Leveraging synthetic promoters to control gene expression in plants

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Understanding gene expression regulation is central to the development of biotechnological solutions for several pressing agricultural problems. The ability to precisely regulate gene expression would allow for man-driven control of plant growth. The advent of synthetic biology methods has opened the door to programmable genetic constructs in plants that confer tunability and spatiotemporal regulation to gene expression. These tools can be applied in a variety of agricultural research projects to create a multiplex of customizable promoters that drive the expression of genes of interest. However, the relationship between promoter elements within the promoter of a gene and the level of that gene's expression is not well defined. To begin tackling this question, we are building synthetic promoters harboring up to ten transcription factor binding sites using GoldenBraid technology and cloning these promoters upstream of a reporter gene. This is done by inserting one to ten copies of a protospacer, a 23bp recognition sequence for the dCas9-based synthetic transcription factor, into a neutral promoter sequence that has no known transcription factor binding sites in plants. We assemble the reporter gene and transiently co-express it along with a dCas9 activation system in *Nicotiana benthamiana* to test the effects of protospacer position, orientation, angular phase shift, copy number, and spacing on reporter expression. Preliminary results show that constructs with protospacers in either the sense or antisense orientation confer comparable levels of gene expression, an increase in protospacer copy number boosts reporter activity, and that there may be a decrease in expression as the distance between the protospacer and the core promoter increases. This work is expected to shed light on the rules of nature dictating promoter architecture that, in turn, determine gene expression levels, ultimately paving the way to the creation of tunable expression systems. We will apply this knowledge to build stimulus-inducible constructs from promoter elements of hormone-inducible genes to confer hormone-regulated expression to genes of interest.