

## Metabolic herbicide resistance gene discovery in wild oat (*Avena fatua*)

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**Summary** Weedy plant species that have evolved resistance to herbicides due to enhanced metabolic capacity to detoxify herbicides are a major issue threatening herbicide sustainability and global crop production. Metabolism-based herbicide resistance (referred to as metabolic resistance) in weeds has not been well characterised at the genetic level. Diclofop-methyl resistant (R) wild oat lines only possessing metabolic resistance were isolated and used for RNA-Seq transcriptome analysis for metabolic resistance gene discovery. Forty contig genes that showed constitutive expression differences between the R and S samples were selected for validation, and these include 22 CYP P450 genes, 9 Glycosyl transferase genes and 9 GST genes related to herbicide metabolism. After validation with RNA-seq samples, the candidate genes were reduced to 31 including 17

CYP P450, 8 Glycosyl transferase family genes, and 6 GST genes. The 31 contigs were then evaluated using additional samples from the R and S lines, and from a single wild oat line segregating R at 4000 or 6000 g ha<sup>-1</sup> and S at 1000 g ha<sup>-1</sup> of diclofop-methyl. Next, a physiological validation experiment was conducted, in which 2,4-D pre-treatment was used to induce gene expression providing diclofop protection in S individuals due to increased metabolism. This way only two candidate genes (CYP450) were significantly induced in the S samples. Therefore these two CYP450 genes (CYP72A15 and CYP71C4) are very likely associated with metabolic resistance to diclofop-methyl in the R wild oat line and prioritised for further characterisation using rice genetic transformation.

**Keywords** RNA-sequencing, wild oat, diclofop, metabolic resistance, CYP450.