Analysis of GMO Plum Plant Culture in System Operations Failure

Dianne Mercado
NASA Kennedy Space Center
Major: Biotechnology
NIFS Fall Session
17/11/2017

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Dianne Mercado[1] and Lashelle Spencer[2]
Kennedy Space Center, FL 32899

GMO plum trees are being evaluated at the Kennedy Space Center as a possible candidate for future space crops. Previously conducted horticultural testing compared the performance of several plum genotypes in controlled environment chambers, resulting in a down-selection to the NASA-11 genotype. Precursory studies determined the water use requirements to sustain the plants as well as the feasibility of grafting non-GMO plum scions onto GMO plum rootstocks of NASA-5, NASA-10, and NASA-11 genotypes. This study follows the growth and horticultural progress of plum trees and *in-vitro* cultures from August 2017 to November 2017, and provides supplemental support for future GMO plum studies. The presence of Hurricane Irma in early September 2017 resulted in the plants undergoing material deterioration from major changes to their overall horticultural progress.

Nomenclature

BA = 6-Benzyladenine
EtOH = ethanol
GMO = genetically modified organism
IBA = indole 3-butyric acid
KSC = Kennedy Space Center
L = liters
NASA = National Aeronautics and Space Administration
NIFS = NASA Interns, Fellows, and Scholars
PtFT1 = Flowering locus T1
SGM = standard growth media
SRM = standard rooting media
USDA = United States Department of Agriculture

I. Introduction

Plum trees (*Prunus domestica*) genetically modified to overexpress the FT flowering gene are being evaluated as a candidate for future space crop selection. This overexpression removes seasonal dormancy requirements and allows the dwarf plants to mature and produce fruit from a young age (12 to 14 months), and exhibit compact continuous growth from seed to fruit. Prior studies have linked phytonutrients found in plums to prevent bone calcium loss in both animal and human models[3,4]. Successful fruit trees present a potentially sustainable and high-impact addition to space food systems, especially those of long-term deep-space missions, in a two-fold form: providing a source of fresh food and different produce variety which contributes to stable psychological well-being, and enhancing nutritional benefits that help counteract one of the largest health-related obstacles of spaceflight missions[3]. Plum trees and *in-vitro* plum tissue cultures of the NASA-5, NASA-6, NASA-10, NASA-11, and Control-5 genotypes are currently growing and monitored at KSC under controlled environments.

[1] NIFS Intern, Engineering Services Contract, Kennedy Space Center, University of Central Florida.
[2] Scientist and NIFS internship mentor, Engineering Services Contract, Kennedy Space Center.

II. Methods and Materials

A. Plum Trees

Modified and unmodified varieties of plum trees (*P. domestica*, "Blue Byrd" cultivar) were kept and monitored in a controlled environment chamber at the Operations and Check Out (O&C) Building at KSC for the duration of the study. In addition to the plums, two tomato plants (*Capsicum annum*, "Red Robin" cultivar) were grown as well to provide a source of comparison to the plums.

Grown specimens of NASA-5, NASA-6, NASA-10, NASA-11 (the down-selected line), and Control-5 were maintained in the chambers under a 16hr:8hr light:dark lighting schedule at temperatures of approximately 22 degrees Celsius and approximately 50% relative humidity. The plants were fertigated 6 times daily with Peter's granulated nutrient mixture solution with an electric conductivity of 1200-1250 µL and pH of 6.00-7.00. An automatic pump irrigation system was developed and used to deliver nutrient solution to plants.

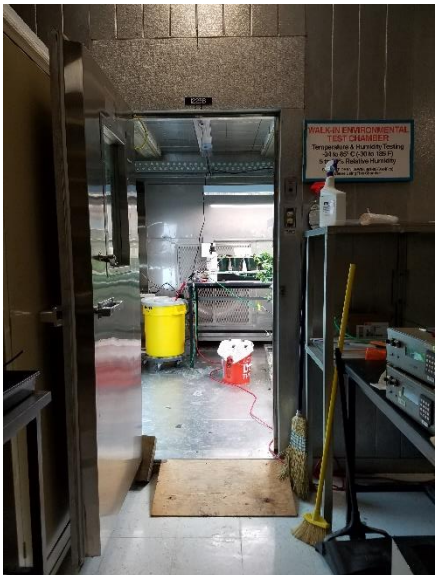

Figure 1. Controlled environment chambers for plum plants.

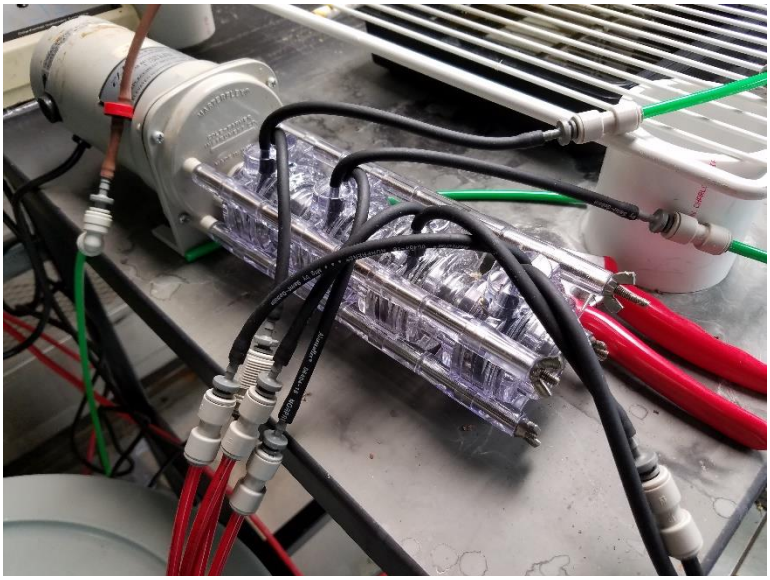

Figure 2. Automatic pump irrigation system for nutrient solution delivery. *Main pumps pulled up intake from manually filled reservoir and auxiliary tubes were attached to growing pots of plums.*

Figure 3. Magenta boxes containing transferred plant material in IBA/BA SGM. *Continuous lightning from AIBC LED Grow Light fixtures and ambient environment temperature were maintained throughout this study.*

Daily checks twice a day, one in the morning (timestamps ranging from 10:00am-11:00am) and the other in the afternoon (timestamps ranging from 1:00pm-2:00pm), were carried out to observe and maintain horticultural progress of the plants, with the exception of the duration of Hurricane Irma in September 2017.

B. Plum Tissue Cultures

Fresh plant material from the four selected GMO plum lines (excluding NASA-6) and an unmodified plum line called Control-5 were transferred aseptically into *in-vitro* Murashige and Skoog-based IBA/BA SGM and had been exhibiting leafy plantlet and callus growth prior to the start of this study. 6-8 node bud tissues for each genotype were transferred into Magenta Plant Culture boxes with IBA/BA SGM and monitored daily under ambient lab temperature (~23°C) and 50 µmol of light.

Figure 4. Magenta box containing three plant material samples from genotype NASA-10 in SGM. *While node tissues were initially used for tissue culture study, calluses (example: sample to the right of the box) were also separated from the main tissue and grown in the same conditions.*

Figure 5. Tissue cultures growing under 24/7 LED light in ambient room temperature. *Cultures were separated on trays by genotype, clonal propagation date, and transfer date based on aseptic transfers into new media every 4 to 6 weeks.*

III. Results and Discussion

A. Plum Plants

Specimens of NASA-5, NASA-6, NASA-10, the down-selected line NASA-11, and Control-5 were kept and maintained in controlled environment chambers at KSC under the specified parameters. Prior to Hurricane Irma in early September 2017, all genotypes exhibit healthy nodal tissues, production of chlorophyll-rich leaves, and growth of secondary and tertiary branches from the main plant. Plants were watered manually while the automatic irrigation system was under development.

Before Hurricane Irma reached main-land Florida and initiated statewide environmental emergency conditions, immediate steps were taken to increase the optimal viability of the plants through the hurricane. Due to the automatic irrigation system undergoing technical complications, as well as facing the possibility of losing access to power and water for up to several weeks, the potted plants were placed in growth trays and submerged in 5 L of nutrient solution for sub-irrigation. The chambers were set to be active throughout the hurricane.

After Hurricane Irma, assessments were carried out to analyze the health of the plants. The chambers had lost power during the hurricane and increased the humidity of the growing environment, resulting in mold and fungal growth on all the plants. In addition, it is estimated that the plants drained their nutrient solution three to four days into the hurricane duration and two to three days prior to any possible initial assessments being made. In the days following Hurricane Irma, the combination of dehydration and fungal growth resulted in all genotypes losing leaves and developing infected tissue.

Figure 6. NASA-11 and Control-5 three days after Hurricane Irma. *While automatic irrigation system was established soon after the hurricane, the plants still suffered from fungal growth which reached the xylem and phloem of the inner stem and resulted in plant deterioration.*

Figure 7. NASA-6 plant three days after Hurricane Irma. *Despite fungal growth and lack of sufficient water supply, NASA-6 remained the healthiest of all tested genotypes and has to date produced flowers.*

Figure 8. NASA-6 plant tissue cultures in petri dish wrapped with parafilm to maintain moisture content. *Daily observations indicated fast (one to two week) nodal growth and development of initial leafy tissue.*

Out of all genotypes, NASA-6 remained the most unaffected by the effects of Hurricane Irma, maintaining 70% of its healthy leaves and nodes. This is most likely due to its heavily modified genetic growth and may be a point of interest to explore in the future. NASA-5 also showed less plant deterioration than NASA-10, NASA-11, and Control-5, with one plant maintaining 60% of its healthy leaves and nodes. Interestingly, the NASA-5 plants were observed to recover faster from the hurricane before the other plum lines, with two NASA-5 plants exhibiting healthy nodes and leaf growth approximately one and a half week after the hurricane. As of the writing of this paper, NASA-10 and NASA-11, the down-selected genotype, have not yet fully recovered.

B. Plum Tissue Cultures

The plum tissue cultures did not undergo any adverse effect from Hurricane Irma. However, due to the plants in the chambers suffering from fungal growth and dehydration which led to wilting and further deterioration, there was a need to preserve and maintain all tissue cultures for future GMO plum studies. Cultures both node-based and callus-based that had outgrown their boxes were cut in half (or in some cases of particularly large growths, three times) and aseptically allocated to additional boxes with SGM. The number of samples were doubled within two to three weeks.

In addition, healthy node tissues were extracted from the NASA-6 plants and processed into petri dishes with SGM for comparison to the other genotypes due to its horticultural performance despite the contextual effects of Hurricane Irma. Stem nodes containing buds were put through a tissue sterilization process: soaking in 70% EtOH for 30 seconds followed by 10% bleach for 10 minutes. Node tissues were rinsed in sterile nanopure water between treatments to decrease oxidative tissue damage as well as chances of microbial contamination, then aseptically transferred to petri dishes with IBA/BA SGM. Approximately 20% of all individual NASA-6 tissue cultures developed microbial contamination, most likely due to the fungal infection remaining in the plant tissue even after sterilization.

Since Hurricane Irma, all tissue cultures have been growing and monitored daily in IBA/BA. At the end of October 2017, 2 to 3 tissue cultures of all genotypes (excluding NASA-6) were processed into SRM. As of November 6, these cultures remained healthy.

IV. Conclusion

Studies have shown GMO dwarf plums to be a potentially high-yield space crop candidate in terms of biomass production and nutrition content. This study points out the protocols used to maintain plum plants in controlled environments, as well as those used to preserve and propagate *in-vitro* tissue cultures, which require less space, materials, and labor to maintain and may be applied to a space food production setting. Developing a more precise and efficient start-to-finish protocol for the *in-vitro* process (taking healthy plant material from the main plant to processing healthy nodal tissues into SRM to develop roots) may help in furthering GMO plum studies. Additionally, there is room for future research on the long-term viability of not only GMO plums but other space crop candidates on the occasion that controlled system operations fail to operate. While Hurricane Irma resulted in major plant material loss and deterioration, it indicates a need for research regarding plant life viability and how that transitions to long-term deep-space food production systems.

Acknowledgments

The author would like to thank Lashelle Spencer for her mentorship and unfailing guidance throughout this project. Additional thanks go to Mary Hummerick, Lawrence Koss, Dr. Christina Khodadad, and the members of the Kennedy Space Center Veggie team for their assistance and input. The author would also like to thank faculty and academic mentors at the University of Central Florida for their professional support and preparation for this opportunity. Funding for this internship was provided by the NIFS program.

References

[3] Spencer, L., Graham, T., Stutte, G., Massa, G., Mickens, M., and Raymond W., "Space Pomology: Dwarf Plums for Fresh Food Production," Oral/Visual Presentation, 2017.

[4] Globus, R. "Dried Plum Protects from Radiation-Induced Bone Loss by Attenuating Pro-Osteoclatic and Oxidative Stress Responses.," Conference Paper, 2015.