



# ASSESSMENT OF ARTIFICIAL CROSSES BETWEEN *Jatropha curcas* ECOTYPES BY USING SSR MARKERS



NAVARRO-MUÑOZ A\*, ALVAREZ-DAGNINO A., YAMAMOTO-NUÑEZ J., HERNANDEZ-IBARRA K., CALDERÓN-VAZQUEZ C.\*

\*Instituto Politécnico Nacional. Centro de Investigación Interdisciplinario para el Desarrollo Integral de la Región (CIIDIR-IPN Unidad Sinaloa). Departamento de Biotecnología Agrícola. Boulevard Juan de Dios Bátiz Paredes. No. 250. Guasave, Sinaloa, México. CP 81101, \*ccalderon@ipn.mx.

\*anavarromu@gmail.com

*Key words: microsatellite markers, ecotypes, polymorphism*

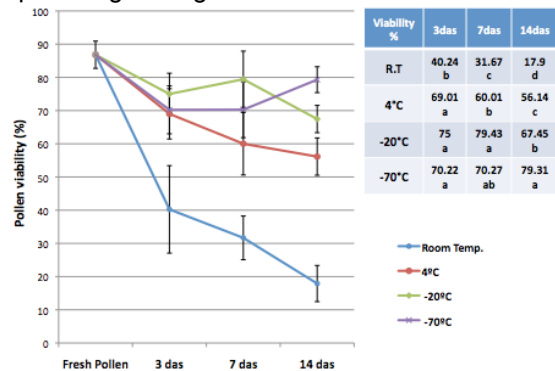
**Introduction.** *Jatropha curcas*, better known as “piñon” is a rapidly emerging biofuel crop attracting a lot of interest and investments because of its high oil seed content (27 to 40%), suitable for biodiesel production. It is known that Mexico and Central America are the centers of origin of *J. curcas* and thus Mexican accessions could possess greater genetic richness than the African or Asian collections, which represents an opportunity to start domestication and breeding programs. For this purpose, accessions from Morelos, Veracruz and Puebla have been selected as promising candidates. For those, setting up a platform for crossing and for generating new allele combinations it is desirable.

This work presents the genotyping results of 3 promising ecotypes by employing 14 SSR markers as well as the monitoring of pollen viability on different storage conditions (4°C, -20°C, -70°C) for 3, 7 and 14 days after sampling.

**Methods.** In order to identify SSR alleles for a parentage analysis, a genetic fingerprinting of 23 putative parentals (Morelos, Veracruz and Puebla) was done by using 14 different SSR markers<sup>1</sup> on a capillary electrophoresis system. Artificial pollination was performed in a experimental field at Guasave, Sinaloa, Mexico making a total of 15 crosses. GeneMapper was employed for the identification and analysis of alleles. For the study of pollen viability, pollen grains were stained with acetocarmine<sup>3</sup> observing and classifying them as fertile or not fertile in an optical microscope under a magnification of 40X. Analysis of variance was performed by using SAS v9.0 (2002). Means were separated by Tukey’s studentized range test at  $P < 0.05$ .

**Results.** The genotyping of 23 parentals with 8 SSR markers<sup>1</sup> showed low polymorphism levels. A total of 11 different alleles were detected. The analyzed markers produced one to two alleles, and 3 of those were polymorphic. All 23 analyzed parentals were homozygous for all the markers and only 2

individuals from Puebla and Veracruz had unique alleles and thus could be used for the parentage assignment.



**Fig.1** Pollen viability percentage under different storage conditions at 3, 7 and 14 days after sampling.

Fresh pollen showed an 86.82 % viability but it decreased significantly to 17.9% at 14 DAS (Days after sampling) under room temperature. The other storage conditions (4, -20, -70°C) showed no significant differences at 3 DAS, but at 14 DAS, the highest levels of viable pollen were observed at -70°C treatment.(Fig.1).

**Conclusions.** The first set of 8 SSR markers assayed revealed low polymorphism and high homozygosity levels.

The pollen viability test showed that -70°C is the best storage temperature in 14 DAS, it remains to evaluate pollen storage conditions at 3 months after sampling.

**Acknowledgements.** Thanks to CONACYT AND COFAA-IPN for funding and also to M.Sc. Luz García-Perez for her valuable assistance in this project.

## References.

1. Basha S.D., G. Francis, H.P.S. Makkar, K. Becker, M. Sujatha. (2009) A comparative study of biochemical traits and molecular markers for assessment of genetic relationships between *Jatropha curcas* L. germplasm from different countries, *P. Sci* 176 pp812–823.
2. Mastan S.G., Pamidimarri D. V. N. S., Rahman H., Ghosh A., Rathore M. S., Prakash C. R., Chikara J. (2012) Molecular characterization of intra-population variability of *Jatropha curcas* L. using DNA based molecular markers. *Mol Biol Rep* 39 pp4383-4390.
3. Radford AE, Dickinson WC, Massey JR, Bell CR. (1974). *Vascular plant systematics*. New York: Harper & Tow Publishing.