GENETIC VARIATION AMONG CLONES OF *Agave tequilana* Weber var. azul SUBJECTED TO DIFFERENT DOSES OF GAMMA RADIATION

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INTRODUCTION

***Agave tequilana* Weber. var. azul is one of the most important cash crops in Mexico, from which the world wide famous distilled bevarage tequila is produced. Intense cultivation has subjected this species to a loss of genetic variation, making the plant highly vulnerable to environmental stress. Gamma radiation has been used previously to increase genetic variability in this species [1]. The mutants are propagated by plant tissue culture techniques, which involves asexual process and, in theory, should result in clonal propagation of true-to-type regenerants. However, epigenetic changes and somaclonal variation are common occurrences in plant tissue culture [2].**

The aim of this study was to evaluate the level of genetic variation among clones of *A. tequilana* from embryogenic callus subjected to different doses of gamma radiation using AFLP molecular markers to confirm or discard variation among plants obtained by plant tissue culture.

METHODS

A total of 66 plantlets obtained from embryogenic callus of *A. tequilana* genotype S7 were evaluated. The calli were irradiated in a gammacell 220 (Canada Ltd), with Co 60 as the gamma radiation source. Doses of 0, 5, 10, 15, 20, 25, 30, 35 and 40 Gy were administered. Five nonirradiated calli were used as control. DNA was extracted from *A. tequilana* leaves following the procedure of Nucleon Phytopure DNA Extraction Kit from Amersham Life Sciences®. DNA fragments were obtained following the protocol of AFLP® Analysis system I & AFLP® starter primer kit from GIBCO, BRL. Six combinations of primers were tested, chosen from the results obtained by Cuevas-Figueroa (2001) [3] based on the highest number of polymorphic fragments. Polymorphic fragments were scored as 1 for presence and 0 for absence across all the polymorphic loci to create a binary matrix.

RESULTS AND DISCUSSION

Of 151 markers obtained from AFLP analysis 87 (57.6%) were polymorphic (Table 1). These polymorphic markers were specific for each dose of radiation used, where the dose of 15 Gy had the highest amount. The combination that produced the greatest number of unique markers was ACG-CAC, suggesting that most of the mutations may be associated with changes in cytosine. Finally, a first genetic map for *A. tequilana,* was obtained from the markers, covering a distance of 1870 cM with 28 homogeneous groups (Fig. 1).

Table 1. Number of fragments obtained from each combination of primers.

Primers Eco RI/Mse I	Number of amplified fragments	Number of polymorphic fragments
AGG/CAC	20	1
ACG/CAC	39	25
AGG/CTT	28	20
AGG/CAA	13	9
ACG/CAA	30	19
ACC/CAG	21	13

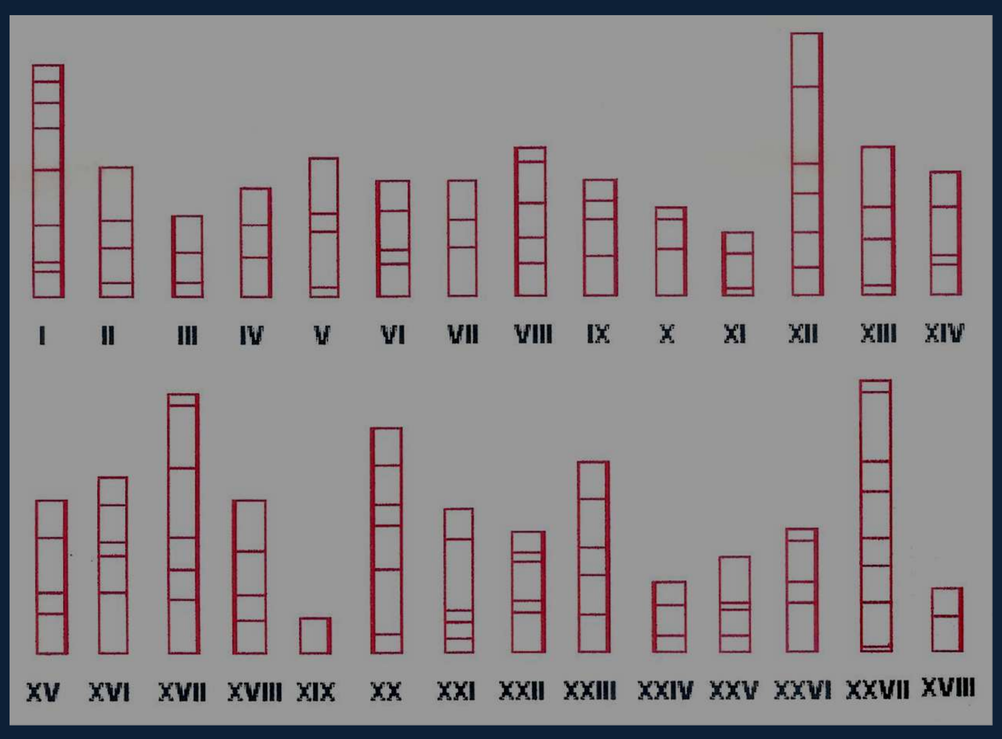

Fig. 1. Genetic map of Agave clones in vitro based on molecular markers AFLP and obtained by Mapmaker version 3.0 with LOD score of 3.5

CONCLUSIONS

AFLP markers are capable of detecting mutations in DNA induced by gamma radiation among clones of *A. tequilana.* These polymorphic markers were specific for each dose of radiation used.

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