

Graft-transmissible resistance of cherry pepper (*Capsicum annuum* var. *cerasiforme*) to powdery mildew (*Leveillula taurica*) is associated with elevated superoxide accumulation, NADPH oxidase activity and pathogenesis-related gene expression

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Abstract We found that resistance to pepper powdery mildew (PM) (*Leveillula taurica*) develops in a sweet pepper (*Capsicum annuum*) cultivar ('Totál') when grafted on a resistant cherry pepper (*C. annuum* var. *cerasiforme*) rootstock (cv. Szentesi). Resistance is manifested both towards PM symptoms and pathogen accumulation. In healthy, uninfected plants PM-resistance can be predicted by enhanced accumulation of the reactive oxygen species (ROS) superoxide ($O_2^{\cdot-}$) and activity of NADPH oxidase, the enzyme mainly responsible for pathogenesis-related superoxide generation. In *L. taurica*-inoculated PM-resistant 'Szentesi' high levels of superoxide and NADPH oxidase activity are sustained even 45 days after inoculation, as opposed to PM-susceptible 'Totál'. This is also true for 'Totál' grafted on resistant 'Szentesi' rootstocks, where PM resistance, enhanced superoxide production and NADPH oxidase activity is likely due to an unknown, graft-transmitted signal. To further elucidate the mechanisms of graft-transmissible PM-resistance we monitored expression of pathogenesis-related (PR) genes in healthy and infected plants. In healthy plants, expression of *CaPR*-

1 is several times higher in leaves of PM-resistant pepper than in sensitive plants, while high expression of *CaPR*-2 (glucanase) does not entirely correlate with PM-resistance, being detectable only in PM-resistant 'Szentesi'. However, during advanced stages of PM-pathogenesis (45 DAI) expression of *CaPR*-1 and *CaPR*-2 is by far the highest in PM-susceptible 'Totál'. Our results suggest that the direct biochemical cause of graft-transmissible PM-resistance in pepper is the enhanced accumulation of NADPH oxidase-generated superoxide. To our knowledge, this is the first report on the role of ROS (superoxide) in graft-transmissible, pathogen-specific disease resistance.

Keywords *Capsicum annuum* var. *cerasiforme* · Graft-transmissible resistance · *Leveillula taurica* · Superoxide · NADPH oxidase · Pathogenesis-related genes

Introduction

Resistance of plants to pathogenic invaders is effective when the result is inhibition/killing of pathogens. Several physiological processes have been shown to be associated with plant disease resistance, including accumulation of antimicrobial compounds, cell wall reinforcement, localized cell/tissue death (hypersensitive response), reactive oxygen species (ROS), etc. (e.g. Jones and Dangl 2006; Spoel and Dong 2012; Künstler et al. 2015). In particular ROS, primarily superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) have been associated and functionally linked to numerous plant disease resistance events (see e.g. Baker and Orlandi 1995; Torres 2010; Dubiella et al. 2013; Lehmann et al. 2015). Importantly, ROS have a dual role during plant defense to infections: high concentrations of ROS may kill both plant and invading pathogen cells,

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while low concentrations act as signals for the induction of antioxidant and pathogenesis-related genes in plant tissues adjacent to infection sites (Levine et al. 1994; Torres et al. 2005; Pogány et al. 2009; Hafez et al. 2012).

Superoxide was the first ROS where a specific involvement in plant disease resistance had been shown. In the 1980s pioneering discoveries demonstrated that resistance of potato and tobacco to an oomycete (*Phytophthora infestans*) and virus (*Tobacco mosaic virus*, TMV) pathogen, respectively, is closely associated with the NADPH oxidase-mediated generation of superoxide in plasma membranes (Doke 1983, 1985; Doke and Ohashi 1988). Ádám et al. (1989) were the first to report superoxide accumulation in tobacco during resistance to bacteria (*Pseudomonas syringae* pv. *syringae*). The functional role of superoxide in pathogen limitation is suggested e.g. by the work of Shang et al. (2010) demonstrating that absence of *Cucumber mosaic virus* in “dark green islands” of systemically infected leaf tissues correlates with superoxide accumulation. In addition, we could elicit symptomless resistance to TMV if susceptible plants were treated with a riboflavin-methionine solution generating superoxide (Bacsó et al. 2011). Furthermore, it has been shown that bacterial and fungal plant pathogens are also sensitive to externally produced superoxide both in vitro and in planta (e.g. Aver'yanov and Lapikova 1988; Jordan et al. 1992; Király et al. 1993; Ouf et al. 1993; El-Zahaby et al. 2004).

The endoparasitic powdery mildew fungus *Leveillula taurica* (anamorph: *Oidiopsis taurica*) is a serious threat to pepper production. Under a temperate climate, heavy epidemics could cause a significant yield loss of up to 2–4 kg/m² in greenhouses (see e.g. Cerkauskas and Buonassisi 2003). Besides extensive fungicide applications, pepper powdery mildew (PM) could be controlled by using resistant cultivars containing resistance (R) genes introgressed from related wild species but this is problematic partly because this race/cultivar specific resistance is eventually overcome by newly emerging pathogen races (see e.g. Zheng et al. 2013a).

Grafting may facilitate stable transmission of certain genotypic and phenotypic traits (e.g. RAPD DNA profiles, fruit shape and color) from pepper rootstocks to scions (see e.g. Taller et al. 1998; Tsaballa et al. 2013). Furthermore, interspecific grafting of e.g. solanaceous species (pepper, tomato and eggplant) has been shown to cause heritable changes in DNA methylation patterns in scions (Wu et al. 2013; Warschefsky et al. 2016). Importantly, several cases are mentioned when plant disease resistance has been transmitted by grafting although primarily for controlling soilborne diseases (King et al. 2008; Louws et al. 2010; Al-Mawaali et al. 2012; Guan and Zhao 2012). Well known examples include grafting of European grapevine (*Vitis vinifera*) onto American (*Vitis labrusca* and other species)

rootstocks to overcome the *Phylloxera* plague (see e.g. Mudge et al. 2009) and grafting of citrus onto sweet orange to combat *Phytophthora* foot rot (Wutscher 1979). Recently, however, grafting has also been reported to improve resistance of e.g. cucumbers to certain foliar diseases such as powdery mildew (caused by *Podosphaera xanthii*) and downy mildew (caused by *Pseudoperonospora cubensis*) (Louws et al. 2010; Sakata et al. 2006). These results imply the existence of graft-transmitted signal(s) that may confer disease resistance in scions but the biochemical/genetic mechanisms are not entirely clear. For example, phenylalanine ammonia-lyase is a defense-related enzyme with a role in biosynthesis of antimicrobial phenylpropanoids and the defense regulator salicylic acid. However, in planta phenolic contents do not always correlate with levels of graft-transmitted disease resistance (Guan and Zhao 2012; Wallis et al. 2013). On the other hand, enhanced tolerance of grafted plants (e.g. tomato, eggplant) to abiotic stresses is coupled to higher antioxidant enzyme activities as compared to self-rooted plants (He et al. 2009; Wei et al. 2009; Guan and Zhao 2012). This could indicate the role of high ROS levels in eliciting tolerance/resistance of grafted plants to abiotic stresses and pathogenic infections, a phenomenon that has not been investigated so far.

A Hungarian cherry pepper (*Capsicum annuum* var. *cerasiforme*) cultivar (‘Szentesi’) bred from selections of wild-grown Mexican genotypes is highly resistant to PM (*L. taurica*) and has been used in production for over several years (Lantos 2011). Our goal was to investigate if the PM resistance of ‘Szentesi’ is graft-transmissible to a susceptible sweet pepper (*C. annuum*) cultivar (‘Totál’) and to clarify the possible role of the ROS superoxide and pathogenesis-related (PR) gene expression in this type of disease resistance.

Materials and methods

To graft PM-susceptible sweet pepper ‘Totál’ scions on PM-resistant cherry pepper ‘Szentesi’ rootstocks (Szentesi + Totál graft) seeds of these two pepper cultivars were sown in a laboratory greenhouse. Grafting was carried out when the first true leaves were fully developed by cutting stems of rootstocks and scions with a razor blade above the cotyledons in a 45° angle and pairing with the aid of grafting clips. Two weeks acclimation (in plastic cages, ca. 25 °C and 90% relative humidity) was allowed for the development of graft unions. Control grafts were also made (Szentesi + Szentesi and Totál + Totál). Grafted and self-rooted plants were 60 days old when used for experiments.

Provocation tests were conducted at two different greenhouse production sites (Szentesi, Southeastern

Hungary). Average day and night temperatures were ca. 28 and 18 °C, respectively, with close to 90% relative humidity, conditions that are optimal for initiating natural PM infections in pepper. For inoculation of pepper plants with PM under controlled laboratory conditions, a *L. taurica* isolate derived from one of the provocation test sites and maintained on ‘Totál’ plants was used. Inoculation was conducted as described by Zheng et al. (2013a, b), except that 60 days old plants were inoculated with a fine paint brush and kept afterwards in growth chambers.

Accumulation of the PM pathogen (*L. taurica*) in inoculated pepper leaves was assessed by quantitative (real time) PCR (qPCR). Total genomic (plant and fungal) DNA was extracted from leaves of PM-inoculated pepper by the REDEExtract-N-AmpTM Plant PCR Kit (Sigma Aldrich, USA). For qPCR a primer pair (LtLV) specific to *L. taurica* ITS sequences was employed: 5'-AGCCGACTAGGCTTG GTCTT-3' (5' primer) and 5'-GCGGGTATCCCTACCTG ATT-3' (3' primer) (Zheng et al. 2013b). A pepper house-keeping gene (actin, GenBank AY572427) was chosen as an internal control, PCR-amplified using the primer pair: 5'-ATCCCTCCACCTCTTCACTCTC-3' (5' primer) and 5'-GCCTTAACCATTCCTGTTCCATTATC-3' (3' primer) as described by Silvar et al. (2008). qPCR for assaying *L. taurica* DNA levels was conducted with the 2× SYBR FAST Readymix Reagent (KAPA Biosystems, USA) in a Bio-Rad CFX-96 real-time thermocycler (Bio-Rad, USA) essentially as described (Zheng et al. 2013b).

Superoxide (O₂⁻) accumulation in healthy and PM-inoculated pepper leaves was detected by histochemical staining with nitro blue tetrazolium chloride (NBT) (Sigma Aldrich, USA), as described earlier for tobacco (Ádám et al. 1989; Király et al. 2008).

NADPH oxidase enzymatic activity in healthy and PM-inoculated pepper leaves was assayed as described by Ádám et al. (1997) and Xia et al. (2009) with modifications. Samples were homogenized in eight volumes of extraction buffer (50 mM Tris-HCl, pH 7.5, 0.25 M sucrose, 1 mM Na₂S₂O₅, 1 mM EDTA, 0.6% PVP) and pellets obtained by ultracentrifugation were resuspended in 0.5 ml extraction buffer.

Monitoring expression of two pathogenesis-related (PR) genes (*CaPR-1* and *CaPR-2*) in healthy and *L. taurica*-inoculated plants was conducted by RT-qPCR. Healthy and inoculated pepper leaves were used for total RNA extraction by a minicolumn kit (Viogene, USA). Reverse transcription (RT) was done with a RevertAidTM H⁻ cDNA Synthesis Kit (Thermo Fisher Scientific, USA). qPCR to determine PR gene expression was conducted as described above for assaying *L. taurica* DNA levels and in Höller et al. (2010) by using the following primer pairs: *CaPR-1* 5'-GTTGTGCTAGGGTTCGGTGT-3' (5' primer) and 5'-CAAGCAATTATTTAAACGATCCA-3' (3' primer);

CaPR-2 5'-ACAGGCACATCTTCACTTACC-3' (5' primer) and 5'-CGAGCAAAGGCGAATTTATCC-3' (3' primer) (Silvar et al. 2008).

For the quantification of PM levels, NADPH oxidase activity and PR gene expression, each biological sample contained at least six leaves collected from three pepper plants, with three technical repeats per sample. Statistically significant differences from susceptible plants were calculated by Student's *t* test (at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$).

Results

Initial provocation tests in greenhouses suggested that resistance of pepper to PM (*L. taurica*) is graft-transmissible. Fully developed, self-rooted susceptible ‘Totál’ sweet pepper uniformly displayed typical symptoms of PM infection [chlorotic flecks on adaxial leaf surfaces and patches of white, powdery fungal growth (mildew) on abaxial leaf surfaces]. When PM-susceptible sweet pepper ‘Totál’ scions were grafted on PM-resistant cherry pepper ‘Szentesi’ rootstocks (Szentesi + Totál) no visible symptoms occurred, except for occasional mild chlorosis and PM on maximum 30% of leaf area of lower, senesced leaves. Fruit yield in these Szentesi + Totál grafts was similar to that of healthy control self-rooted ‘Totál’. As expected, more than 95% of self-rooted ‘Szentesi’ cherry pepper plants did not display visible PM symptoms (data not shown).

To demonstrate the graft-transmissibility of PM-resistance under controlled laboratory conditions, we have inoculated the pepper plants mentioned above with *L. taurica*. 45 days after inoculation (DAI) self-rooted ‘Totál’ displayed typical PM symptoms, ‘Szentesi’ did not display any visible PM symptoms, while Szentesi + Totál grafts displayed only occasional mild symptoms on lower (senesced) leaves, similarly as observed in provocation tests (Fig. 1a).

To confirm that the absence of PM symptoms in pepper is indeed associated with a significant reduction in pathogen levels (i.e. a bona fide PM-resistance), accumulation of *L. taurica* was assayed by qPCR. In an advanced stage of PM-pathogenesis (45 DAI), levels of *L. taurica* genomic DNA were significantly lower in pepper displaying resistance to PM symptoms (‘Szentesi’ and Szentesi + Totál grafts) as compared to susceptible ‘Totál’ plants (Fig. 1b). These results clearly show that ‘Szentesi’ is indeed resistant to *L. taurica* and that this type of PM-resistance is graft-transmissible.

To assess the possible role of the ROS superoxide in the graft-transmissible PM-resistance of pepper, superoxide accumulation in healthy and PM-inoculated (45 DAI) pepper leaves was detected by histochemical staining with

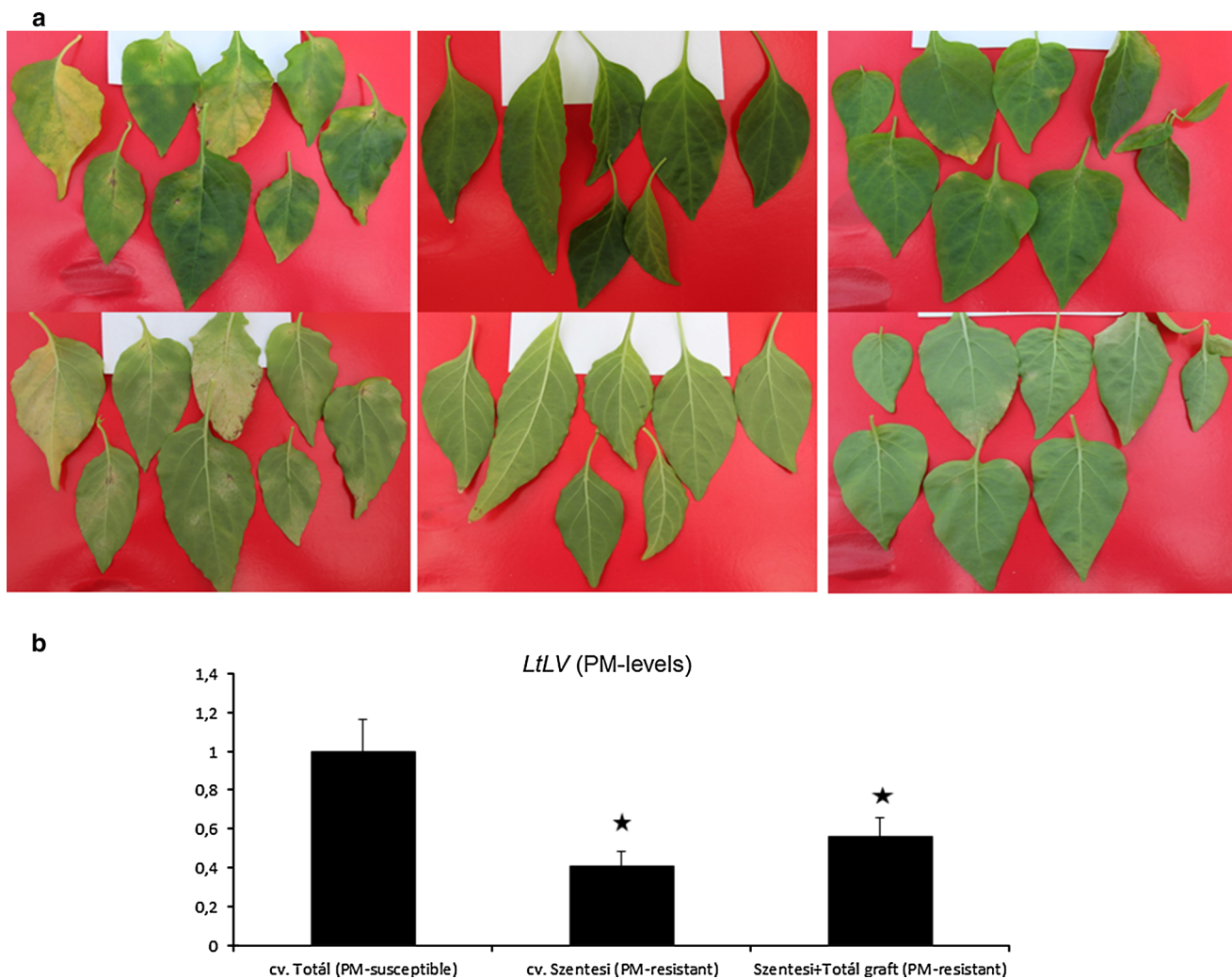
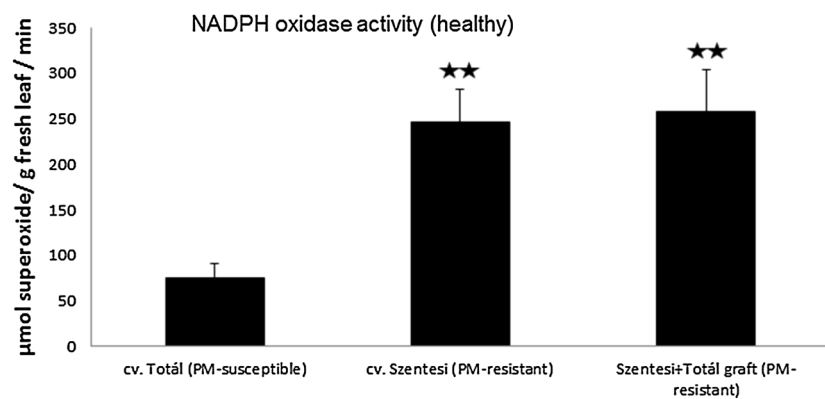
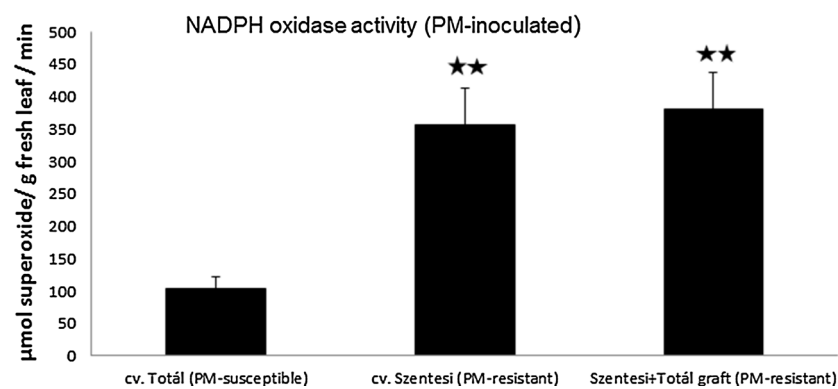
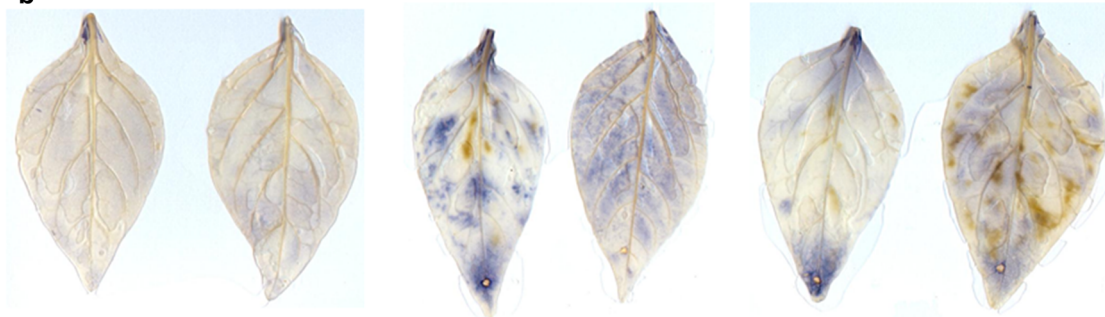


Fig. 1 Resistance of cherry pepper (*Capsicum annuum* var. *cerasiforme* cv. Szentesi) to pepper powdery mildew (PM) (*Leveillula taurica*) is graft-transmissible: resistance induced in susceptible sweet pepper (*C. annuum* cv. Totál) grafted on resistant cherry pepper cv. Szentesi. Typical symptoms of powdery mildew infection in pepper leaves 45 days after inoculation (DAI) (a). Upper panels: chlorotic flecks on adaxial leaf surfaces; lower panels: patches of white, powdery fungal growth (mildew) on abaxial leaf surfaces. Left, middle and right panels: leaves of susceptible (cv. Totál) and resistant

(cv. Szentesi and Szentesi + Totál graft) pepper, respectively. Repetition of the experiment led to similar results. Quantification of PM levels in inoculated pepper leaves at 45 DAI by qPCR using the LfLV primer pair (see details in text) (b). A relative value of 1 represents levels of LfLV-amplified PM genomic DNA in susceptible (cv. Totál) plants. Columns represent mean \pm SD from three independent biological experiments. * indicate statistically significant differences from susceptible plants at $p \leq 0.05$ (Student's *t* test)

NBT. In healthy pepper, negligible amounts of superoxide were present in PM-susceptible 'Totál' leaves, while a pronounced superoxide accumulation was apparent in leaves of PM-resistant 'Szentesi' and Szentesi + Totál grafts (Fig. 2a upper panels). Interestingly, in *L. taurica*-inoculated pepper, overall superoxide levels increased slightly, i.e. still very little superoxide accumulated in PM-susceptible 'Totál' leaves, while high superoxide levels were essentially retained in PM-resistant leaves (Fig. 2b upper panels). These findings suggest that superoxide accumulation is a marker and a possible functional component of this graft-transmissible PM-resistance.

Fig. 2 Graft-transmissible resistance of cherry pepper (*Capsicum annuum* var. *cerasiforme* cv. Szentesi) to pepper powdery mildew (PM) (*Leveillula taurica*) is associated with superoxide ($O_2^{\cdot-}$) accumulation and elevated NADPH oxidase activity in both healthy (a) and PM-inoculated (45 DAI) (b) plants. Upper panels: superoxide accumulation in healthy/PM-inoculated pepper leaves as visualized by nitro blue tetrazolium chloride (NBT) tissue staining. Left, middle and right panels: leaves of susceptible (cv. Totál) and resistant (cv. Szentesi and Szentesi + Totál graft) plants, respectively. Repetition of the experiment led to similar results. Lower panels: enzymatic activity of NADPH oxidase in healthy/PM-inoculated pepper leaves. Columns represent mean \pm SD from three independent biological experiments. ** indicate statistically significant differences from susceptible plants at $p \leq 0.01$ (Student's *t* test)

a**b**

Since pathogenesis-related, plasma membrane-derived superoxide accumulation in plants has been primarily associated with the activity of NADPH oxidases (see e.g. Marino et al. 2012; Kaur et al. 2014; Kadota et al. 2015), we thought that the elevated superoxide accumulation associated with graft-transmissible PM-resistance of pepper could be also a consequence of NADPH oxidase activity. To test this hypothesis, NADPH oxidase enzymatic activity was assayed in healthy and PM-inoculated (45 DAI) pepper leaves. In healthy pepper, a basal NADPH oxidase activity was present in PM-susceptible ‘Totál’, while a significantly higher activity was apparent in PM-resistant ‘Szentesi’ and Szentesi + Totál grafts (Fig. 2a lower panels). In *L. taurica*-inoculated pepper, overall NADPH oxidase activities increased but high activities were retained in PM-resistant leaves (‘Szentesi’ and Szentesi + Totál grafts) as compared to PM-susceptible ‘Totál’ (Fig. 2b lower panels).

To further elucidate the mechanisms of graft-transmissible PM-resistance of pepper we monitored expression of

two pathogenesis-related (PR) genes in healthy and *L. taurica*-inoculated (45 DAI) plants by RT-qPCR. We have chosen *CaPR-1* and *CaPR-2*, since activities of these two PR genes have been associated with disease resistance (Sarowar et al. 2005; Silvar et al. 2008). In healthy plants, expression of *CaPR-1* was several times higher in leaves of PM-resistant pepper than in susceptible ‘Totál’, although *CaPR-1* expression was by far the highest in PM-resistant ‘Szentesi’, while it was markedly lower in PM-resistant Szentesi + Totál grafts. On the other hand, high expression levels of *CaPR-2* did not entirely correlate with PM-resistance, being detectable only in PM-resistant ‘Szentesi’ but neither in PM-resistant Szentesi + Totál grafts nor in susceptible ‘Totál’ (Fig. 3a). Interestingly, however, in *L. taurica*-inoculated pepper (45 DAI) PR gene expression patterns were reversed, since high *CaPR-1* and *CaPR-2* transcript levels were associated with PM-susceptibility of ‘Totál’, while negligible expression of both genes was apparent in PM-resistant ‘Szentesi’ and Szentesi + Totál grafts (Fig. 3b).

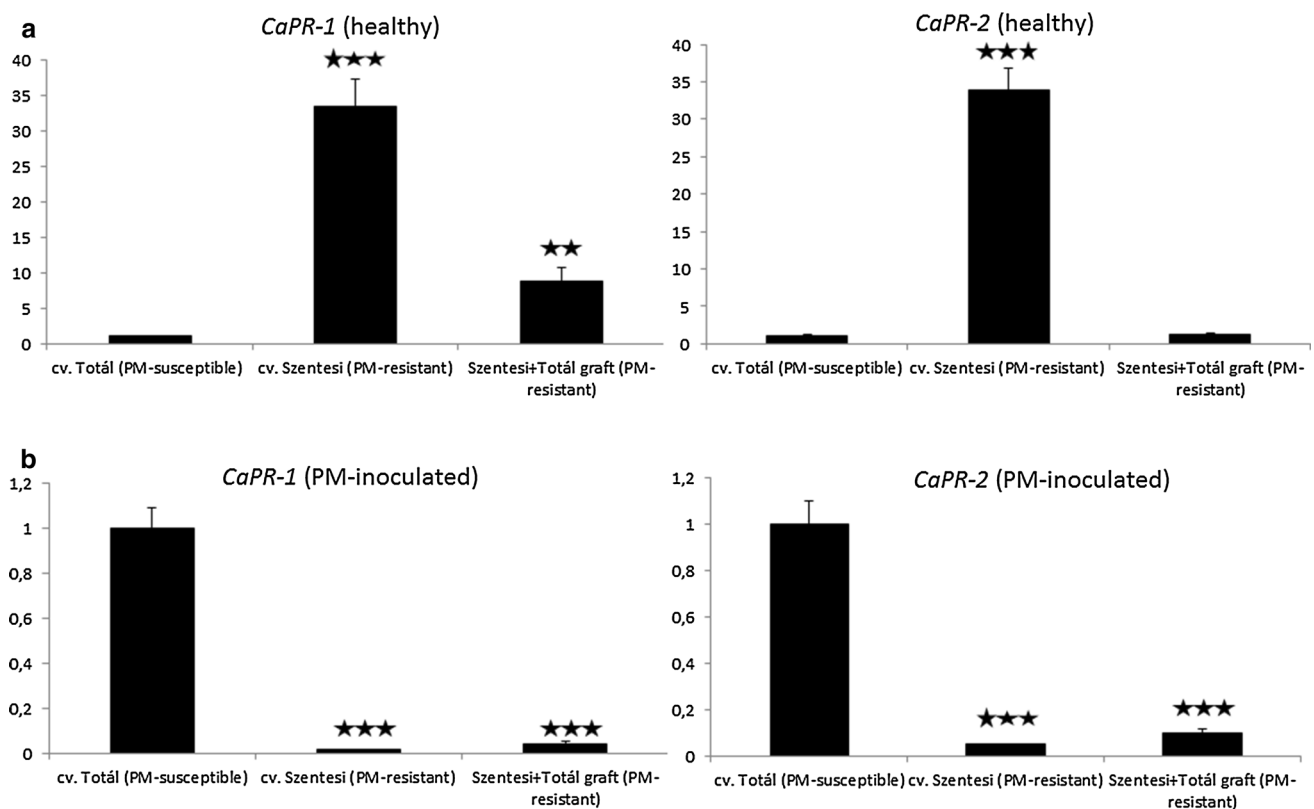


Fig. 3 Expression of pathogenesis-related genes (*CaPR-1* and *CaPR-2*) is a marker of graft-transmissible resistance of cherry pepper (*Capsicum annuum* var. *cerasiforme* cv. Szentesi) to pepper powdery mildew (PM) (*Leveillula taurica*) in healthy (a) but not in PM-inoculated (45 DAI) (b) plants. Expression of *CaPR-1* and *CaPR-2* as assayed by RT-qPCR in healthy/PM-inoculated leaves of susceptible

(cv. Totál) and resistant (cv. Szentesi and Szentesi + Totál graft) pepper, respectively. A relative value of 1 represents gene expression in susceptible (cv. Totál) plants. Columns represent mean \pm SD from three independent biological experiments. ** and *** indicate statistically significant differences from susceptible plants at $p \leq 0.01$ and $p \leq 0.001$, respectively (Student's *t* test)

Discussion

We have demonstrated that resistance to pepper powdery mildew (PM) (*Leveillula taurica*) develops in a sweet pepper (*Capsicum annuum*) cultivar ('Totál') when grafted on a resistant cherry pepper (*C. annuum* var. *cerasiforme*) rootstock (cv. Szentesi). The only documented case of graft-transmissible PM-resistance so far is the resistance of cucumber scions to PM symptoms caused by *Podosphaera xanthii* (Sakata et al. 2006). These authors have shown that certain, but not all, PM-resistant rootstocks may confer resistance or tolerance to cucumber scions, even in mature plants. In the present study, we have demonstrated for the first time a similar phenomenon to occur in a solanaceous plant (pepper), showing that graft-transmissible resistance is effective not only against PM symptoms but also in limiting the pathogen, *L. taurica*.

Our results show that elevated accumulation of NADPH oxidase-generated superoxide is associated with the graft-transmissible PM-resistance of pepper described in this study. In barley leaves artificial superoxide generation by external treatment with e.g. riboflavin/methionine confers resistance to barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) (El-Zahaby et al. 2004). In addition, sufficient expression of a NADPH-oxidase gene responsible for superoxide production is required for penetration resistance of barley to its powdery mildew pathogen (Proels et al. 2010). Observations that NADPH oxidases are mainly responsible for superoxide formation during plant disease resistance (e.g. Doke 1985; Király et al. 2008; Dubiella et al. 2013; Kadota et al. 2015) are supported by studies showing that absence of expression of NADPH oxidase genes may confer enhanced susceptibility to hemibiotrophic pathogens, e.g. *Phytophthora infestans* (Yoshiooka et al. 2003; Hajianfar et al. 2016) and biotrophic pathogens (i.e. fully preferring live host tissues) like powdery mildews. The latter was shown, e.g. in the pathosystems *Arabidopsis thaliana*/*Golovinomyces cichoracearum* (Berrocal-Lobo et al. 2010) and *Hordeum vulgare*/*B. graminis* f. sp. *hordei* (Proels et al. 2010). Importantly, these findings indicate that NADPH oxidase-derived superoxide indeed has a role in limiting these pathogens by inducing pathogen and/or host cell death (Király et al. 1993; El-Zahaby et al. 2004) and promoting cell wall reinforcements in attacked host cells (Proels et al. 2010).

We found that in healthy pepper capable of graft-transmissible PM-resistance, elevated expression of PR genes (*CaPR-1* and *CaPR-2*) may be associated with preformed defense responses (e.g. NADPH oxidase-generated superoxide accumulation), although elevated *CaPR-2* expression is not graft-transmissible. In planta-produced

superoxide can be converted to hydrogen peroxide inducing e.g. PR gene/protein expression, processes which lead to further plant defense responses and resistance to pathogenic infections (see e.g. in Van Loon et al. 2006; Torres 2010; Lehmann et al. 2015). *CaPR-1* is encoding for a basic PR-1 protein and shows elevated expression in pepper during resistance to the oomycete *Phytophthora capsici* (Silvar et al. 2008). Overexpression of *CaPR-1* in tobacco enhances tolerance to oomycete and bacterial pathogens (Sarowar et al. 2005). *PR-1* genes may also contribute to penetration resistance of barley to powdery mildew (*B. graminis* f. sp. *hordei*), as shown by transient silencing of *PR-1b* in barley epidermal cells (Schultheiss et al. 2003). Although the functional role of PR-1 proteins in plant disease resistance is not exactly known, it has been demonstrated that in broad bean (*Vicia faba*) a basic PR-1 protein inhibits differentiation of rust (*Uromyces fabae*) infection hyphae (Rauscher et al. 1999). The basic PR-1 protein encoded by *CaPR-1* could play a similar role in pepper PM-resistance, considering that both pathogens (*U. fabae* and *L. taurica*) enter plant leaves through stomatal pores.

CaPR-2 encodes for a basic β -1,3-glucanase, hydrolyzing β -1,3-glucans of fungal/oomycete cell walls (see e.g. Van Loon et al. 2006). Interestingly, however, *CaPR-2* expression following *P. capsici* infection is markedly induced only in certain resistant pepper cultivars and a significant gene induction also occurs during successful pathogenesis (Silvar et al. 2008). This is in line with our results showing that in an advanced stage of PM-pathogenesis (45 DAI) elevated expression of *CaPR-1* and *CaPR-2* in pepper is associated with susceptibility, rather than resistance. Similarly, in barley exposed to *Bipolaris sorokiniana* or its culture filtrate, *PR-1b* expression correlated with susceptibility (Király et al. 2002; Schultheiss et al. 2003). Furthermore, enhanced accumulation of *PR-1b* transcripts/protein occurred in barley and rice successfully infected with different fungal pathogens (*Drechslera teres*, *Magnaporthe grisea*, *B. sorokiniana*) (Reiss and Bryngelsson 1996; Manandhar et al. 1999). Therefore, PR gene expression might be a marker and/or functional component of preformed resistance of pepper to *L. taurica* but likely does not have a role in maintaining defenses during advanced stages of pathogenesis.

In conclusion, this study is the first to show that resistance of cherry pepper 'Szentesi' to PM (*L. taurica*) is graft-transmissible to susceptible sweet pepper and associated with elevated superoxide accumulation, NADPH oxidase activity and pathogenesis-related (PR) gene expression. Our results suggest that the direct biochemical cause of graft-transmissible PM-resistance in pepper is the enhanced accumulation of NADPH oxidase-generated

superoxide which, unlike elevated PR gene expression, is maintained even during advanced stages of pathogenesis and effectively transferred by unknown signal(s) from rootstocks to scions. In principal, the mobile, graft-transmitted signal(s) of PM-resistance could be ROS themselves. Although ROS are sensitive to degradation by e.g. antioxidants, exposure of plant tissues to abiotic stresses initiates enhanced ROS production, triggering a systemic, autoproducting ROS producing wave dependent on NADPH oxidase and traveling to distal plant parts at a rate of up to 8.4 cm/min (Miller et al. 2009; Mittler et al. 2011; Gilroy et al. 2014). The existence of similar systemic ROS waves seems also likely during elicitation/translocation of disease resistance responses, since several studies report that stimulation of ROS-accumulation in plant tissues induces ROS synthesis and resistance in distal non-treated plant parts (Alvarez et al. 1998; Fodor et al. 2001; Dubiella et al. 2013). However, other graft-transmissible signal(s) identified in the phloem could be also involved in translocating resistance responses from rootstocks to scions (or vice versa), including mRNAs, small RNAs, defense-related proteins, phytohormones, etc. (Golecki et al. 1998; Lough and Lucas 2006; Park et al. 2007; Kehr and Buhtz 2008; Guan and Zhao 2012; Warschefsky et al. 2016). In particular, plant small RNAs can move across graft unions and initiate epigenetic modifications in recipient cells (Molnar et al. 2010). Future research should determine the mobile biochemical/genetic signals of graft-transmissible PM-resistance in pepper.

Author contribution statement All authors conceived and designed laboratory experiments, RA and FL designed and performed provocation tests, RA, AK, AA and LK performed laboratory experiments, RA and LK wrote the paper.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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