

# **Generation and evaluation of a mutation-line collection useful for enhancing resistance for potyviruses and major diseases of watermelon**

## **Annual Report to National Watermelon Association --- Grower Summary**

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### **Introduction**

In this study we proposed to implement an experimental procedure to develop a library of watermelon mutant lines derived from the United States Plant Introduction accession (PI 595203, known to have resistance to several potyviruses, including *zucchini yellow mosaic virus* (ZYMV), *papaya ring spot virus* (PRSV), and *watermelon mosaic virus* (WMV). We hypothesize that additional gene mutations in this genotype (PI 595203) could enhance resistance to potyviruses, including resistance to the *squash vein yellowing virus* (SqVYV) that causes watermelon vine decline (WVD).

The mutant library should provide a plethora of lines that in the future will be used by the watermelon research community for screening for resistance for major diseases or viruses of watermelon. This is a long term project that could take five years of intensive work and will need the cooperation of the watermelon community, including scientists from ARS, Universities, and seed companies. The proposed mutant library should be of significant benefit the watermelon industry. In addition to potential disease or virus resistance, the library should be useful for screening and identifying mutant lines that produce over-dominance (heterosis) in yield and fruit quality, as has been shown in other crop plants (Semel, 2006).

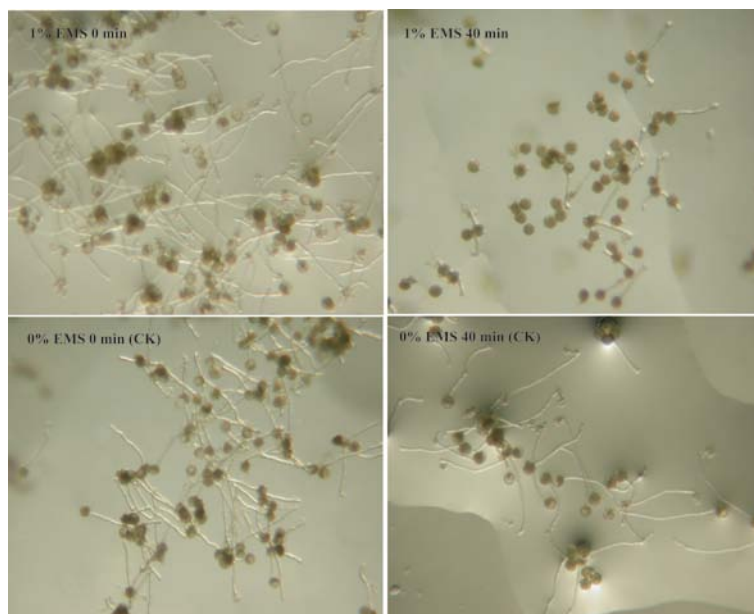
Our major objectives in the first stage of this project were: 1) to optimize the mutagenesis procedure for watermelon using ethyl methane sulfonate (EMS), and 2) generate and develop a mutation population.

**Initiation of Project.** Forty watermelon plants (PI 595203) were grown in the greenhouse and over 10,000 seeds were generated and prepared for further mutagenesis experiments.

**Mutagenesis Experiments with Pollen.** Forty watermelon plants were successfully grown in the greenhouse. Male flowers were collected and pollen grains were used in mutagenesis experiments with EMS. Three key factors were investigated: treatment length (15 min - 1 hr), temperature (25°C - 30°C) and EMS concentration (0.03 – 1.50%). EMS solution was mixed

with paraffin oil. The results in these experiments indicated that treating the watermelon pollen grain with 1% EMS for 40 minutes at 30°C is optimal for obtaining mutated but viable pollen grains. Figure 1 shows viability of pollen tubes grown on a medium following a brief exposure (less than 1 minute) to EMS versus 40 minutes exposure to EMS. The long exposure to EMS resulted in a lower, but sufficient number of viable pollen grains.

**Pollination of female flowers with mutagenized pollen grain.** Every morning before 8 am, male flowers were collected from plants and exposed for 40 minutes to EMS. Then, the pollen grains were immediately used for pollinating the female flowers using a fine brush. At this time we have about 500 mutated seed. These seeds are being kept in a cold storage room (-18C) at the USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, GA and their germination will be tested in a greenhouse at the U.S. Vegetable Laboratory in Charleston, SC.



**Figure 1.** Shows viable pollen (having pollen tubes) grown on a medium following a brief exposure (less than 1 minute) to EMS (upper left) versus 40 minutes exposure to EMS (upper right). The lower photos show pollen tubes that have been treated for less than 1 minute (lower left) and for 40 minutes (lower right) with a solution that does not contain the mutagenic compound EMS. The upper right photo shows that about half of the pollen tubes are viable (having pollen tubes germinating), while the other pollen grains are not able to generate pollen tubes, possibly as the result of excessive exposure (40 minutes) to EMS.

## **Mutagenesis Experiments for Seeds**

As mentioned above, we have generated over 10,000 seeds of PI 595203. At present we are conducting experiments to optimize mutagenesis conditions with EMS for the watermelon seeds. Following optimization of mutagenesis conditions, seeds will be treated in the spring of 2011 and upon treatment will be germinated and resulting plants will be morphologically evaluated at the U.S. Vegetable Laboratory in Charleston, SC.

## **Progress**

During the first year we have optimized the mutagenesis procedure for watermelon pollen grains and produced 500 seeds with putative mutations. These seeds are being kept at a cold storage room at USDA-ARS Station in Griffin, GA and will be germinated, and resulting plants will be evaluated in a field in Charleston, SC during the spring and summer of 2011.

We have generated over 10,000 seeds of PI 595203. Experiments for optimizing mutagenesis for watermelon seeds are being conducted by Dr. Ming-Li Wang and his team at the USDA-ARS, Griffin, GA. Additional watermelon seed will be treated with EMS using or optimized procedure and morphological evaluation and seed increase for every mutant plant will be conducted in a field in Charleston, SC.

## **Future Work**

Our long term objective is to have a watermelon mutant-lines library that should be useful for the watermelon research community, including the public sector and industry. In addition to elucidating and identifying potential disease or virus resistance, the library should be useful for identifying mutant lines that produce over-dominance (heterosis) in yield and fruit quality, as has been shown in other crop plants (Semel, 2006).

## **Budget Report**

Of the \$11,000 given ARS \$1,200 (11%) were used by ARS as overhead.

Dr. Ming Li Wang received \$9,800 for his project. These funds have been used for hiring a part time assistant for the project. To date, \$8528.78 have been used and the rest (\$1271.22) will be used for the assistant employment through the end of the year.

A temporary employee was hired and one supporting scientist (half-time) also involved in this project. Supplies were purchased by ARS, and the experiment was conducted in Griffin molecular lab, greenhouse, cold frame house, and field.