

to suboptimal temperatures is among the neglected areas of potentially useful research and development. Sensitivity to chilling in many important crop plants limits their climatic distribution for economic production. Even in areas normally suited to these crops, this sensitivity causes losses through injury suffered from sporadic low temperatures.

Our laboratory has been exploiting the existing genetic diversity in wild tomato germplasm for plant breeding to reduce crop losses and allow expansion into environments too hostile for economic production. As a result, we have improved chilling tolerance in the cultivated tomato through the technique of pollen selection.

Our strategy has been to test whether selection among haploid male gametophytes (pollen grains) might have a positive, correlated effect on the vegetative (sporophytic) generation resulting from directed selection of genes expressed in both stages. The critical requirement of this overlapping model is that specific genes that are expressed in the pollen grains and confer a particular physiological trait also function in the vegetative plant. Hence, selection for these genes in the pollen phase could have a positive effect on the success of the sporophytic phase.

In practice, virgin stigmas are pollinated and exposed to a low temperature challenge. Under these controlled conditions, only those pollen genotypes conferring germinability



Scanning electron micrograph of pollen grain germinating on surface of stigma.

and pollen tube elongation under low temperatures would effect fertilization. Therefore, only the selected pollen genotypes would contribute their genes to the subsequent generation. We have demonstrated, in fact, that the haploid (pollen) genome can modify the fertilization ability of individual gametes in a manner reflecting their genotype. Furthermore, the "low-temperature" genes amenable to pollen selection also confer low-temperature tolerance to the resultant sporophytic progeny.

Using the pollen selection technique, we have successfully transferred chilling tolerance from a wild tomato species to the cultivated tomato. This was accomplished, without recourse to laborious and expensive progeny testing, by applying pollen selection during each subsequent backcross. We now have ad-

vanced backcross progeny carrying selected genetic characters that enable them not only to grow and reproduce in environments where cultivated tomatoes would experience severe chilling injury, but also to survive temperatures just above freezing that kill cultivated types.

The correlated response established between chilling tolerance in tomato pollen and the sporophyte is the first of its kind and permits the plant breeder to select with great economy in the haploid generation. The pollen selection technique developed in this program may prove useful in selecting for improved physiological efficiencies in numerous crops under a host of stress conditions.

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Embryo callus hybrids

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Hybridization between cultivated species and related wild species has been of great value for cultivar improvement. Genes that enhance the survival of the wild species by providing disease or insect resistance, salt tolerance, cold tolerance, and the like, often confer the same trait on the cultivar to which they are transferred. Fertility barriers restrict the number of wild species that can contribute genes to any particular cultivated species through normal sexual crosses, but these fertility barriers can often be overcome through the use of special procedures.

The cultivated tomato, *Lycopersicon esculentum*, hybridizes with all but two of the wild tomato species in the same genus by normal sexual crosses. *Lycopersicon peruvianum*, the most distantly related species in the genus, does not produce viable seeds when hybridized with cultivated tomatoes. Hybrids between these two species have been obtained

only with great difficulty by embryo culture. The undeveloped seeds produced were carefully dissected to search for the rare embryos (approximately 1 in 10,000) that had developed enough to then continue their development into plants on appropriate culture media.

Hybrids between *L. esculentum* and *L. peruvianum* can be obtained much more efficiently when whole, undeveloped seeds are placed on a culture medium suitable for the growth of callus. Under these conditions, up to 1 in 10 (but sometimes only 1 in 200) of the seeds form callus. All plants regenerated from this callus have clearly derived from hybrid embryos rather than endosperm or maternal cells in the seed. All of the regenerated plants were diploid or tetraploid, whereas endosperm cells are triploid. Traits of the regenerated plants that resemble the male parent (*L. peruvianum*), such as anthocyanin content (the female parent used was anthocyaninless), form of leaves, flowers, and fruits, inability to self-pollinate, and the isozyme migration rate in gel electrophoresis, proved that the plants derived from hybrid embryos

and not maternal seed tissues or maternal self-pollinated embryos.

The embryo callus method may become a valuable tool for tomato breeders. F₁ hybrids have been obtained easily for 8 out of 11 *L. peruvianum* strains tested. More work will be needed to determine more precisely what proportion of *L. peruvianum* strains are susceptible to hybridization by this technique. The first backcross of some F₁ hybrids to *L. esculentum* has also been achieved via embryo callus. Subsequent backcross generations are expected to arise by means of normal seeds, so the embryo callus method appears sufficient to completely overcome the fertility barriers between the two species.

Although the success rate is somewhat variable, indicating that more work will be needed to determine the optimum plant growth conditions to use, the embryo callus technique is clearly more efficient than embryo culture for wide crosses between wild and cultivated tomato species.

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