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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