Final Scientific Report

Cover Page

BARD Project Number: IS-4017-07

Date of Submission of the report:

Project Title: Role of polyploidy in vine cacti speciation and crop domestication

Investigators
Principal Investigator (PI): Noemi Tel-Zur

Institutions
Ben-Gurion University of the Negev

Co-Principal Investigator (Co-PI): Jeff J. Doyle
Cornell University

Collaborating Investigators: ---

Keywords allopolyploidy, autopolyploidy, breeding program, cytomixis, fruit quality, Hylocereus, interploidy-interspecific hybridization, molecular phylogenetics, ploidy level, polyploid origins, Selencereus

Abbreviations commonly used in the report, in alphabetical order:
BC1: first-back crosses; IH: interploid F1 hybrids; PMCs: pollen mother cells; nrDNA ITS: internal transcribed spacers 1 and 2 of nuclear ribosomal DNA

Budget: IS: $115,000     US: $157,000     Total: $272,000

Research & Development Authority
Ben-Gurion University of the Negev
P.O. Box 635, Beer-Sheva 84105, Israel

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Appendix G6a
**Final Scientific Report**

**Publication Summary (numbers)**

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**Postdoctoral Training:** List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

**Cooperation Summary (numbers)**

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**Description Cooperation:**

Synergistic and complementary cooperation existed between the two laboratories throughout the entire four-year span of this research project. Dr. Tel-Zur carried out phenotypical and cytological characterizations and genome size measurements using flow cytometric cell sorting, and she studied pollen and seed viabilities. Prof. Doyle and his students conducted molecular systematic studies using nuclear and chloroplast genes. DNA was sent from Israel to the USA to support the joint pursuit of the project goals. Molecular technologies and protocols were transferred to the Tel-Zur laboratory by the Israeli Ph.D. student, Aroldo Cisneros, who visited Doyle's laboratory during January-February, 2009. Prof. Doyle, in turn, visited the Tel-Zur laboratory and presented a seminar at the Blaustein Institutes for Desert Research (BGU) – Sede Boqer campus, during November 2009.
## Patent Summary (numbers)

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Appendix G6b
1. **Abstract:** Over the past 25 years, vine cacti of the genera *Hylocereus* and *Selenicereus* have been introduced into Israel and southern California as new exotic fruit crops. The importance of these crops lies in their high water use efficiency and horticultural potential as exotic fruit crops.

Our collaboration focused on the cytological, molecular and evolutionary aspects of vine cacti polyploidization to confront the agricultural challenge of genetic improvement, ultimately to improve success of vine cacti as commercial fruit crop plants.

More specifically, we worked on the:

1. Identification of the putative ancestor(s) of the tetraploid *H. megalanthus*;
2. Determination of the number of origins of *H. megalanthus* (single vs. multiple origins of polyploidy);
3. Cytogenetic analysis of BC₁ and F₁ hybrids;
4. Determination of important agricultural traits and the selection of superior hybrids for cultivation.

The plant material used in this study comprised interspecific *Hylocereus* F₁ and first backcross (BC₁) hybrids, nine *Hylocereus* species (58 genotypes), nine *Selenicereus* species (14 genotypes), and four *Epiphyllum* genotypes.

Two BC₁ hexaploids (BC-023 and BC-031) were obtained, a high ploidy level that can be explained only by a fertilization event between one unreduced female gamete from the triploid hybrid and a balanced gamete from the pollen donor, the diploid *H. monacanthus*. These findings are scientific evidence that support the possibility that “hybridization followed by chromosome doubling” could also occur in nature.

Cytomixis, the migration of chromatin between adjacent cells through connecting cytoplasmatic channels, was observed in vine cacti hybrids and may thus imply selective DNA elimination in response to the allopolyploidization process.

Evidence from plastid and nrDNA internal transcribed spacers (ITS) sequences support the placement of *H. megalanthus* within a monophyletic *Hylocereus* group. Furthermore, both plastid and ITS datasets are most consistent with a conclusion that this tetraploid species is an autopolyploid, despite observations that the species appears to be morphologically intermediate between *Hylocereus* and *Selenicereus*. Although the possibility of very narrow allopolyploidy (i.e., derivation from parents that are barely diverged from each other such as closely related species in the same genus) cannot be ruled out entirely based on our data (in part due to the unavailability of *Hylocereus* species considered to be morphologically the closest relatives of *H. megalanthus*), the possibility of *H. megalanthus* representing an intergeneric cross (i.e., *Hylocereus × Selenicereus*) seems extremely unlikely. Interestingly, the process of homogenization of ITS sequences (concerted evolution) is either incomplete or lacking in both *Hylocereus* and *Selenicereus*, and the inclusion of several artificial hybrids in the molecular study revealed the potential for biparental plastid inheritance in *Hylocereus*.

The most important agricultural implication of this research project was the information collected for F₁ and BC₁ hybrids. Specifically, this project concluded with the selection of four superior hybrids in terms of fruit quality and potential yields under extreme high temperatures. These selected hybrids are self-compatible, avoiding the need for hand cross pollination to set fruits, thus reducing manpower costs. We recently offered these hybrids to growers in Israel for prioritized rapid evaluation and characterization.
2- Achievements

Significance of main scientific achievements: We evaluated a total of 109 hybrids over three consecutive years; 40 were interspecific interploid F₁ hybrids (IH), and 69 were first back crosses (BC₁). Ploidy estimation (2C-DNA content) was carried out using flow cytometry. The 2C-DNA of the plants ranged from 3.66 pg for IH-051 to 11.44 pg for BC-023, showing diploid, triploid, tetraploid and hexaploid levels. Ploidy levels higher than 3x, observed in the hybrids under study, were probably due to unreduced gametes produced by the mother (triploid) plant or by the pollen donor in the case of the tetraploid *H. megalanthus*, or by both. In BC₁, we expected to find ploidy levels ranging from 2x to 4x, i.e., based on the ploidy of the pollen donor, but never 6x, as was obtained for BC-023 and BC-031. In these two cases, we assumed that the genome was duplicated following a fertilization event between one unreduced \((2n)\) female gamete from the triploid female parent and a normal reduced \((n)\) male gamete from the diploid *H. monacanthus*.

During observations of pollen mother cells (PMCs) of the F₁ and BC₁ generations, multiple chromatin bridges, which allow chromatin transfer between cells, were observed extending between anywhere from two to four microsporocytes in Prophase I. These observations of PMCs showed that the cytomixis process was more frequent in interspecific interploid hybrids that resulted from a cross between the allotriploids S-75 and 12-31 as the mother parent, and the diploid *H. undatus* as the pollen donor. The occurrence of this phenomenon in these hybrids is most likely because of the convergence of three different genomes (*H. monacanthus*, *H. megalanthus* and *H. undatus*), and it may thus imply selective DNA elimination, a key component of the allopolyploidization process, as a response to hybridity. Another interesting phenomenon observed was the formation of vesicle-like objects in the cell walls of the PMCs. Chromosome segments were observed in and around some of those vesicles, indicating that the latter may be part of a mechanism to remove DNA from the PMCs before the meiotic division is completed. To the best of our knowledge, the literature contains no previous reports on similar vesicle-like formation.

The morphological traits of the F₁ and BC₁ generations showed variations in fruit form (from round to elongated ellipse), number of flowers per hybrid per year, and fruit weight. Comparing these results with the parental genotypes, the majority of
the hybrids showed partial dominance or co-dominance of the studied morphological traits. For example, fruit flesh color was a co-dominant trait, and spiny peel, inherited from *H. megalanthus*, was a dominant trait showing classical Mendelian inheritance. Two hybrids, IH-003 and IH-052, showed the highest potential yields of 3.25 and 3.72 kg/plant/year, respectively. These yields were probably underestimated and will increase when the plants fully mature.

Quantification of the number of viable seeds/fruit revealed a high percentage of viable seeds (more than 85%) in most of the $F_1$ and $BC_1$ representatives we studied. In spite of previous reports about the correlation between seed number and fruit size in Cactaceae, we found no such correlation in our study.

The flowering season of the $F_1$ hybrids and of $BC_1$ was from August to November. The time to full ripeness varied widely among the studied plants. Ripening time was found to be a genotype-specific trait with considerable variability among the IH and $BC_1$ lines. These results showed that the harvest season could be expanded using different hybrids with extended flowering and ripening periods.

Myriad taxonomic speculation surrounds *H. megalanthus* due to observations that the species appeared to be morphologically intermediate between *Hylocereus* and *Selenicereus*. Britton and Rose (1920) placed the species in a new genus, *Mediocactus*, in an effort to account for its putative intermediacy. However, Bauer (2003) argued that *H. megalanthus* was, in all ways, like other *Hylocereus* members, and that the presence of spiny fruit, though more common in *Selenicereus*, was not unheard of in *Hylocereus*. Nevertheless, the tetraploid status of *H. megalanthus* evoked a compelling hypothesis of intergeneric hybridization between *Hylocereus* and *Selenicereus* followed by genome duplication. Cytological observations (Lichtenzveig et al. 2000) and evidence from FISH and GISH (Tel-Zur et al. 2004a) and RAPDs (Tel-Zur et al. 2004b), while contributing to our general understanding of *Hylocereus* and *Selenicereus*, could not conclusively answer the question of whether *H. megalanthus* is of allo- or auto-polyploid origin. To investigate this issue further, we conducted a molecular phylogenetic study using three chloroplast markers and nrDNA ITS 1 and 2 sampled from 101 individuals representing eight of the 14 *Hylocereus* species, seven of the 11 *Selenicereus* species, three genera of closely related epiphytic cacti, and three genera of more distantly related columnar cacti. Chloroplast and ITS data suggest that *H. megalanthus* is nested within a monophyletic *Hylocereus* group, supporting Bauer’s (2003) placement of this species in *Hylocereus*.
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4. A. Cisneros and N. Tel-Zur. Characterization and genetic relationships among interspecific-interploid hybrids and back crosses in *Hylocereus* species (Cactaceae) revealed by phenological and molecular markers. *In preparation*
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