Cryogenic germplasm storage and recovery in US hemlocks: Can it be applied for tropical trees?

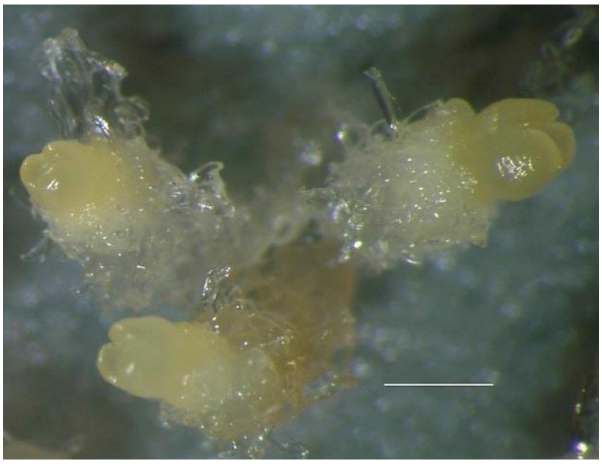
Two hemlock trees (Tsuga canadensis and T. caroliniana) are vital components of Northern Hardwood Forest and southern Appalachians in the US. Millions of individuals of both the tree species were destroyed due to infestation by a deadly insect, Adelges tsugae. At this juncture researchers were left with two major tasks, one is to plant individuals exhibiting natural resistance for the pest and the other is to successfully store disease-free germplasm for longer periods. This was achieved by a research team from University of Georgia. They devised a protocol to initiate disease-free somatic embryos through tissue culture, preserved the materials in cryogenic storage and established a successful recovery. A similar conservation approach can be applied for several tropical trees exhibiting restricted distributions in their natural range due to a number of infestations.

There are only two native hemlock species in the eastern U.S.--eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*T. caroliniana*). Both are in terrible danger from an insect that first appeared in Virginia in the 1950s. *Adelges tsugae*, commonly known as hemlock woolly adelgid (HWA) is a pest native to East Asia that kills hemlocks possibly by injecting a toxin into the trees while feeding on sap. It has spread from Virginia and virtually exploded in the Appalachians. A team of researchers headed by Prof. Merkle from Warnell School of Forestry and Natural Resources, University of Georgia had found a solution. Prof. Merkle is also the lead author of the research paper published in the journal 'Trees' recently. While the loss of eastern hemlock will cause severe ecological consequences on forest composition, wildlife and nutrient cycles, loss of Carolina hemlock would dilute the value of the Appalachian forest. Unfortunately, researchers are not yet sure how the insect is even killing hemlocks.

Infested Appalachian hemlock forests

A number of researchers are studying ways to fight the infestation, including the possibility of introducing some sort of biocontrol or predator to eat the adelgids. Prof. Merkle looked at ways to not only introduce natural resistance to newly planted hemlocks, but also to successfully store hemlock germplasm cryogenically to conserve it in case a solution isn't found before they are wiped out. Long-term storage of hemlock germplasm has been hindered because seeds from the trees have lost viability after being stored under refrigeration after two to four years, meaning that once planted, they won't germinate and grow into thriving plants. The U.S. Forest Service is growing collections of hemlocks outside the range of the adelgid to conserve their germplasm, but this approach requires maintenance of the trees in areas where hemlocks are not found naturally.

Merkle and his research team took a different approach to standard storage methods: Using seeds from surviving trees, they created *in vitro* cultures of a number of eastern and Carolina hemlocks that they then froze in liquid nitrogen at -196 degrees Celsius --something no one else had successfully done. The researchers cryofroze several samples from different hemlock lines from around the Southeast for several months, then thawed them out, allowed them to re-grow and began to produce trees from them. Of the five hemlock lines they tested, all samples of three Carolina lines and one eastern hemlock line grew well after coming out of cryostorage.



Somatic embryos of eastern hemlock (8 weeks culture)

Merkle says the ability to cryostore and recover hemlock cultures, followed by production of new trees from them, provides a practical approach for storing the germplasm of a large number of trees indefinitely, so that the species can be repopulated once a system to deal with the adelgid is in place. Being able to grow them after they're thawed won't make them resistant to the woolly adelgid, Merkle said, but it does mean that if need be, researchers might be able to save samples of the hemlocks from extinction. These cultures also allow the researchers to assist with testing other methods of introducing resistance to the insect, including trying to create clones of hybrids with Asian hemlocks that do have a natural resistance to the woolly adelgids.

A Carolina hemlock somatic seedling

Some individual hemlock trees in the U.S. appear to be naturally resistant, Merkle said, so he and his team are working on ways to determine if the resistance is genetically based. They'll do that by collecting seeds from these trees that have survived the insect infestation and creating embryogenic cultures that they can then use to clone single trees. Because these surviving trees are all genetically different researchers can't really tell what key factor makes them resistant to the woolly adelgid. But if 20 trees are planted that are all members of the same clone, clonal testing can be done to try to narrow down why particular trees can fight the pest. And once a genetic line is identified that is resistant, Merkle said, "You can then supply seedlings to people for planting."

The embryogenesis and cryostorage systems developed in the study are already being integrated with hemlock breeding efforts to develop clones that are resistance or tolerance to HWA. Such initiatives can be applied to tropical trees that face infestations due to numerous insect pests. For instance, the dipterocarp *Shorea robusta* (sal) occurs in plenty in forest of central and northern India. This tree of high commercial importance faces severe epidemic of a cerambycid beetle (*Hoplocerambyx spinicornis*) almost throughout its natural range. In this case, trees exhibiting natural resistance should be identified and seeds must be collected after intra-specific crosses among them. Such seeds may be used to as explants for somatic embryogenesis. Thus this approach of combining somatic embryogenesis and cryopreservation with breeding programs will serve as a potential tool for conservation of tree genetic resources in natural habitats through reforestation.

Source:

http://www.sciencedaily.com/releases/2015/02/150203112127.htm