

Identifying Candidate Genes Associated with Cold Hardiness in Coastal Douglas-fir Using DNA Microarrays

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Cold hardiness is an adaptive trait with considerable variation and importance in Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco). We are working towards identifying genes associated with cold hardiness in coastal Douglas-fir using gene expression profiling. In the first year, we grew one-year-old seedlings outdoors in Corvallis, Oregon, and harvested them in the fall, winter, and spring (from bud set to bud burst) for RNA extraction. RNA samples were pooled and used to make three cDNA libraries (cold acclimating, maximum cold hardiness, and cold deacclimating). A fourth cDNA library was constructed from actively growing seedlings. In the second year, we studied cold hardiness in seedlings from two Douglas-fir populations. The Coos Bay (CB) population is from a coastal, low elevation site in Oregon, whereas the Yakima (YK) population is from an inland, high elevation site in Washington. RNA extractions and artificial freezing tests were conducted on the seedlings from these two populations at various times during cold acclimation, maximum cold hardiness, and cold deacclimation. Damage to needles, stems, and buds was assessed using visual inspection, and needle damage was also measured using electrolyte leakage. The cold hardiness of the two populations was significantly different for all tissues. For example, in late October, differences in predicted damage at -7°C were as large as 48% for needles (i.e. CB=50% and YK=2%), 47% for stems (i.e. CB=50% and YK=3%), and 35% for buds (i.e. CB=36% and YK=0%). Correlations between needle, stem, and bud damage were stronger in the fall than in the spring. RNA isolated from these two populations will be used to examine differences in gene expression associated with cold hardiness. Our four cDNA libraries have produced >20,000 ESTs that will be used to construct custom oligonucleotide microarrays for gene expression profiling. Genes that show differential expression (1) throughout the winter and (2) between populations will be classified as cold hardiness candidate genes and integrated into a larger association study of adaptive traits in Douglas-fir populations from Oregon and Washington.