Researchers discover key gene for toxic alkaloid in barley

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All plants mediate their environmental interactions via chemical signals. An example is the alkaloid gramine produced by barley, one of the world's most widely-grown cereals. Gramine provides protection against

herbivorous insects and grazing animals and inhibits the growth of other plants. Despite long-standing interest, the key gene for the formation of gramine remained elusive.

The researchers discovered a cluster of two genes in barley for gramine biosynthesis. The first gene (HvNMT) had already been discovered 18 years ago. In their study, the researchers from IPK and the Leibniz University Hannover now identified a second gene (AMI synthase, HvAMIS), and found out that both genes are located in proximity of each other on the same chromosome. With this discovery, the pathway of gramine biosynthesis is now fully elucidated. The findings are published in the journal *Science*.

"We discovered that AMIS is an oxidase enzyme that carries out an unusual cryptic oxidative rearrangement of tryptophan, allowing us to revise the previous biosynthetic proposal from the 1960s," says Dr. John D'Auria, head of IPK's research group Metabolic Diversity.

Prof. Dr. Jakob Franke, head of the group Biochemistry of Plant Specialized Metabolites at Leibniz University Hannover, adds, "We were very surprised by the so far unknown enzyme mechanism by which gramine is formed. At the same time, we now have the possibility to produce biologically active alkaloids with sustainable biotechnological methods."

The research team could produce gramine in yeast and model plants (Nicotiana benthamiana, Arabidopsis).

"In contrast to many other protective metabolites from plants, production of gramine requires only two genes. Therefore, using our findings for practical applications is relatively straightforward," said Ling Chuang from Leibniz University Hannover, one of the first authors.

"Furthermore, genetic engineering of barley allowed us to produce gramine in a non-gramine producing barley variety, and eliminate gramine production in a gramine producing barley variety by genome editing," explains the other first author Sara Leite Dias.

"The results set the basis to produce gramine in organisms without the native ability to synthesize it for purposes such as a natural plant protection agent, or to eliminate gramine from barley and other grasses to reduce toxicity towards ruminants," says Dr. John D'Auria.

"Our findings set the ground for improving barley to increase its resistance to pests, reduce its toxicity to ruminants and contribute to sustainable weed management."

More information: Sara Leite Dias et al, Biosynthesis of the allelopathic alkaloid gramine in barley by a cryptic oxidative rearrangement, *Science* (2024). DOI: 10.1126/science.adk6112. www.science.org/doi/10.1126/science.adk6112

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