Special Research Report # 308: Engineering Fungal Resistance in Bedding Plants Using a Gene for Mannitol Dehydrogenase, Part II

Funding Generations of Progress Through Research and Scholarships

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BACKGROUND

Activated forms of oxygen and molecules derived from their reaction with cell components act as signals and direct defenses against attacking pathogens in plants. To neutralize these defenses, many fungal plant pathogens secrete mannitol, an antioxidant, as a part of their infection process. However, when the fungi's ability to make mannitol is disrupted, either, either by a naturally occurring mutation or by genetic manipulation, their ability to infect plants is greatly reduced.

On the plant side of this interaction, we have discovered a gene for a plant enzyme that converts mannitol to the non-quenching sugar mannose. This enzyme, mannitol dehydrogenase (MTD), is surprisingly not only found in plants like celery or parsley that normally make mannitol, but is also present in plants that do not make mannitol. In the latter case, it is only turned on when the plant is attacked by a pathogen. When over-expressed in *Nicotiana*, this enzyme confers increased resistance to the mannitol secreting fungal pathogen *Alternaria*. This resistance was presumably due to the plants increased ability to metabolize fungal mannitol.

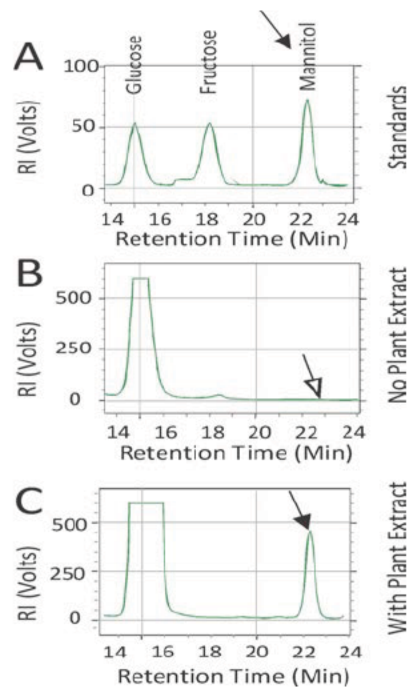

Fig. 1. Botrytis cinerea secretes mannitol in the presence of plant extract.

In the current project, we over-expressed MTD in zonal geraniums (*Pelargonium ×hortorum*) and looked for potential changes in fungal resistance. Initially, we assessed resistance to *Botrytis*, because it is not only an important pathogen of many floriculture crops, but also is a pathogen for which no specific immunity has been reported.

EXPERIMENTAL METHODS

First, we needed to see if *Botrytis cinerea* can secrete mannitol. We grew the fungus in culture with or without plant extracts, and tested for appearance of mannitol in the growth medium.

Then, we tested the impact of MTD over-expression on resistance. Zonal geraniums were transformed with a DNA construct for MTD that is expressed in all growing tissues. Plants

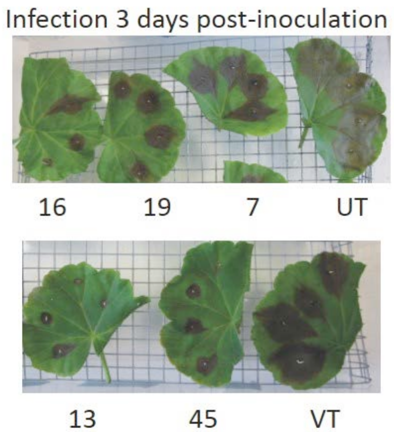

Fig. 2. MTD over-expressing zonal geranium had increased Botrytis resistance in detached leaf assays.

confirmed to produce MTD from this construct were assayed for relative resistance to *Botrytis* by inoculation of detached leaf material maintained in a controlled humidity chamber (detached leaf assay).

RESULTS

We found that while mannitol was not secreted by *Botrytis* in medium lacking plant extracts (Fig 1.), it was secreted in media with plant extracts. This suggests that *Botrytis* uses mannitol to suppress host immunity.

In addition, zonal geranium plants (Fig. 2) that expressed significant amounts of MTD were found to be more resistant to *Botrytis* in detached leaf assays.

Symptoms shown are 3 days after inoculation on plants expressing varying amounts of MTD. Plants 13, 16 and 45 expressed high levels of transgenic MTD. Plants 7 and 19 expressed medium levels. Untransformed (UT) and vector transformed plants (VT) expressed low levels of an endogenous MTD.

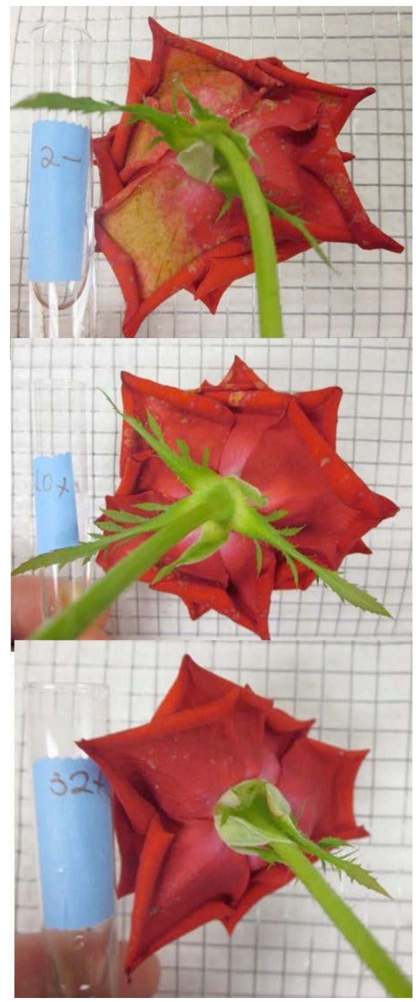

Fig. 3. Roses overexpressing MTD. Symptoms are 4 days after spraying Botrytis spores on flowers of a low MTD expressing plant (2-), medium expressing plant (10+), and a high expressing plant (32+).

CONCLUSIONS

(1) *Botrytis cinerea* makes and secretes mannitol. In combination with the observation that a number of other fungal leaf and flower pathogens also secrete mannitol, this suggests that this is a fairly common strategy for suppressing plant immunity.

(2) Over-expression of MTD appears to increase resistance to mannitol secreting fungal pathogens such as *Alternaria* and *Botrytis*.

Complete results of this study can be found in: Williamson JD, Dasai A, Krasnyanski S, et al. (2013) *Plant Cell Tiss Org. Cult*. 115:367-375.

INDUSTRY IMPACT

By using a gene from a plant that is used for food (celery), to modify plants we do not eat, we avoid a number of common barriers to the acceptance of genetically modified plants. The MTD gene, in fact, occurs naturally in a wide variety of plants, but is not expressed at a high enough level to confer effective resistance. Thus, this research not only directly provides the grower with an ecofriendly way to manage fungal diseases, but also provides the breeder with a proven molecular marker for screening breeding populations for naturally occurring, high levels of MTD expression.

FUTURE DIRECTIONS

Considering these studies as well as earlier ones, we are assessing resistance to *B. cinerea* in the popular cut rose 'Charlotte' that has been transformed to overexpress MTD. This is an important extension of the present research because roses have limited resistance to *B. cinerea*. This could be an asset to all segments of the floricultural industry, including the consumer. Initial experiments (Fig. 3.) indicate that MTD over-expression might also provide useful protection in roses.

The primary barrier to applying this type of approach, and, thus, a major challenge for the future is the current inability to transform a number of elite floriculture crops at economical efficiencies.

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