Microbiological/Horticultural Internship Final Abstract

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Nomenclature

GMO	=	genetically modified organism
NASA	=	National Aeronautics and Space Administration
KSC	=	Kennedy Space Center
NIFS	=	NASA Interns, Fellows, and Scholars
USDA	=	United States Department of Agriculture
PtFT1	=	Flowering locus T1
SGM	=	Shoot generation media
SRM	=	Shoot regeneration media
2,4-D	=	A synthetic auxin plant growth regulator
WPM	=	Woody plant media
TDZ	=	thidiazuron, a synthetic cytokinin plant growth regulator
EtOH	=	Ethanol

Abstract

GMO dwarf plum (*Prunus domestica*) is being evaluated as a candidate food crop for long duration space flight missions. A project was undertaken to develop a protocol for transferring selected genetic lines of GMO plum (previously maintained in pots and propagated by cuttings at NASA's Kennedy Space Center in Florida) into *in vitro* tissue culture. *In vitro* culture may reduce the space, materials, and labor required to maintain the current lines of GMO plum and better preserve them for future study.

Fresh plant material from three selected GMO plum lines ('NASA-5', 'NASA-10', and 'NASA-11') and a non-modified control line ('Control-5') were processed aseptically into *in vitro* culture on four separate occasions. The impact of multiple treatments on the successful growth of GMO plum tissue *in vitro* were tested: Parent explant tissue type (leaf petioles, stem nodes containing buds and internodes without buds), tissue sterilization method [soaking in 10% bleach only (5 min for petioles or 10 min for nodes/internodes), or soaking in 70% EtOH (30 sec) followed by 10% bleach (5 min for petioles and 10 min for nodes/internodes)], and media type [three Murashige and Skoog-based medias (SGM, SRM, and SRM+2,4-D) and one recipe containing woody plant media (WPM)].

22.2% of the plates containing tissue sterilized with bleach alone developed microbial contamination after two weeks, while only 11.8% of plates containing tissue sterilized sequentially with EtOH and bleach developed contamination. Explant pieces rinsed with sterile water in between the EtOH and bleach treatments suffered less oxidative tissue damage and survived better than those that were not rinsed between treatments.

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Node bud tissue from all four genetic lines of plum produced leafy plantlets on SGM and SRM media after 4-6 weeks, however the most numerous and well-developed plantlets were present on SGM. Upon reaching a suitable size, these plantlets were transferred to larger media containers for further growth. Some node bud growth occurred on SRM+2,4-D and WPM 2.5 weeks after plating, however as of yet no pieces on SRM+2,4-D have developed into leafy plantlets suitable for transferring. Some tissue pieces from 'NASA-5' plated on WPM are currently developing leaves and will be ready for transferring soon. Internode tissue lacking bud meristem failed to produce any plantlets on any plates. Petiole tissue also failed to produce any plantlets on any plates, however they developed large masses of undifferentiated callus tissue on the SRM+2,4-D media. These callused pieces were then transferred to SRM+TDZ media, which resulted in even larger callus growth but no differentiation.

Nodes from 'NASA-5' and 'NASA-10' lines produced the most numerous and well-developed leafy plantlets *in vitro*, while those from 'NASA-11' and 'Control-5' were generally smaller, slower growing and less numerous. Genetic differences between the plum lines may influence their varying responses to the *in vitro* culture procedures.

Several node pieces from the GMO lines produced flower buds and one plantlet from NASA-11 produced an open bloom *in vitro*. This response is consistent with the precocious flowering behavior of plum plants modified to overexpress the PtFT1 gene, and results in a terminal stem that does not grow further. Thus it would be beneficial to minimize precocious flowering of the GMO plum lines in tissue culture and encourage vegetative-only growth.

All four selected plum lines were successfully transitioned into *in vitro* culture. The best method overall was to use young stem node tissue with buds, surface sterilize the pieces sequentially with 70% EtOH and 10% bleach, and then plate them onto SGM media. Other future areas of study will include introducing additional genetic lines of GMO plum into *in vitro* culture, attempting to induce shoot growth in petiole callus tissue, testing methods (such as cold storage) that extend the time interval between transferring explants into new media, and testing viability of plantlets transferred from *in vitro* culture back to traditional pot culture.

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