Final Report, DE-FG02-98ER20318

Wick, Meeley and Carter: Reverse Transposon Tagging of Maize Tubulin Genes;
Wick: Growth and Development of Maize that Contains Mutant Tubulin Genes

This project considered the role of specific maize β tubulins. The goal was to identify maize plants in which certain β tubulin genes (*tub*) have been disrupted by the insertion of a *Mutator* (*Mu*) transposon (*tub::Mu*) and to determine what effects the disruptions have on plant development and response to chilling stress. Genes of interest for response to chilling were *tub1*, *tub6*, and *tub7*, all of which we predicted would increase chilling sensitivity, such that a reduction in expression of these genes could lead to increased tolerance of temperatures near the basal growth temperature of maize (between 9 and 12^{o} C, depending on variety). Based on the highly preferential use of β tubulins 2 and 4 during gametogenesis and pollen germination, we likewise predicted that reducing the expression of *tub2* and *tub4* would lead to reduced fertility of maize.

The first several years of the project were spent in identifying lines carrying *tub::Mu* by PCR and Southern blotting and in outcrossing plants with wt inbred lines to reduce the number of extra copies of *Mu* in other genes. Whenever possible, we have tried to work with at least two independently derived lines that contain a *Mu* insertion into a given *tub*. After several generations of outcrossing and continual screening to cull only those plants containing *tub::Mu* for the next round of crossing, attempts were made to produce plants that were homozygous mutant for a particular *tub*. As we identified further *tub::Mu* genes, we also attempted to create plants that contained an insertional mutation in more than one *tub*, e.g.*, tub2::Mu,tub4::Mu*; *tub1::Mu,tub6::Mu*; *tub1::Mu,tub7::Mu; tub1::Mu,tub6::Mu,tub7::Mu.* Plants with these genotypes were produced at lower than expected frequency, suggesting that disrupting the β tubulin composition to this extent was detrimental to plant survival and growth.

Seeds collected from plants that contained one or more disrupted copies of *tub1, tub6, and tub7* were germinated at room temperature before transfer to growth chambers in which the temperature was held at 9^{o} or 12^{o}C, and growth of roots and shoots were monitored for up to three weeks. The first time through the measurements were done manually, but thereafter, seedlings were photographed every two to three days and measurements were done with a computerized program to avoid excessive handling of seedlings. Seedlings were then transferred to potting soil and allowed to grow at room temperature until there was substantial leaf growth. Leaves were harvested and frozen at -80oC. Meanwhile, plants with *tub2::Mu, tub4::Mu, and tub2::Mu,tub4::Mu* were grown in the field until flowering, and general appearance of tassels and ears was noted, relative to siblings with wt copies of *tub2 and tub4*. Very hot and dry weather during flowering time for two summers in a row caused flowering abnormalities among all genotypes, but the few mutant *tub* plants available to study displayed greater frequencies of late or small ears and tassels that didn't shed pollen. The little pollen available on mutant plants was used for reciprocal crosses whenever possible.

At this point, DOE funding ended. Undergraduate students conducting directed research projects or funded by internal sources have continued the project at a slow pace. Leaves of cold-grown seedlings were freeze-dried, DNA was extracted, and the *tub::Mu* status of each plant is being determined. This will allow plant-by-plant comparison of cold growth characteristics with genotype. Determining the male and female fertility of plants with *tub2::Mu, tub4::Mu, and tub2::Mu,tub4::Mu* relative to wt siblings is also underway.