

Evaluating and Validating Seedlessness Strategies for Citrus

Project Leader:

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Seedy citrus fruit are a major quality issue and of significant concern to the citrus industry. Elimination of seed formation would be a valuable trait for many citrus cultivars, especially the mandarin varieties and seedy lemon varieties. In this project we will evaluate the role of ovule specific regulation of auxin to induce parthenocarpy in citrus.

Parthenocarpy is the formation of fruit in the absence of fertilization, which occurs in plants and results in fruit that bear no seeds. The regulation of auxin will be manipulated in a tissue-specific context focusing on the ovule tissues via regulation of auxin synthesis or responsiveness. Proof of concept experiments will be done in tomato prior to transforming citrus.

Objective 1: Seedlessness induced by ovule specific regulation of auxin synthesis: Parthenocarpy will be induced via the expression of an auxin biosynthesis gene *iaaM* encoding a tryptophan monooxygenase from *Agrobacterium tumefaciens* (At) regulated by an ovule-specific promoter, *DefH9*, from *Antirrhinum majus* (Am) or an OS promoter from *Arabidopsis thaliana* (AT).

We have successfully cloned the *iaaM* genes from the reference At strain C58 and confirmed that the sequence was identical to that which is available in the GenBank database. DNA was extracted from seedlings of *Antirrhinum majus* and used to clone the *DefH9* promoter. The OS promoter is highly regulated in ovule tissues of *Arabidopsis*. Expression cassettes were successfully constructed containing *DefH9-iaaM* and *OS-iaaM* in our shuttle vector. These expression cassettes were then moved in to our previously constructed binary vectors to create the binary vectors pDU04.1602 for the expression of *DefH9-iaaM* and pDU04.1004 for the expression of *OS-iaaM* (Figure 1).

These vectors were successfully transformed into MicroTom tomato, and transgenic plants obtained were evaluated for seedlessness. About 25-27% of the transgenic MicroTom lines obtained with pDU04.1602 or pDU04.1004 showed no seed formation, and another 17-27% had just a few seeds with the remaining showing greatly diminished seed production. Transformation of these with another commercial tomato variety cv MoneyMaker are in progress.

Objective 2: Seedlessness induced by ovule specific modulation of auxin response: The auxin response modulating *rolB* gene from *Agrobacterium rhizogenes* (Ar) was introduced and regulated by the ovule specific promoters *DefH9* or OS. Expression of the *DefH9-rolB* or *OS-rolB* specifically in the ovule will result in the modulation of the sensitivity of this tissue to the phytohormone IAA such that the endogenous levels of IAA present in the tissue will be sufficient to induce parthenocarpy and the formation of seedless fruit.

We have successfully cloned the *rolB* gene sequences from the A4 reference strain of *Ar*. Expression cassettes were successfully constructed containing *DefH9-rolB* and *INO-rolB* in our shuttle vector. These expression cassettes were then moved in to our previously constructed binary vectors to create the binary vectors pDU04.4001 for the expression of *DefH9-rolB* and pDU04.4522 for the expression of *OS-rolB* (Figure 1). These vectors were successfully transformed into MicroTom tomato, and transgenic plants

have been obtained and are currently being evaluated for seedlessness. Transformation with these vectors of another commercial tomato variety cv Moneymaker are in progress.

Contact Citrus Research Board for figure.

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