MICROSATELLITES ANALYSIS OF GENOMIC PROFILING OF *Jatropha curcas* ACCESIONS

Ana Ramos-Díaz[1,3]; Abelardo E. Navarrete-Yabur[2], Guadalupe López-Puc[1,3,4]

[1]Centro De Investigación y Asistencia en Tecnológica y Diseño del Estado de Jalisco A.C., Unidad Sureste.

[2] Agroindustria Alternativa del Sureste S.P.R de R.L. de C.V.

[3] Present address: Calle 30 No. 151, Col. Garcia Gineres (Interior CANACINTRA) C.P. 97070, Mérida, Yucatán, México.

[4] Corresponding author E-mail: glopez@ciatej.net.mx

ABSTRAC

Microsatellites are molecular genetic markers that do not depend on the physiological or morphological information, ideal markers for genetic analysis because they are not affected by natural selection or environmental changes, are specie specific, but differ in the number of repetitions between individuals. They are highly polymorphic, these markers identify both homozygous and heterozygous individuals, thus are considered codominant markers. The feasibility of this type of genomic analysis to analyze genetically different genotypes of *J. curcas*, is considered a breakthrough for genetic improvement.

The present investigation was undertaken to assess the genetic diversity among 26 selected accessions of J. curcas by advantageous characteristic agronomic, that are used as parental for the generation of new varieties. Of 25 primers tested initially, eight were selected for genomic analysis, that amplified a pattern consistent and repeatable, between four and ten bands, the position of each band amplified by each primer was considered as a character for the phylogenetic analysis. Phylogenetic analysis of 52 characters is expressed with a distance matrix and a phylogenetic tree which groups individuals according to their genetic proximity.

RESULTS AND DISCUSION

Figure 1. *Jatropha c.*

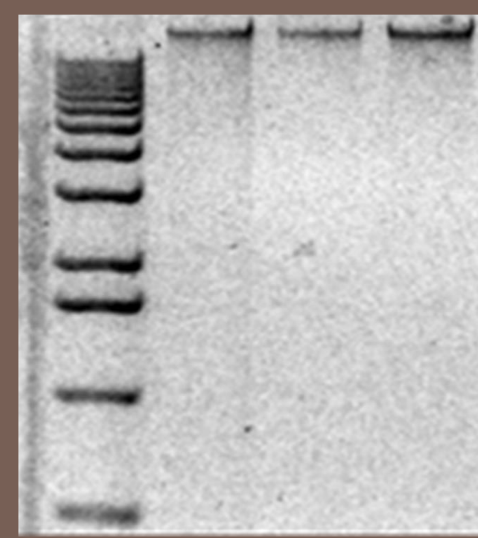

Figure 2. ADN *Jatropha c.*

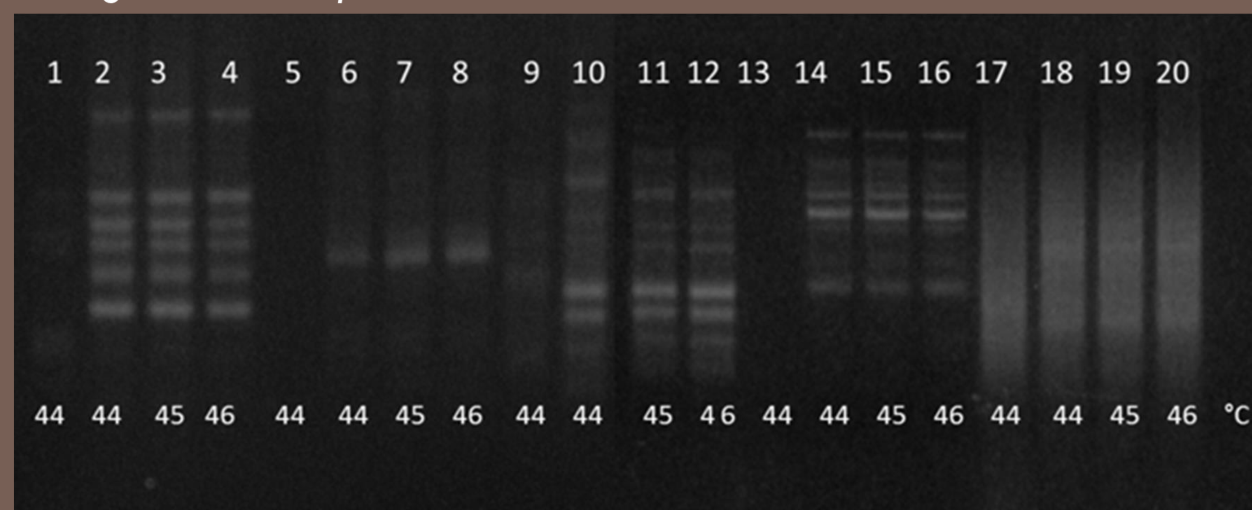

Figure 3. DNA amplification accession J. curcas with oligonucleotides: oligonucleotide 13 (lines 1-4), oligonucleotide 8 (lines 5-8), oligonucleotide 12 (lines 9-12); oligonucleotide 25 (lines 13-16) and oligonucleotide 16 (lines 17-20). Amplifications were performed at 44, 45 and 46 ° C. Lines 1, 5, 9, 13 and 17 correspond to oligonucleotides corresponding reactions without the addition of genomic DNA.

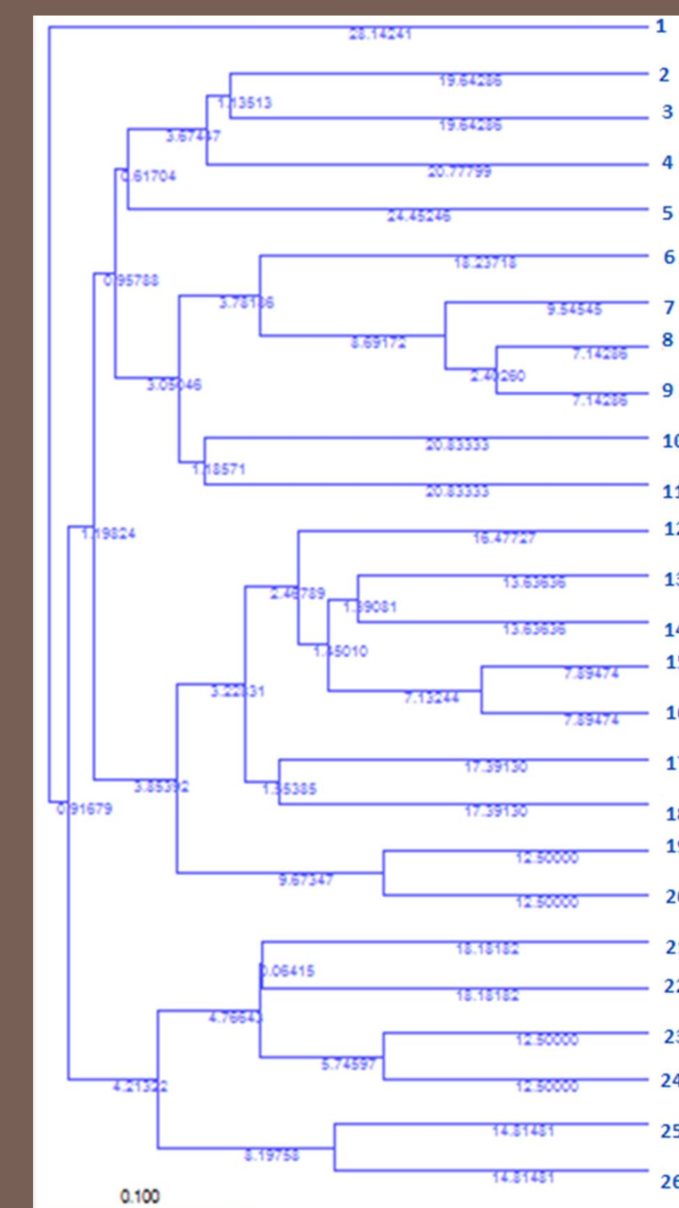

Figure 4. Dendrogram generated using UPGMA analysis, which shows the relationship between 26 accessions of J. curcas (numbers represent quantitative probability assigned to each branch leaves).

Genomic DNA was isolated from young leaves of plants J. curcas (figure 1) using the methodology reported by Dellaporta et al. (1983) with modifications(figure 2). DNA use for amplification temple five accessions of J. curcas , three plants were analyzed for each type of accession . In the figure 3, observed band patterns obtained in the amplification of genomic DNA from an accession alignment three different temperatures (44, 45 and 46 ° C) , using the oligonucleotides called ISSR 8, 12, 13, 25 and 16 , where the first four amplified repeatable banding patterns , oligonucleotide 16 was discarded because I can not identify the separated bands were obtained at temperatures only sweeps amplifications evaluated .

The second criterion for selection of oligonucleotides was polymorphism range , allowing to select a group 10 of the remaining 14 oligonucleotide . For genomic analysis each amplified band is considered a loci, and corresponds to a quantifiable nature , where the presence of the band in a position (relative molecular mass) was given the value 1 and the absence of the same in another sample given the value of 0 (lack of expression of the character).

After the PCR reaction , the bands were separated in agarose gel 1.5% , the pattern of amplified bands were visualized and documented in fotodocumentador gels. Digital file was analyzed with Gel Quest software for estimating the position of each band and obtaining a binary matrix ,

The number of bands amplified by each primer was insufficient and unreliable to estimate the genetic closeness between two individuals , so we decided to work with eight oligonucleotides produced a total of 52 loci quantifiable , similar results were reported by Sun et al. (2008) , where ISSRs analysis showed low genetic diversity among 58 accessions of *J. curcas* from India.

Information distance matrix was subjected to analysis "cluster " with " Method for Unweighted Pair Group Arithmetic Mean (UPGMA), and a dendrogram was generated using the program ClusterVis in the dendrogram (Figure 4). In future, this analysis can be applied to identifying characteristics distinticas accessions as toxic and nontoxic as reported by Tanya et al. (2011) , where he discussed the use of ISSR for classifying Mexican accessions of this species in toxic and non-toxic .

SAGARPA

Acknowledgments: Al Fondo sectorial SAGARPA-CONACYT, por los recursos para realizar el proyecto 163502 del cual forma parte el presente trabajo.

CONCLUSION

ISSR markers proved to be efficient for detection of genetic variability *J. curcas* that can be used in conjunction with field data, plan crosses that increase genetic diversity and thereby increase the chances of obtaining new accessions with desirable agronomic characteristics.

BIBLIOGRAPHY

-Dellaporta S.l.; Wood, J., Hicks J.B. (1983). A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. 1 (14): 19-21.
-Sun Q., Lib L., Lib Y., Wua G., Gea X. (2008). SSR and AFLP Markers Reveal Low Genetic Diversity in the Biofuel Plant *Jatropha curcas* in China. Crop Science48: 1865-1871.
-Tanya P., Taeprayoon P., Hadkam Y., Srinives P. (2011) .Genetic Diversity Among Jatropha and Jatropha-Related Species Based on ISSR Markers. Plant Mol Biol Rep