Production of genetically modified Citrus plants from juvenile explants at Citrus Transformation Facility-The benefits for Florida Citrus Industry

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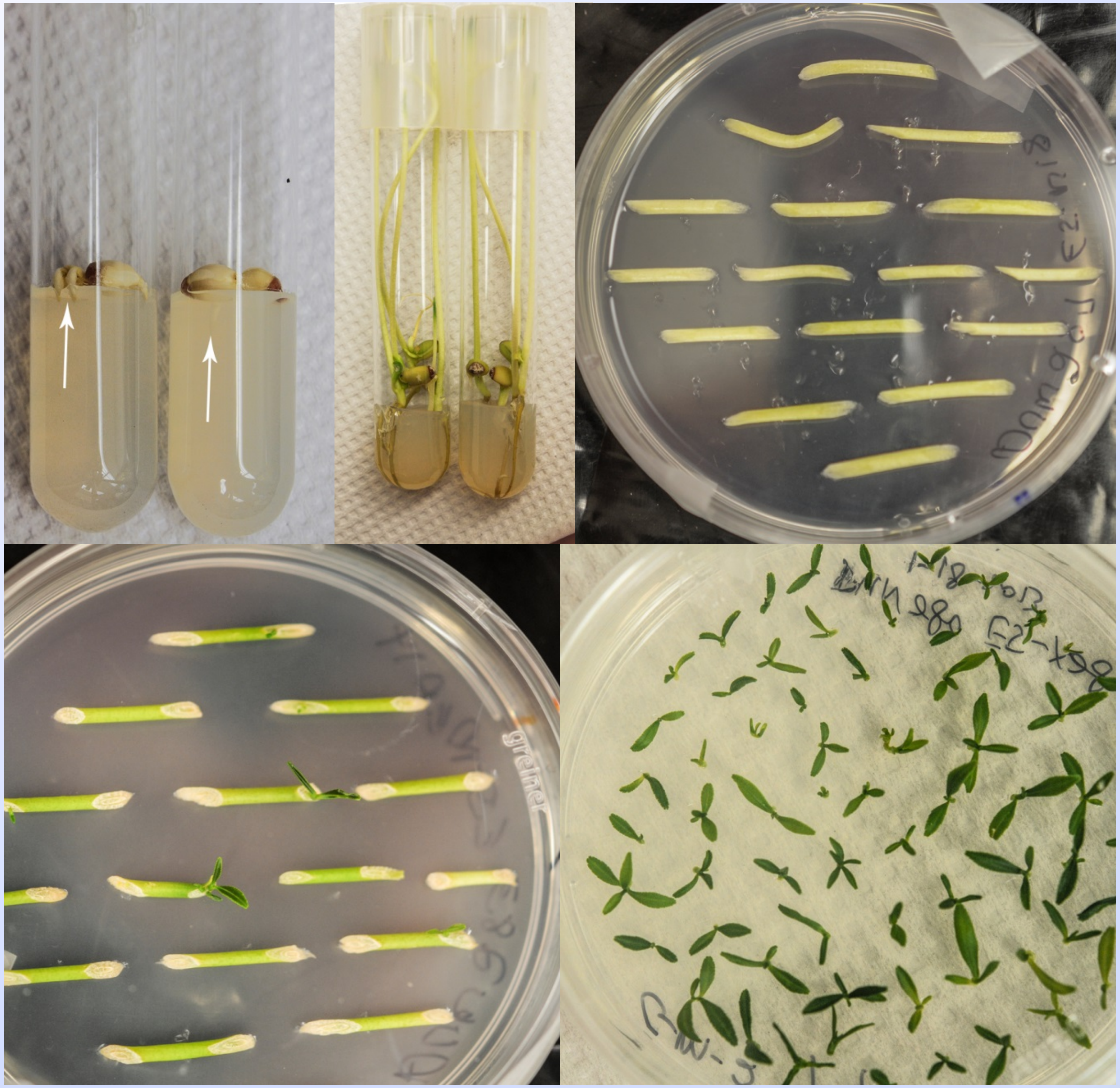
What is it that we do?

- CTF produces transgenic plants belonging to many Citrus cultivars that include sweet oranges, grapefruits, limes, rootstocks, and others.
- CTF offers researchers STEADY service-we are active throughout whole year and try to produce transgenic plants irrespective of picking season for some cultivars.
- Turnaround time is relatively short (6-8 months) although it varies with cultivar and introduced gene.
- For the last few years, we produced on average about 250 transgenic plants per year.

How do we produce transgenic citrus plants?

- We employ a procedure that requires action of pathogenic bacteria *Agrobacterium tumefaciens*. During the years, we fine tuned this procedure and added some steps to facilitate selection of transgenic plants.
- Our starting material for transformation is juvenile tissue obtained from seedlings. The major benefits are ample availability and low cost. Transformation rate is much higher than for mature tissue.
- In one week we process in average 3000 explants (~150,000 per year). These explants (stem segments) are cut from about 1000-1300 seedlings germinated from about 750-1000 seeds.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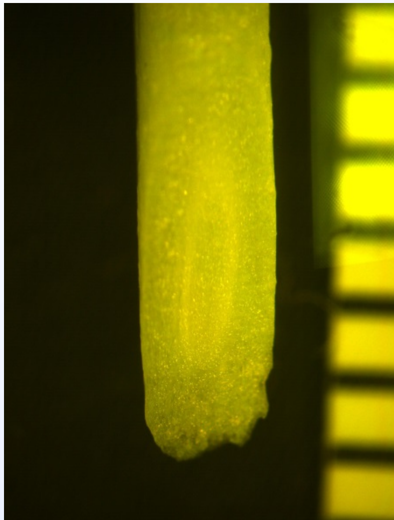
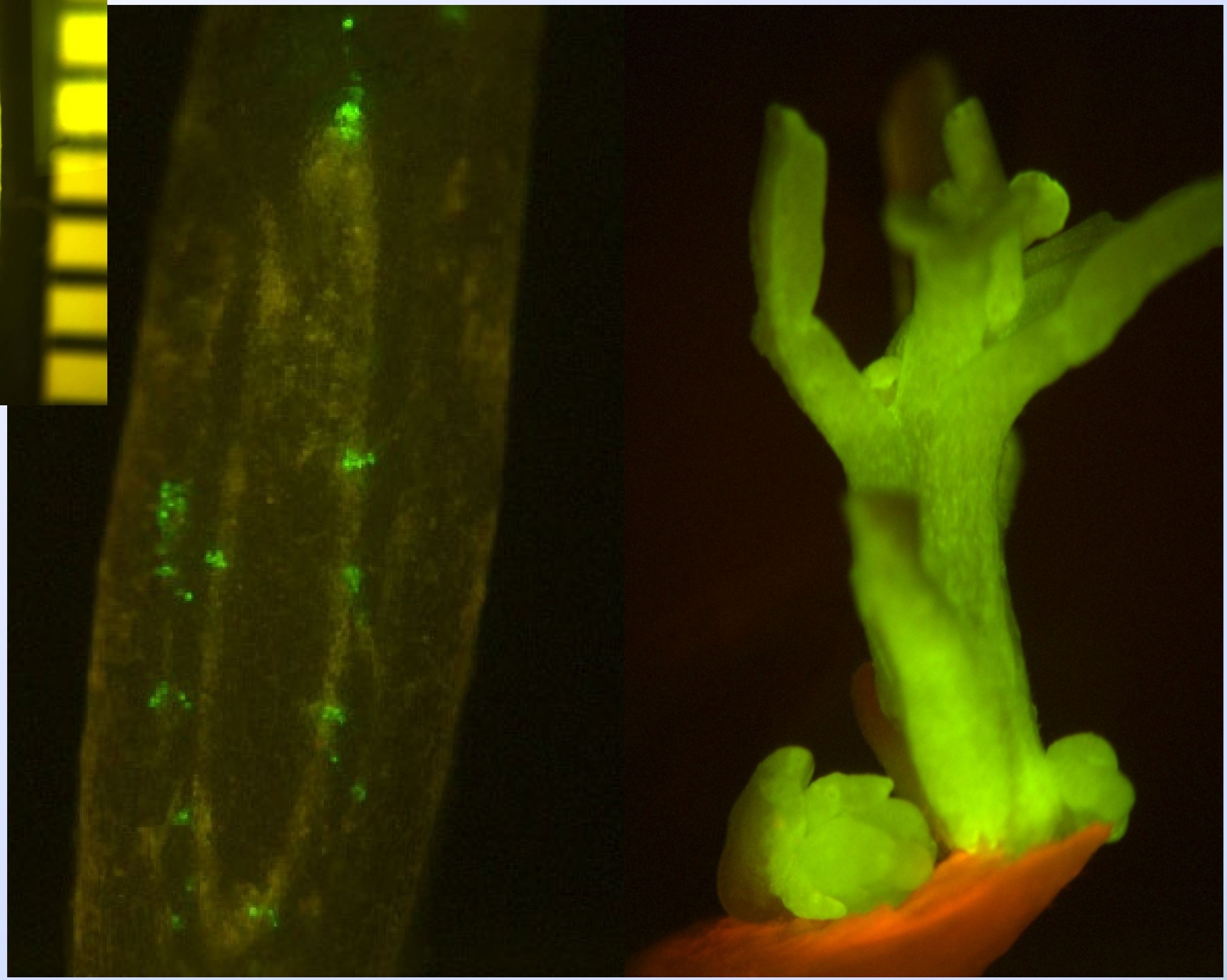
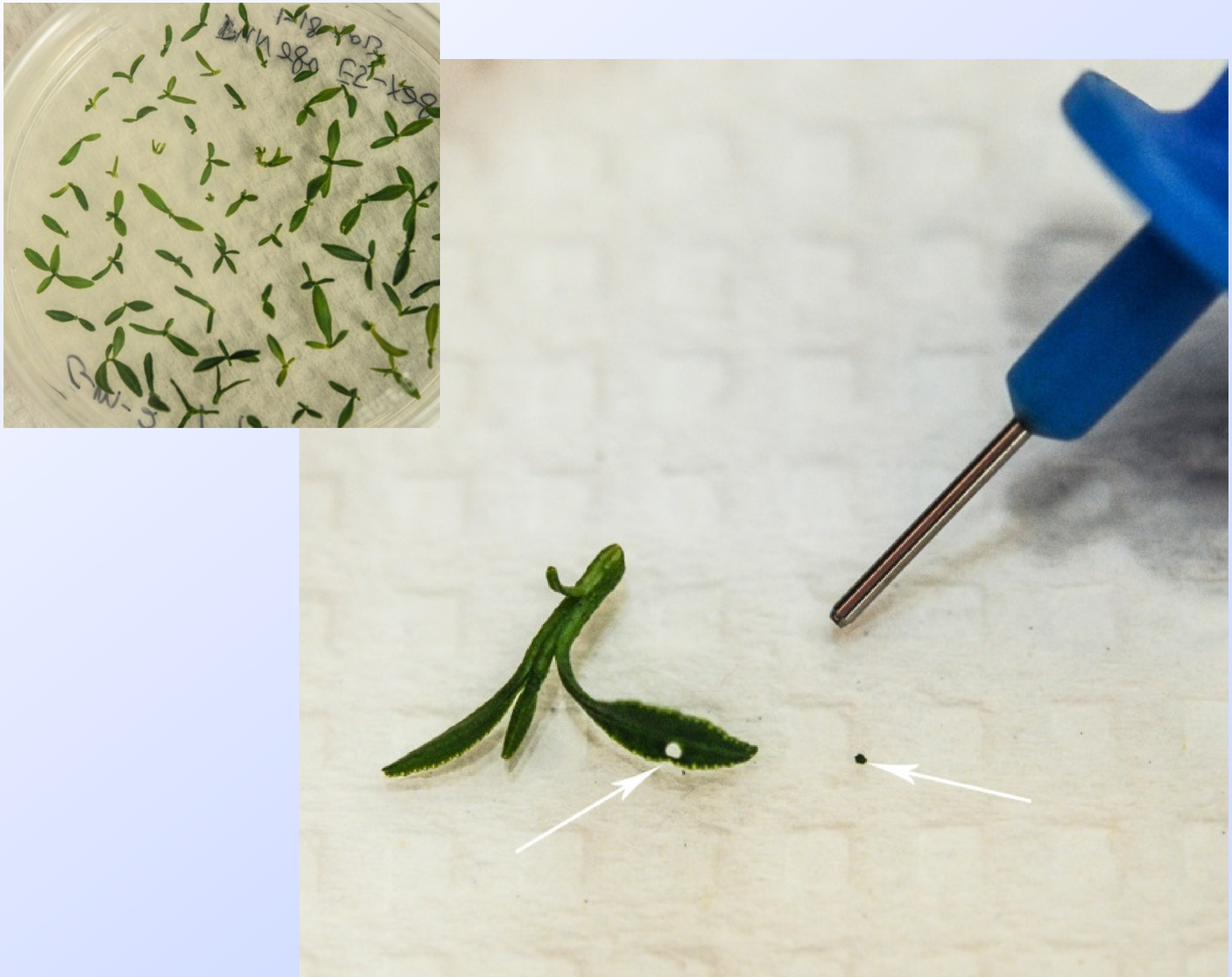
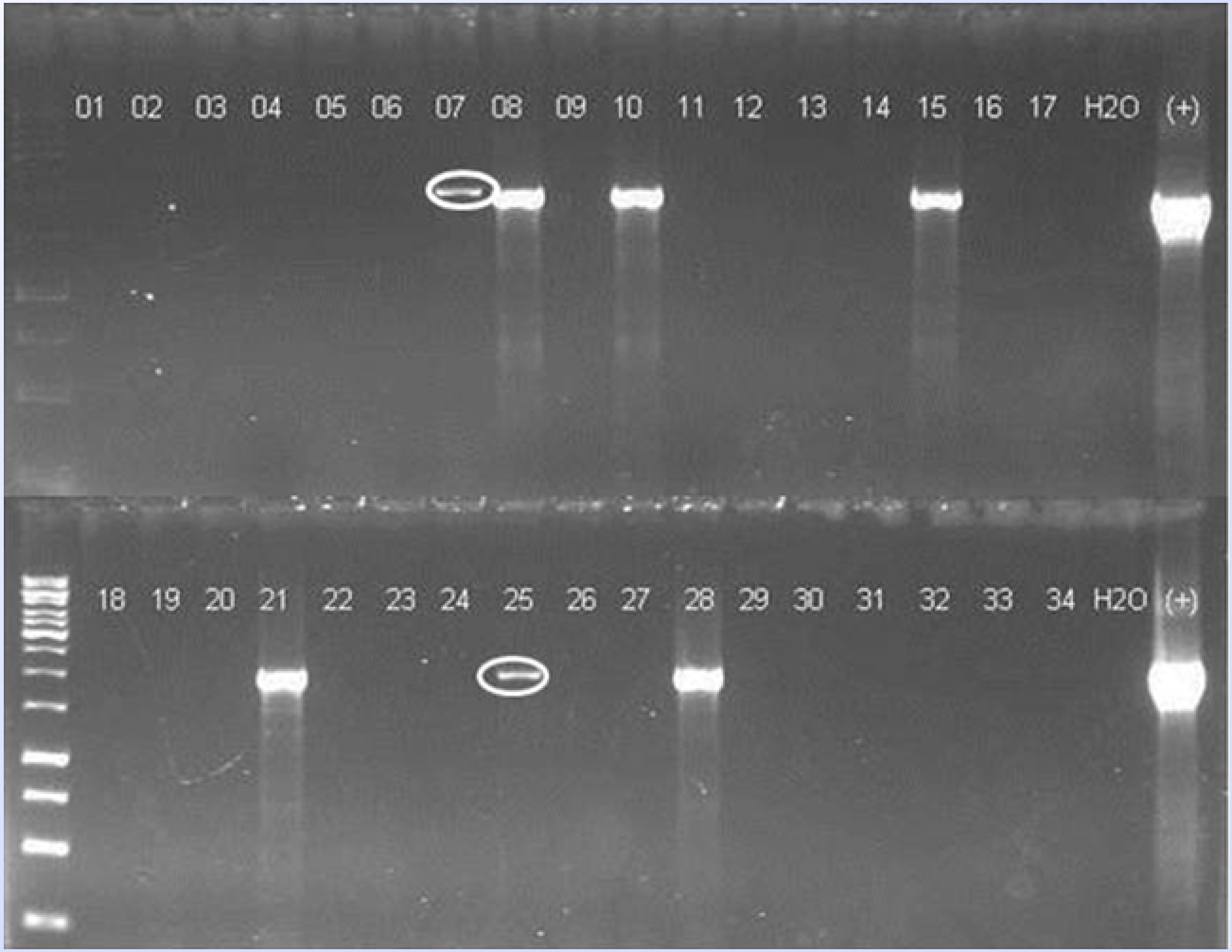
How do we produce transgenic citrus plants?

- Each week we inspect from 500 to 2000 shoots for GFP fluorescence (“reporter gene” presence).
- In average, we test about 100-150 shoots with PCR per week.
- Both GFP fluorescence in the plant and presence of PCR product in the reaction suggest transgenic nature of tested shoots.

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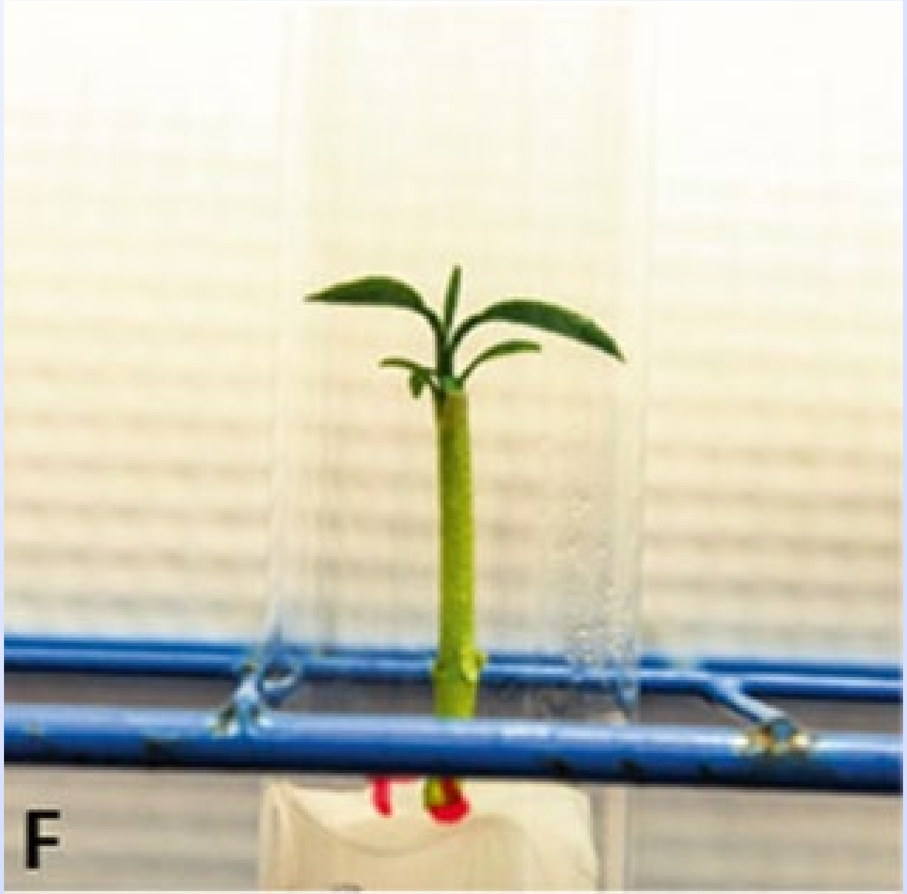
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What is the purpose of our work?

- Researchers come up with ideas on how citrus trees may be tolerant or resistant to diseases, but they may not have the knowledge or are not ready to invest time into production of plants that have altered traits that will improve their performance against diseases. The mission of CTF and the purpose of our work are to remedy this situation. We take orders and produce those modified plants. Such approach saves time and money by cancelling the need to hire new employees to do transformation in multiple labs.

Idea → CTF (proof of concept, confirmation of the benefit of the introduced gene for the plant against pathogen) → Propagation or Mature Tissue lab

What is the money granted by CRDF used for?

- In the new funding cycle, 98% of money would be used for salaries of staff-four OPS employees at different FTEs (range from $11.50 to $14.50). Other operating expenses will be covered from USDA grants attracted to CTF by facility's manager.
- When CTF was first funded by CRDF in 2008, that grant represented 95% of the operating budget. The money we are requesting now from CRDF for operation of the CTF will be close to the half (50%) of the budget.
- For our work we charge a fee of $1500/order. For this fee, we commit to production of 10 plants that are unique for the trait client wanted to introduce or alter. In average, CTF gets 25-30 orders per year. If all were paid for it would come to $37,500-45,000. This money is used for repairs, consumables, etc.

What is the benefit for Florida Citrus Industry?

- National Academy of Sciences recently concluded that solution for HLB will probably include the use of transgenic technology and genetically modified plants. This type of work was being done previously and is proceeding at fast pace at present time. Because of the work done by the CTF, research towards possible solution to HLB and other citrus diseases will stay in-interrupted in many labs that use our services.
- The high majority of our clients are local, associated with the University of Florida or USDA Centers in Florida. Even those clients who are not from Florida, are focused on genes that may mitigate HLB and Citrus canker.

What is the benefit for Florida Citrus Industry?

- Here are the examples of work done in CTF that contributed to some new concepts to come to the forefront of fight against HLB:

1. CRISPR mediated genome editing in citrus (done for the first time in CTF);
2. Production of intragenic citrus plants-modified plants that have citrus DNA introduced into genome (done for the first time in CTF);
3. Control of transgene expression by the application of exogenous chemicals (done for the first time in CTF);

- During the nine year period of CRDF funding, CTF worked on 264 orders and produced about 2500 transgenic plants.

What is the benefit for Florida Citrus Industry?

- The list of publications where transgenic plants made at CTF were described:

- Robertson, C., Zhang, X., Gowda, S., Orbovic, V., Dawson, W., Mou, Z. (2018) Overexpression of the Arabidopsis NPR1 protein in citrus confers tolerance to Huanglongbing. Journal of Citrus Pathology,
- Levy, A., El Mochtar, C., Wang, C., Goodin, M., Orbovic, V. (2018) A new toolset for protein expression and subcellular localization studies in citrus and its application to citrus tristeza virus proteins. Plant Methods, 14:2.
- Clark, K., Franco, J.Y., Schwizer, S., Pang, Z., Hawara, E., Liebrand, T.W.H., Pagliaccia, D., Zeng, L., Gurung, F.B., Wang, P., Shi, J., Wang, Y., Ancona, V., van der Hoorn, R.A.L., Wang, N., Coaker, G., Ma, W. (2018) An effector from the Huanglongbing-associated pathogen targets citrus proteases. Nat Commun 9:1718.
- Jia, H., Xu, J., Orbovic, V., Zhang, Y., Wang, N. (2017) Editing citrus genome via SaCas9/sgRNA system. Frontiers in Plant Science, 8:2135.
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- An, C., Orbovic, V., Mou, Z. (2013) An efficient intragenic vector for generating intragenic and cisgenic plants in citrus. American Journal of Plant Sciences, 4:2131-2137.
- Chen, X., Jinyoung, B., Sreedharan, A., Huang, X., Orbovic, V., Grosser, J.W., Wang, N., Dong, X., Song, W-Y. (2013) Over-expression of the Citrus gene CtNH1 confers resistance to bacterial canker disease. Physiological and Molecular Plant Physiology, 84:115-122.
- Orbovic, V., Gollner, E.M., Soria, P. (2013) The effect of arabinogalactan-proteins on regeneration potential of juvenile Citrus explants used for genetic transformation by Agrobacterium tumefaciens. Acta Physiologiae Plantarum, 35:1409-1419.
- Orbovic, V., Soria, P., Moore, G.A., Grosser, J.W. (2011) The use of citrus tristeza virus (CTV) containing a green fluorescent protein gene as a tool to evaluate resistance/tolerance of transgenic citrus plants. Crop Protection, 30:572-576.
- Orbovic, V., Dutt, M., Grosser, J.W. (2011) Seasonal effects of seed age on regeneration potential and transformation success rate in three citrus cultivars. Scientia Horticulturae, 127:262-266.
- Zhang, X., Francis, M.I., Dawson, W.O., Graham, J.H., Orbovic, V., Triplett, E.W., Mou, Z. (2010) Over-expression of the Arabidopsis NPR1 gene in citrus increases resistance to citrus canker. European Journal of Plant Pathology, 128:91-100.
- Pasquali, G., Orbovic, V., Grosser, J.W. (2009) Transgenic grapefruit plants expressing the PAPETALA3-IPTgp gene exhibit altered expression of PR genes. Plant Cell, Tissue, and Organ Culture, 97:215-223.
- Dutt, M., Orbovic, V., Grosser, J.W. (2009) Cultivar dependent gene transfer into citrus using Agrobacterium. Proceedings of Florida Society for Horticultural Science Annual Meeting, 122:85-89.
- Grosser, J.W., Gmitter, F.G. Jr., Orbovic, V., Moore, G.A., Graham, J.H., Soneji, J., Gonzalea-Ramos, J., Mirkov, T.E., Kayim, M. (2008) Grapefruit. In: Kole, C. and Hall, T.C. (eds.). A Compendium of Transgenic Crop Plants. Volume 5: Transgenic Tropical and Subtropical Fruits and Nuts. Wiley-Blackwell. pp 63-76.
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- Ananthakrishnan, G., Orbovic, V., Pasquali, G., Ćalovic, M, Grosser, J.W. (2007) Transfer of Citrus Tristeza Virus (CTV)-derived resistance candidate sequences to four grapefruit cultivars through Agrobacterium-mediated genetic transformation. In Vitro Cellular and Developmental Biology-Plant, 43(6):593-601.
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