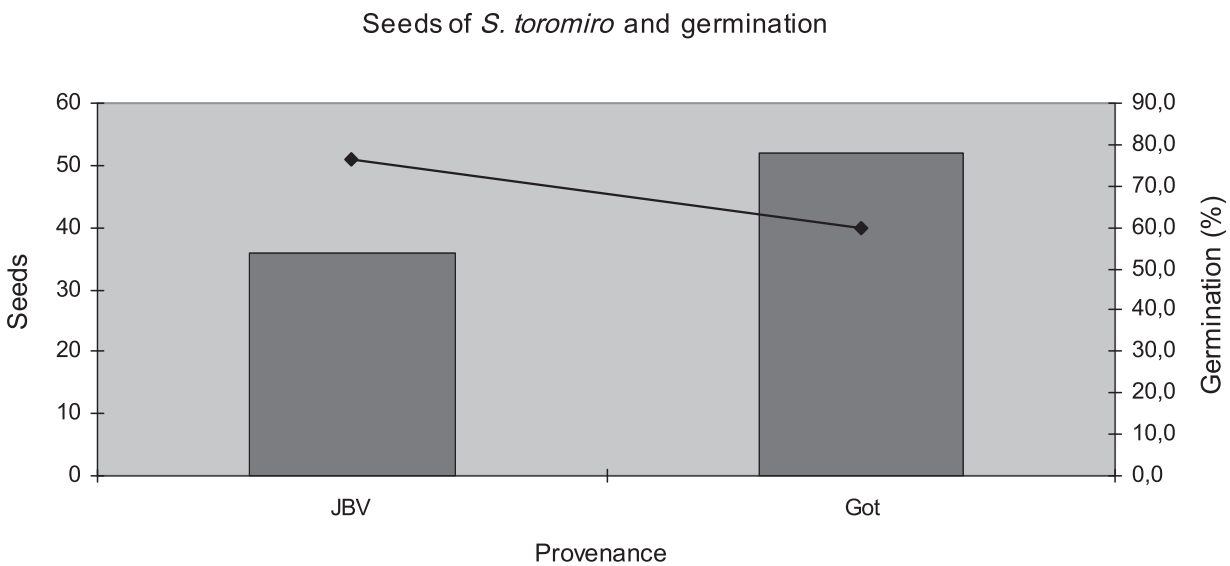

Figure 1. Number of seeds and germination produced by control crosses in the 2010/2011 season.

Figure 2. (a) Close-up of the graft area in the stem of *S. toromiro.* (b) and (c) Development stages of mini grafts.

their conservation status is listed as endangered by Chilean environmental authorities.

A great advantage of choosing the species *S. cassioides* is associated with micro-organisms such as bacteria of the genus Rhizobium and Mycorrhizae (Zúñiga 1996). This is of paramount importance for the establishment and survival of the grafts in the current conditions of the soil of Easter Island.

The results to date deliver a 71% survival or set for the cleft graft technique (Table 2). This type of graft calls for the insertion of a scion (semi-lignified) with a similar diameter to the rootstock, previously cut at its end (in the form of a bevel), and finally, the sealing of the junction scion-holder with graft Parafilm™. Actions for ex-situ germplasm collections or other lines, such as Melbourne, are considered to increase the variability of the future composition of the CSO.

Flowering Pattern and Seed Germination

The flowering of this species, as recorded inside greenhouses, starts in mid-August and finishes in November. The post grafting earliest flowering has already occurred in the Titze line and was observed for three years for the JBV and the Got lines. *S. toromiro* blooming has a similar pattern found in the phenology of the other *Sophora spp.* growing in Chile, in which the peak occurs in the Southern Hemisphere spring.

In relation to reproduction strategy mechanisms, apparently *S. toromiro* presents protandry; however, studies are required to clarify the foregoing, because it is an imperceptible change in the morphology of the stigma that is also quite small and undifferentiated. To this, we can add that exudates are not detected. Studies concerning the timing of receptivity verify that with support agents, stigmatic receptivity markers will be made for present and future seasons according to protocols proposed by Dafni (1992) and Kearns & Inouye (1993). Parallel management studies are considered: pollen collection, germination tests, viability, and storage.

In the 2010 season, self-pollinations were performed using anthers (with pollen dehiscence presence) which were removed with the aid of fine forceps and placed on the stigma. This work was performed every three days during the flowering period of the grafts. The use of this methodology allowed the development of pods and seeds, as indicated in Figure 1.

The seeds obtained were soaked with sulfuric acid (95%) for 20 minutes according to the protocol reported for this species (Gonzalez et al. 2008). Then the seeds were washed in water and placed in Petri dishes lined with moistened paper for a period of two weeks in conditions of stable temperatures (25ºC ± 2) and light (photoperiod: 16/8H; 2.000 Lux) in the laboratory. The average germination obtained was of 68%.

Establishment

In addition to establishing grafts on Rapa Nui, a second back up orchard should be made in benign environmental conditions in continental Chile. All grafts must be established in boxes with the support of gel, fertilizers and irrigation. The selected sector must be free of weed competition and ideally in a wind-protected area. This can be provided by planting next to established plantations of other species or by using topographic depressions such as an area between two hills. Alternatively, we may consider the establishment of *manavai* (traditional stone vegetation shelters) within the canopy to leverage common plants in these structures as in the case of *Musa* spp. in the town of Hanga Roa on Rapa Nui. Previous techniques known as Inter-Situ (Maunder et al. 2004) can deliver higher spot information for *S. toromiro* development and indications of future pilot plantations.

Orthopedics and Induction

Once the material is established and showing good adaptability to environmental conditions (in the 2nd to 3rd year), ramets should undergo orthopedic work in the crown in order to obtain a greater number of points to generate multiple buds to be induced with Paclobutrazol. This chemical known as PP333 (2RS, 3RS) - (4-chlorophenyl) -4,4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3OL) has shown efficacy in a large number of fruit and forest genera, especially *Eucalyptus*, and should be tested (Sedgley & Griffin 1989). There are different techniques of application and the most effective is soil-applied and is introduced to the plant via the root system.

Conclusions

S. toromiro conservation does not rescue the ecosystem, biodiversity, or lessen complex interactions with other species, *per se*. This effort is only an approximation in the rescue of a portion of collective memory for Rapa Nui.

Seed production by maintaining a CSO and subsequent establishment must use integrated strategies and techniques used in both horticulture and forestry along with some ancestral practices proven by the ancient settlers of the island. This proposal will only succeed with the proactive interaction of different parties such as private companies, government agencies, NGOs and the community of Rapa Nui.

The foregoing appears as valid and necessary for restoration of this species. This is still in the development stages and should be monitored for early feedback and to validate the initial proposals. In our view, Rapa Nui is presented as an opportunity that can be extrapolated for particular cases of species that are in survival-limiting conditions both in the wild and ex-situ.

Acknowledgements

The authors acknowledge the suggestions and corrections of Ana Betty Haoa Rapahango and Sonia Haoa Cardinali and two anonymous reviewers. This document corresponds to the oral presentation made in "Evolution of Life on Pacific Islands and Reefs: Past Present and Future" presented in Honolulu, Hawai'i, 2011. Additionally, J. Espejo wishes to thank Forestal Mininco for its support and the execution of all actions of the *S. toromiro* rescue program.

References

Dafni, A. 1992. *Pollination Ecology. A Practical Approach*. Oxford: Oxford University Press.

Englert, S. 1988. *La Tierra de Hotu Matu'a*. 4th Edition. Santiago: Editorial Universitaria.

Espejo, J., J. Harbard, & R. Griffin. 2001. *Consideraciones operacionales de cruzamientos controlados masales (OSP) de Eucalyptus globulus (Labill) en cuatro temporadas en Forestal y Agrícola Monte Aguila S.A.* Valdivia: Actas Desarrollando el Eucalipto del Futuro. Simposio Internacional, IUFRO.

Guerrant, E.O. 1996. Designing Populations: Demographic, Genetics, and Horticultural Dimensions. In *Restoring Diversity: Strategies for Reintroduction of Endangered Plants*. D.A. Falk., C. Millar & M. Olwell (eds.):171-207. Washington, D.C.: Island Press.

Gonzalez, M., I. Quiroz, E. Garcia & B. Guitierrez. 2008. Escarificacion química con acido sulfúrico como tratamiento pregerminativo para semillas de Toromiro (*Sophora toromiro* Skottsb.) *Ciencia e Investigacion Forestal* 14(1):111-118.

Ibañez, P., A. Bordeau & M. Rauch. 2001. Árboles, Recursos Naturales y Comunidades Indígenas en Chile. *Lecturas. Corporación Forestal Nacional.* 36-44.

Kearns, C.A. & D.W. Inouye. 1993. *Techniques for Pollination Biologists*. Boulder: University Press of Colorado.

Maunder, M., A. Culham, A. Bordeau, A. Guillames & M.Wilkinson. 1999. Genetic Diversity and Pedigree for *Sophora Toromiro* (Leguminosae): A Tree Extinct in the Wild. *Molecular Biology* 8:725-738.

Maunder, M., A. Culham, B. Alden, G. Zizka, C. Orliac, W. Lobin, A. Bordeau, J.M. Ramirez & S. Glissmann-Gough. 2000. Conservation of the Toromiro Tree: Case Study in the Management of a Plant Extinct in the Wild. *Conservation* 14(5):1341-1350.

Maunder, M., E.O. Guerrant, K. Havens & K.W. Dixon. 2004. Realizing the Full Potential of Ex Situ Contributions to Global Plant Conservation. In *Ex Situ Plant Conservation: Supporting Species Survival in the Wild.* E.O. Guerrant, K. Haens & M. Maunder (eds.):389-418. Washington, D.C.: Island Press.

Mehrhoff, L.A. 1996. Reintroducing Endangered Hawaiian Plants. In *Restoring Diversity: Strategies for Reintroduction of Endangered Plants*. D. Falk., C. Millar & M. Olwell (eds.):101-120. Washington, D.C.: Island Press.

Myers, N., A.R. Mittermayer. C.G. Mittermayer, G.A.B. da Fonseca & J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.

Moreira-Muñoz, A. 2007. Plant Geography of Chile. An Essay of Postmodern Biogeography. Unpublished PhD Thesis. University of Erlangen-Nuremberg.

Oyarzun, A. 1921. Toromiros de la Isla de Pascua. *Revista Chilena de Historia Natural* XXV:133-140.

Palmer, J. L. 1870. A Visit to Easter Island, or Rapa Nui, in 1868. *Proceedings of the Royal Geographical Society of London* 14(2):108-120.

Pinart, A. 1877. Voyage to Easter Island – 1877. In *Early Visitors to Easter Island 1864-1877. The Reports of Eugene Eyraud, Hippolyte Roussel, Pierre Loti and Alphonse Pinart*. G. Lee, A.M. Altman & F. Morin (eds.):106-136. Los Osos: Easter Island Foundation.

Rivera, Fernandez, E. 2003. El Componente Forestal en los Sistemas de Producción de los Pequeños Propietarios de la Etnia Rapa Nui de Isla de Pascua, V Región. Unpublished thesis. Facultad de Ciencias Forestales, Departamento de Manejo de Recursos Forestales. Universidad de Chile.

Sedgley, M. & R.Griffin. 1989. *Sexual Reproduction of Tree Crops*. Waltham: Academic Press.

Toromiro Management Group. 1998. Minutes of 5th International Toromiro Management Group Meeting. Available at http://www.kew.org/conservation/cpdu/Toromiro/tor_min1.htm

Zizka, G. 1991. Flowering Plants of Easter Island. In *Palmarum Hortus Francofurtensis, Scientific Reports* 3:1-108. Frankfurt am Main: Palmengarten.

Zúñiga, E. 1996. Asociaciones simbióticas tripartitas en *Sophora*. Unpublished thesis. Conducente a Licenciatura en Biología, Universidad Austral de Chile.

This article has been peer-reviewed.
Received 1 July 2013; accepted 25 August 2013.