

## Shoot production from cotyledons of *Prosopis glandulosa* var. *torreyana* cultured *in vitro*

The genus *Prosopis* includes 44 species, nine of which are found in Mexico (Rzedowski, 1988). The genus is used for a variety of purposes and has a long tradition of use in the arid and semi-arid zones where it grows (Felker, 1993). However, misuse of this resource is causing deforestation and irreversible loss of genetic diversity. In planning sustainable use of plants threatened by genetic depauperation it is important to select elite individuals, propagate them clonally, and establish plantations of these individuals making depredation of the resource unimportant. The technique of micropropagation makes possible the clonal mass propagation of elite individuals; however, *Prosopis* species show a certain degree of recalcitrancy to *in vitro* culture (Harris, 1992). In this short communication we present preliminary observations on the response of *P. glandulosa* var. *torreyana* cotyledons to *in vitro* culture.

Pods with seeds were collected from wild populations of *P. glandulosa* Torr. var. *torreyana* (L. Benson) M.C. Johnst, located at San Matias, Baja California (México). Taxonomic identification was performed by M.C. Jesús Castellón (Universidad Autónoma de Baja California, Ensenada, México) and verified at the Herbarium of the Natural History Museum of San Diego, California (USA). Seeds were germinated in humidified cotton after scarification treatment (30 min. in concentrated H<sub>2</sub>SO<sub>4</sub>) and deinfestation by immersion in ethanol 70%, 1 min. and in sodium hypochlorite 10% for 10 min. Seeds were carefully washed in running tap water before germination.

Ten-day-old seedlings were used as the source of explants. Cotyledons with hypocotyl were extruded and cultured on MS basal solid medium (Murashige and Skoog, 1962) enriched with the growth regulators naphthalen-acetic acid (NAA) at 0.0, 0.01, 0.1, 0.5 and 1.0 (mg/l) combined with Kinetin (KIN) at 0.0, 0.01, 0.05, and 0.1 (mg/l) giving a total of 20 different combinations. The pH of the medium was adjusted to 5.6, after which it was supplemented with 0.8% agar and 3 % sucrose, poured into baby food jars and autoclaved for 15 min. at 20°C. Four explants per jar were inoculated in a sterile area and incubated at 27°C ± 2 in a growth chamber with 16 hours photoperiods with 3.2 w.m<sup>-2</sup> light and 8 hours darkness. Observations were recorded every week over 3 months, and four replicas

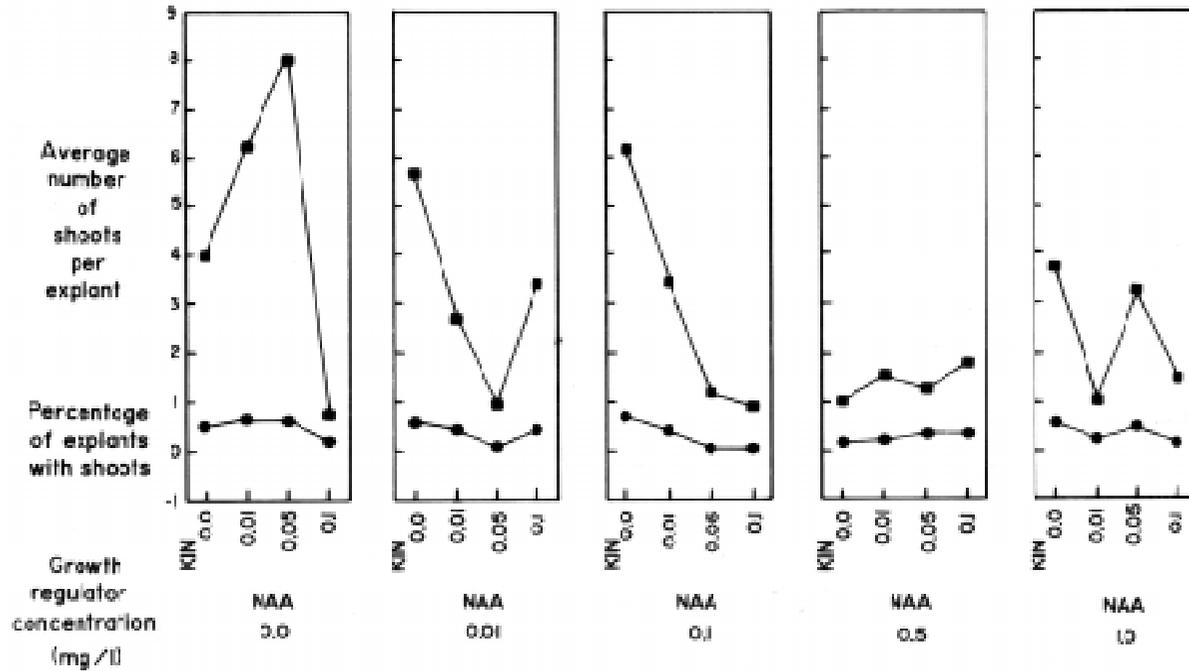


Fig. 1. Shoot formation after 3 months in culture of *Prosopis glandulosa* var. *torreyana* cotyledons with hypocotyl exposed to twenty combinations of KIN/NAA (mg/l). Percentage of explants with shoots is expressed as 1=100% (-O-). Average number of shoots per explant (—)



Fig. 2. Cotyledons with hypocotyl of *P. glandulosa* var. *torreyana* after 3 months of *in vitro* culture in MS enriched with KIN 0.05 mg/l. Note the absence of callus.

were performed per treatment regime. Shoot formation on the twenty treatments analyzed was subjected to an ANOVA and a Fisher LSD test using a Statistica w/4.1 program.

All twenty of the treatments utilized produced vigorous shoots, even the control (no growth regulators added). Two variables were recorded to quantify this growth: percentage of explants with shoots and number of shoots per explant. Figure 1 shows the relationship between both of them; however, differences arose between all the applied treatments. ANOVA showed an interaction for the KIN/NAA, also the variable percentage of explants with shoots ( $P=0.018$ ) and number of shoots per explant ( $P=0.0076$ ). The LSD Fisher test also shows differences between the treatments in both cases. The analysis of the percentage of explants with shoots exhibited two groups (data not shown); however, no correlation was detected between growth regulator concentrations, with the best response (77.5%) being found at 0.0/0.1 mg/l of KIN/NAA (Fig. 1). In the case of average number of shoots per explant, the LSD Fisher test discriminated at least three groups (data

not shown), but again without any definite correlation with the concentrations of the growth regulators. The best response was attained at 0.05/0.0 mg/l of KIN/NAA, with an average of 8 shoots per explant (Fig. 1). This results agrees well with the findings of a study of several explants of *P. cineraria* by Nandwami and Ramawalt (1993), which found that the maximum number of shoots was produced from the cotyledonary explants. Nandwami and Ramawalt concluded that cotyledons play an important role in shoot production from seedling explants which supply endogenous growth regulators to cultures. To our knowledge the literature contains no mention of attempts to achieve *in vitro* regeneration of *P. glandulosa* var. *torreyana*; only the successful use of *in vivo* cuttings of this species has been described (Felker and Clark, 1981).

It is noteworthy that in the present study no callus formation was detected in any of the shoots produced in any of the applied treatments (Fig.2). This observation is important for the clonal propagation of *Prosopis* species because callus production has been mentioned as a cause of genetic instability in these species (Jordan, 1996). On the other hand, we applied some of the treatment combinations outlined in this report to other *Prosopis* species (*P. juliflora*, *P. palmeri*, *P. tamaulipana*, and *P. laevigata*) but observed no morphogenetic response in any of them and only callus formation was detected. In conclusion, our results along with the findings of most other studies on *Prosopis* (Harris, 1992; Jordan, 1996) make it clear that a priority area of strategic research should be to carry out a fundamental study of the reasons underlying the poor performance of *Prosopis* tissue in culture.

**Acknowledgements.** The authors wish to thank CONACYT proyect 27759-B for partial support of this research. Mr. Felipe Villegas for art work and M.en C. Eulogio de la Cruz for critical reading of the manuscript.

### Literature cited

- FELKER, P. AND P. R. CLARK. 1981. Rooting of mesquite (*Prosopis*) cuttings. *Journal of Range Management* 34: 466-468.
- FELKER, P. 1993. Review of applied aspects of *Prosopis*. In: J. C. Temari, N. M. PASIECZNIK AND P. C. C. HARRIS (eds.) *Prosopis species in the arid and semi-arid zones of India*. *Prosopis Society of India*. Jodhpur, Rajasthan, India, 11-14.
- HARRIS, P. J. 1992. Vegetative propagation of *Prosopis*. In: Dutton, R. W (ed.) *Prosopis species: aspects of their value, research and development*. Proceedings of the Symposium University of Durham, U. K., 175-191.
- JORDAN, M. 1996. Métodos de propagación biotecnológicos y convencionales de leguminosas de usos múltiples para zonas áridas,. In: J. Izquierdo, y G. Palomino (eds.) *Técnicas convencionales y biotecnológicas para la propagación de plantas de zonas áridas*. Oficina Regional de la FAO para la América Latina y el Caribe, Santiago, Chile, 111-150.
- MURASHIGE, T. AND F. SKOOG . 1962. A revised medium for rapid growth and bioassay with tobacco cultures. *Physiologia Plantarum* 15: 473-479.

- NANDWAMI, D. AND RAMAWALT, K.G. 1993. *In vitro* plantlets formation through juvenile and mature explants in *Prosopis cineraria*. *Indian Journal of Experimental Biology* 31: 156-160.
- RZEDOWSKI, J. 1988. Análisis de la distribución geográfica del complejo *Prosopis* (Leguminosae, Mimosoideae) en Norteamérica. *Acta Botanica Mexicana* 3: 7-19.

Recibido: 15-10-2001

Aceptado: 18-04-2002

ABRAHAM RUBLUO, ELIZABETH ARRIAGA E INGRID BRUNNER, Jardín Botánico, Instituto de Biología, UNAM. Apartado postal 70-614. Del. Coyoacán. 04510, México, D.F.; JUANA JUÁREZ, Colegio de Postgraduados, Montecillo, México; AGUSTÍN VARGAS, FES-Iztacala, UNAM, Av. de los Barrios s/n Los Reyes Iztacala, Edo. de México.