A PRELIMINARY PROTOCOL TO PROPAGATE TRUE-TO-TYPE AND CLADODE SWELLING DISEASE-FREE CACTUS PEAR STOCK PLANTS

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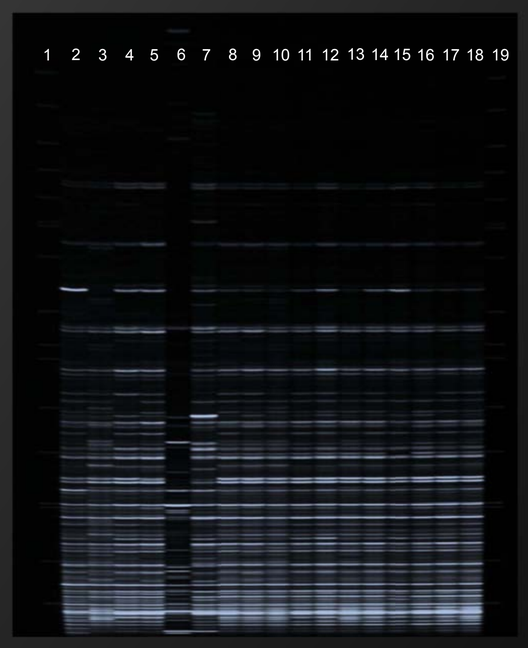
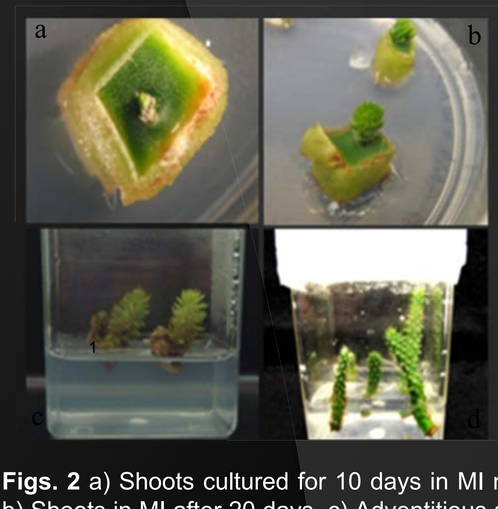
INTRODUCTION: **C**actus pear is the 6th most important fruit crop in Mexico, as vegetable and forage crop is gaining attention from growers and ranchers from semiarid areas. All orchards are using cuttings as planting stock, generally obtained from pruning waste, rendering them susceptible to diseases. "Cladode swelling" (CS) is one of the most important diseases which is spreading rapidly due to the lack of control measures, according to ( ) CS is associated to phytoplasms, recent studies conducted by Felker (2009) state that an umbravirus hosted by the cowpea aphid correlated with the spreas of CS in a commercial orchard of Salinas CA, USA. The lack of a nursery stage on cutting production is promoting its rapid spread in Mexico. Typical symptoms of CS are: thickening of cladodes due to increase in volume of the spongy parenchyma, phloem obstruction, apparent thickening of mucilage, bud break arrest with the consequent reduction of the number of new cladodes and flowers, therefore, affecting plant productivity. Fruits affected by CS are small, short and with thick peel, considered non-commercial (Fig. 1). CS dispersion on the field seems to be related to the presence of the disease on the donor orchard and the expertise of the worker collecting cuttings. CS expression on the plant is irregular, sometimes affecting individual cladodes, branches and in the worst case the entire plant. At plants at the latter stage do not recover, remaining unproductive for years, however there are no reports of plants killed by the disease.

Fig. 1. Symptoms of cladode swelling in whole plant, cladodes and fruits in cultivated cactus pear (Roja Lisa).

MATERIALS AND METHODS

This study was conducted under the assumptions that infection through seed is not feasible and apomictic plants preserve the genetic identity of the mother plant. Seeds from apparently healthy plants of 10 varieties; Roja Vigor, Milpa Alta, Reyna, Burrona, Roja Lisa, Copena F1, Atlixco, Copa de Oro and Cristalina were collected from selected commercial orchards. They were scarified with sulfuric acid, then planted in commercial germination mix and grown in the greenhouse. Once germinated, apomictic plants separated by AFLPs (Vos *et al.*, 1995) were used as initial explants for the micropropagation process. Explants were placed on induction medium (IM) which consisted on Gamborg medium with 3 % sucrose and 4 mg/L bencilaminopurine to induce adventitious shoot formation from vegetative buds. Five pairs of coligonucleotides were designed based on a comparative study between cactus phytoplasms and the sequence of *Xillela fastidiosa* consulted in the NCBI database. The non-homologous sequences of both organisms were identified generating specific oligonucleotides specific for the phytoplasm sequence. Infected cladodes of Cv. Roja Lisa were blended and homogeneaized in liquid nitrogen. DNA was extracted, Doyle y Doyle [3] and used to perform direct PCR on 3 tissues; parenchyma, colechyma and colenchyma secretion using the kit Phire® Plant Direct PCR Kit (FINNZYMES), with 5 different oligonucleotide pairs of designed nucleotides Fito F1/Fito R1, Fito F2/Fito R2, Fito F3/Fito R2, Fito F4/Fito R4, Fito F5/Fito R5). Amplification conditions were: initial desnaturalization at 95 ºC for 3 min, 40 cycles of 95 ºC for1 min, 45 ºC for 2 min and 72 ºC for 1 min with a final extensio phase of 72 ºC por 3 min





Figs. 2 a) Shoots cultured for 10 days in MI media. b) Shoots in MI after 20 days. c) Adventitious shoots 25 days after vegetative buds formation. d) Shoots in elongation media.
Left: AFLP gel lane 1 Cristalina, a group of Cristalina seedlings compared to xoconostle (lane 6).

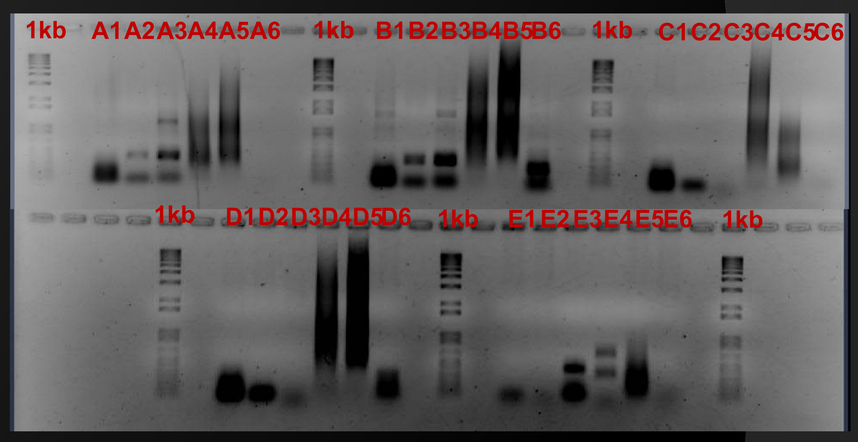


FIG. 3. Amplification of several plant parts with designed oligonucleotids. A: Sample1: Cladode AND Plant 1; B: Sample 2 DNA de Cladode; C: Cholénchyma; D: Parénchyma; E: Colénchyma Secretion. Lanes 1 to 5 indicate oligonucleótide pairs of Fito F1/R1 to Fito F5/R5 respectively, lane 6 tests oligonucleotides16R758F/16R1232R (universal for phytoplasms). (*Experiment in progress*).

RESULTS:

AFLPs were useful to separate individuals of apomictic from sexual origin sexual Fig 2. Buds were formed at the base of the initial shoot 15 days after culture in IM. Adventitious shoots developed 20 days after buds formation. Approximately 20 shoots formed and developed in each subculture. Shoots that reached 7 cm height were transferred to elongation medium (IM with no growth regulators) for subsequent transfer to greenhouse. Amplificatión showed several sizes of amplicons, oligonucleotids Fito F1/ Fito R1 y Fito F2/ Fito R2 showed amplification in most of the tissues tested. Oligonucleotids Fito F3/ Fito R2 and Fito F4/ Fito R4 amplified on colénchyma secretión, but only the former amplified, while Fito F5/ Fito R5 did not. Using this protocol is possible to obtain a large number of stock or mother plants with the same genetic identity than the original plant and CS-free in 70 to 75 days.

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