Rational design and testing of osmotic-stress inducible synthetic promoters from poplar *cis*-regulatory elements

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Crops are frequently subjected to abiotic stresses including water deficit, salinity, and heat. Here we show the performance of a set of rationally-designed osmotic stress-inducible synthetic promoter in hybrid poplar, a strategic bioenergy crop. We mined poplar transcriptome data for signature promoter motifs that are putatively drought- or salt-responsive. *De novo* motif-detecting algorithms yielded 30 water stress- and 34 salt stress candidate DNA motifs using *E*-value threshold of 0.001 from the respective promoters of drought- and salt-responsive co-expressed genes from poplar data sets. In drought responsive motifs, a novel domain was comprised of 3 to 9 conserved motifs (SD1-9) found in 16 co-expressed gene promoters. A newly-identified well-conserved motif (SS16) for salt-response was discovered. Fifteen synthetic promoters using mined sequence were fused to a green fluorescent protein (GFP) gene. These promoters were screened by transient expression assays using poplar leaf mesophyll protoplasts and agroinfiltrated *Nicotiana benthamiana* leaves under osmotic stress condition. Twelve of these synthetic promoters induced GFP expression in both transient expression systems. Especially, two SD (SD3-1 and 6-2) and three SS (SS16-1, 16-2, and 16-3) synthetic promoters responded significantly to low water content and high salinity, respectively, in agroinfiltration test. These five synthetic promoters were then selected for generating stable transgenic *Arabidopsis* to validate the inducibility in plants. SD3-1 and 6-2 responded to water deficiency, while SS16-1, SS16-2, and SS16-3 responded to high salinity in *Arabidopsis*. Orthogonal synthetic promoters for multiple crops is a ‘grail’ of biotechnology. The current results appear to provide multiple expression profiles and choices to deploy osmotic stress-inducible promoters that may be effective in multiple plant species. The design-build-test strategy appears fruitful for tuning abiotic-stress tolerance responses in the next steps of the research in engineered poplar trees.

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