

## Urease affects soybean susceptibility to fungi

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The soybean ubiquitous urease, encoded by *GmEu4* gene, is known for recycling metabolically-derived urea. However, additional biological roles of plant ureases have been recently demonstrated, especially in defense against fungi and insects attack. In the present study, the relevance of *GmEu4* in soybean response to fungi was investigated through overexpression and silencing approaches. First, *GmEu4* expression profile over the course of *Phakopora pachyrhizi* infection was determined by RT-qPCR. A differential expression pattern was found in susceptible and resistant genotypes, especially 24 h after inoculation. Transcript levels were up-regulated in resistant genotype and down-regulated in susceptible one. *GmEu4* full length ORF was cloned into pH7WG2D, designed for gene overexpression in plants. Soybean somatic embryos were submitted to transformation via particle bombardment and bombardment/*Agrobacterium* system. A total of thirty adult, transgenic soybean plants, representing seven independent transformation lines, were obtained. A single transgenic line exhibited the intended overexpression and enhanced ureolytic activity. Most transgenic plants showed *GmEu4* co-suppression and decreased ureolytic activity. Progeny was obtained from *GmEu4* co-suppressed plants. The growth of *Rhizoctonia solani*, *Phomopsis* sp., *Fusarium solani*, *Colletotrichum gossypii* and *Penicillium herquei* in media containing crude protein extract from either transgenic or non-transgenic leaves was evaluated. Protein extracts from the overexpressing plant inhibited fungal growth compared to extracts from non-transgenic plants, while in extracts from co-suppressed plants fungal growth was higher than extracts from non-transgenic controls. Additionally, when infected by *P. pachyrhizi* uredospores, detached leaves of *GmEu4* co-suppressed plants developed a significantly higher number of lesions, pustules and erupted pustules than leaves containing normal levels of the enzyme (non-transgenic plants). Altogether our results confirm the importance of soybean ubiquitous urease in plant defense against a wide range of fungi and suggest that genetic manipulation of this gene represents an alternative for soybean resistance to fungi.

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