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Special Research Report # 307: Plant Breeding & Genetic Engineering
Engineering Fungal Resistance in Bedding Plants using a Gene for Mannitol Dehydrogenase.

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BACKGROUND

Plants use activated forms of oxygen as a defense against pathogens. There is increasing evidence that fungi use the reactive oxygen quencher mannitol to counter these defenses. Fungal plant pathogens including rusts (Uromyces), leaf molds (Cladosporium), and leaf spots (Alternaria) all secrete mannitol as a part of the infection process. Furthermore, when the fungi's ability to make mannitol is disrupted 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 found a gene encoding a plant mannitol dehydrogenase (MTD). This is an enzyme that converts mannitol to the non-quenching sugar mannose in plants like celery or parsley that normally make mannitol.

Surprisingly, this gene is also present in plants that do not make mannitol, but it is only turned on when the plant is attacked by a pathogen. When we over-expressed this gene in *Nicotiana*, the enzyme conferred increased resistance to pathogenic, mannitol- producing *Alternaria*. This increased resistance was presumably due to the plants increased ability to convert the fungal mannitol to mannose.

In the current project, we over-expressed MTD in petunia (Petunia ×hybrida) and zonal geranium (Pelargonium ×hortorum) and looked for potential changes in fungal disease resistance. We first looked at resistance to Botrytis, because it is an important pathogen of many floriculture crops for which no specific immunity has been reported.

Experimental Methods

To see if mannitol is secreted by *Botrytis*, we grew the fungus in liquid culture with or without plant extracts. The amount of mannitol secreted into the medium in each case was measured.

To test the impact, if any, of MTD over-expression, both petunia and zonal geranium were transformed with a gene construct for MTD that is expressed in all growing tissues.

Lines confirmed to produce MTD from this construct were checked for potential changes in resistance to *Botrytis*. Initial assessment of resistance was performed by inoculation of detached leaf material maintained in a controlled humidity chamber (detached leaf assay).

Results

Initially, we confirmed that *Botrytis* does secrete mannitol (Fig. 1) in response to plant extracts, and, thus, presumably uses mannitol to suppress host immunity.

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

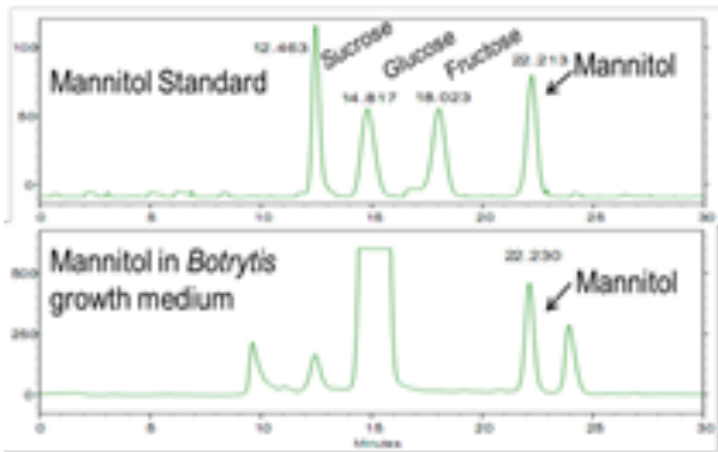


Fig. 2. ***MTD over-expressing Petunia had decreased symptoms 2 and 4 days after inoculation with* Botrytis**.

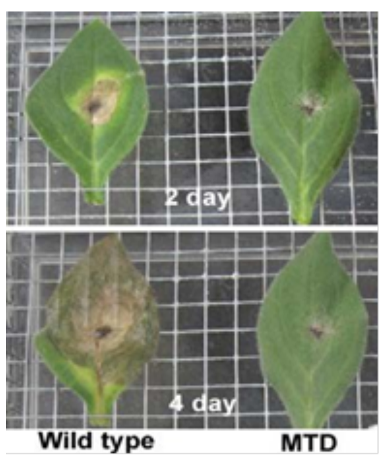
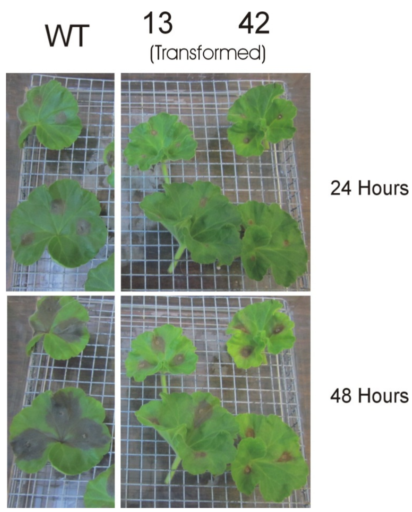


Fig. 3. ***MTD over-expression confers* Botrytis *resistance in detached petal assays. The flowers on the left (A-C) are from non- transformed petunia. The flowers on the right (B, D) are from MTD over-expressing transgenic plants. Symptoms were scored 2 days (A, B) and 4 days (C, D) after inoculation with* Botrytis.**

In turn, both petunia (Figs. 2 and 3) and zonal geranium plants (Fig. 4) that expressed significant amounts of MTD appeared to be more resistant to *Botrytis* in detached leaf and petal assays. In fact the geranium used in these experiments ('Multibloom Red') was significantly more susceptible to *Botrytis* than was petunia, making increased resistance easier to detect.

Fig. 4. ***MTD over-expressing zonal geranium (lines 13 and 42 shown here) have decreased sensitivity to* Botrytis *1 and 2 days after inoculation.***



Conclusions

1) *Botrytis* makes and secretes mannitol. Together with the observation that a number of other fungal leaf and flower pathogens secrete mannitol, it suggests that this mechanism is fairly common for suppressing plant immunity.

2) Over-expression of MTD appears to provide a general means for creating plants that have increased resistance to mannitol secreting fungal plant pathogens such as *Alternaria* and *Botrytis*.

Impact on the Industry

Because we are using a gene from a plant that is currently cultivated for food (celery or parsley), and putting it into plants we do not eat, we avoid a number of common concerns with genetically modified organisms. In fact, our research has found that the gene for

MTD occurs naturally in a wide variety of plants, but is often expressed either too late or at a level that is too low to provide effective resistance.

Thus, this research provides the grower with an ecofriendly means of controlling fungal diseases. Growers would also have reduced production expenses due to lower pest-management costs and decreased shrinkage caused by plant mortality and aesthetic damage from fungal disease.

For the consumer, over- expression of this naturally occurring plant enzyme could result in healthier plants with lower maintenance needs in the landscape, and, thus, lower environmental impact.

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