

Freezing-Stress-Responsive Genes and Their Expression in Barley

B.C. Koo^{1)*}, B.S. Bushman²⁾, I.W. Mott²⁾, R.R. C. Wang²⁾, N.J. Chatterton²⁾

¹⁾National Institute of Crop Science, RDA, Suwon, Korea

²⁾USDA-ARS Forage and Range Research Laboratory, USU, Logan, Utah

Purposes

Objective of this research was to identify genes from the Dicktoo variety with transcript changes associated with freezing tolerance, which can be used for downstream functional genomics and understand the mechanism of freezing stress and tolerance by analysis of responsive genes for freezing stress.

Experimental

Injury of barley plants by spring freezing is a major cause of crop loss, but most cold tolerance research has focused on cold acclimation, which confers freezing tolerance upon exposure to low nonfreezing temperatures. In order to address freezing tolerance *per se*, we have chosen a cold sensitive (*cv* Keunal) and a cold tolerant (*cv* Dicktoo) barley (*Hordeum vulgare*) variety, where only the latter is capable of survival at -3°C for 30 hours. The varieties were subjected to cold acclimation (4°C for two days), cold acclimation plus freezing (4°C for two days followed by -3°C for 6 hours), freezing treatment without prior cold acclimation (-3°C for 6 hours), and control treatments. Our objective was to identify genes from the Dicktoo variety with transcript changes associated with freezing tolerance, which can be used for downstream functional genomics. Treatments were applied and RNA extracted in the stem elongation stage, and the barley genome array (Affymetrix) was used to survey for variable transcript levels. Forty-six percent of the genes identified across all comparisons and treatments were known to be involved in cold-related or other stress metabolism.

Results

In both varieties there was disjunction between genes that were variable in response to acclimation (with or without freezing) and those that were variable upon freezing only. Upon comparison of freezing vs. control, 53 genes were variable in Dicktoo, and 46 in Keunal. However, only 4 of those 99 genes were common between the two varieties, suggesting different mechanisms may exist for tolerating cold and freezing temperatures.

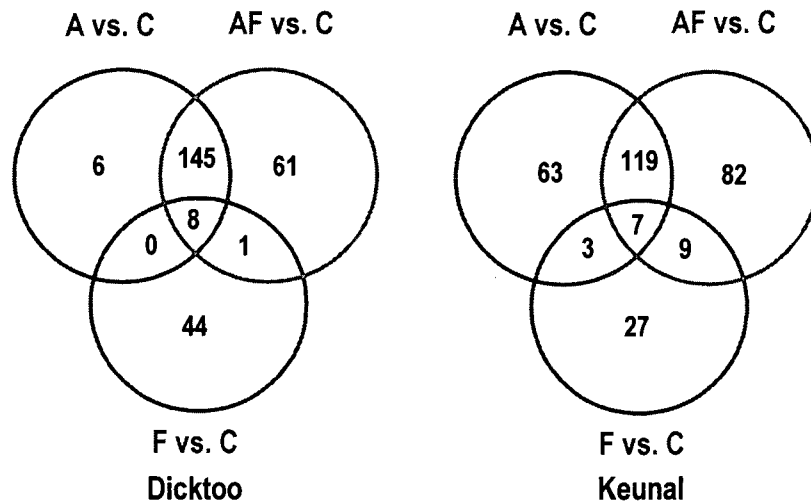


Figure 1. Venn diagram of differentially expressed probe sets of freezing tolerant (cv Dicktoo) and sensitive (cv Keunal) barley varieties. Lists were composed of probe sets that had 3-fold differential expression with $P < 0.0001$. **Compared to controls, freezing without prior acclimation (F) had few expression polymorphisms in common with acclimation (A) or acclimation + freezing (AF); in both cultivars.**

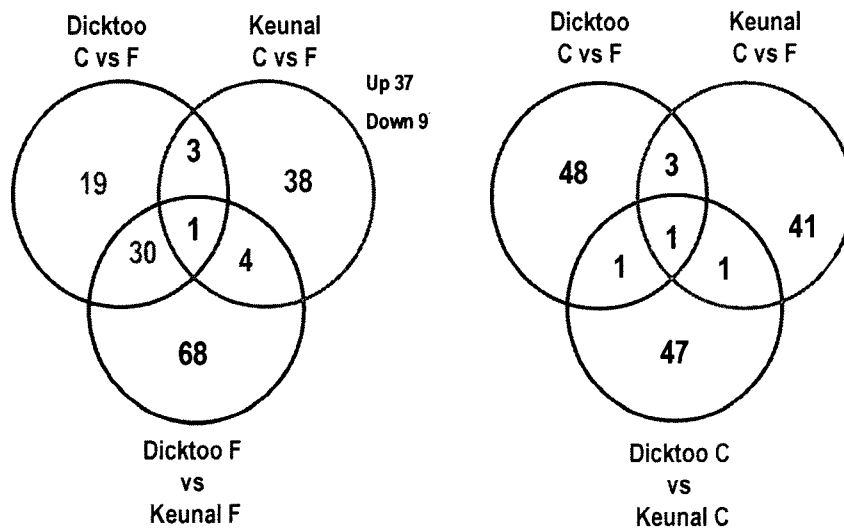


Figure 2. Venn diagram of differentially expressed probe sets of freezing tolerant (cv Dicktoo) and sensitive (cv Keunal) barley varieties. Lists were composed of probe sets the differential expression with $P < 0.0001$. **In our model, candidate gene choice for freezing tolerance was variable between control and freezing in either cultivar, and variable in freezing between the two cultivars (highlighted area).**