

## Postharvest Resistance Through Breeding and Biotechnology

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Postharvest losses due to infections by microorganisms can be prevented or reduced by several means. Commonly used practices are proper harvesting and handling of materials to minimize infection of susceptible plant parts, treatment with chemicals, and storage in a controlled atmosphere (5). An alternative is the incorporation of resistance in the host to the potential invaders. This way one can eliminate the need for special chemical or atmospheric treatments. In this paper I address the problem of obtaining genetic resistance to storage diseases.

At first glance, the problem of obtaining resistance to storage diseases appears to be similar to other forms of resistance breeding. One must identify the disease and the organism responsible for the disease, devise tests for resistance, detect resistance in breeding lines or wild species, breed resistance into the crop lines, and then select plants with superior resistance. However, on further analyses some real difficulties can be seen with each step of the procedure. For example, the organisms causing preharvest and postharvest disease may be quite different. Thus, a whole new set of diseases may need to be considered. Also, the susceptibility to infection may occur only after the ripening process; before ripening the plant material may be quite resistant. Altering resistance may interfere with the desired ripening and ruin the food value of the crop. Finally, disease resistance is only one of many characters that one must select while producing a new cultivar and, at times, it appears that one can only obtain a particularly desirable trait at the expense of another. In this paper I discuss a number of these problems. I describe some of our work with interspecific somatic hybridization of potato with wild *Solanum* species that may provide some new resistances to tuber soft rot caused by *Erwinia* spp., a major postharvest problem of potatoes.

### DISEASES OF PLANTS IN THE FIELD AND IN STORAGE

A number of fungi from several genera cause problems with fruits and vegetables in storage. Among these are representatives of *Botrytis*, *Penicillium*, *Mucor*, *Alternaria*, and *Aspergillus* on pome fruits (7). Problems on stone fruits can be caused by *Monilinia*, *Rhizopus*, *Botrytis*, *Gloeosporium*, *Geotrichium*, *Penicillium*, and others (6). Some vegetables such as tomatoes are also infected by species of *Botrytis*, *Rhizopus*, and *Mucor*. These fungi, which often infect and cause damage only after the harvesting of fruits and vegetables, are only a subset of organisms that cause diseases of plants. Consider, for example, the problems of potato in the field. Important diseases include black leg, ring rot, bacterial wilt, Verticillium wilt, early blight, and late blight. Viruses such as potato leaf roll virus (PLRV), and potato virus Y (PVY) or nematodes such as *Globodera* spp. and *Meloidogyne* spp. can also cause severe losses. These are major problems that

plant breeders must face if the plants are even to survive to produce a crop for storage. Of the above, only late blight and black leg (tuber soft rot brought on by *Erwinia* spp.) are very important in storage. Thus, it is clear that the breeder is faced with many disease problems that must be solved before postharvest problems can be addressed.

Resistances to the many possible diseases in the field are only some of the many factors that must be considered by the breeder. With potato, for example, yield, uniformity of tubers, skin color, flesh color, tuber shape, tuber size, sugar content, and maturity all are critical for a cultivar. In fact, the agronomic and processing characters are so important that disease resistances are often relegated to a low priority if chemical applications can be used to protect the crop.

In spite of the many other factors that breeders must consider, breeding for disease resistance of crop plants in the field has been highly successful and is an important part of cultivar development. To date, however, breeding for resistance to postharvest diseases has not received as much effort. In fact, an important question is whether there is any real evidence of genetic control of resistance to storage diseases. Fungi such as *Botrytis* and *Penicillium* spp. seem to be widespread and affect many fruits. Is there any hope of finding resistance to these invaders? Based on several lines of evidence, the answer to this question seems to be a qualified yes. First, it appears that although of some genera attack many different crop species, the fungal species that attack the various crops may differ. Thus, *Penicillium expansum* is the major member of this genus that attacks apple in storage, whereas *P. digitatum* is the major problem on citrus fruits (6). *P. cyclopium* is a major pathogen of onion, whereas *P. corymbiferum* is a problem on garlic (15). These results indicate that some resistance to the noninvading species is still maintained by the plant material. It may be worthwhile to examine the reasons for this resistance and to consider the possibility of exploiting it, if the resistance is due to an intrinsic property of the plant rather than incompetence or avirulence of the pathogen.

In other cases, a single species may cause a problem on many crops. Damage from *Botrytis cinerea*, for example, is widespread on many crops. Can it be that, with this organism, our only hope is proper cleanliness and good storage conditions? Yoder and Whalen (16), however, found variation in susceptibility of stored cabbages to *B. cinerea*. Thus, there may be resistance to exploit even with this pathogen. In the same vein, some differences in susceptibility of peaches to *Monilinia fructicola* have been reported (9) and Dennis (4) cites a number of differences in resistance of strawberry and raspberry to both *Rhizopus* spp. and *B. cinerea*.

It appears, therefore, that resistances to storage disease may exist in various crops. Whether the observed resistances are sufficiently effective to substitute for special storage conditions is another question entirely. Also, whether wild species have resistances that might be used to improve cultivars is an open question. However, there appears to be enough evidence to

warrant careful evaluation of the possibilities of breeding plants with improved resistances to postharvest diseases.

Those diseases that are problems both in the field and in storage are good candidates for investigating the possibility of improving resistances to postharvest diseases by breeding. With potato, for example, tuber soft rot and black leg are both caused by *Erwinia* spp. Also, late blight of potatoes in storage could possibly be decreased by planting cultivars with foliar resistance to *Phytophthora infestans*. In the United States, where fungicide application is so prevalent, late blight may not be a serious postharvest problem. Still, the disease can be serious in other areas of the world and may become a problem in the United States if restrictions on fungicide use become more common or fungicide-resistant mutants of *P. infestans* arise more frequently. However, to obtain resistances to soft rot or late blight, it is necessary to have good sources of resistance that can be integrated into breeding lines. At this point, therefore, I turn to the potential of wild species as sources of disease resistances and a demonstration of how biotechnology may help us use these resistances.

### RESISTANT PLANTS FROM INTERSPECIFIC SOMATIC HYBRIDIZATION

Wild species related to a crop species have traditionally been good sources of disease resistance and other economically important characters. The wild species is often crossed with a breeding line, desirable progeny are then selected and further crossed with breeding lines until a cultivar results that contains the character from the wild species. In certain cases, however, incompatibilities that prevent crosses can make the standard breeding pathway difficult. Consider, for example, the wild *Solanum* species and the very desirable disease resistances listed in Table 1. Unfortunately, none of these species can be crossed directly with potato. They are diploid ( $2n = 2x = 24$ ) lines, whereas commercial potato lines are tetraploid ( $4x = 4n = 48$ ). Equalizing the ploidy of the plants, either by doubling the chromosome number of the wild species or by halving the chromosome number of the potato breeding line, does not result in compatibility, however. These wild species are what Johnston et al (14) have called one endosperm balance number (1 EBN) species. Even if there are no stylar or other barriers to fertilization, successful development of the endosperm appears to take place only when they are crossed with other 1 EBN species. Thus, tetraploid North American potato lines (4 EBN), or even diploids extracted from these potatoes (and, thus, 2 EBN), are incompatible with the wild species, a situation that renders the genes unavailable to the breeder using standard techniques. By a combination of biotechnology and breeding, however, the genes for resistance can be made available. The technique that we have used to circumvent this incompatibility is interspecific somatic hybridization of protoplasts from the wild species with protoplasts from potato.

Initially, we set out to obtain resistance to PLRV by fusing protoplasts from potato with *S. brevidens*, a diploid South American species that does not form tubers. *S. brevidens* is

TABLE 1. Potentially valuable disease or stress resistances in some diploid wild *Solanum* species that are sexually incompatible with potato\*

<i>Solanum</i>	Resistance or immunity
<i>S. brachistotrichum</i>	Late blight, peach aphid
<i>S. brevidens</i>	PLRV, frost and <i>Erwinia</i> spp.
<i>S. bulbocastanum</i>	<i>Verticillium</i> , <i>Meloidogyne chitwoodii</i>
<i>S. cardiophyllum</i>	Black leg and late blight
<i>S. chancayense</i>	<i>Verticillium</i> , potato aphid
<i>S. etuberosum</i>	PVX, PVY immune, PLRV resistant
<i>S. fernandezianum</i>	Golden nematode
<i>S. jamesii</i>	<i>Verticillium</i> , root knot resistant
<i>S. mochicense</i>	Immune to <i>Verticillium</i>
<i>S. pinatisectum</i>	Late blight, ring rot
<i>S. polyadenium</i>	<i>Globodera pallida</i> , late blight
<i>S. trifidum</i>	Wart, late blight, <i>Verticillium</i>

\*Information obtained from Hanneman and Bamberg (12).

resistant to PLRV. Our potato line, like many potato cultivars, is susceptible to PLRV. To have a marker for the potato genome, we chose a tetraploid late blight differential line for our potato parent. *S. brevidens* is highly susceptible to race 0 of *P. infestans*, whereas PI 203900, the differential line chosen for this study, is resistant to race 0 but susceptible to race 4. Using procedures developed for protoplasts from several *Solanum* species (11), and a fusion procedure used to fuse *S. brevidens* with another potato line (1), we obtained hexaploid somatic hybrids. The hybrids expressed the resistance to PLRV of the *S. brevidens* parent and the resistance to late blight of the potato parent (13). As a bonus, the somatic hybrids were fertile (8).

The somatic hybrids and others obtained from fusion experiments were planted at the Hancock Experimental Station in the central sands area of Wisconsin, about 140 km north of Madison. There we have observed the materials in the field and recorded flowering times, flower colors, pollen shed, leaf shape, yields, tuber shapes, etc. (2). At harvest we found that the tubers of the potato parent, as well as seed pieces of other potato cultivars, had rotted away during the summer. However, the mother tubers of the somatic hybrids were almost intact and almost as firm as the new crop. Since rotting by *Erwinia* spp. is a major cause of seed piece decay, we tested the tubers from the somatic hybrids for their ability to withstand rotting by *Erwinia carotovora* subsp. *atroseptica*, *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi*. We found that the somatic hybrids were resistant to all three bacterial pathogens, whereas the potato used in the somatic hybridization was susceptible (3).

The hexaploid somatic hybrids have been crossed with the tetraploid cultivar, Katahdin. Progeny of the cross segregate for resistance to *Erwinia* spp. Some are clearly resistant, others are clearly susceptible; Katahdin tubers included in these tests were all very susceptible (3). Since *S. brevidens* does not form tubers, we can only infer that the resistance came from that species, although it is difficult to explain the results otherwise. We are examining the genetics of these materials in detail at this time.

### CONCLUSIONS

Bypassing sexual incompatibility through the use of somatic hybridization is but one way in which biotechnology may enhance our genetic resources to improve crops. It is clearly not as elegant as the insertion of a single gene or a few genes for resistance into the genome of a susceptible plant. Also, a number of crosses may be required before the new lines with resistances acquired through somatic hybridization are satisfactory in other economic traits such as yields, processing quality, etc. However, to insert a gene for resistance, one must have the DNA fragment that confers resistance. For the most part, molecular biologists are not even close to having the appropriate DNA pieces to insert. In contrast, somatic hybridization, as with conventional breeding, can be done at the level of phenotypic expression. Also, physical identification of the gene(s) of interest need not be done.

The soft rot resistance example cited above is not unique. We have found that resistance to PLRV can be detected in somatic hybrids of *S. brevidens* with diploid potato lines (1). Also, tubers from plants obtained from the fusion of *S. brevidens* and the potato cultivar Russet Burbank are highly resistant to *Erwinia* spp. (3). Thus, mixing the genomes of two different species by somatic hybridization can result in the acquisition of potentially valuable traits from wild species even though these species cannot be crossed with potato lines. Since many wild *Solanum* species have potentially useful resistances (12), somatic hybridization could provide new access to resistant germ plasm for diseases in the field as well as in storage. Attempts to obtain somatic hybrids of other crop species with their wild relatives might, therefore, be useful for improving resistances in crops other than potato.

Finally, it is appropriate to mention the role of serendipity in finding resistance to diseases. Gabelman and his co-workers found that in the process of developing hybrid onions that all matured at the same time, they greatly decreased the incidence

of neck rot (10). In the case of soft rot resistance in somatic hybrids, who would have looked for resistance to tuber soft rot in a plant that formed no tubers? Perhaps other, still undetected genes will be found when they are put into a different background where they can be expressed.

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