



Improved Drought Stress Tolerance in Maize

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Water availability is the primary limiting factor of global crop yields. Consequently, considerable effort is devoted to converging breeding and technological research to develop crops with improved performance under water-limiting conditions. Transgenic plants tolerant to abiotic stress are being developed through the introduction of multiple genes; however, successfully producing stress tolerant crops remains a challenging task.

Tolerance to abiotic stresses is improved by the expression of bacterial cold shock proteins (CSP). A small set of *E. coli* cold shock proteins (CSPs) exhibits a prototypical cold shock domain (CSD) during cold treatment. Expression of related cold shock proteins from bacteria—CspA from *Escherichia coli* and CspB from *Bacillus subtilis*—promotes stress adaptation in multiple plant species.

Both CSPs and CSDs are found in bacteria and eukaryotes, including plants. In bacteria, CSD proteins (7 – 10 kD) contain sufficient nucleic acid binding activity to function as RNA chaperones. RNA chaperones—often called “protein chaperones”—are proteins that aid RNA folding by preventing or resolving misfolded species of RNA, in contrast to proteins that assist protein or RNA folding by catalyzing steps along the folding pathway or by stabilizing the final folded protein or RNA structure. RNA chaperones in bacteria favor active transcription, translation, and/or ribosome assembly. Research on CSPs and CSDs confirms that the endogenous function of CSPs in plants depends on RNA binding/chaperone activity through the CSD, and CSPs regulate stress responses through a post-transcriptional mechanism.

Recently, Monsanto Company researchers showed that bacterial CSPs can confer improved stress adaptation in multiple plant species by demonstrating improved stress tolerance in both *E. coli* and maize, and reported further ‘proof of concept’ in dicots and monocots—*Arabidopsis*, rice, and maize. Expression of bacterial CSPs improves cold tolerance in transgenic *Arabidopsis* when compared to nontransgenic controls. Similar experiments expressing CspA and CspB in transgenic rice show improved plant growth rates in plants exhibiting an increased tolerance to a number of abiotic stresses like cold, heat, and water deficit.

Further abiotic stress testing of transgenic CspA and CspB in maize demonstrates that transgenic expression by CspB improves vegetative growth performance. Twenty-two CspB transgenic events were evaluated under water limited field trials using commercial grade corn in an environment that received no rainfall during the target period. The best performing events show a growth rate increase of 12% and 24% under water deficit conditions. The CspB-expressing plants also demonstrate significant improvements in chlorophyll content by 2.5%, increasing the photosynthetic rates by 3.6% across all events. Transgenic maize plants expressing CspA under greenhouse conditions were also tested.

Increases in plant growth rates, chlorophyll content, and photosynthetic efficiency are key indicators of plant productivity and are expected to improve the overall yield of the plant. Therefore, the reproductive performance of CspB-expressing maize plants was also evaluated by harvesting all kernel-bearing ears from six replicates (34 plants per replicate) for each of six events selected for harvest, based on the magnitude of their improved vegetative performance. An across-event analysis demonstrates significant improvements were made in the number of plants showing an increased number of kernels per plant. These improvements are congruent with the expected results based on the timing of limited-water treatment, which was provided during the late vegetative stages and early immature ear development, and was relieved with sufficient water during the pollination and grain-fill periods.

Grain yield trials were also carried out under water deficit stress and non-stress conditions on 10 CspA- and 10 CspB-positive events that had demonstrated superiority in vegetative performance. Grain yield data were collected from four field sites where water was limited during the late vegetative phase of development, a treatment similar to the initial water deficit trial. An across-event analysis demonstrates that CspA transgenic lines contribute to a yield increase of 4.6% under water stress, with the two best performing events showing 30.8% and 18.3% improvement. CspB transgenic plants show 7.5% improved yield averages over controls; the best two performing events, CspB-Zm events 1 and 2, demonstrate yield improvements of 20.4% and 10.9%, respectively. These are the same two events that demonstrate significant improvements in leaf growth, chlorophyll content, and photosynthetic rates, indicating that these improvements in vegetative productivity translate into improvements in reproductive performance and grain yield.

To further investigate the ability of the CspB gene to provide tolerance to maize under water deficit conditions, CspB-Zm event 1 was deployed into three hybrid backgrounds and evaluated under two distinct stress treatment conditions at



five replicated locations. The two treatments result in a decrease of overall yield of approximately 50%, relative to well-watered treatments. When compared to controls, the CspB transgenics demonstrate improved yields by at least 0.5 t/ha across 12 out of 15 reproductive stress treatments. The multi-year analysis with CspB-Zm event 1 shows the stability of yield advantages across locations under water-limited conditions, which proves the utility of this technology across the US maize growing regions.

The performance of CspB-Zm event 1 was also assessed by combining yield performance data from three hybrid test crosses collected over four years. The event yielded a 10.5% average benefit across the four years. CspB-Zm event 1 was also tested under western dryland maize conditions without supplemental water. Under these conditions when compared to the non-transgenic control, the CspB transgenic event contributed a yield benefit of up to 0.75 t/ha or 15%.

In summary, constitutive expression of *E. coli* CspA and bacterial RNA chaperones can confer abiotic stress tolerance in transgenic *Arabidopsis*, rice, and maize. This technology also provides a stable yield improvement under water limiting conditions.

Sources

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