AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2018 – 2020

TITLE: Varietal Improvement in Mint Using Chromosome Doubling

RESEARCH LEADERS: Kelly Vining, Ryan Contreras

COOPERATORS: Kim Hummer, Nahla Bassil **(**USDA National Clonal Germplasm Repository, Corvallis, Oregon).

EXECUTIVE SUMMARY: The goal of this project is to produce mint plants that can be compatible in inter-species sexual crosses. Mint DNA is packaged in chromosomes, which come in sets of 12. Different mint species can have two, four, six, or eight sets of chromosomes. We are working with wild mint plants that have two sets of chromosomes, termed “diploid”. Chromosome doubling involves treating plants with a chemical that interrupts normal chromosome segregration into daughter cells during cell division.

OBJECTIVES: Starting with mint plants containing two sets of chromosomes (diploid, two genome copies), induce chromosome doubling to obtain mint plants containing four sets of chromosomes (tetraploid, four genome copies).

PROCEDURES:
With the goal of developing a consistent protocol for chromosome doubling in mint plants, initial experiments were performed with application of oryzalin onto plant apical meristems. These efforts proved to be labor intensive and did not produce the desired results. To achieve high throughput while increasing the percentage of recovered tetraploid plants after treatment, a new approach was employed combining steps from several protocols as described below..

Stem cuttings of four different diploid mint plants were rooted in soil mix in a growth chamber for 2 weeks. Stems of the rooted young plants were collected, leaves were removed without damaging the axillary meristems, and the stems were washed and surface sterilized. Cleaned stems were then cut into pieces, each bearing two axillary meristems. The stem pieces were divided into two groups: treatment and control. For the treatment group, ~50 stem pieces were transferred into an Erlenmeyer flask with 125 ml of 0.15% aqueous colchicine solution. Stem pieces in the control group were transferred to a flask containing 125 ml sterile water without colchicine. Flasks with stem pieces were kept on shaker at low speed for 24 hours at room temperature. After incubation, rinsed pieces were transferred to sterile magenta boxes with shooting media for recovery. Recovery was 71 to 91% for control and 25 to 90% for treatment. After shoots grew for 3 to 4 weeks they were transferred onto rooting media for an additional 3-4 weeks after which each shoot was separated and moved to soil. For protection during adaptation, plants were covered with plastic domes for one week. Domes were then removed to let air flow. Once plants grew to the flowering stage, pollen size and ploidy levels

were assayed by flow cytometry. Plants were either uniformly tetraploid, or “mixaploid”, indicating a mixture of diploid and tetraploid cells. Three out of four diploid accessions had at least one plant that was mixaploid or tetraploid. In tetraploid plants, pollen size was increased by ~30%. Propagation of the plants that showed tetraploid or mixaploid characteristics is ongoing by planting stem cuttings and collecting and germinating seeds obtained from flowering individuals. Propagated plants will be assayed phenotypically and by flow cytometry to confirm that their ploidy is stable.

SIGNIFICANT ACCOMPLISHMENTS:
The new protocol is more efficient, enabling high throughput. In addition, the protocol increases survival and successfully produced tetraploid and mixaploid plants.

BENEFITS & IMPACT:
The capability to consistently perform chromosome doubling in mint is essential to the OSU mint breeding program. The most important breeding goal is to achieve resistance to the fungal disease Verticillium wilt. We have identified wilt resistance sources among USDA accessions representing several different mint species with different ploidy levels. These plants are currently being crossed to produce interspecific hybrids. Diploid mint plants cannot be directly crossed with octoploid plants(eight sets of chromosomes), but tetraploid plants can. Successful chromosome doubling means that breeding can pyramid different sources of resistance in cultivars currently under development.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: Oregon Mint Commission, 2019.

FUTURE FUNDING POSSIBILITIES: Oregon Mint Commission, Mint Industry Research Council