Awakening of the native *PHYTOENE SYNTHASE 1* promoter by correcting near-miss *cis*-acting elements activates carotenoid biosynthetic pathway in embryogenic rice callus

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Resumen *Metabolic engineering in plants typically involves the introduction of transgenes and/or the mutation or silencing of endogenous genes. An alternative approach is promoter modification where small changes in the promoter sequence allow genes to be switch on or off in particular tissues. We screened promoters for near-miss cis-acting elements that can be converted into functional endosperm-specific regulatory motifs by changing 1–4 nucleotides. Such minimal interventions would awaken "silent latent endosperm-enabled promoters" (SLEEPERs). To test this hypothesis, we chose rice PHYTOENE SYNTHASE 1 (PSY1), encoding the enzyme responsible for the first committed step in the carotenoid biosynthesis pathway, because it is not expressed in rice endosperm. Sequence analysis of the promoter region identified six motifs within a 120-bp region, upstream of the transcriptional start site, which differed from endosperm-active elements by up to four nucleotides. We mutated four motifs to match functional elements in the endosperm-active BCH2 promoter, and this promoter was able to drive GFP expression in callus and in seeds of regenerated plants. The 4 M promoter was not sufficient to drive PSY1 expression, so we mutated the remaining two elements and used the resulting 6 M promoter to drive PSY1 expression in combination with a PDS transgene, which encodes phytoene desaturase, the subsequent enzyme in the pathway. Callus transformed with the corrected PSY1 construct and PDS resulted in deep orange tissue indicating the accumulation of carotenoids, which was subsequently*

confirmed by targeted metabolomics analysis. PSY1 expressioncontrolled by the uncorrected or 4 M variants of the promoter plus a PDS transgene produced callus that lacked carotenoids. These results confirm that the correction of promoter elements can facilitate the ectopic activation of endogenous plant promoters in rice callus and endosperm and most likely in other tissues and plant species.

Palabras clave: Callus · Carotenoids · *cis*-acting element · Endosperm · Metabolic engineering · Promoter

Participación preferida: oral