Genetically Engineered Plant Foods: Tomatoes

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Calgene is a leading United States plant biotechnology company organized in 1980 to develop and commercialize new crop varieties and plant products developed through the use of biotechnology. Plant biotechnology offers the opportunity for both the proprietary protection of genes which are isolated and patented, and the development of new plant cultivars which are produced using these genes. In some cases, researchers will be able to develop multiple products from a single gene, while in others the developmental cycle of new plant products will be shortened given the tools of biotechnology. Calgene currently focuses on three crops: rapeseed for use in the production of both edible and industrial oils; cotton for improved fiber quality, herbicide safening and insect resistance; and tomatoes for both the fresh and processing markets.

The work described here presents the current status of Calgene's anti-sense polygalacturonase tomato. Polygalacturonase (PG) is a pectin-degrading enzyme found in ripening tomatoes which has been correlated to tomato fruit softening and rotting by numerous investigators in the past. Polygalacturonase was selected as a target for the genetic engineering process of tomato fruit quality improvement based in part on characteristics found in naturally-occurring mutant tomato lines which lack substantial levels of PG activity. Historically, tomato ripening mutants such as the Never Ripe *(Nr)* and ripening inhibitor *(rin)* have been used for many years in tomato breeding programs based on certain characteristics determined by these mutations. Two of the most obvious characteristics are very slow softening and extended shelf life. The difficulty with these mutations

has been that they are pleitropic in nature, as demonstrated by the fact that genotypes containing these mutations do not evolve ethylene nor do they develop color to any significant degree. These pleitropic effects have limited the utility of these mutant lines in commercial production.

The antisense approach to eliminating polygalacturonase in tomatoes seeks to avoid the pleitropic effects associated with ripening mutants and create a product that is more than just an extended firmness tomato, but is a *better quality* tomato in both fresh market and processing applications. On the fresh market side, this means a tomato that will provide the grower, shipper and packer with reduced spoilage and improved shelf life. It will allow the grower to vine ripen the tomato. It will allow the packer the option of eliminating refrigeration and/or ethylene treatment of the vine-ripened material, enhancing the flavor of the product over the mature green or gassed tomato commonly produced today. On the processing side, inhibiting pectin degradation results in improved field holding of the ripe tomatoes and increased serum viscosity in processed product. Extended field storage will allow the grower to more accurately time the harvest and eliminate waste due to rot. Improved serum viscosity translates into a higher quality processed product.

The antisense approach to regulation of gene expression involves cloning of the gene of interest and transforming that gene into the plant in the reverse orientation. Analysis of mRNA, protein and enzyme activity of transformed plants has confirmed the utility of this approach. Selected transformants have reductions of PG mRNA levels and subsequent enzyme activities of over 99 percent relative to non-transformed controls. Calgene has produced a number of lines of transgenic tomato to test the utility of these new genotypes relative to both the naturally-occurring ripening mutants and non-transgenic controls. To accomplish this evaluation it has been necessary to produce this material on a large scale in the field. This has been accomplished in cooperation with the Campbell Institute for Research and Technology.

Our first field evaluation was planted in Guasave Sinaloa, Mexico, during the winter of 1988-89. There were several objectives of this trial: morphological evaluation, a test of field holding, and an evaluation of fruit processing characteristics. Morphological evaluation confirmed the lack of any of the commonly observed pleitropic effects associated with the known ripening mutants. Results of the field holding experiments indicated a significant difference in the ability of the transgenic fruit to hold

up under adverse field conditions, and processing evaluation indicated a highly significant improvement in the serum viscosity and juice consistency of transgenic fruit versus non-transgenic controls.

To confirm these results, as well as make additional selections from numerous transgenic lines, a second field trial was planted during the summer of 1989 in Yolo County, California. Fruit from selected lines was processed for analysis. Results indicated that in all cases, for each non-pectin related parameter measured, (total solids, soluble solids, pH, titratable acidity, and color), there were no differences between transgenic and non-transgenic controls. In the case of the pectin-related parameters examined, very significant positive differences were observed in serum viscosity (Ostwald test). Additional observations made both in the laboratory and in the field indicated the possibility of enhanced resistance of the transgenic material to certain fungal pathogens that are normally encountered both in the field and post-harvest during commercial production. Results of initial laboratory experimentation demonstrated enhanced resistance of transgenic fruit to two common post harvest pathogens, namely *Rhizo-f>us stolotiifer* and *Geotrichum canadum.* While initial results are encouraging, additional research needs to be carried out to determine the range of enhanced resistance which exists as well as the mechanism involved.

Our most recent field evaluation was conducted in Ruskin, Florida, during the winter of 1990. Material grown included third generation transformation events of three genotypes, CIR1, CIR2, and Rutgers. The focus of this experiment was to evaluate transgenic fresh market tomatoes homozygous for the antisense PG gene for their ability to withstand commercial packing and handling practices at different stages of ripening relative to non-transgenic controls. The trial was planted in February and harvested in late May. Fruit was harvested by hand following standard commercial practices in Florida, sorted depending upon developmental stage (mature green, pink, red) and packed into 25 pound boxes for storage. The finished harvest totaled just over five thousand pounds. Harvested fruit was transported by truck to a commercial packing shed were it was treated as follows: mature green fruit was gassed with ethylene for five days while being stored at 65°F and 65 percent relative humidity and then removed from the gas rooms and stored as described above for an additional five days with the exception of the ethylene gas. Fruit harvested pink and red was stored for ten days as described with no ethylene treatment. After the ten day storage period, the three genotypes, CIR1, CIR2, and Rutgers, were

evaluated for firmness relative to non-transgenic controls by measuring deformation of the fruit under a 500 gram load for a period of 15 seconds. In all, more than 900 fruit were evaluated and the results of the analysis indicate that in all cases, the transformed material was firmer and more intact after ten days of storage as described. Currently, further experimentation is underway to evaluate the utility of this trait in hybrid combination as well as to expand the breeding effort into commercial lines.