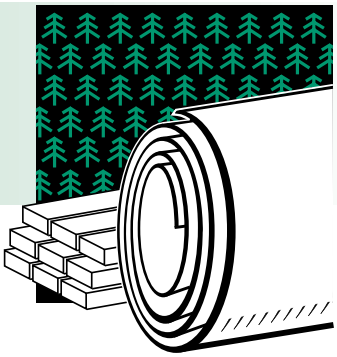
FOREST PRODUCTS

Project Fact Sheet



Dominant Negative Mutations of Genes for Genetic Engineering of Sterility in Forest Trees

Benefits

- Development of unique method for engineering sterility in plants
- Production of stable sterility by the Dominant Negative Mutations
- Identification of sterile lines before they reach flowering age
- Avoidance of adverse effects sometimes seen with other methods of inducing sterility (e.g., introduction of cytotoxin genes)
- Dispersal of transgenes limited to asexual propagules
- Potential for transfer of method for producing sterility in poplar to other angiosperms and conifers

Applications

The anticipated results of this project are the availability of "elite" genetic lines of sterile poplars into which other commercially desirable genetic traits may be simultaneously introduced. The sterile genotypes will be prepared and field tested until they meet the standards set by government regulatory agencies. Minimal further testing will be required as new traits are introduced into these genotypes before they can be offered to the commercial marketplace.

Unique Method for Inducing Sterility Will Facilitate Use of Transgenic Trees

Commercially desirable traits are being introduced into poplars and other forest trees via transformation—the insertion of novel genes (DNA) by non-sexual processes. However, use of "transgenic" (i.e. genetically modified) trees in plantations raises complex environmental, legal, and/or political concerns due to the potential for wide dispersal of the introduced genes (transgenes) via pollen and seed. A reliable method for engineering sterility within transgenic populations would mitigate these concerns by limiting transgene movement to asexual propagules.

The function of a native gene can be inhibited by introducing an altered form of the gene that contains a dominant negative mutation (DNM). In the DNM version, an essential portion of a gene is deleted or otherwise modified, thus rendering it non-functional. Potent DNM forms of floral homeotic genes, which control various aspects of reproductive development, could be a highly effective method for inducing sterility. Most floral homeotic genes belong to the MADS-box family of transcription factors (sequences that regulate the expression of other genes) and contain the same four domains (a highly conserved motif: Figure 1). Deletion of one of these regions (the C-terminal or downstream region) from the *Arabidopsis* floral homeotic gene *AGAMOUS* generated a DNM, which, when expressed in transgenic plants, led to steritlity (Mizukami et al., Plant Cell 8: 831 - 845, 1996). Because protein domain and DNA sequence information is highly conserved among floral homeotic genes from diverse species, it is likely that the other DNMs generated from poplar genes can be used to produce sterility.

Schematic Presentation of DNM Sterility Engineering

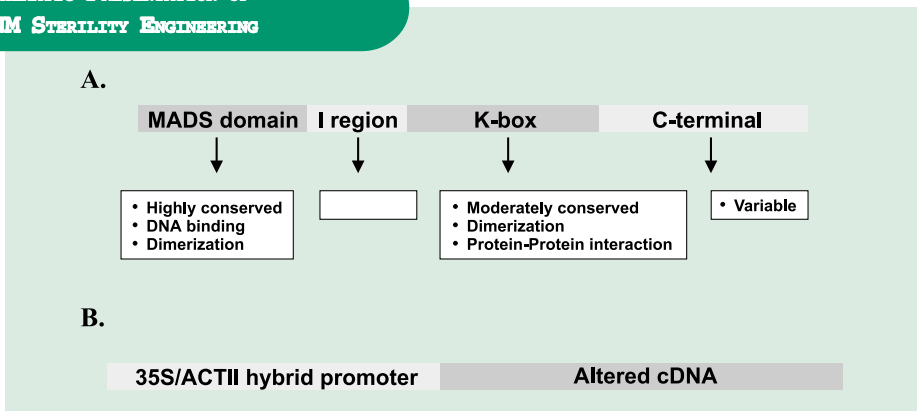


Figure 1. Production of DNM constructs for engineering reproductive sterility . (A), domains of the plant MADS-box genes, defined by sequence conservation and protein structure. (B), poplar cDNAs encoding MADS domain proteins or the poplar LEAFY protein will be modified to generate a DNM, placed under the control of a promoter, such as 35S enhancer/ACTIN 11, that is strongly expressed in floral meristems, and inserted into a binary vector for Agrobacteriun-mediated transformation of poplar clones.

Project Description

Goal: To produce lines of sterile poplars by introducing altered forms of the genes (dominant negative mutations) that control reproductive development.

Previously, four *Populus trichocarpa* genes homologous to well-studied *Arabidopsis thaliana* floral homeotic genes (i.e., *LEAFY, APETALA3, AGAMOUS*) were isolated and characterized. The overall objective of this effort is to study engineered sterility by introducing DNM forms of these poplar floral homeotic genes, both singly and in combination, into early-flowering transgenic poplars and non-transgenic commercial clones. An additional poplar floral homeotic gene will also be isolated for use in this study.

Progress & Milestones

- Investigators isolated two closely related genes that are clearly poplar homologs of *AP1* (another floral homeotic gene). Both genes are expressed in a pattern similar to AP1's expression pattern (floral tissue-specific in *Arabidopsis*), suggesting that they act redundantly to specify a similar function in poplar.
- Production of initial DNM floral homeotic gene constructs is complete.
- 6 poplar DNM constructs were co-transformed into a male poplar clone and at least 20 independent lines for each DNM construct were verified to contain both the DNM transgene and 35S-*LFY*. The latter has been shown to induce early flowering (within 4-6 months of transformation) in this clone of poplar. Surprisingly, only a few lines have flowered to date.
- Researchers are successfully propagating a naturally early-flowering clone from Italy in the hopes that it will provide a much-improved system for testing future sterility constructs.
- All 6 DNM constructs were introduced into *Arabidopsis* and more than 20 T_1 plants (plants germinated from seeds produced by the original transgenic plants) were produced for each construct. Most lines showed floral abnormalities, though these varied in degree.
- Since investigators have not observed the desired loss-of-function phenotypes for either DNMs produced from either poplar or *Arabidopsis* transgenes, researchers are generating additional DNM constructs.
- The PTLF-1 (a full-length version of the poplar homolog of the *LEAFY* gene from *Arabidopsis*) construct was transformed into both *Arabidopsis* and tobacco. 46 transgenic *Arabidopsis* lines were identified by kanamycin selection and seed was collected. 30 trasgenic tobacco lines were verified.
- Additional DNM constructs will be evaluated in early-flowering poplar.
- Researchers will introduce various constructs into both female and male commercial cottonwood clones.
- Transgenic plants will be selected, propagated, and field-tested to observe their phenotype (physical appearance).

Project Partners

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