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Short communication

Peroxidase activity in roots and root exudates of strawberry – linked to the resistance to root pathogens?

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*We investigated the role of peroxidases (POX) in roots and root exudates of strawberry (*Fragaria x ananassa*) by quantitatively evaluating POX in the roots of different cultivars differing in their susceptibility to root diseases and qualitatively evaluating POX in the exudates. We found a significantly higher POX activity in the roots of the less susceptible cultivar 'Daroyal' than of the medium susceptible cultivars 'Alba', 'Asia' and 'Queen Elisa' and the highly susceptible cultivar 'Elsanta'. In addition, we clearly confirmed the exudation of POX from strawberry roots into the surrounding medium. We hypothesize that the higher POX activity in the roots leads to enhanced exudation of POX which may implicate an enhanced formation of highly reactive ortho-quinones in the soil inhibiting fungal enzymes. This could be one resistance mechanism against different root pathogens, worth studying profoundly in more genotypes.*
Keywords: Fragaria x ananassa, ortho-quinones, polyphenols, rhizosphere

Korreliert die Peroxidaseaktivität in Wurzeln und Wurzelexsudaten von Erdbeeren mit der Resistenz gegenüber Wurzelpathogenen? *In dieser Arbeit wurde die Rolle der Peroxidase (POX) in Wurzeln und Wurzelexsudaten von Erdbeeren (*Fragaria x ananassa*) untersucht. Bei einem Vergleich von fünf Sorten, die sich in der Widerstandsfähigkeit gegen Wurzelkrankheiten unterscheiden, wurde eine signifikant höhere Peroxidaseaktivität in der wenig anfälligen Sorte 'Daroyal' als in den mittelmäßig anfälligen Sorten 'Albal', 'Asia' und 'Queen Elisa' und in der stark anfälligen Sorte 'Elsanta' festgestellt. Zusätzlich konnte in einem in vitro-Versuch gezeigt werden, dass Erdbeerwurzeln Peroxidase exsudieren. Die höhere Peroxidaseaktivität in den Wurzeln könnte zu vermehrter Exsudation von Peroxidasen führen, die Polyphenole zu hochreaktiven ortho-Chinonen oxidieren können, welche wiederum die Fähigkeit haben, pilzliche Enzyme zu inhibieren. Dies könnte einen Resistenzmechanismus gegen verschiedene Wurzelpathogene darstellen, der an mehr Genotypen weiter untersucht werden sollte.*
Schlagwörter: Fragaria x ananassa, ortho-Quinone, Polyphenole, Rhizosphäre

L'activité peroxydase dans les racines et dans les exsudats racinaires de fraises est-elle en corrélation avec la résistance aux pathogènes racinaires ? *Dans le présent travail, le rôle de la peroxydase (POX) dans les racines et dans les exsudats racinaires de fraises (*Fragaria x ananassa*) a été étudié. Lors de la comparaison de cinq variétés se distinguant par leur résistance aux maladies racinaires, on a constaté une activité peroxydase significativement plus élevée dans la variété peu fragile 'Daroyal' que dans les variétés de fragilité moyenne 'Albal', 'Asia' et 'Queen Elisa' et dans la variété très fragile 'Elsanta'. En outre, il a pu être démontré dans un essai in vitro que les racines des fraises exsudent de la peroxydase. L'activité peroxydase plus élevée dans les racines pourrait mener à une augmentation de l'exsudation de peroxydases qui peuvent oxyder les polyphénols en orthoquinones très réactives qui, de leur côté, ont la capacité*

d'inhiber des enzymes fongiques. Cela pourrait représenter un mécanisme de résistance aux différents pathogènes racinaires qu'il faudrait continuer à analyser sur la base d'un plus grand nombre de génotypes.
Mots clés : Fragaria x ananassa, orthoquinones, polyphénols, rhizosphère

In previous on-farm experiments, we found large differences between strawberry cultivars in their susceptibility to root diseases (Weissinger et al., 2011). The most important strawberry diseases in Central Europe are Verticillium-wilt (*Verticillium dahliae*), Phytophthora crown rot (*Phytophthora cactorum*), Phytophthora red stele root rot (*Phytophthora fragariae*), and black root rot (*Rhizoctonia fragariae, Pythium sp., Pratylenchus penetrans* etc.). Cultivars completely resistant to *Verticillium*-wilt, crown rot and black root rot are not known. Cultivars differ in their field resistance to these diseases which is due to the interaction of many genes (Korbin, 2011) and in the case of *Verticillium*-wilt due to a combination of resistance and tolerance mechanisms; cultivars differ both in the extent of fungal colonization and in the tolerance to the fungus (Shaw et al., 2010). Genetic factors or physiological processes controlling resistance are widely unknown (Klosterman et al., 2009), which complicates resistance breeding.

The aim of this study was to investigate the peroxidase (POX) activity in roots of several cultivars differing in their susceptibility to root diseases and to examine if strawberry roots also exude POX which could lead to not yet well-described defence reactions in the rhizosphere.

Plant peroxidases are important enzymes in plant defence since they trigger the reinforcement of the cell walls, lead to oxidative burst and catalyse the synthesis of numerous phytoalexins whereby they often use phenolic compounds as substrates (Almagro et al., 2009). Plants do not only contain but also exude enzymes and phenolics into the rhizosphere (Bertin et al., 2003; Lanoue et al., 2010). These secreted compounds may play an important role in plant defence, but the specific functions are often not understood yet (Bais et al., 2004; Weston et al., 2012). Hoffmann et al. (2012) demonstrated the in situ biosynthesis of flavan-3-ols in roots and their exudation in form of border cells in several *Rosaceae-species*, among them in *Fragaria x ananassa*. In contrast, the exudation of POX has not been investigated in strawberry to our knowledge so far. If more knowledge about the specific functions of POX in planta and in the rhizosphere is available, POX could maybe used as a biochemical marker for resistance breeding.

Material and Methods

Five strawberry cultivars, differing in the grade of field resistance to root pathogens, namely 'Elsanta' (highly susceptible), 'Albal', 'Asia', 'Queen Elisa' (medium susceptible) and 'Daroyal' (less susceptible) (Weissinger et al., 2011) and grown on native soil at the experimental orchard of the University of Natural Resources and Life Sciences (Jedlersdorf/Vienna, Austria), were used to investigate POX activity. In early September 2008, strawberry stolons from the experimental orchard in Jedlersdorf/Vienna were rooted in 0.5 litre plastic pots with substrate (sand: substrate ED 63 (Einheitserde und Humuswerke, Sinntal-Altengronau, Germany) 1:1 (w/w)). In late November 2008, five plants per cultivar were removed from the substrate, and small pieces of roots were frozen with liquid nitrogen and ground. 1 g roots, 0.5 g Polyclar AT, 0.5 g quartz sand and 5 ml buffer A (0.01 M Tris; 0.0068 M EDTA; 0.01 M $Na_2B_4O_7$ x 10 H_2O in H_2O were homogenised in a mortar, filled in tubes and centrifuged (10 min, 10,000 x g, 4 °C). 400 µl of the supernatant were passed through a G-25 sephadex column and eluted with 400 µl buffer B (0.1 M Na_2HPO_4 adjusted to pH 6.5). For the POX enzyme assay, 1090 µl buffer C (0.1 M KH_2PO_4 adjusted to pH 6.5 containing 10^{-4} % H_2O_2), 10 µl o-dianisidine solution (1 % (w/v) in methanol) and 10 µl sample were mixed, and the POX activity was measured photometrically at 460 nm by the change of extinction per minute (ΔE/min) (modified according to Worthington and Teller, 1959). Protein content was determined by a modified Lowry procedure (Sandermann and Strominger, 1972) using crystalline bovine serum albumine as a standard. POX activity (ΔE/min, measured at 460 nm) was expressed per gram protein content. For statistical analysis, SPSS 15.0 (one-way ANOVA, S-N-K test, $P < 0.05$) was used.

For investigating the root exudation of POX, seeds of cultivar 'Selma-Herz' (Samen Mauser, Winterthur, Switzerland) were surface sterilized for 15 min in 1 ml sterilization solution (10 mg Bayrochlor dissolved in

100 µl sterile water and 900 µl 95 % ethanol) and rinsed afterwards three times with 95 % ethanol. Seeds were sowed on agar growth medium (2.2 g/l Murashige & Skoog medium, 10 g/l sucrose, 14 g/l agar agar; pH 5.8 with KOH). For the detection of POX activity, the strawberry plants were gently removed from the agar together with their roots. Subsequently, 1100 µl buffer C with 10 µl o-dianisidine solution (1 % (w/v) in methanol) were pipetted on the agar surface. During incubation for approximately 2 h at room temperature, the o-dianisidine oxidizes to its red-coloured form if a POX activity is present. The experiment was carried out on three dates with five plants in total.

Results and discussion

When we measured the POX activity in roots of different strawberry cultivars, the less susceptible cultivar 'Daroyal' showed a significantly higher POX activity than the medium susceptible cultivars 'Alba', 'Asia' and 'Queen Elisa' and the highly susceptible cultivar 'Elsanta' (Fig. 1).

Many studies proved that an increased POX activity in roots is linked to a higher resistance to plant pathogens (e.g. Smit and Dubery, 1997). This is explained by several functions of POX in plant defence: generation of reactive oxygen species (ROS), polymerisation of cell wall compounds (lignification) and regulation of H_2O_2 levels (Passardi et al., 2005; Almagro et al., 2009).

Additionally, POX may also be exuded into the rhizosphere what was already proven by Gramss et al. (1999) and Muratova et al. (2009) for several plant species. We could clearly confirm the exudation of POX also for strawberry (Fig. 2).

Gramss et al. (1999) described the main function of exuded POX as the oxidative degradation of certain soil constituents, among them aromatic lignin components, phenolic groups of the humus polymers, and the oxidation of plant root-exuded phenolics. According to Elstner et al. (1996), POX can trigger the oxidation of phenolic compounds to highly reactive ortho-quinones which can interact with NH_2- or SH-groups of proteins, e. g. enzymes of pathogens (cellulases, pectinases) and so cause toxicity to these pathogens during infection. So, in addition to phytoalexin synthesis in planta, POX may also contribute to phytoalexin synthesis in the rhizosphere, using plant-derived or even soil-derived substrates. We also did preliminary experiments to see if POX activity in the root

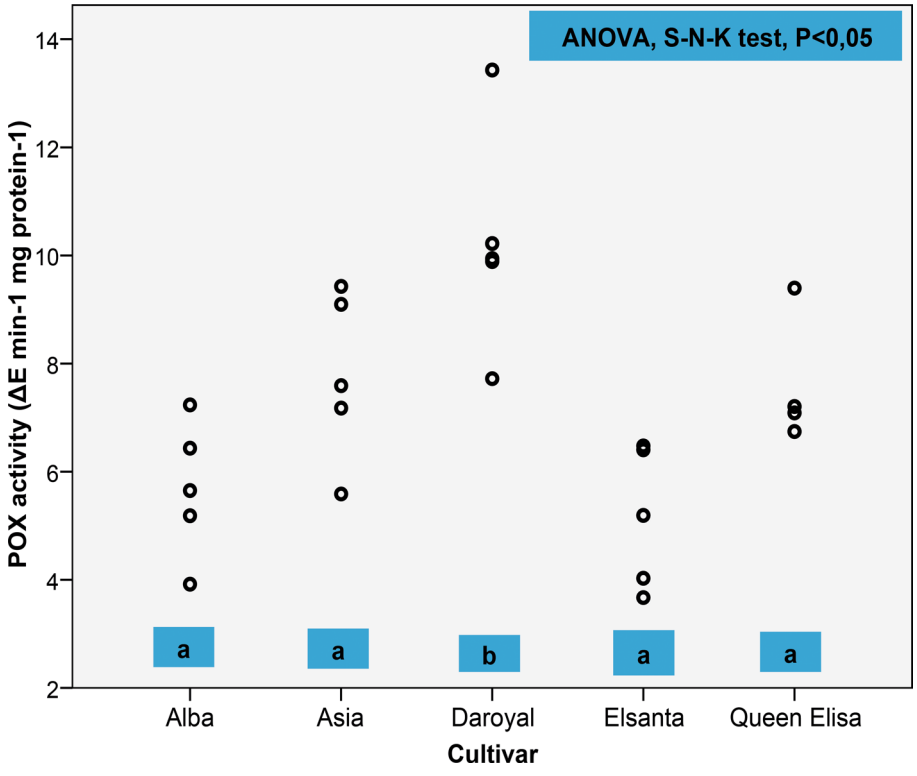

Fig. 1: POX activity in the roots of five strawberry cultivars

Fig.2a

Fig.2b

Fig.2c

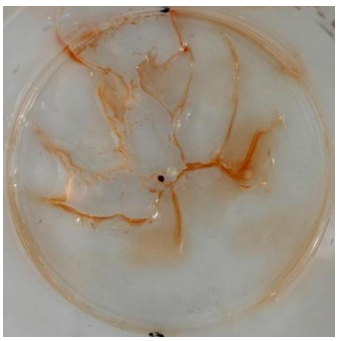

Fig. 2: In vitro experiment; plant on agar (a) with leaves and (b) after removing the leaves (c); red stain of oxidized o-dianisidine caused by POX exuded from the roots into the agar

exudates correlates with susceptibility to root diseases in the same five strawberry cultivars. We could see a similar pattern as for the POX activity in the roots, but at a very low level and with high variation between the single plants (data not shown). Nevertheless, we hypothesize that the higher peroxidase activity in the roots leads to enhanced exudation of POX which implicates a higher formation of highly reactive ortho-quinones in the soil contributing to field resistance to different root pathogens.

The next research steps are then to validate the correlation between field resistance and POX root activity respective POX activity in root exudates for a broader range of strawberry genotypes, and to investigate the exudation of phenolics with and without pathogen infections. In addition, other roles of POX in strawberry resistance to root pathogens should be examined, especially the lignification process in relation to plant pathogenesis.

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