

ONTARIO TOMATO RESEARCH INSTITUTE

DEVELOPMENT OF TRANSGENIC TOMATO WITH RESISTANCE TO NEMATODES 2001

By

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EXECUTIVE SUMMARY

The long-term objectives of this research are to: 1. Provide nematode-resistant lines of tomato that can be immediately incorporated into tomato breeding programs; 2. Identify additional targets for engineering nematode resistance; and, 3. Identify alternative or complementary strategies for the field management of nematodes, thereby reducing requirements for, and human and environmental hazards associated with the use of nematicides; and, increase the production and security of the food supply.

The short-term objectives are to: 1. Genetically engineer tomato plants with elevated levels of an inhibitory neurotransmitter known as gamma aminobutyric acid (GABA), using sense expression of tomato GAD and/or antisense expression of tomato GABA transaminase (GABA-T); and, 2. Test the potential, under closed environment conditions, for using these transgenic lines to reduce crop damage attributed to root-knot and/or root-lesion nematodes.

In the first year, we began to research the prevention of GABA catabolism in 'Micro-Tom' tomato by the enzyme GABA transaminase (GABA-T) using antisense technology. To achieve this objective, identification of the various GABA-T genes present is required. Recently, we cloned a pyruvate-dependent GABA-T cDNA from *Arabidopsis*, which enabled identification of 20 sequences with 75-91 % identity from the tomato EST database available at the National Center For Biotechnology Institute. These were divided into three distinct classes with overlapping regions. This information provided the basis for design of class- specific primers for amplification by polymerase chain reaction (PCR) of the GABA-T sequences. The use of RT-PCR verified the presence and transcription of each putative gene in 'Micro Tom' tomato, and the use of RACE-PCR enabled retrieval of the full-length sequences. The predicted amino acid sequences of the three GABA-T isoforms possessed 458-520 amino acids, 76-80 % identity to each other, and 73-81 % identity to the *Arabidopsis* GABA-T, but only 21-28 % identity to non-plant GABA-Ts from human, bacterial and yeast sources. A distinct location for each isoform (mitochondrion, endoplasmic reticulum and cytosol) was indicated by the sequences. This study represents the first molecular evidence of multiple GABA-T isoforms plants, animals, bacteria or yeast. Currently, the three putative GABA-T genes are being

expressed in *Escherichia coli* to investigate the specificity of the recombinant protein for two different amino acceptors, pyruvate and 2-oxoglutarate. These studies will provide unambiguous information about the identity of the genes and indicate whether it is necessary to inhibit some or all of the putative GABA-Ts to elevate the GABA levels.