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

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Summary:

We propose to implement the 'targeting induced local lesion in genome' (TILLING) experiments to develop a library of 2,000 mutant lines derived from the watermelon PI 595203, known to have resistance to several potyviruses, including ZYMV, PRSV, and WMV. We assume that having additional gene mutations in this genotype (PI 595203) could result in enhanced resistance to potyviruses, such as the *squash vein yellowing virus* (SqVYV) that cause watermelon vine decline (WVD). The library will 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 will 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 could, benefit the watermelon industry in the long run. In addition to potential disease or virus resistance, the library should be useful for screening and identifying mutant lines that produce overdominance (hetrosis) in yield and fruit quality, as have been recently shown in other crop plants (Semel, 2006). We ask the NWA for funds ($13,500) to initiate this project.

Project Background: As a result of thousands of years of human selection for favorable fruit qualities, with only limited thought to disease and pest resistance, watermelon cultivars are susceptible to a large number of diseases and pests. Watermelon has suffered major losses from aphid and whitefly-transmitted viruses. The aphid-transmitted potyviruses, including zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), papaya ring spot virus (PRSV), and cucumber mosaic virus (CMV) are considered the most important viruses of watermelon (Lecoq et al. 1998). Recently, the whitefly transmitted *squash vein yellowing virus* (SqVYV) was identified as the causal agent of watermelon vine decline (WVD) that has caused severe damages to the watermelon industry in Southwest Florida (Adkins et al., 2007). These viral diseases are difficult to control due to their transmission by insect vectors (aphid or whitefly) from field to field. Watermelon growers in Florida alone lost 60-70 million US dollars in the 2003-2004 seasons due to the WVD caused by SqVYV (Huber, 2006). Watermelon producers are extremely concerned that the SqVYV will spread to neighboring states with the migration of the B-biotype sweet potato whitefly, *Bemisia tabaci* (Gennadius).

The U.S. Plant Introduction (PI) 595203 (Figure 1) was found to be resistant to ZYMV, PRSV, and WMV. Several mechanisms for resistance to potyviruses in cucurbit species were elucidated in recent studies (Ling et al., 2009). These studies have shown that mutations in the eukaryotic translation initiation genes (i.e., eIF4E, eIF(iso)4E, or eIF4G) confer resistance to specific RNA viruses (Maule et al., 2007). In a recent study (Ling et al. 2009) we have identified point mutations in the eukaryotic translation initiation gene eiF4E that could confer resistance to the ZYMV-FL in PI 595203. We hypothesize that inducing point mutations in the eukaryotic translation initiation

Figure 1. PI 595203 resistant to ZYMV (left) and the watermelon cultivar 'Calhoun Gray' susceptible to ZYMV (right).

genes could produce resistance to potyviruses in watermelon. The "Targeting Induced Local Lesion In Genome (TILLING)" is a powerful technique for identification of mutations (point mutation and small insertion or deletion) and elucidation of gene function for traits of interest.

Implementation of TILLING in watermelon will open a new avenue for screening and identifying mutations conferring disease or virus resistance. Here, we propose to use the TILLING procedure to produce a collection of mutation lines from the watermelon PI 595203. The mutation lines produced in this proposed project will be screened for enhanced resistance to potyviruses, including, ZYMV, PRSV, WMV and the SqVYV that cause watermelon vine decline. The mutation lines will be valuable for the watermelon community, and will be useful for plant pathologist and breeders searching for resistance to major diseases of watermelon, including fusarium wilt, gummy stem blight, and bacterial fruit blotch.

Objectives:

1) Generate and develop over 2,000 mutant lines for the watermelon PI 595203 using EMS mutagenesis.
2) Maintain and screen the mutant library by TILLING for resistance to potyviruses and other major disease of watermelon.
3) Examine association of candidate genes with resistance to potyvirus and major diseases of watermelon.

Procedures for generating and developing mutation lines: Application of the TILLING technique to any crops can be divided into three main steps: (1) generation and development of the mutation populations, (2) identification and validation of mutations on the DNA sequence level, and (3) association of a specific genotype (gene mutation) with a phenotype.

The PI 595203 will be used for TILLING. To assure purity of the line used in TILLING, we have conducted a single seed descent (SSD) procedure (Goulden, 1939) derived from a single PI 595203 plant. Seed batches will be tested for morphological phenotype in the greenhouse. Mutagenesis will be conducted by ethyl methanesulfonate (EMS) treatment at the USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, GA. The best treatment conditions that include EMS concentrations (0.1 to 1.0%, v/v), treatment length, and recovery time will be determined (McCallum et al., 2000). Due to greenhouse and field space limitation, a small-scale morphological observation will be conducted at Griffin, GA, while large-scale morphological observation and generation advancement will be conducted in the following year at North Carolina State University, Raleigh, NC (by Todd Wehner and his team).

Two-thousand M_2, M_3, and M_4 lines will be generated over three consecutive years using a procedure optimized for watermelon breeding lines by Todd Wehner and his team to increase seeds by self-pollinating a large number of individual lines (Wehner et al., in preparation). Seeds will be extracted using a screen tray to separate the seeds from the fruit flesh, and will be rated for their size and color (using a procedure optimized by Wehner et al., in preparation). Since it is critical to obtain pure SSD M_4 lines, students and workers in this project will be trained and supervised carefully to avoid cross-contamination errors in pollen or seed.

Research Plan

1) During the first year we will optimize the mutagenesis procedure for watermelon and generate over 2,000 mutation plants that in the following years will be used for developing stable mutation lines.
2) During the second, third, and forth year we will grow the 2,000 plants and self-pollinate each plant using the single seed descended (SSD) procedure to produce stable mutant lines.
3) During the fifth year will maintain the mutant library and screen the mutant lines for resistance to potyviruses, particularly for resistance to the SqVYV that cause watermelon vine decline.

Expected Results and Benefits

We expect to develop a mutation-line collection (2,000 lines) that can be a useful source for disease or virus resistance breeding lines and gene loci that can be incorporated into watermelon cultivars. A narrow genetic diversity exists among watermelon cultivars. Enhancing the genetic diversity in watermelon using the TILLING technology could benefit the whole watermelon industry and produce a plethora of lines and genes useful for researchers and plant pathologists interested in reducing pathogen infestation and in enhancing watermelon production and quality. The lines produced in this study will be screened for disease and potyvirus resistance with first priority to the SqVYV that cause sudden vine decline in watermelon.

Budget Proposed

This is a long term project than can take about five years of intensive work and cooperation among the watermelon researchers and breeders to develop and maintain the mutant-line population. We are asking the NWA for $13,500 for the first year to initiate the project.

The funds will be used for conducting experiments to optimize the EMS mutation procedure for watermelon and generate 6,000 mutant seeds that will be used for developing 2,000 mutant lines. The experiments will be conducted by Dr. Ming-Li Wang (ARS, Griffin, GA) with the assistance of Drs. Levi, Ling and Wechter (ARS, Charleston, SC). These funds will be used for greenhouse and laboratory supplies and for employing a part time student to assist with this project.

At the end of the first year we will provide a report to the NWA and if the work produces promising results, we will ask for additional support and ask the seed companies, and University and USDA researchers to assist in In-Kind support to increase the mutant-line population.

Budget: Year 2010

Part time student employed for a nine-month period….. $9,200

Greenhouse supplies ……. $1,800

Laboratory Supplies ……. $2,500

Total funds requested ……$13,500