Pepper DNA transfer to eastern white pine enhances capacity to withstand prolonged periods of drought

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Summary

Christmas tree production in North Carolina can be increased if more species can be grown in the eastern part of the state where higher mean temperatures and drought conditions prevail. We have been working on research to understand how conifers tolerate drought as well as using new biotechnologies to improve drought tolerance in Christmas trees. Our approach is to use both tissue culture cloning and gene transfer technology that we have developed in our laboratory to genetically modify Christmas trees so that they can be more tolerant of drought stress. It has been shown by other researchers that certain types of DNA known as transcription factors play an important role in most plant stress tolerance responses. It appears that they have a similar functional significance in Christmas trees. Transcription factor DNA has been isolated from a pepper plant (Capsicum annuum) and we have used both cloning technology and gene transfer technology to transfer this pepper DNA into Christmas trees. We have discovered that the introduction of pepper DNA into eastern white pine (Pinus strobus L.) results in a dramatic increase in tolerance of the white pine to drought stress. These results show that the enhanced ability of genetically transformed plantlets to drought stress tolerance in eastern white pine confirms the postulated function of this pepper DNA in regulating other genes in providing tolerance to environmental stresses. These results demonstrate that pepper DNA may be useful in genetically engineering Christmas tree species for drought stress tolerance.

Introduction

Christmas tree production in North Carolina can be increased if more species can be grown in the eastern part of the state where higher mean temperatures and drought conditions prevail. We have been working on research to understand how conifer trees tolerate drought as well as using new biotechnologies to improve drought tolerance in Christmas trees. Our approach is to use both tissue culture cloning and gene transfer technology that we have developed in our laboratory to genetically modify Christmas trees so that they can be more tolerant of drought stress. It has been shown by other researchers that certain types of DNA known as transcription factors play an important role in most plant stress tolerance responses. It appears that they have a similar functional significance in Christmas trees.

Environmental stresses such as drought, freezing, and salt are major environmental factors that limit plant growth and crop productivity worldwide. Plants have the unique capability to perceive and respond adaptively to harsh environmental conditions. Indeed, it has been shown that internal plant stress signalling to drought comprises a complex network in which there is extensive cross talk among different metabolic pathways. Pieces of DNA known as transcription factors which regulate multiple gene expression have been reported previously. These factors induce the expression of drought-regulated genes and promote drought tolerance.

In this study, we have studied the function of the pepper (Capsicum annuum) transcription factor DNA when it is introduced into eastern white pine (Pinus strobus L.). We show that this foreign DNA increases tolerance of eastern white pine to drought and allows it to withstand prolonged periods of drought stress.

continued on page 10

Results

Plant materials and gene transfer

Eastern white pine (Pinus strobus L.) seeds were used and tissue pieces (young leaves) from the embryos were isolated from them (Figure 1A). Pepper DNA transfer to the white pine tissues embryos is accomplished by inserting it first in a bacterium known as Agrobacterium (Figure 1A). The bacterium with the pepper DNA is cultured in liquid broth, and the broth is then placed on the white pine tissues whereby the bacteria infect the tissue and transfer the pepper DNA into the cells (Figure 1A). The genetically modified tissues are subjected to a sterilization treatment and the bacteria are killed, while the white pine tissue cells containing the pepper DNA remain alive.

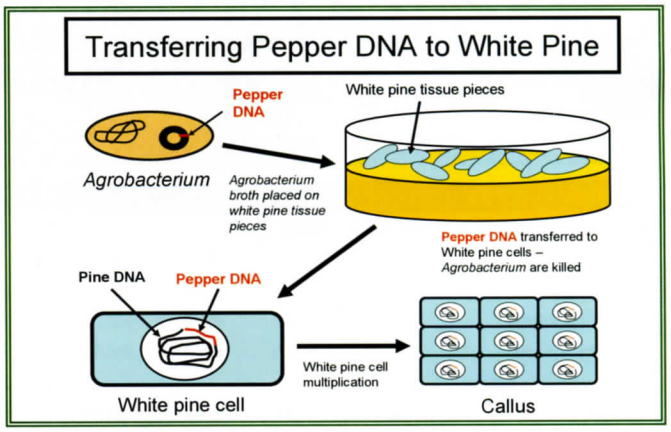

Figure 1A. Schematic representation of transferring pepper DNA into eastern white pine. We inserted the pepper DNA into the Agrobacterium DNA. The bacterium infects the white pine tissue, and transfers the pepper DNA to the interior of the white pine cells where it is integrated in with the white pine DNA. The bacteria are killed, the tissue is sterilized, and the white pine cells with the pepper gene begin to multiply and produce callus.

Cells multiply into callus tissue

The genetically modified tissues were placed on a nutrient medium and induced to form a callus tissue (Fig 1A). The callus stage is shown in Figure 2B. More than 300 independent groups of callus were generated. Using molecular biology techniques of identification, it was determined that these calli were comprised of cells that contained the newly transferred pepper DNA (Figure 1A). These genetically-modified callus lines were transferred into fresh growth medium tri-weekly for 3 months to produce more calli from each line.

Genetically modified white pine plantlets are produced

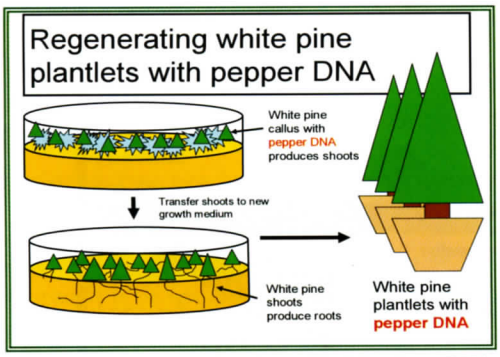

Figure 1B. Eastern white pine calli with pepper DNA regenerates multiple shoots. The shoots are transferred to growth medium which induces root formation on their bases. The regenerated plantlets with the pepper DNA are transferred to a soil medium in pots.

The 3 month-old genetically-modified calli were placed on a new medium containing a hormone called a cytokinin (Figure 1B). Cytokinins cause shoots to form on the callus surface (Figure 2C). After another 3 months, shoots are transferred to a different growth medium for 3 months and allowed to grow and elongate (Figure 2E). The genetically-modified elongated shoots are then transferred to a growth medium containing a root-forming hormone (indole-butyric acid) (Figure 1B). After three months, the shoots have sufficient root development and are now designated as plantlets (Figure 2F), whereby the genetically modified plantlets then can be transferred to a soil medium (Figure 1B). Next, the plantlets are grown in plastic pots filled with a perlite/peat-moss/vermiculite (1:1:1 v/v) mixture (Figure 2G, 2H, 2I). The genetically-modified plantlets are grown for 4 weeks in this mixture before the drought-stress tests are initiated.

Expression of the transferred pepper DNA enhances tolerance to drought-stress in genetically modified white pine plantlets

Genetically modified eastern white pine plantlets expressing pepper DNA (Figure 1B) were tested to see if they tolerated drought more than those plantlets not receiving the foreign DNA. Water was withheld from plantlets growing in soil. First, plantlets were irrigated for 3 months, and then irrigation ceased for 30 days; thus water was withheld from the plantlet roots immersed in the soil medium. After this, plantlets were irrigated again for a week and were then evaluated for their capacity to recover from this prolonged drought period. (We found no apparent effect of the transferred gene on the overall growth and morphology of the plantlets). We tested both non-genetically modified control plantlets and several genetically modified

plantlets. We observed that the non-genetically-modified control plantlets died, and the genetically modified plantlet lines with the transferred pepper DNA survived. In three series of trials of drought stress treatments with repeated irrigation withdrawals, the pepper DNA carrying plantlets reproducibly exhibited high rates of survival after the treatment, which demonstrated that the transferred DNA conferred significant drought tolerance to eastern white pine plantlets.

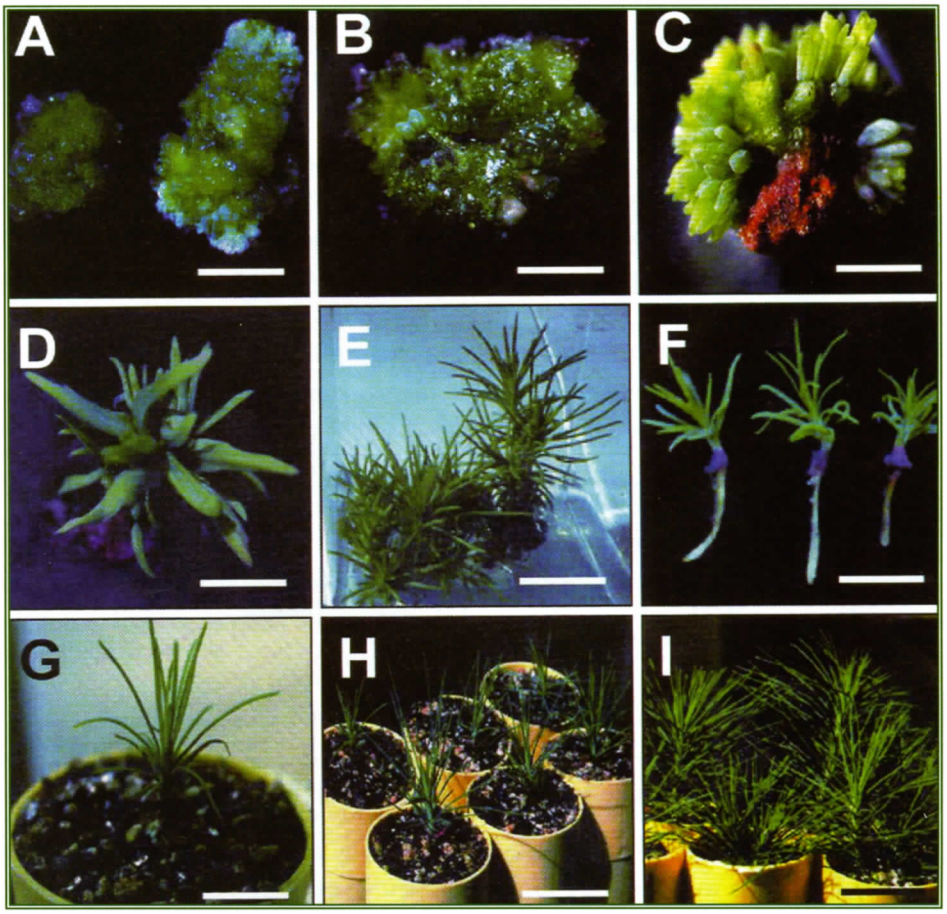

Figure 2. Regenerating eastern white pine: the tissue culture cloning system used to transfer pepper DNA into eastern white pine. **A.** Tissue pieces (young leaves) placed on solid growth medium for 3 weeks. **B.** Callus derived from tissue pieces on solid growth medium for 6 weeks. **C.** Several clusters of shoots on shoot formation medium for 9 weeks. **D.** A cluster of shoots cultured on shoot formation medium for 12 weeks. **E.** A cluster of shoots that have grown and elongated. **F.** Rooting of shoots cultured on rooting medium for six weeks. **G.** Regenerated plantlets established in soil mixture for 1 week. **H.** Regenerated plantlet established in soil mixture for 3 weeks. **I.** Regenerated plantlets established in soil mixture for 3 months. **[Figures adapted from: Tang, W. and Newton, R.J. (2005). Plant regeneration from callus cultures derived from mature zygotic embryos in white pine (*Pinus strobus* L.). *Plant Cell Rep.* 24, 1-9.]**

Discussion

The pepper DNA appears to play an important role in the drought stress responses in that it enhances tolerance to white pine plantlets when they experience drought. The adaptive processes include changes in gene expression patterns, which ultimately lead to biochemical, cellular, developmental, and physiological changes. In other plants, DNA similar to that of pepper used here, has been shown to regulate the expression of a variety of genes during their response to drought stress. Transcriptional DNA regulators such as the pepper DNA mediate the stress-responsive gene expression, and thus they play an important role in stress adaptation in many plant types. Whatever the tolerance mechanism may be, we clearly show that the expression of the pepper enhances tolerance to drought stress.

Thus, it is possible to develop Christmas trees with enhanced resistance to environmental stress using this unique pepper DNA and tissue culture technology. Eastern white pine is an important Christmas tree and forest species. Because of its highly efficient regeneration capacity, eastern white pine has great potential to become a useful model plant for basic and applied research purposes with Christmas trees. However, one of the goals of our research is to select and make fir Christmas tree species more compatible with the drought-prone environment of eastern North Carolina. The use of this technology and the transfer of DNA similar to that of the pepper DNA to fir species may help in providing drought tolerance to firs, so that they can be grown in a "choose and cut" farm in the eastern part of the state. The tissue culture cloning technology described here for eastern white pine is now being developed in our laboratory for Christmas tree fir species. Furthermore, in cooperation with other researchers, we have isolated a specific DNA element from Aleppo pine which we think has characteristics similar to that of the pepper DNA. The Aleppo pine DNA is now being studied by transferring it into Christmas tree pine species.

COVER PHOTOS:

Wayne Ayers and son, Steve, presented Governor Mike Easley a North Carolina Fraser Fir for his private office. Steve's family was also present: Sherry and sons Austin and Eli. Wiley and Andrea Gimlin also presented Governor Easley a wreath for his private office door. The Ayers and the Gimlins also provided trees and wreaths to help decorate the State Capital building for Christmas 2005. These families won this honor by winning the NCCTA Tree and Wreath Contests at the Summer Meeting in Spruce Pine.