Engineered and Conventional Approaches
To Develop Potatoes for Sub-optimal Irrigation Conditions

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**Statement of Challenge**
For the past 15 years the MSU potato breeding program has been developing cultivars that combine disease and insect resistance with key market limiting traits. Abiotic stresses such as drought, cold and salinity have not been concerns in potato production because of intensive management of the crop. As a consequence, potato production is a heavy user of groundwater for irrigation during the summer months. Use of water by the industry is beginning to come under greater scrutiny by the public and legislature.

Genetic engineering offers the opportunity to introduce new genes into our cultivated germplasm that otherwise would not be exploited. Recent research has shown that is now possible to use transgenic approaches to improve abiotic stress tolerance with few traits than originally anticipated (Zhang et al. 2001). One strategy is to express heterologous $CBF1$ (C-repeat/dehydration-responsive element binding factor 1) genes in plants to improve environmental stress resistance (Thomashow 1999). $CBF1$ genes are referred to as master switches that activate expression of $COR$ genes, increasing stress tolerance in the absence of cold stimulation. Recently Hsieh, et al. (2002) reported that heterologous Arabidopsis $CBF1$ gene can confer water deficit resistance in transgenic tomato plants.

**Objectives**
1. Engineer vector constructs to express the $CBF1$ regulatory element under stress conditions, then transform potato and characterize resulting transgenic lines.

2. Evaluate the agronomic performance, marketable maturity and adaptability of advanced selections and new releases in Michigan and other public and private breeding programs with emphasis on yield and quality under sub-optimal irrigation.

**Results and Accomplishments**
1. Our strategy was to use regulated over-expression of the $CBF1$ gene using the ABA-inducible promoter which drives the gene for cytosolic expression. The $COR78$ promoter was placed upstream from the $CBF1$ gene and nptII was used as the selectable marker. The initial set of events (38 lines) was selected and advanced for molecular characterization. Four lines were advanced for growth chamber, greenhouse and field testing based upon insertion of the gene and normal plant growth and appearance (Figure 1). Growth chamber experiments are currently underway and field plants are being grown for seed tuber production. Another $CBF1$ construct using the $COR15$ promoter
was created and transformations have been made. These plants may have a different expression pattern for the drought response because of the promoter employed.

2. Field experiments were conducted at the Montcalm Research Farm during the 2003 and 2004 field seasons to evaluate the agronomic performance of 60 advanced potato clones from 6 US breeding programs under dry land, sub-optimal and standard irrigation practices. As expected, the yield was lower, but the internal quality was greater for the non-irrigated plots. From this study we were able to identify 10 clones for further evaluation. Two MSU-bred cultivars, Boulder and Michigan Purple, performed well under non-irrigated conditions. Other advanced clones that yielded well and had good internal quality were from Texas, Michigan and Colorado breeding programs. Some of these clones yielded relatively better under dry land vs. standard, while others ranked similarly under dry land and standard.

![Image](image-url)

**Fig. 1. CBF1 transgenic plants prior to greenhouse and growth chamber studies. Normal plant growth is observed.**

**Impacts**

We have created CBF1-expressing transgenic potato lines that can be further characterized under field and growth chamber conditions for their tolerance to water deficit conditions. Because of the nature of CBF1, these lines can also be evaluated for cold and salt tolerance. These lines should be a unique and important germplasm resource for further breeding efforts to reduce abiotic stress in potato.

We also have evaluated advanced germplasm for dry land performance in sandy loam soils (typical of Michigan production systems for potato). The clones with greater tolerance to drought stress conditions will be studied further to understand the contributing physiological mechanisms (i.e. increased root growth, stomatal closure, osmotic adjustment and synthesis of stress proteins). Concurrently, this more drought tolerant germplasm can be introgressed into the potato breeding variety development program. A few of the more drought tolerant lines identified are being considered for release by the breeders of these lines.
New variety development is the key to the health and growth of the Michigan potato industry. The chip-processing market can be strengthened and the tablestock market can expand. With the breeding program we continue to address market and production limiting traits. Some of these traits are chip quality from storage, scab resistance, late blight resistance, potato early die resistance, beetle resistance, bruise resistance and solids. The ability to integrate this applied biotechnology approach with conventional breeding strengthens the overall effort to develop superior potato cultivars. Addressing drought stress facing potato through breeding and biotech approaches will position the industry with tools to manage sub-optimal water management.

Progress from this research was reported at the 2004 Potato Association of America annual research meetings and 2004-5 North Central Regional Potato Breeding and Genetics technical meetings. The field trials were shown at the 2004 Montcalm Field Day.

A 2-year grant with USDA Egypt Science and Technology funds was awarded July 2005 to continue this research.

**Summary Statement**
We have created CBFI-expressing transgenic potato lines that can be further characterized under field and growth chamber conditions for their tolerance to water deficit conditions and we identified potato lines that are comparatively better yielding under non-irrigated conditions. Addressing the abiotic stresses facing potato through breeding and biotech approaches will position the industry with tools to manage sub-optimal water management.

**Funding Partnerships**
Douches received two grants for approximately $55,000 per year from the National Potato Council for funding leverage during this period for other transgenic research. The Michigan Potato Industry Commission has supported the program at $24,000 per year.

Partial funding from the Potato Special Grant for Potato Breeding and Variety Development will be used to support the research. Kirk receives approximately $23,000, while Douches receives approximately $60,000 of the $145,000 grant to Michigan State University.

**References**
