

Manipulating Ethylene Levels in Tomatoes to Increase Their Disease Resistance, Firmness and Field Storage

Report submitted To the Ontario Tomato Research Institute Nov 19/2001

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Plant diseases cause significant reductions in processing tomato yields and diseases that colonize the fruits reduce their field storage capability, firmness and quality. The development of new strategies for disease control, based on a plant's own defence mechanisms, would contribute to the profitability of this industry, enhance its position of concern for a healthy environment and ensure that the produce is free of agrochemicals. In previous work we produced plants with greatly reduced levels of ethylene by transforming them with the 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase gene from a plant growth promoting bacterium and showed that the transgenic tomato seedlings had significantly better survival rates after infection with *Fusarium* or *Verticillium* than nontransformed plants. Also, symptom spread on detached leaves inoculated with *Fusarium* or *Xanthomonas* was significantly less than in nontransgenics but interestingly, for some of the transgenics disease resistance was the highest if they were given a short pulse of ethylene at the time of infection. From work by others, we expect that the transgenics with reduced ethylene production would be firmer at maturity and may have better field storage capacity. **The objective of the work is to examine the susceptibility of tomatoes with altered ethylene levels to fruit pathogens and to assess the impact of the genetic changes on the quality of the tomatoes.**

Experiments were initiated to examine the following questions:

1. Do the existing ACC deaminase transgenics have enhanced resistance to fruit rots that occur during storage?
2. Do the existing ACC deaminase transgenics have enhanced storage properties?
3. Do the existing transgenics have enhanced compositional characteristics?
4. Will the overproduction of an ACC synthase in the existing ACC deaminase transgenics lead to an enhanced pattern of ethylene production and better disease resistance than the existing ACC deaminase transgenics?

Because the level of funding obtained was significantly less than requested we did not employ a postdoc, rather the work was carried out in part by a grad student and technical help on a part-time basis. We also obtained matching funding through the MII program at Ag Canada to cover growth room and consumable costs.

Results

1. Fruit rot tests. Transgenic (R) and nontransgenic (S) tomato plants (four 35S transgenic, four 35S nontransgenic, four rolD transgenic and four rolD nontransgenic, two Pprprotein transgenic and two Pprprotein nontransgenic and wt nontransformed) lines were grown in a greenhouse and fruit produced by the plants were harvested at the breaker stage and selected for uniform color, shape and size. The fruit were wounded with a sharp pointer and inoculated with spores of *Colletotrichum coccodes* (anthracnose) or *Botrytis cinerea* (gray mold). The diameters of the lesions that developed from the wound-site was assessed after 5 – 7 days incubation under a high humidity condition at 23 °C. Nine pairs of fruits harvested from transgenic (R) and nontransgenic (S) plants have been evaluated against *C. coccodes* (anthracnose).

Our preliminary results showed that fruits from individual R- and S- plants have a similar level of resistance ($P \leq 0.05$), except that average lesion size of R39 fruit was greater than that of S39. In comparison with fruit from wild type plants, lesions on fruits of R27, R39 and R213 were larger, but those on fruits of R31 were smaller. Gray mold (*B. cinerea*), lesions on fruits of R317 and R319 were larger than that of S317 and S319, respectively. There was no significant difference between fruits of R-plants and of S- plants for the other pairs. Compared with fruits of wild type plants, lesions on fruits of R27, S27 and S319 were significantly smaller.

The evaluations with other fungal as well as bacterial pathogens are ongoing. We plan to expand the analyses of those transgenic plants that have smaller lesions (R31, R27).

2. Enhanced Storage. Unfortunately, we have not been able to precede with these tests yet.

3. Compositional properties of existing transgenics. Headspace Solid-phase Microextraction –GC/MS was used to compare aroma compounds given off by the fruit. Tomatoes at breaker stage were used in this study. Before sampling, one or two tomatoes were put into a 125 mL Mason jar. After 3, 6 and 16 hours of equilibration at room temperature (22 °C), headspace volatile compounds were measured by solid-phase-micro-extraction (SPME) (Supleco, Bellefonte, PA) followed by gas chromatography-mass spectrometry (GC-MS) on a GCQ Ion Trap operated in the electron ionization mode at 70 eV (ThermoQuest Finnigan, San Diego, CA). Eight pairs of tomatoes have been analyzed. Although we did see some differences among the “promoter” groups, the results are still too preliminary to identify trends. Work is continuing in this area.

4. Next generation transgenics. To further manipulate ethylene levels through transgenesis we initiated two tomato gene isolation projects. The objective of the first project was to clone a full length ACC synthase from tomato that responds to stress. Transgenics with an extra copy of this gene might be expected to have a boost of initial ethylene synthesis following infection. The ACC synthase gene was cloned with its own promoter and it was used to transform tomato. Eight independent transgenic plants were produced that have an extra copy of the gene. These plants have short internodes and the leaves senesce early but their fruit production is normal. They will be crossed with the first generation tomatoes (containing ACC deaminase) in an attempt to produce tomatoes that respond quickly to a pathogen by synthesizing a burst of ethylene followed by a prolonged reduction in ethylene.

The objective of the second gene cloning project was to isolate a gene and its promoter that responds to pathogen attack within 72h. We hope to use the promoter in the construction of future transgenics to drive the expression of ACC deaminase or ACC synthase, thus allowing us to manipulate ethylene levels in the plants in response to pathogen attack. From a comparison of the genes expressed in *Verticillium*-infected tissue with uninfected tissue, a gene which is a homologue of a human regulator of chromosome condensation (RCC1) was identified. The coding region and a portion of the promoter was cloned and sequenced. The gene may be important in the control of cell cycle regulation, mRNA processing and transport. The putative promoter region had many potential regions for regulatory proteins to bind, including DNA sequences that may be involved in ethylene regulation.

Summary. We have made good progress on most of the proposed areas of research but some of the results are too preliminary for us to determine whether altering the ethylene levels of tomatoes has beneficial effects on fruit quality.