The role of photosynthetic activity in the regulation of flg22-induced local and systemic defence reaction in tomato

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Abstract

Flagellin (flg22) induces rapid and long-lasting defence responses. It may also affect the photosynthetic activity depending on several internal and external factors, such as the phytohormone ethylene or the day/night time. Based on the results, flg22 treatment, neither in the light phase nor in the evening, caused any significant change in chlorophyll fluorescence induction parameters in the leaves of wild-type and ethylene-receptor mutant Never ripe tomato plants measured the next morning. However, flg22 in the light phase decreased the effective quantum yield and the photochemical quenching both locally and systemically in guard cells. In parallel, the production of reactive oxygen species and nitric oxide increased, which contributed to the stomatal closure and a decrease in CO₂ assimilation the next day. A decrease in sugar content and elevated hexokinase activity measured after flg22 exposure can also contribute to local defence responses in intact tomato plants.

Keywords: assimilation; flagellin; mycotoxin; photosystem II; stomatal conductance.

Introduction

The presence or absence of light and the circadian clock mediate various molecular, biochemical, and physiological processes in living organisms such as the defence mechanisms (Chen et al. 2004, Roberts and Paul 2006, Ballaré 2014, Reddy and Rey 2014). The light-driven photosynthesis serves the generation of energy and reducing power not only under normal conditions but also contributes to the successful defence mechanism, e.g., by partitioning assimilates or by the production of chloroplastic reactive oxygen species (ROS) during the day (Dodd et al. 2005, Berger et al. 2007, Kangasjärvi et al. 2012). It can be crucial because the day/night time of

Highlights

- Flg22 decreased the effective quantum yield of PSII in tomato guard cells
- Flg22 induced local and systemic stomatal closure which was dependent on ethylene
- Hexokinase activity and expression of SlHXK3 were elevated locally by flg22

Abbreviations: ABA – abscisic acid; Chl – chlorophyll; ET – ethylene; Flg22 – flagellin 22; Fₐ – minimal fluorescence yield in dark-adapted state; Fₐ/Fₘ – maximal fluorescence yield in dark-adapted state; FM – fresh mass; Fₐ/Fₘ – maximum quantum yield of PSII; HXK – hexokinase; JA – jasmonic acid; NO – nitric oxide; NPQ – nonphotochemical quenching; Nr – Never ripe; PAM – pulse amplitude modulation; Pₖ – net photosynthetic rate; qₚ – photochemical quenching coefficient; ROS – reactive oxygen species; SA – salicylic acid; WT – wild type; Yₑ – effective quantum yield of PSII photochemistry.

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The infection can determine the outcome of the successful defence reaction of plants. Interestingly, reduced and delayed defence reaction (Krupinski et al. 2003) and lesion formation were found in response to avirulent pathogens in the dark (Zetter et al. 2004, Grieben and Zetter 2008). The dynamics of the energy-producing processes during the day and especially in the dark phase could be very significant (Poort et al. 2021). In plants, the degradation of photosynthetic products such as starch, which provides an entry site to pathogens (Melotto et al. 2003), and the maintenance of carbohydrate availability until the next anticipated dawn and sustain plant productivity (Gral et al. 2016, Lu et al. 2017). In addition, other photosynthetic products, the soluble sugars (glucose, fructose, and sucrose), as well as ROS interacting with defence-related photosynthetic enzymes, such as lactic acid (SA) and ethylene (ET), play an important role in regulating stress responses in complex- and daytime-dependent manner (Coose et al. 2006, Rosa et al. 2009, Wind et al. 2010, Ballaré 2014). The photosynthetic activity of mesophyll cells and the photosynthesis of guard cells in the epidermis play a crucial role in the regulation of defence against various bacterial pests (Lawson 2009). It is well known that stomata serve the transpiration and CO2 accumulation for plants but also provide an entry site to pathogens (Malloiu et al. 2017). The regulation of stomatal pore size is under strong light and circadian control (Chen et al. 2012). Blue light stimulates the opening of stomates and together with red light facilitates transpiration and CO2 uptake for photosynthetic CO2 fixation during the light period in C3 plants (Nakamura et al. 2020). During the dark periods, stomata are closed, providing the first line of defence against several pathogens, which are mostly infective in the dark (Roberts and Paul 2006). At the same time, long-term defence responses can be regulated in a reaction mixture containing 2 mM Ca(NO3)2 (Broekgaarden et al. 2015) by promoting local (Zhang et al. 2021) and systemic stomatal closure (Czékus et al. 2021a), regulating photosynthesis (Müller and Murchie 2019) by the main components of the circadian clock (Bosch et al. 2012). Based on these findings, understanding the role of ET in local and systemic responses of plants to pathogens is of high importance. However, it is not yet known how photoperiodic signals are transduced to increase photosynthesis from the stomata (Korneli 2012). The light phase (Devireddy et al. 2020). This stomatal closure as a part of the systemic phase upon stress orather pathogen infection is also a crucial step of systemic signalling under high-resolution digital camera (Zeiss Axiowert 200 M). The fluorescence intensity of ROS and NO production was measured by using a photoelectronically at 630 nm (Konstru, Milano, Italy) after reaction with anthrone using glucose (Normapar, VWR Int., Leuven, Belgium) dissolved in 80% ethanol as a standard. Three samples from four different plants were measured in the case of all treatments which were repeated three times (t = 3). Means ± SE were calculated based on all data of the three biological repetitions. The fluorescence intensity of ROS and NO production was measured by using Axiowision Rel. 4.8 (Carl Zeiss) and analyzed using a standard. Three samples from four different plants were measured in the case of all treatments which were repeated three times (t = 3). Means ± SE were calculated based on all data of the three biological repetitions. The fluorescence intensity of ROS and NO production was measured by using Axiowision Rel. 4.8 (Carl Zeiss) and analyzed using a standard. Three samples from four different plants were measured in the case of all treatments which were repeated three times (t = 3). Means ± SE were calculated based on all data of the three biological repetitions. The fluorescence intensity of ROS and NO production was measured by using Axiowision Rel. 4.8 (Carl Zeiss) and analyzed using a standard. Three samples from four different plants were measured in the case of all treatments which were repeated three times (t = 3). Means ± SE were calculated based on all data of the three biological repetitions.
In the elicitor-treated WT leaves (Fig. 6A). In addition, relative transcript levels of SlHXK3 decreased in Nr leaves (Fig. 6D). In other cases, the gene expression of tomato HXKs did not change significantly in either WT or Nr leaves upon flg22 in the next light phase (Fig. 6E).

Discussion

Chloroplasts not only play a crucial role in photosynthesis but also the synthesis of several phytohormones and the generation of ROS. Thus, chloroplasts significantly contribute to the successful defence responses of plants locally and systemically, respectively (Lindbo et al. 2021). Pathogen infection alters the normal molecular and physiological processes in the host plants influencing the photosynthetic activity which is vital for plants (Kuznicki and Kopczewski 2020). In this work, flg22 was used to study the long-term daytime- and ET-dependent effects of bacterial pathogens in intact leaves of tomato plants focusing on the local and systemic effects of the elicitor and the role of photosynthesis in this process.

Bacteria- and flg22-induced signalling and plant defence responses are also highly dependent on the presence of the light during day/night time (Zeier et al. 2004, Griebel and Zeier 2008, Sanò et al. 2014). Two closest time points were selected for the treatments in the late light and early dark period of the day (17:00 and 21:00 h) based on our previous work (Czekus et al. 2021b) to distinguish the direct effect of external light/darkness from the internal effect of circadian rhythm on plants and measurements were accomplished in the next light phase at 09:00 h. These mimic the natural environmental conditions and make it possible to compare and describe plant defence responses under natural light/dark conditions instead of artificial darkening. Natural light/dark conditions have crucial importance from the aspect of defence as most of the plant bacteria are more active at night (Santamaría-Hernando et al. 2018). At 17:00 h, stomata are open and the accumulation of photoassimilates in usually finished (Lawson 2009). At 21:00 h (3 h after the end of the light period), the light-dependent processes of photosynthesis and active phytochrome signalling are already inactivated (Graf et al. 2010, Medrzyńska et al. 2013). At the same time, these time points for treatments were chosen to each other providing almost the same availability of carbohydrates and starch for metabolic processes.
and photosynthesis is active at this time point (Czékus et al. 2020).

In our previous work, it was found that H₂O₂ contents increased locally within 30 min by flg22, and superoxide production was significantly higher in systemic leaves of WT tomato plants after 1 h in the light period of the day promoting the rapid stomatal closure in both leaf levels (Czékus et al. 2021b). At the same time, not only ROS but also ET, JA, and SA accumulation was observed after 1 h in flg22-treated WT tomato leaves while such changes were not detected in Nr plants and in the case of the night treatments at 21:00 h (Czékus et al. 2021b). These rapid changes in leaves can determine the long-lasting defence responses of plants. Nevertheless, long-term- and day/night-time-dependent effects of flg22 on physiological responses of intact plants in the morning of the next day have not been investigated. In this context, the role of photosynthesis has neither been examined thus we focused on that in this manuscript. Changes in photosynthesis are crucial under pathogenesis because it serves energy and reducing power to the successful defence process of plants (Dodd et al. 2005, Berger et al. 2007). At the same time, pathogens can suppress photosynthesis and the photosynthesis-related gene expression as was observed in the case of Pseudomonas syringae (Bonfig et al. 2006) or Xanthomonas oryzae infection (Yu et al. 2014). In addition, the presence or absence of light highly influences photoinhibition and photodegradation in the infected leaves. It was found that the damage of the photosynthetic apparatus was greater in the dark after the 3-d-long P. syringae infection in tobacco leaves (Cheng et al. 2016). However, more rapid changes (30 min) were observed in photosynthesis-related genes and the rapid induction of various phytohormone-mediated signalling components were observed after flg22 treatments in the light or dark. Similar results were observed after the application of photosynthesis-inhibiting inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) suggesting that photosynthesis plays a role in controlling the light-dependent expression of flg22-inducible defence genes (Batto et al. 2014). Göhre et al. (2012) investigated firstly the short- and long-term effects of flg22 on the photosynthetic activity using Arabidopsis seedlings grown in liquid media. A rapid and significant decrease in the NPQ was
observed already after 20 min, reached a minimum after 1 h but then recovered after 4 h despite the constant presence of flg22 in the liquid media (Göhre et al. 2012). In parallel, flg22 induced high ROS production with the maximum after 20 min (Göhre et al. 2012). Interestingly, the authors detected the long-term adaptation to flg22 in Arabidopsis seedling and found that V_{md} significantly decreased while NPOQ significantly increased after 7 d upon flg22 (Göhre et al. 2012). These results suggested that flg22 has long-term effects on the photosynthetic activity of seedlings mediating plant defence responses but the day/night-time or systemic effects of flg22 treatments were not investigated. We first measured the effects of different day/night-time applications of flg22 on the photosynthetic activity of various leaves of intact tomato plants in the morning of the next light period. Surprisingly, flg22 treatments did not induce any significant changes in the photosynthetic activity of mesophyll cells based on the analysis of Chl fluorescence parameters. This suggests that the PSII activity of mesophyll is less sensitive or later affected by the effects of flg22 in leaves of intact and developed plants as compared to the plants exposed to the constant presence of flg22 (Göhre et al. 2012).

There are some important differences between chloroplasts of guard cells and palisade mesophyll cells such as the lower number and size of chloroplasts in guard cells as compared to the palisade mesophyll cells which can affect their photosynthetic activity. Other important differences can be found in the case of starch metabolism of guard cell chloroplasts influencing the stomatal movement during the day (Lawson 2009). At the same time, guard cells localized in the epidermis are exposed to flg22 first, thus the effects of flg22 on the photosynthetic activity of guard cells could be very significant in determining the long-lasting stomatal closure as a part of the defence responses of plants to the pathogen attack which resulted in the significantly decreased Y_{md} and q_{p} in guard cells of local and systemic leaves of WT plants treated in the light period (17:00 h). In parallel, NPOQ increased only slightly in the guard cells of these plants. This suggests that stomatal photosynthetic activity is much more sensitive to flg22 locally and systemically which can contribute to stomatal closure and the limitation of CO2 assimilation at the whole plant level systemically, as well as to the inhibition of pathogen entry in the morning of the next day. These effects of flg22 on stomatal photosynthetic activity can be dependent due to the rapid ROS production within minutes mediated by NADPH oxidase (Zhang et al. 2008, Ranft et al. 2011, Thor et al. 2017) and later to the ROS production by chloroplasts in a self-amplifying loop (Kangasjärvi et al. 2012) which contributed to the rapid stomatal closure and which cannot be detected in the dark (Czékus et al. 2021b). At the same time, these changes in the photosynthetic parameters were neither observed in the case of the evening treatment in the dark nor in WT leaves suggesting the daytime (light)- and ET-dependent effects of flg22 on stomatal photosynthesis. Based on these results daytime (light) and ET can play a role in the regulation of the early detection of flg22 by FSLS2 and the rapid signaling of flg22 mediated by ROS (Mersmann et al. 2010, Czékus et al. 2021b) in the absence of which there is no long-term effect of flg22 on the photosynthetic activity. This is in a good correlation with the results of Borbély et al. (2019) who found that ET in a time- and concentration-dependent manner can induce a rapid production of superoxide and H2O2 in the leaves and parallely can decrease Y_{md} and increase NPOQ. Moreover, ET production, sensing, and signalling are not only highly dependent on the developmental stages of plants but also on the light (Harkey et al. 2019). Our previous result also confirmed that flg22 induced rapid ET production and signalling only during the day which was not detectable at night in the dark (Czékus et al. 2021b). These differences between the two-time points confirm the long-term effects of flg22 in the next light phase at 9:00 h.

Treatment with flg22 at 17:00 h not only induced significant changes in the photosynthetic activity of stomata but also induced high stomatal ROS production which can promote chloroplastic ROS generation in a self-amplifying loop contributing to the inhibition of photosynthetic activity (Kangasjärvi et al. 2012) and thus maintaining the long-lasting and systemic stomatal closure in the next treatments. Moreover, flg22 exposure induced significantly high NO production in the guard cells of the flg22-treated leaves in the light period which was not detected in guard cells of WT leaves. At the same time, NO plants accumulate higher HXK as compared to WT plants whose NO contents were not influenced by flg22, thus suggesting a potential role of ET in this process in the regulation of increased ROS and NO production by flg22 in guard cells promoted the activation of plasma membrane-localized Ca2+ channels and subsequently of the SLAC1 anion channel and aquaporin PIP2;1 in guard cells contributing to stomatal closure after the bacterial elicitor treatments locally in the light phase of the day (Zhang et al. 2008, Ranft et al. 2011, Thor and Peiter 2014, Deges et al. 2015, Toun et al. 2015, Rodrigues et al. 2017). However, this investigation confirmed the long-term effects of flg22 on stomata of intact plants in the next light phase. Mersmann et al. (2010) found that flg22 did not induce stomatal closure mediated by ROS in ET-insensitive Arabidopsis mutants. Here, we measured also the absence of stomatal closure in the case of ET-receptor mutant Nr plants whose closure depended on the ET-light- and ET-dependent ROS and NO production. At the same time, flg22 did not induce ET production and ET signalling at night in the dark (Czékus et al. 2021b) which can also contribute to the lack of ROS wave in flg22-treated plants at this time and the inhibition of stomatal closure in the next morning. Stomatal closure induced by flg22 at 9:00 h could contribute to the significant decrease in the net photosynthetic rate of tomato leaves which was measured by us. This decrease in CO2 assimilation can negatively influence the Calvin cycle and sugar biosynthesis resulting in the reduction of biomass production. Göhre et al. (2012) observed also growth inhibition and biomass reduction upon 7-d-long flg22 exposure in Arabidopsis. This process is also dependent on the light-dark phase and the active ET signalling mediated by ROS/NO production based on our measurements.

Recently, it was found that plant HXKs not only play a role in sugar metabolism (Graon et al. 2013) or in the regulation of stomatal closure (Shen et al. 2021) or in mitochondrial cell death by regulating ROS (Point et al. 2019) but also influence plant immune reactions via responding to glucose (Ving et al. 2020). Sugars such as glucose not only play a role as an energy source but also as a signalling molecule interacting with phytohormones such as ET (Steen 2014, Li and Shen 2016). Reduction in disease symptoms of Pseudomonas syringae was found in the hek1 Arabidopsis mutant, indicating that HXKs play role in plant immune responses (Ving et al. 2020). We measured that flg22 treatment in the light phase of the day elevated HXK activity and the expression of mitochondrial SSX3 in the treated leaves of WT tomato plants, but changes were not detected systematically in or Nr leaves. Our results suggest that a decrease in sugar content and elevated HXK activity in a light and ET-dependent manner could contribute to local stomatal closure and the activation of defence in intact tomato plants.

In conclusion, flg22 is responsible for the induction of rapid and long-term defence reactions in plants but its effects on photosynthesis are less studied especially in the case of stomata. Based on our results, it can be concluded that flg22 treatment neither in the light phase nor in the evening caused any significant change in chlorophyll fluorescence induction parameters of tomato plants measured in the next morning at the beginning of the next light cycle. However, treatment in the light phase of the day increased ET production and the photochemical quenching (q_p) both locally and systemically in guard cells of epidermal peels prepared from the flg22-treated leaves. In parallel, the production of ROS and NO increased in these guard cells, which may have contributed to the next day’s long-term stomatal closure and a decrease in CO2 assimilation in these plants. These processes can be dependent on the daytime (light) and ET, which take part in the regulation of flg22 sensing and signalling via the rapid ROS and NO production in guard cells. Our results demonstrated also that flg22-induced ET- and light-dependent decrease in sugar content and elevated ET activity could contribute to local defence responses in intact tomato plants.

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