**10.16 P**

## Breaking citrus juvenility by modulating endogenous miR156 and miR172 levels

Jiang, Y.[1] and Gabriel, D.W.[1,2]

[1]Integrated Plant Genetics, Inc., Alachua, FL, USA
[2]Plant Pathology Dept., University of Florida, Gainesville, FL, USA

The ability to either transform mature citrus directly or to transform juvenile citrus and also induce it to flower and set fruit within a few years is critical for evaluation of fruit quality, quantity and of horticultural performance of any transgenic trees. In plants, the transition from juvenile to adult stage is regulated by the sequential and complementary action of microRNAs miR156 and miR172. miR156 suppresses the expression of specific transcriptional factors that would otherwise promote the juvenile to adult phase transition, including factors that activate miR172, which directly promotes the transition. Here, we created a target mimic for miR156 to attempt to sequester miR156 and reduce its levels in juvenile citrus. We cloned the nonprotein coding gene IPS1 from Arabidopsis and replaced its native microRNA target with the predicted citrus target of citrus miR156, resulting in a citrus miR156 mimic gene, cMIM156. Five sweet orange (Hamlin) seedling transgenic plants expressing cMIM156 were produced and the endogenous miR172 levels were monitored over time. All five transgenic plants showed enhanced expression levels of miR172 (two exhibited 10X higher levels) compared to non-transgenic control plants regenerated from explants at the same time as transformants. By 1 year after transformation, miR172 levels were still 5X less than that observed using mature sweet orange, and no flowering has yet been observed. The levels of miR172 expression have increased over time, and we are attempting to establish a timeline for flowering of 3 years after transformation.
Funding: USDA-APHIS CPHST