Transgenic virus resistant plums poised for release

in

- Genetically Engineered Trees [1]

On 9 September 2004 the Agricultural Research Service of the United States Department of Agriculture (USDA) petitioned that virus resistant transgenic plums be given non-regulated status. That petition is still under consideration but should be available for public comment soon. The proposed commercial release is, I believe, the patented plum variety "Honey Sweet". plum developed jointly by USDA, the Institut National de la Recherche Agronomique, Paris France and Cornell University. The plum tree has the plum pox virus (PPV) coat protein gene incorporated to provide resistance to the major plum pest PPV. The female parent of the plum is "Bluebyrd" (named for Senator Robert Byrd) while the pollen parent is "unknown". The plant is not self fertile, a pollinator is required. The variety is propagated by bud grafting to standard rootstocks. The plum fruit is a typical drupe in which the skin and flesh of the fruit contain only maternal genes, the seed embryo and endosperm contain both paternal and maternal genes. The seeds of the transgenic plum are viable and could produce viable plants. In the event that the pollen was produced on the PPV transgenic plum the flesh of the fruit produced when a normal plum is pollinated would not contain the PPV gene but the seed would.

The transgenic plum contained the PPV coat protein gene, along with the selectable markers NPTII (Kanamycin resistance) and GUS (β-Glucuronidase). There were multiple copies of the PPV coat protein gene linked at the insertion site. The genetic modification of the plums was done using a gene cassette containing the NPTII gene driven by the relatively weak nos promoter from Agrobacterium and terminated by the nos terminator. The PPV-CP was driven by the Cauliflower mosaic virus (CaMV) promoter and transcription was terminated by the nos terminator gene from Agrobacterium finally the GUS gene was driven by the CaMV promoter and transcription was terminated with the nos gene. Analysis of the genes inserted into the plum clone C5 showed that there was a second insertion of the PPV gene insert that was "unlinked" to the primary NPTII, GUS, PPV-CV gene insert. Fragments of the NPTII gene and the GUS gene were also detected in the transgenic plum. The multi copy PPV inserts behaved like single genes in crosses indicating that they were relatively close together on a chromosome. The PPV-CV inserts were found to be methylated unlike the promoters or the GUS gene. There was a high level of PPV-CP gene transcription in the plum cell nucleus, there was a low level of PPV-CP messenger RNA in the cytoplasm and very little detectable coat protein produced. Attempts to infect the transgenic plum with large inocula of PPV showed that little virus was produced in the plum. The resistance to PPV in the transgenic plum was based on a process called post transcriptional gene silencing. Post transcriptional gene silencing is a cellular based sequence specific post transcriptional RNA degrading system that is programmed by the transgene encoded RNA sequence. RNA viruses produce double stranded RNA during replication and these replicating virus is rapidly degraded by the enzyme complex programmed by the transgene RNA.

The insertion of the PPV-CP gene cassette into the plum is necessary but not sufficient to produce strong stable resistance to PPV. For example plum transformation events C2, C3 and C4 accumulated high levels of PPV-CP messenger RNA and coat protein but were not resistant to PPV, in contrast event C5 produced little PPV-CP messenger RNA and barely detectable coat protein. Event C5 PPV resistance was stable in open field trials including controls either without transgenes or the
transformation events that were not virus resistant such as event C3(6). The reason that event C5 was stably resistant to PPV appears to be the duplication and methylation of the PPV-CP gene in event C5.

One problem with the C5 event in the environment may be the transfer of the NPTII gene to soil bacteria and in turn horizontal transfer of the gene to animal pathogens. The NPTII gene was shown to be extensively transferred to a soil bacterium, Actinobacter, from transgenic sugar beet (7). Even though the root stock for the C5 plum was not transgenic and not able to transfer the NPII gene the autumn leaves, shed bark and flowers of the plum would certainly deliver a good quantity of the antibiotic resistance gene to the soil.

In 2004 the United States EPA published a Scientific Advisory Panel (SAP) report on Plant Incorporated Protectant , specifically those based on viral coat proteins (PVCP-PIPS). The report provided extensive discussion of concerns such as the spread of virus resistance to weedy relatives but did not deal with the special consideration of the implications of post transcriptional gene silencing. Viral interaction including recombination between a viral transgene and an invading virus (either the same or a different virus from which the transgene was recovered), heterologous encapsidation (adding the transgenic coat protein to the capsid of an unrelated invading virus) and synergy (synergy is illustrated by viral suppressors of post transcriptional gene silencing). The panel believed that heterologous encapsidation and synergy were relatively unimportant in PVPCP-PIPS and felt that the recombination could be prevented by removal of the three prime (tail end of the gene construct) un-translated end of the gene construct even though there was limited support for that conclusion. The panel concluded that eating transgenic viral coat protein should be considered safe (without experimental verification) because people have been eating virus infected plant material for a long time (8). This SAP report like many others tends to long on speculation and discourages full and adequate experimentation in areas where safety experiments are few and inadequate.

The SAP report on PVCP-PIPS provides poor guidance for the PPV-CP plum. There are well known post transcriptional gene silencing suppressors in the poty viruses related to PPV and within PPV. The extent of homologous recombination between PPV and the PPV-CP transgene has not been adequately investigated. Furthermore, even the small amount of PPV coat protein produced in the transgenic plants should be evaluated for human consumption because the PPV-CP gene has been manipulated and is the product of a nuclear gene not a virus. The SAP report, essentially ignored phenomena such as the read through effects associated with the nos transcription terminator in roundup ready soy (9) The read through produced transcription variants and potentially unsuspected fusion proteins.

In conclusion, transgenic plum pox resistant Honey Sweet Plum is based on posttranscriptional gene silencing. It evaluation prior to the application for non-regulated status does not appear to be fully adequate. USDA may have acted peremptorily in seeking release of a novel but poorly tested variety of a fruit tree. Given the outlook of USDA the PPV resistant fruit tree may be imposed on all plum producers in the near future to contain the spread of PPV. The period of public consultation may near at hand and it seems wise to comment on the petition for un-regulated status.

References
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