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CLONING THE BARLEY NEC3 DISEASE LESION MIMIC MUTANT USING COMPLEMENTATION BY SEQUENCING

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Disease lesion mimic (DLM) or necrotic mutants display necrotic lesions in the absence of pathogen infections. They can show improved resistance to some pathogens and their molecular dissection can contribute to revealing components of plant defense pathways. Although forward-genetics strategies to find genes causal to mutant phenotypes are available in crops, these strategies require the production of experimental cross populations, mutagenesis or gene editing, and are time- and resource-consuming or may have to deal with regulated plant materials. In this study, we described a collection of 34 DLM mutants in barley (*Hordeum vulgare*) and applied a novel method called complementation by sequencing (CBS) which enables the identification of the gene responsible for a mutant phenotype given the availability of two or more chemically mutagenized individuals showing the same phenotype. CBS relies on the feasibility to obtain all induced mutations present in chemical mutants, and on the low probability that different individuals share the same mutated genes. By CBS, we identified a cytochrome *P450 CYP71P1* gene as responsible for orange blotch DLM mutants, including the historical barley *nec3* locus. By comparative phylogenetic analysis we showed that *CYP71P1* gene family emerged early in angiosperm evolution but has been recurrently lost in some lineages including *Arabidopsis thaliana*. CBS is a straightforward cost-effective approach to clone genes controlling phenotypes in a chemically mutagenized collection. The TILLMore (TM) collection will be instrumental for understanding the molecular basis of DLM phenotypes and to contribute knowledge about mechanisms of host-pathogen interaction.