Molecular mechanisms of the$Hs1^{pro-1}$-mediated nematode (Heterodera schachtii) resistance and its potential for genetic engineering of plant disease resistance

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The beet cyst nematode *Heterodera schachtii* Schm. is an obligate sedentary endoparasite causing great losses in agriculture. The nematode is mainly active in temperate regions of the world attacking most of the Chenopodiaceae and Brassicaceae species including sugar beet (*Beta vulgaris*), spinach (*Spinacea oleracea*) and oilseed rape (*Brassica napus*). Cyst nematodes can completely penetrate the roots of susceptible plants intracellularly as motile infective second-stage juveniles (J2) and induce changes in a number of host cells to form highly metabolically active feeding cells sustaining the nematode throughout its life cycle. Nematicides are available but often not allowed because of their environmental and mammalian toxicity. In this context resistant varieties provide the most economically and environmental friendly opportunity to prevent plants from nematode attack. Furthermore, sugar beet suffers heavily from diseases like powdery mildew (*Erysiphe betae*), *Cercospora* leaf spot (*Cercospora beticola*), *Rhizoctonia* root and crown rot (*Rhizoctonia solani*) and Rhizomania (BNYVV).

The $Hs1^{pro-1}$ locus confers resistance to the beet cyst nematode *H. schachtii* in sugar beet (*Beta vulgaris*). The gene $Hs1^{pro-1}$ had been cloned from the resistant sugar beet line, but the resistance mechanism still remains obscure.

By use of transcript profiling strategy, the gene *BvGLP-1* was identified from the sugar beet genome. It encodes for an oxalate oxidase-like germin protein and is highly upregulated in resistant, but not in susceptible sugar beet in response to nematode infection. For functional analysis, we transferred *BvGLP-1* into sugar beet roots and Arabidopsis plants and challenged them with the beet cyst nematode. While the expression of *BvGLP-1* in nematode feeding cells (syncytia) of both sugar beet roots and Arabidopsis plants was sufficient to initiate nematode resistance, knockout of the homolog gene of *BvGLP-1* in Arabidopsis significantly increase plant susceptibility to nematode infection. In addition, we found that *BvGLP-1* functions as an oxalate oxidase generating hydrogen peroxide (H_2O_2) in plant cells and regulate the expression of pathogenesis-related proteins suggesting that *BvGLP-1* plays a central role in regulating plant nematode resistance.

Of great interest is the establishment of transgenic plants with resistance against a broad-spectrum of pathogens, especially these with a wide host range such as *Rhizoctonia solani*. To check the potential of *BvGLP-1* in conferring resistance against fungal phytopathogens, we analyzed *BvGLP-1* in infection assays with *V. longisporum* and *R. solani* as well as with the beneficial endophytic fungus *Piriformospora indica*. As a result, the expression of *BvGLP-1* in Arabidopsis resulted in significant resistance to the two fungal pathogens, but does not affect the beneficial interaction induced by *P. indica*. Thus, we conclude that *BvGLP-1* regulates plant defense responses following a specific signaling route that diverges from that induced by the beneficial fungus *P. indica*.

In addition, three RGA sequences, cZR-3, cZR-7 and cZR-9 were investigated in respect of their potential for initiating resistance in transgenic Arabidopsis. The three RGAs all belong to the CC-NBS-LRR resistance protein family and share high sequence and structure similarity to a set of recently cloned resistance proteins, suggesting their potential role in nematode resistance. Transgenic *A. thaliana* plants expressing each of the RGAs were challenged with *H. schachtii*. We found that transgenic Arabidopsis expressing cZR-3 or cZR-7, respectively, showed a significant anti-nematode effect compared to the control plants whereas knockout of a homolog gene of cZR-3 in Arabidopsis drastically increased the susceptibility to nematode infection. These results strongly suggest an active role of both RGAs in nematode resistance. In addition, the expression of cZR-3 and cZR-7 in Arabidopsis elevates the transcript levels of *RAR1* and *SGT1* but not of *NPR1* and *EDS1* and consequently upregulates the expression of a set of PR proteins. Therefore, we conclude that cZR-3 and 7 are involved in the $Hs1^{pro-1}$ mediated nematode resistance following a signaling route specific for CC-NBS-LRR resistance proteins. It is worth speculating that the interference of PR proteins may represent an important aspect of the mechanism underlying the $Hs1^{pro-1}$ mediated nematode resistance.

An efficient transformation protocol for oilseed rape (*Brassica napus* L.) was also established in this study. It is a two-step shoot regeneration protocol from hypocotyl explants of oilseed rape, thus providing efficient tools to transfer the gene of interest into the oilseed rape genome by Agrobacterium-mediated transformation in the future.

Taken together, the results obtained from this study provide a deep insight into the molecular mechanism of the $Hs1^{pro-1}$ mediated nematode resistance but also novel strategies for genetic engineering of plant disease resistance e.g. by use of various natural resistance mechanisms.