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The role of glutathione in the symplast and apoplast in the PVY^{NTN} interplay with a potato host with different resistance levels to the virus

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INTRODUCTION & AIM

Plant viruses are very dengerous plant pathogens. One of the most impactus of plant viruses is Potato virus Y ^{NTN} (PVY^{NTN}). Therefore, searching the elements which could modulate pro- or antiviral response are crucial to invastigate. The glutathione as molecule could be a crucial cell molecule during plant–pathogen interplay response via its role in the direct control level of ROS also in host-plant virus interaction. The proper regulation of ROS creates a line between susceptibility (with high damage to cell components) and host resistance in interaction between plant and pathogen. Therefore, the aim of the study was to analyzed the role of glutathione forms (GSH/GSSG), as well as their cellular localization, in compatible and incompatible (HR reaction) potato cultivars infected with Potato virus Y ^{NTN} (PVY^{NTN}) during interaction in the symplast and apoplast with the use of microscopic and HPLC methods.

RESULTS

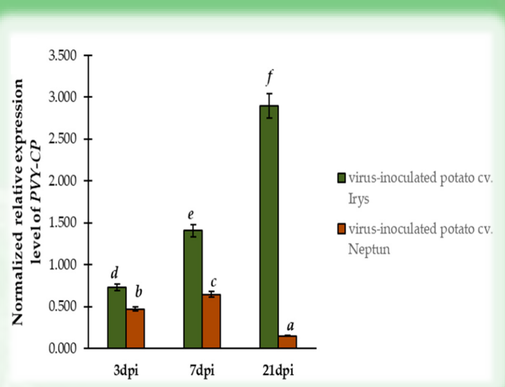

Figure 1. Normalized relative expression of PVY-CP calculated based on mean expression of StEf1α and Stsec3 reference genes. The statistical significance of differences was assessed at p < 0.05 using ANOV6A with post hoc Tukey's HSD (marked by letters above the bars).

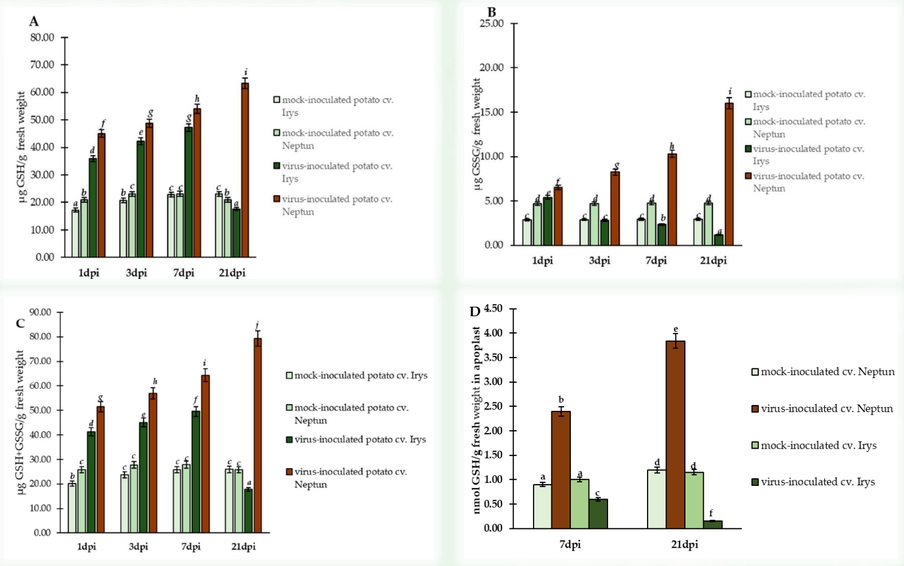

Figure 2. The mean concentration of reduced (GSH) and oxidized (GSSG) glutathione in the leaves of PVY^{NTN} and mock inoculated susceptible Irys potato and resistance Neptun potato plants. (C) The mean of summary concentration of reduced (GSH) and oxidized (GSSG) glutathione in the leaves of PVY^{NTN} and mock inoculated Irys and Neptun potato plants between 1 and 21 dpi. (D) The mean concentration of apoplastic GSH of PVY^{NTN} and mock inoculated susceptible Irys potato and resistance Neptun potato plants. Using ANOVA and Tukey's HSD test, the mean concentrations of GSH and GSSG were calculated at p < 0.05.6 The statistically significant values are marked by letters above the bars.

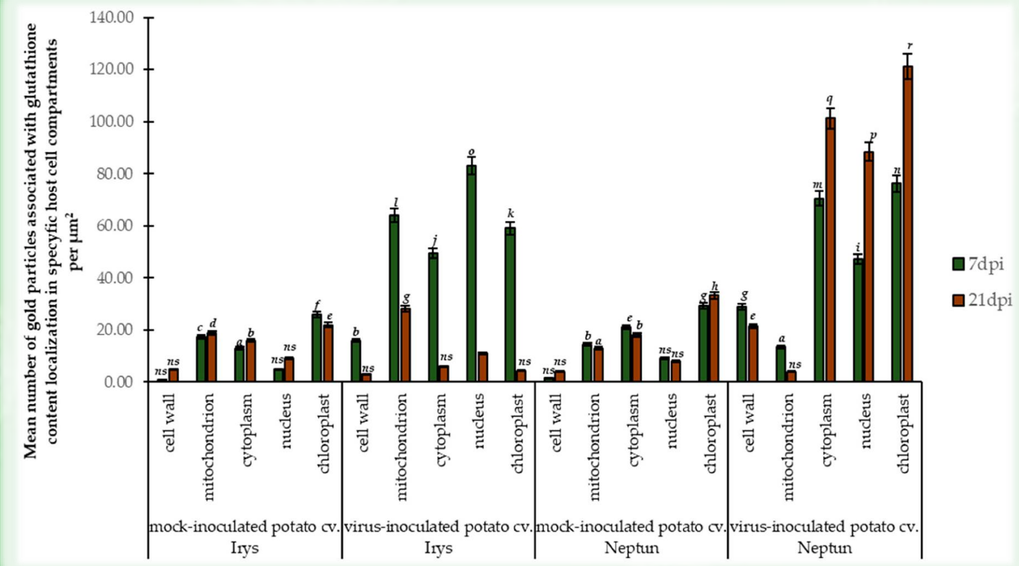

Figure 3. Quantification of immunogold labeling of glutathione content in mock and PVY inoculated Irys and Neptun potato leaves. The figure presents the mean number of gold particles localized in specific compartments per µm2 at 7 and 21 dpi in mock and virus inoculated leaves. Statistical validation of immunogold localization was performed using ANOVA. The mean values were calculated at p < 0.05 with post hoc Tukey's HSD test. Statistically significant values are marked with letters above the bars. Nonsignificant values are marked as ns.

We analysed the normalized expression of PVY^{NTN}-CP in susceptible- Irys and resistant- Neptun host plants based on two reference genes- StEf1α and Stsec3 (**Figure 1**). The PVY^{NTN}-CP expression in the Irys (susceptible) cultivar was increasing along with further post inoculation periods. In the virus inoculated Neptun (resistant) plants, PVY-CP expression significantly increased between 3 and 7 dpi, but between 7 and 21 dpi a sudden downregulation was observed (**Figure 1**). It suggested that in Neptun plants, the resistance reaction began from 7 dpi. We correlated this results with changes in concentration of various glutathione forms and total gluthatione in both cultivars at different stages of infection. The GSH levelwas chcked inside cell and in apoplast. At early stages, induction of GSH content has been observed in both cultivar's plants, compared to mock inoculated plants. In PVY^{NTN} inoculated Irys plants, the concentration of GSH increased to the highest level at 7 dpi and then downregulated. It indicated that after 7 dpi susceptible plants cannot overcome the oxidative stress. In Neptun plants the level of GSH was continuously increasing from 1 to 21 dpi. It shows that, Neptun plants better react to the oxidative stress. Changes in concentration of GSSG were different. In Irys potato plants, the concentration of GSSG decreased from 1 to 21 dpi, while in Neptun plants increased in the same period. It defines that infection development of PVY^{NTN} leads to the direct change in glutathione forms. Moreover, modulation of glutathione forms can cause significant changes in overall glutathione content (**Figure 2C**). In the susceptible Irys potato plants, the values of glutathione content in summary increased before 7 dpi and then significantly decreased. Differently, in virus inoculated Neptun plants the general concentration of glutathione rised between 1 and 21 dpi. The simmilar situation was observed in apoplast wheras level of GSH was also iduced during ongoing resistance reaction against PVY in Neptun plants (**Figure 2D**) and decreased in suceptible cultivar.

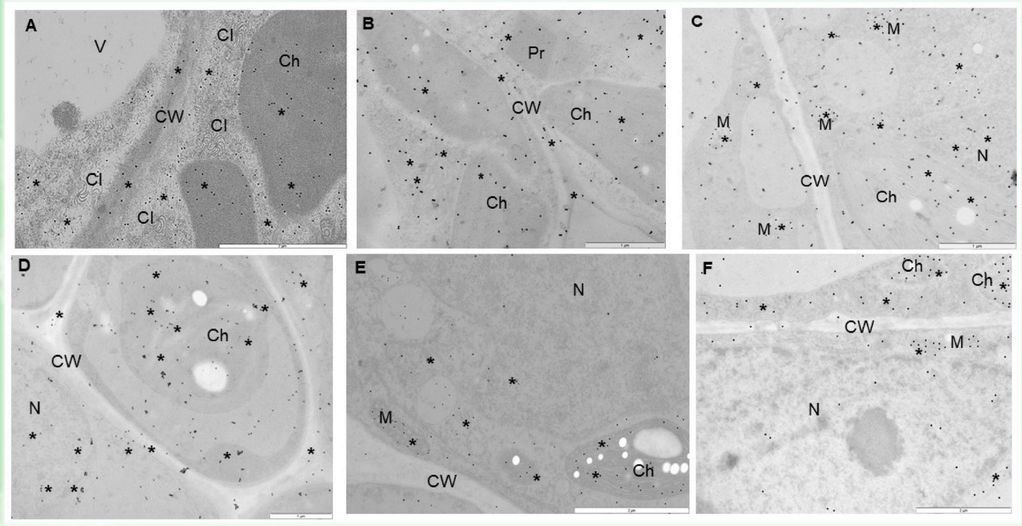

Figure 4. Immunogold labeling of glutathione content in the leaves of PVY and mock inoculated susceptible Irys (A,B,E) and Neptun (C,D,F) potato plants at 7 dpi. (A) Glutathione (*) in chloroplast (Ch) and cytoplasm in pallisade mesophyll cell. Virus cytoplasmic inclusions (CI) presented near the cell wall (CW). Bar = 2 µm. (B) Glutathione (*) in chloroplast (Ch) and cytoplasm in spongy mesophyll cell. Gold deposition also found in cell wall (CW) and peroxisomes (Pr). Bar = 1 µm. (C) Gold granules (*) indicated glutathione in mitochondria (M), chloroplast (Ch), and nucleus (N) in Neptun potato mesophyll cells. Bar = 1 µm. (D) Glutathione (*) in chloroplast (Ch) and nucleus (N) in Neptun potato phloem parenchyma cells. Gold granules also present in cell wall. Bar 1 = µm.(E) Glutathione localization in chloroplast (Ch), mitochondria (M), and cytoplasm in phloem of potato Irys mock inoculated cells. Bar = 2 µm. (F) Glutathione localization in chloroplast (Ch), mitochondria (M), and cytoplasm in the phloem of potato Neptun mock inoculated cells. Bar = 2 µm.

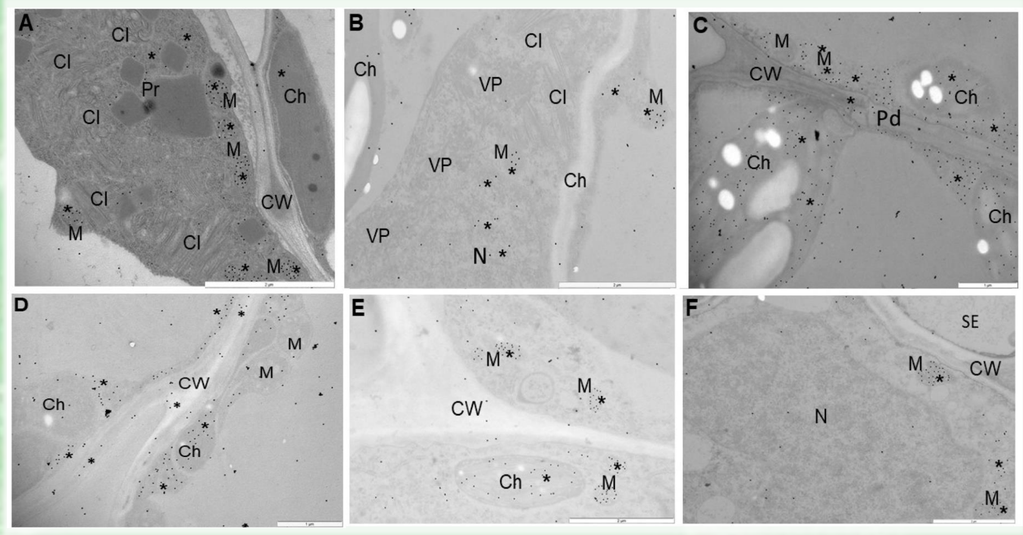

Figure 5. Immunogold labeling of glutathione content in the leaves of PVY and mock inoculated susceptible Irys (A,B,E) and Neptun (C,D,F) plants at 21 dpi. (A) Glutathione (*) in mitochondria in palisade mesophyll cells. Virus cytoplasmic inclusion (CI) present in cytoplasm. Bar = 2 µm. (B) Gold granules (*) indicated glutathione in mitochondria (M) and cytoplasm. Virus particles (VP) and cytoplasmic inclusion present in cytoplasm of spongy mesophyll cell. Bar = 2 µm. (C) Glutathione localization in cytoplasm, chloroplast (Ch), and mitochondria (M) in mesophyll cells. A few gold granules in cell wall around plasmodesmata (Pd). Bar = 1 µm. (D) Glutathione localization (*) in cytoplasm and chloroplast (Ch) in phloem parenchyma cells. A few gold granules in cell wall. Bar = 1 µm. (E) Glutathione localization (*) in mitochondria (M) and chloroplast (Ch) in phloem parenchyma cells of mock inoculated Irys potato. Bar = 2 µm. (F) Weak glutathione localization (*) in nucleus (N) and mitochondria (M) in phloem parenchyma cells of mock inoculated Neptun potato. Bar = 2 µm.

Changes in GSH and GSSG content measured using HPLC indicated that the leaf summary glutathione content and glutathione usage were elevated during HR to PVY^{NTN} in Neptun cultivars, especially from 7 dpi. However, this result did not confirm the subcellular redistribution of glutathione, which could be crucial in the reaction to PVY^{NTN} inoculation.Therefore, we performed validated (**Figure 3**) immunogold localization (**Figure 4A–F** and **Figure 5A–F**) to determine the exact localization of glutathione content in infected cells. Glutathione deposition was induced after virus inoculation in susceptible as well as resistant potato, but the deposition was lower in susceptible potato tissues. Moreover, analysis of localization in the mesophyll and vascular tissues of the susceptible Irys potato indicated that localization in nucleus, mitochondrion, and chloroplast at 7 dpi was higher, in comparison to mock inoculated plants (**Figure 3** and **Figure 4A,B,E**). Virus infection was also accompanied by the formation of virus cytoplasmic inclusions (**Figure 4A**), whereas, in the resistant Neptun potato, the induction in chloroplast, cytoplasm, and nucleus at 7 dpi was more intense in comparison to mock inoculated plants (**Figure 3** and **Figure 4C,D,F**). Moreover, in the resistant Neptun potato, the induction of glutathione deposition in the cell wall at 7 dpi was more intense than in susceptible Irys and mock inoculated tissues (**Figure 3** and **Figure 4D,E**). In the resistant Neptun potato, glutathione localization in mitochondrion at 7 dpi was at a similar level to mock inoculated tissues. Interestingly, glutathione distribution significantly changed in both susceptible and resistance potato plants at 21 dpi. In Irys plants, PVY infection was fully developed, causing an extreme decrease in glutathione localization. Virus particles and cytoplasmic inclusions were observed in mesophyll and vascular tissues (**Figure 5A,B**). The level of glutathione was statistically significant only, respectively, in the mitochondrion and nucleus at 21 dpi (**Figure 3** and **Figure 5A,B**). This suggested that, at further stages of infection, the susceptible cultivar could not precisely redistribute glutathione and protect the crucial cell compartments from oxidative stress. On the contrary, glutathione deposition was upregulated compared to the control and even compared with that observed at 7 dpi. Different localization patterns were noticed in the resistant Neptun potato than in the susceptible Irys potato at 21 dpi (**Figure 3** and **Figure 5C,D,F**). In the resistant Neptun potato, localization was most frequently noticed in the chloroplast, cytoplasm, and nucleus, and the most dynamic increase in glutathione deposition was noticed in the chloroplast. On the other hand, a decrease in localization was observed at 21 dpi only in the mitochondria and cell wall in the resistant Neptun potato.

CONCLUSION

Our observations indicated that glutathione is an important component of signaling as well as the regulatory network in the PVY^{NTN}–potato pathosystem. In resistance responses to PVY^{NTN}, this metabolite activates plant defenses by reducing potential damage to the host plant cell, causing a reduction in virus concentration, while it can also be involved in the development of PVY^{NTN} elicited symptoms, as well as limiting oxidative stress, leading to systemic infection in susceptible potato plants.