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The reactive oxygen species are involved in resistance responses of wheat to the Russian wheat aphid

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Summary

The effect of Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), infestation on the hydrogen peroxide (H_2O_2) content and NADPH oxidase (EC 1.6.3.1) activity was studied in the resistant (cv. *Tugela DN*) and near-isogenic susceptible (cv. *Tugela*) wheat (*Triticum aestivum* L.). The objective of this study was to investigate the involvement of the reactive oxygen species (ROS) during the resistance responses against the RWA. Infestation significantly induced an early accumulation of the H_2O_2 and increase of NADPH oxidase activity to higher levels in the resistant than susceptible plants. Results of inhibitory studies using diphenylene iodonium (DPI), a suicide inhibitor of NADPH oxidase, strongly suggested a possible signalling role for H_2O_2 during RWA resistance response by activation of downstream defence enzymes [intercellular peroxidase (EC 1.11.1.7) and β-1,3-glucanase (EC 3.2.1.39)].

Introduction

The Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), is a serious pest of wheat in South Africa and elsewhere in the world (Du Toit and Walters, 1984; Smith et al., 1991). Plant resistance is a cost effective and environmentally friendly alternative for insecticides to control the disease. RWA-resistant cultivars have been released in South Africa (Du Toit, 1989). The biochemical mechanism

Abbreviations: DPI, diphenylene iodonium; EDTA, ethylenedinitrilo tetraacetic acid; HR, hypersensitive response; IWF, intercellular washing fluid; O_2^-, superoxide anion; PR, pathogenesis related; PVP, polyvinylpyrolidone; ROS, reactive oxygen species; RWA, Russian wheat aphid; SOD, superoxide dismutase; UV, ultraviolet

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of resistance, however, still needs to be resolved. A better understanding of the wheat plant's defence mechanism against the RWA could eventually contribute to more effective breeding or manipulation of resistance, thereby reducing the dependency on chemical control.

The downstream defence or secondary defence responses in the RWA-wheat interaction have been studied quite extensively. It was found that the RWA-resistance response is a typical hypersensitive response (HR) induced by RWA infestation. It included the induction of the intercellular β-1,3-glucanase (van der Westhuizen and Pretorious, 1996; van der Westhuizen et al., 1998b, 2002), peroxidases and chitinases (van der Westhuizen et al., 1998a), which highly resembles the defence responses during pathogenesis. In the case of pathogenesis, it has been recognized for some time that one of the earliest events during the HR is the generation of the reactive oxygen species (ROS) such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (Alvarez et al., 1998; Bolwell, 1999; Fath et al., 2002). These ROS are directly protective and also drive oxidative cross-linking of the cell wall (Lamb and Dixon, 1997). Moreover, ROS induce arrays of cellular protectant and defence genes and also initiate the collapse of challenged cells (Levine et al., 1994; Orozco-Cárdenas et al., 2001; Hancock et al., 2002). In several model plant systems studied, the oxidative burst and the H_2O_2 accumulation appear to be mediated by the activation of a membrane-bound NADPH oxidase complex (Doke et al., 1996; Potikha et al., 1999; Pei et al., 2000).

Little is known about the signalling events leading to the induction of the secondary defence reactions in the RWA-wheat interaction. In an attempt to unravel more of the RWA resistance mechanisms, especially the early events, the objective of the study reported here was to establish whether the ROS production, specifically H_2O_2, forms part of the signalling events during the RWA resistance response in wheat.

Materials and methods

Resistant [cv., *Tugela DN*, containing the *Dn 1* (PI 137739) resistance gene (Du Toit, 1989)] and the near isogenic susceptible (cv., *Tugela*) wheat (*Triticum aestivum* L.) plants were grown under greenhouse conditions in trays, at temperatures of 24 °C ($\pm$2 °C). Culture conditions and infestation procedures were as described by Du Toit (1988). Plants were infested in the early three-leaf stage by scattering the RWAs (*Diuraphis noxia*, Mordvilko), approximately 20 RWAs per plant, on the leaves. Another set of plants (resistant and susceptible) was left uninfested as control. Plants were harvested after specific time periods and frozen immediately in liquid nitrogen. For all assays performed in this study, two separate experiments (i.e. planting new sets of resistant and susceptible plants) were conducted and within each experiment, assays were done in triplicate.

Treatment of plants with diphenylene iodonium

Resistant plants were treated with diphenylene iodonium (DPI), an inhibitor of NADPH oxidase, according to the method of Orozco-Cárdenas et al. (2001). Plants were excised at the base of the stem at the early three-leaf stage with a razor blade. For inhibitor treatment, excised plants were placed in a 10 mM potassium phosphate buffer (pH 6.0) containing 100 μM DPI for 2 h while control excised plants were put in the buffer alone under the same growth conditions described above. After these treatments, the plants were infested and transferred to distilled water. After 6 h of infestation, the leaves were used for the determination of NADPH oxidase activity and H_2O_2 content. The intercellular washing fluid (IWF) was collected from the leaves after 48 h of infestation. To investigate the effect of longer treatment, some excised plants remained in the DPI solution for the duration (48 h) of the experiment.

Treatment of plants with a H_2O_2-generating mixture of glucose and glucose oxidase

Resistant plants were treated with glucose plus glucose oxidase according to the method described by Orozco-Cárdenas et al. (2001). Plants were excised at the base of the stem and then placed in 10 mM potassium phosphate buffer (pH 6.0) containing (50 mM) glucose plus glucose oxidase (2.5 units/mL) for 2 h. Control excised plants were placed in buffer alone. Thereafter, plants were transferred to distilled water and after 6 h, the leaves were used for H_2O_2 assay. These treatments were performed in the green house under the same conditions described above. The IWF was collected from the leaves after 48 h.

Collection of the intercellular washing fluid (IWF)

Leaves were cut in 10 cm long pieces, thoroughly rinsed in distilled water, and then vacuum infiltrated