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Modifying *Myxococcus xanthus* protoporphyrinogen oxidase to plant codon usage leads to a high level of oxyfluorfen resistance in transgenic rice despite decreased protein expression

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Objectives

Transgenic rice plants expressing an *Mx* Protoporphyrinogen oxidase (Protox) confers a higher herbicide resistance by overexpressing the *Mx* Protox protein in both chloroplasts and mithochondria with 5- and 12-fold increase in Protox activity, respectively, over the wild-type rice. However, codon usage in the *Mx* Protox gene differs significantly from that of plant Protox genes that the bacterial Protox gene might not be properly expressed in plants. Therefore, we altered the codon usage to more closely resemble that of plants without changing the *Mx* Protox amino acid sequence by synthesizing the full-length coding sequence for *Mx* Protox, and we used this synthetic gene to create transgenic rice plants to compare the transgenic rice plants expressing the native *Mx* Protox gene on Protox expression and herbicide resistance.

Materials and Methods

Using plant-preferred codons, we designed a synthetic gene encoding the amino acid sequence for *Mx* Protox. The codon usage was based primarily on that of the Protox gene from plastid *Arabidopsis* (GenBank accession number D83139) because this *Arabidopsis* Protox has been utilized as a representative gene to develop many Protox inhibitor-resistant crops.

Results and Discussion

Protox of *Myxococcus xanthus* (*Mx* Protox) is a 49-kDa membrane protein that catalyzes conversion of protoporphyrinogen IX (Protogen IX) into protoporphyrin IX (Proto IX). Upon heterologous expression in transgenic rice plants, *Mx* Protox is dually targeted into plastids and mitochondria, increasing resistance against the herbicidal Protox inhibitor oxyfluorfen. Here, we describe the chemical synthesis of the *Mx* Protox gene by assembling several small, synthetic DNA fragments derived by ligation-PCR. Codon usage in the resulting 1416-bp gene was modified to correspond to that of the *Arabidopsis* Protox gene, a change that resulted in a decrease in G+C content from 71 to 49%. Modified *Mx* Protox gene was used to generate transgenic rice plants *via Agrobacterium*-mediated transformation. Integration, expression, and inheritance of the transgenes were demonstrated by Southern, northern, and western blot analyses. In plants transformed with the modified, low G+C-content *Mx* Protox gene, levels of Protox expression and enzyme activity were low compared to the levels observed for plants transformed with the native *Mx* Protox gene. However, compared to the native gene, the modified gene conferred a higher level of resistance to the herbicides oxyfluorfen and acifluorfen when judged by germination testing. This result is attributable to the decreased accumulation of Proto IX either in the plastids or in the cytoplasm.

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