IN VITRO PROPAGATION OF *HELIANTHEMUM BYSTROPOGOPHYLLUM* SVENT., A RARE AND ENDANGERED SPECIES FROM GRAN CANARIA (CANARY ISLANDS).

ISABEL SANTANA LÓPEZ[1], J. C. LUIS[2], MARGARITA RAVELO ARMAS[2] & FRANCISCO VALDÉS GONZÁLEZ[2]

[1] Jardín Botánico Canario "Viera y Clavijo". Apto. 14 de Tafira Alta. 35017. Las Palmas de Gran Canaria. Canary Islands.Spain (isantana@grancanaria.com).
[2] Universidad de La Laguna, Facultad de Farmacia, Departamento de Biología Vegetal, UDI Biología y Fisiología Vegetal. Avenida Astrofísico Francisco Sánchez s/n, C.P. 38071 La Laguna, Tenerife, Spain. Phone number: 922 31 84 53; e-mail: jcluis@ull.es

Recibido: Marzo 2004

Palabras claves: Cistaceae, micropropagación, *Helianthemum bystropogophyllum*, especies amenazadas, multiplicación por segmentos nodales, enraizamiento, segmentos apicales.

Key words: Cistaceae, endangered species, *Helianthemum bystropogophyllum*, micropropagation, rooting, shoot multiplication.

SUMMARY

An efficient micropropagation protocol has been developed for *Helianthemum bystropogophyllum* Svent., an threatened species from Gran Canaria (Canary Island, Spain). Shoot tips and nodal segments isolated from seedlings were used as primary explants. Multiple shoot production was observed in MS medium supplemented with different concentrations of BA. Rooting was achieved in MS medium by adding IBA or without any plant growth regulators. An 82% survival rate was obtained in the acclimatisation process.

RESUMEN

En el presente trabajo se da a conocer un protocolo de micropropagación rápido y eficiente desarrollado para *Helianthemum bystropogophyllum* Svent., una de las especies más amenazadas de la flora endémica de Gran Canaria (Islas Canarias). Como explantos iniciales se utilizaron yemas apicales y segmentos nodales aislados de plántulas obtenidas *in vitro* a partir de la germinación de semillas. La multiplicación de yemas fue alcanzada en medios MS suplementados con diferentes concentraciones de BA. El enraizamiento se llevó a cabo en el mismo tipo de medio, sin reguladores o con la adición de IBA. Durante el proceso de aclimatación se obtuvo un 82 % de supervivencia.

Abbreviations: BA – benzyladenine, Kint –kinetin, IBA – indole-butiric acid, MS – Murashige & Skoog, NAA – naphthalenacetic acid.

ISSN 0211-7150

INTRODUCTION

Helianthemum bystropogophyllum Svent. (Cistaceae) is a rare endemic plant from Gran Canaria, in the Canary Islands (Spain). Nowadays, the populations of this species is reduced in numbers and they appear to be found only in the Macizo de Inagua (Montaña de los Hornos y Montaña de Las Brujas) (ALMEIDA & MARRERO 2004), in the south-west region of the island. Therefore, *Helianthemum bystropogophyllum* has been catalogued as "Critical" by the IUCN (V V. A A., 2000). and also included in national lists of threatened plants such as "Catálogo Nacional de Especies Amenazadas" and "Catálogo de Especies Amenazadas de Canarias". In vitro techniques can be used in the propagation and conservation of endangered species either to produce new plant material or as intermediate or long-term storage systems (CLEMENTE MUÑOZ, 1995). When they are used of a suitable way, they can be useful in species that have a reduced number of individuals, since it is possible to produce a great amount of plantlets in a short time from a small quantity of plant material, without affecting the natural populations by over collection. In this paper, we present a protocol for the *in vitro* micropropagation of *Helianthemum bystropogophyllum*, which showed to be very efficient, as exhibited by the results obtained.

MATERIALS AND METHODS

Plant material

Seeds of *Helianthemum bystropogophyllum* were collected from plants in the "Reserva Natural Integral de Inagua" in the south-west of Gran Canaria (Canary Islands, Spain).

Germination tests

Numerous examples of heat treatments to induce germination on Cistaceae have been reported in the bibliography (IRIONDO *et al*., 1995; CORRAL *et al*., 1990). In this experiment, three different treatments were used: dry heat (100ºC), immersion in hot water (100ºC), and immersion in hot water (100ºC) followed by immersion in cold water (4ºC). The heat exposition times in all three treatments were 10, 30 and 60 minutes. After sowing the seeds, they were incubated at 20ºC with a 16/8 hour photoperiod. The percentage of germination was measured after 30 days of incubation.

Sterilisation protocol

Seeds were rinsed in running water and Tween 20 for several minutes and then immersed in ethanol (70%) for 30 seconds, followed by a 10 minutes rinse in Tween 20 and commercial bleach (2.5%). Finally, all the seeds were rinsed three times with sterile water before been sown in MS medium.

In vitro propagation

Seedlings were isolated and sown in tubes with 20 ml of basal MS medium (MURASHIGE & SKOOG, 1962). Shoot tips and nodal segments were excised

from germinated plants and propagated with various concentrations of Kint or BA. One centimetre or longer healthy shoots were rooted by using MS medium with different concentrations of IBA or without any plant growth regulators. All plant material were maintained at 20ºC with 16/8 hours of photoperiod and subcultured for 30 ± 5 days.

Healthy, well-developed, and rooted plants were transferred to the greenhouse for their acclimatisation. They were carefully washed in water to eliminate excess agar and rinsed with a benomilo solution (fungicide, 1 g/l). Thereafter, they were placed into 7.5 cm diameter pots filled with a sterilised soil:peat:vermiculite mix (2:1:1), and then introduced into trays covered with transparent plastic. During acclimatisation, aeration was progressively increased by the partial removal of the plastic cover.

Twenty replicates were used for each treatment and all the experiments were repeated twice. Analysis of Variance and Duncan's Multiple Test were performed on the data (SPSS for Windows version 6.1.3.).

RESULTS AND DISCUSSION

Germination Tests

After four weeks of incubation the best results obtained for *in vitro* germination were observed in the hot water tests, specially, the 30 minutes immersion treatment (Fig. 1). Neither treatments with hot water nor treatments with hot water followed by cold water during both, 10 and 60 minutes, had any significant results. Similarly, dry heat treatments showed no results at all.

	In vitro germination tests	% Rooting
1	10' heat water (100 ºC)	1
2	30' heat water (100 ºC)	28
3	60' heat water (100 ºC)	1
4	10' heat water (100 ºC) + 24 h 4 ºC	0
5	30' heat water (100 ºC)+ 24 h 4 ºC	12
6	60' heat water (100 ºC)+ 24 h 4 ºC	0
7	10' dry heat (100 ºC)	0
8	30' dry heat (100 ºC)	0
9	60' dry heat (100 ºC)	0
10	Reference	0

Figure 1. - Percentage of germinated seeds of *Helianthemum bystropogophyllum* under different heat treatments.

Other experiments with endemic species of *Helianthemum* (SANTANA LÓPEZ *et al*., unpublished) provided, unlike results in this work, excellent results on germination without heat treatments. This shows a great variability in the germination ability of these endemic species, which could be related to the seeds physiological conditions.

Multiplication

In vitro micropropagation of *Helianthemum bystropogophyllum* was studied by using MS medium with different concentrations of BA or Kint (Fig. 2). Best results were obtained with BA at 1 mg/l, observing a multiple shoot production. Lower concentrations of BA such as 0.15 mg/l or 0.25 mg/l showed no significant differences between both concentrations. However, they showed lower shoots per explant when compared with the 1 mg/l concentration of BA. In addition, when Kint was used instead of BA all concentrations tests exhibited no significant differences between them.

These results contrast with those obtained for *Helianthemum almeriense* Pau (MORTE & HONRUBIA, 1992), where best results are achieved on media supplemented with Kint in very low concentrations (0.1 and 0.2 mg/l). On the other hand, IRIONDO *et al.* (1995) obtained for *Helianthemum polygonoides* Peinado *et al*. similar results than those observed in this paper for 1 mg/l of BA, but with a higher concentration of BA (5.0 mg/l) or in combination with NAA (1.0 or 0.5 BA + 0.1 NAA).

BA	kint	Shoots / explant
0	0	1,38 a
1,0	0	4,05 b
0,25	0	1,65 a
0,15	0	1,50 a
0	1,5	1,58 a
0	1,0	1,54 a
0	0,5	1,05 a
0	0,25	1,15 a
0	0,15	1,06 a

Figure 2. - Effect of BA and Kint on the formation of shoots per explant. Shoots per explant values followed by the same letter are not significantly different ($p<0.05$) as determined by Duncan's Test.

In a different experiment we analysed the influence of lower concentrations of BA on the explants obtained with BA 1 mg/l (Fig. 3). Reducing BA to half of the concentration showed no significant differences with the previous medium. However, the shoots length was increased and the formation of hiperhidric tissues

was reduced. This test exhibited an efficient shoot production system omitting the elongation step, subsequently allowing us the immediate rooting of the material.

Growth regulators (mg/l)	Shoots / explant
BA 1	4,05 a
BA 0,5 from BA 1	3,35 a
BA 0,25	1,65 b
BA 0,15 from BA 0,25	2,07 b

Figure 3. - Effect of reducing BA to half of the concentration in the second subculture. Shoots per explant values followed by the same letter are not significantly different ($p<0.05$) as determined by Duncan's Test.

Rooting and acclimatisation of the plant material

Shoot tips produced by reducing the concentration of BA were rooted in MS medium adding different concentrations of IBA or without any growth regulator. The absence of IBA from the medium showed a higher number of roots per explant with a higher length. However, when different concentrations of IBA were used, only the higher ones (4 and 4.5 mg/l) exhibited similar results (Fig.4).

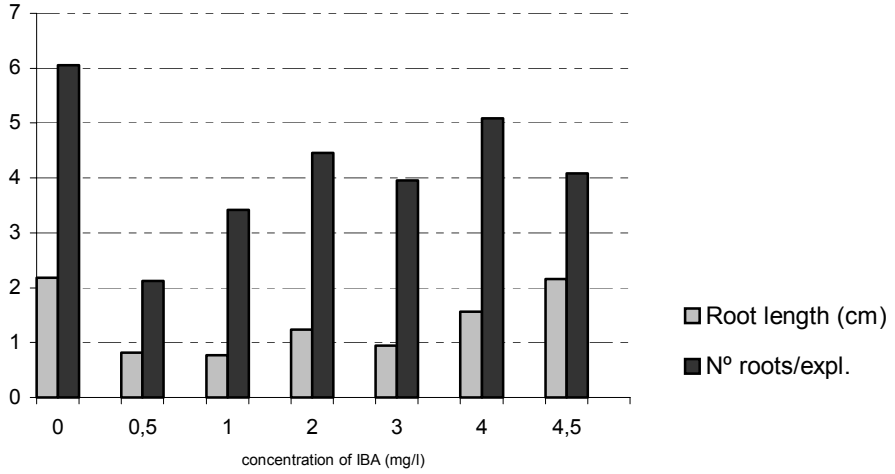

Figure 4. - Influence of the concentration of IBA on the number and length of roots.

The results obtained in the stage are consistent with the results from IRIONDO *et al.* (1995) with *Helianthemum polygonoides*, where the percentage of rooted plants

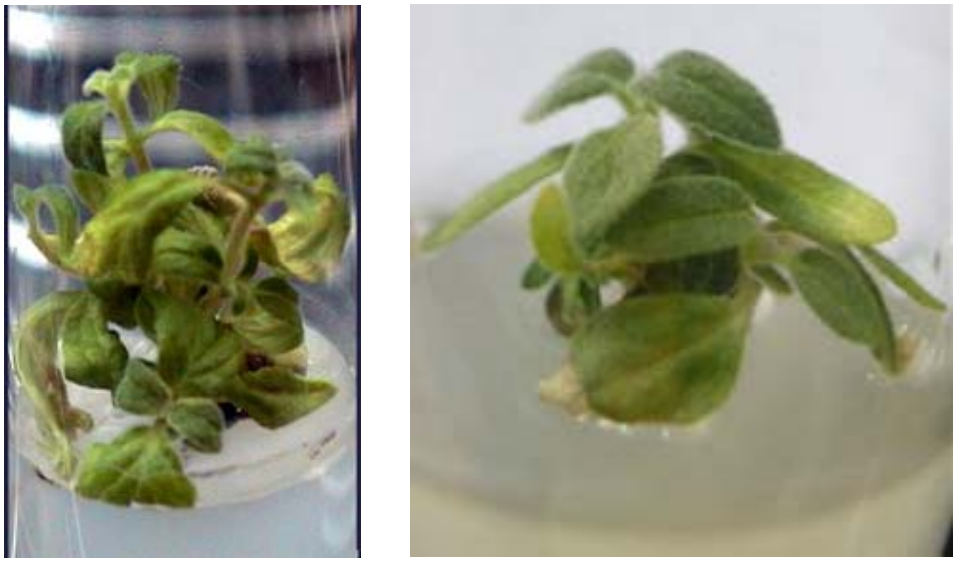

Figure 5. - Shoot multiplication on MS medium supplemented with 1 m/g of BA (left) and 1.5 mg/l of Kint (right).

Figure 6. - In vitro rooted plant before transfer to soil (left) and plants growing at the Jardín Canario, one year after their acclimatisation (right).

increased with the higher concentrations of IBA. The absence of IBA, allowed, however, an efficient rooting system for *Helianthemum bystropogophyllum.*

Well-rooted and healthy plants were transferred to the greenhouse and later to an appropriate area in the botanical garden of Gran Canaria, where the conditions were similar to their natural habitat. An 82% survival was observed after the acclimatisation process. (Fig. 6)

CONCLUSIONS

In summary, the results of this research showed an efficient method for the micropropagation of *Helianthemum bystropogophyllum* Svent. This method, allowed us to start with a relative small amount of plant material, obtaining at the end of the acclimatisation process an 82% survival. However, some aspects such as the seed germination ability and the physiology of this process in different species of *Helianthemum* should be studied in detail in a near future.

ACKNOWLEDGEMENTS

We want to thank you the Cabildo of Gran Canaria for the post-graduate studentship given to the Jardín Botánico Canario “Viera y Clavijo”, which made this work possible. We are also grateful to David Bramwell and José Naranjo Suárez (Jardín Botánico Canario “Viera y Clavijo”) for their support and help throughout the process. The collection of seeds was made thanks to the efforts and dedication of Roque López González (Cabildo de Gran Canaria).

REFERENCES

ALMEIDA, R. S. & A. MARRERO, 2004.- Adiciones y precisiones a la corología de H*elianthemum bystropogophyllum* Svent. *Bot. Macaronésica* 25: 177-186.

CLEMENTE MUÑOZ, M., 1995.- Micropropagation of endangered species. *Ecología Mediterránea* XXI (1/2), 291-297.

CORRAL, R., PITA J. M. & PÉREZ-GARCÍA, F., 1990.- Some aspects of seed germination in four species of Cistus L. *Seed Sci. & Technol*., 18, 321-325.

IRIONDO, J. M., MORENO, C., & PÉREZ C., 1995.- Micropropagation of Six Rockrose (Cistus) Species. *Hort Science* 30 (5), 1080-1081.

-, PRIETO, C. & PÉREZ-GARCÍA, 1995.- F. In vitro regeneration of Helianthemum polygonoides Peinado et al., an endangered salt meadow species. *Botanical Gardens Micropropagation News*, Vol 2 Part 1 September.

MORTE, M. A. & HONRUBIA, M., 1992.- In vitro propagation of Helianthemum almeriense Pau (Cistaceae). *Agronomie* 12, 807-809.

MURASHIGE T. & SKOOG F., 1962.- A revised médium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol. Plant*. 15, 473-497.

V V. A A., 2000.- Lista Roja de la Flora Vascular Española (valoración según categorías UICN). *Conservación Vegetal* 6 (extra): 11 – 38.