Different roles of Ca^{2+} and chitohexose in peanut (*Arachis Hypogaea*) photosynthetic responses to PAMP- Immunity (#90414)

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Different roles of Ca^{2+} and chitohexose in peanut (*Arachis Hypogaea*) photosynthetic responses to PAMP- Immunity

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Background: ~~There are recognition receptors on the surface of plant cells that stimulate immune responses~~. The conserved components of pathogenic microorganisms that can be recognized by such receptors are called pathology-related molecular patterns, e.g. flagellin 22 (FLG22) and chitohexose. This immune triggering pattern is relatively conservative and is the result of long-term plant evolution. Leaf disease is a common disease of peanut in late growth period. It has been widely accepted that Ca^{2+} signal transduction pathway involved into almost all plant physiological processes. Meanwhile, the regulation of photosynthesis by immune pathways of triggered by PAMPs in peanuts remains unclear. Methods: In the present study, we try to assess the role of PAMPs in peanut photosynthesis, especially, the interaction between PAMPs and Ca^{2+} signal transduction pathway. **Results:**Both FLG22 and chitohexose significantly promoted the expression of PR-4 and PR-10 genes, and Ca^{2+} enhanced the expression of these two genes. Ca^{2+} is involved in the downregulation of the PSII reaction center activity induced by FLG22 immune response, but the role of chitohexose is not obvious. Additionally, Ca^{2+} significantly reduced non-photochemical energy dissipation in FLG22 and chitohexose - induced immune response. **Conclusion:** These results indicated that both FLG22 and chitohexose can trigger peanut immune pathways through Ca^{2+} signal transduction, but their regulation of PSII reaction center activity is different.

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Abstract

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Methods: In the present study, we ~~try to assess~~ the role of PAMPs in peanut photosynthesis, especially, the interaction between PAMPs and Ca^{2+} signal transduction pathway.

Results: Both FLG22 and chitohexose significantly promoted the expression of PR-4 and PR-10 genes, and Ca^{2+} enhanced the expression of these two genes. Ca^{2+} is involved in the downregulation of the PSⅡ reaction center activity induced by FLG22 immune response, but the role of chitohexose is not obvious. Additionally, Ca^{2+} significantly reduced non-photochemical energy dissipation in FLG22 and chitohexose -induced immune response.

Conclusion: These results indicated that both FLG22 and chitohexose can trigger peanut immune pathways through Ca^{2+} signal transduction, but their regulation of PSⅡ reaction center activity is different.

Keywords: Peanut, PAMP, Photosynthesis, ROS, Ca^{2+} signal transduction pathway

Introduction

Peanut (*Arachis Hypogaea*) is an important oil crop in China, even in the world. Besides being used for oil and food, peanut can be used as raw materials for poultry and aquatic feed processing since the by-products of peanut processing are rich in protein.

In natural environments, plants inevitably expose to all kinds of microorganisms, especially disease-causing microbes, which will infect plants to achieve their own growth and reproduction. When plants are infected, the aging and shedding of leaves will be accelerated. In order to prevent the further spread of pathogenic microorganisms, the plants themselves will cause the rapid programmed death of cells around the infection site. This phenomenon is called hypersensitivity, which is a common mechanism of plant immune response. Plants recognize the essential components of microbial conservation through pattern recognition receptors (PRR) to quickly start the immune pathway (*Lu et al., 2010*). The pathogenic microbial conservation components that can be recognized by this receptor are called pathogen-associated molecular patterns (PAMP), and then release a variety of signaling molecules, such as calcium ions (Ca^{2+}), reactive oxygen species (ROS) and various plant hormones. This mechanism evolved from the long-term confrontation between pathogenic microorganisms and plants, so the immunity triggered by pathogen-related molecular patterns is the key to our study of plant immunity (*Jie et al., 2007*).

Plants use light energy to synthesize CO_2 and H_2O into organic matter in chloroplasts and release oxygen, which is known as photosynthesis. Photosynthesis is divided into light reaction and dark reaction. The light reaction is mainly involved in Photosystem Ⅱ (PSⅡ) and Photosystem I (PSI) proteins to convert light energy into chemical energy, while the dark reaction uses the energy and substances generated by the light reaction to convert CO_2 into organic substances, which is the Calvin cycle. Plant photosynthesis is responsible for most of the production of oxygen and the fixation of biomass on Earth (*Hankamer et al., 1997*). Therefore, when plants are stressed, their cells will devote a lot of energy to resist stress. In this case, the physiological and biochemical indexes of plants will inevitably decrease, including those of photosynthesis.

Flagella is the motor organ of bacteria, which enables bacteria to respond to stimuli (*Hajam et al., 2017*). Flagellin is derived from the conserved N-terminal or C-terminal of the flagella of

various bacteria. Animal and plant cells are able to recognize flagellin and respond to bacterial infections in advance. In 1999, *Felix et al.,* purified flagellin from *Pseudomonas syringae* and synthesized a highly conserved amino acid residue sequence at the N-terminal as a stimulus to treat plant cells (*Felix et al., 1999*). They found that plant cells could rapidly produce reactive oxygen species and other substances, and identified FLG22 as a key stimulus factor for some plants to recognize bacteria and produce immune substances (*Felix et al., 1999*). Gómez-Gómez et al., further identified the receptor FLS2 with a transmembrane domain involved in the recognition of FLG22 by screening Arabidopsis mutants (*Gómez and Boller 2000*), while FLG22 recognizes its extracellular domain (*Dunning et al., 2007*). Besides FLG22, chitosan has been widely used in agriculture to increase the ability of plants to resist stress. Although some studies have found that chitosan can induce plant cells to produce a defense response, and even cause programmed cell death, more studies believe that chitosan can stimulate a series of defense responses and enhance plant resistance to stress (*Katiyar et al., 2015; Jia et al., 2019; Khan et al., 2003*).

Environmental signals trigger rapid and transient increases in cytosolic Ca^{2+}. The alteration in the level of Ca^{2+} triggers a full range of signal transduction pathways involved in many physiological and biochemical processes in response to abiotic and biotic stresses in plants (*Bowler and Fluhr, 2000; Schreiber et al., 1994*). Our previous studies have shown that Ca^{2+} signaling pathway plays a regulatory role in photosynthesis, regulating the turnover of PSⅡ reactive center protein components and non-photochemical quenching of chlorophyll fluorescence (NPQ) (*Brunner, 2002*). It is well known that there are many common signaling molecules in the immune and photosynthetic pathways of plants, such as Ca^{2+} and ROS. In this study, FLG22 and chitohexose, were used to study the induction of immune response in peanut leaves as well as photosynthesis. The results showed that both FLG22 and chitohexose could improve the immune function of peanut by inhibiting the photo-cooperation of peanut leaves to improve the disease resistance of peanut plants. In short time, Ca^{2+} synergies FLG22 and chitohexose to promote the inhibition of photosynthesis and enhance FLG22 and chitohexose as enhancers of plant immune response.

Materials and Methods

Plant Materials, Growth Conditions and Treatments

Huayu 25, a peanut (*Arachis Hypogaea*) cultivar variety, was used as material in this study, which was cultured in small plastic pots containing quartz sand. The upper diameter of the pot is 9 cm, the lower diameter is 6.5 cm and the height is 8 cm. The seedlings incubated without Ca^{2+} were marked as NC, and those incubated with Hoagland nutrient solution were marked as CA. The plants were grown at 25/20°C (day/night) under a 14 h photoperiod [300 μmol m^{-2} s^{-1} photon flux density (PFD)] for 20 d in a greenhouse. Calcium nitrate tetrahydrate was completely removed from Hoagland nutrient solution and balanced nitrogen with ammonium bicarbonate in NC. After about 20 days of cultivation, peanut functional leaves were taken and placed in a petri dish with deionized water for dark adaptation 4 h. The initial data of CA and NC groups were measured, and then incubated with 1 μM FLG22 and 200 g ml^{-1} chitohexose for 1 h, 2 h and 4 h, respectively.

Chloroplast fluorescence measurement

Determination of Chlorophyll fluorescence with a portable fluorometer (FMS2, Hansatech, UK) according to the protocol described (*Zargar et al., 2015*). A Handy PEA was used to measure photosynthetic parameters after dark adaptation. Photosynthetic parameters were calculated with reference to Strasser (*Zargar et al., 2015)*. After fully dark adaptation of isolated leaves in each group, $PI_{(abs)}$, a performance index based on absorbed light energy, was measured in the whole process by a continuous excitation fluorescence analyzer (Handy PEA), which was attached to the leaf clip on the probe. NPQ was estimated as NPQ = Fm/Fm'^{-1} according to (*Yang et al., 2015b*), where Fm was measured after dark adaptation for more than 2 h at room temperature prior to stress, Fm′ is the maximum intensity of fluorescence in light-acclimated leaves.

Determination of ROS

Hydrogen peroxide (H_2O_2) concentration was measured according to the method of (*Yang et al., 2015a*) with modifications. The leaf samples (0.5 g) were homogenized with 3 ml phosphate buffer (50 mM, pH 6.8). The homogenate was centrifuged at 6 000 × *g* for 25 min. Extracted solution (3 ml) was mixed with 1 ml of 0.1% titanium sulfate in 20% (v/v) H_2SO_4 and the mixture was then centrifuged at 6 000 × *g* for 15 min. The intensity of the yellow supernatant

was measured at 410 nm. H_2O_2 level was calculated using an extinction coefficient of 0.28 $\mu mol^{-1}\ cm^{-1}$ according to the standard curve plotted with known H_2O_2 concentration.

The assay for superoxide anion ($O_2^{\bullet-}$) was performed as described by (*Yang et al., 2015a*). Fresh leaves without midrib were thoroughly ground in an ice bath in a grinding medium containing 0.05 M phosphate buffer (pH 7.8). The homogenate was centrifuged at 5 000 × *g* for 10 min at 4°C. The supernatant with phosphate buffer (pH 7.8) and 10 mM hydroxylammonium chloride was incubated at 25°C for 20 min, then 17 mM p-aminobenzene sulfonic acid and 7 mM α-naphthylamine were added, and the mixture was incubated at 25°C for 20 min. Finally, ethyl ether was added into the mixture that was centrifuged at 1 500 × *g* for 5 min. The water phase was used to determine the absorbance at 530 nm. The $O_2^{\bullet-}$ generation was calculated per g fresh mass of leaves.

The total RNA extraction and Real Time PCR

Total RNA was extracted from the peanut leaves with the RNA simple kits (TIANGEN BIOTECH, China) according to the manufacture's protocol. cDNAs were reverse transcribed using the PrimeScript™ first-strand cDNA synthesis kit (K1622 Thermo scientific). The PCR was amplified following the instruction of SYBR *Premix Ex Taq*™ (TaKaRa, Inc., Dalian, China) with the qRT-PCR amplification instrument (ABI 7500, USA). The *TUA5* gene was used as control to calculate the relative expression level.

The sequences of *PR-4*, *PR-10*, *PsbO* and *PsbP* genes were obtained from NCBI, and the accession numbers were XM_025747762.2, EU661964.1, XM_025833053, XM_025809804, respectively. Primer sequences were as follows: *TUA5*-F (5'-3'): CTGATGTCGCTGTGCTCTTGG; *TUA5* –R (5'-3'): CTGTTGAGGTTGGTGTAGGTAGG; *PR-4* F (5'-3'): ACTGCTTTCTGTGGGCCTGTTG; *PR-4* R (5'-3'): GCCCTCCATTGCTGCACTGATC; *PR-10* F (5'-3'): TTGAGGGAAACGGTGGTCCT; *PR-10* R (5'-3'): GAGCCACTCCTCCAACAACG.

Statistical Analysis

Statistical significance between groups was evaluated with one-way analysis of variance (ANOVA) followed by Duncan's multiple range test in SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA). The mean ± standard error was calculated from three biological replicates per

177 treatment group for each assay. Differences were considered statistically significantly at $p < 0.05$.
178

179 ## Results

180 **FLG22 and chitohexose induced PR gene expression**

181 PAMPs or pathogenic microorganisms can trigger immune response in plants. Not only ROS-
182 mediated signaling but also mitogen-activated protein kinase (MAPK) is activated upon FLG22
183 to regulate the change in the expression of pathogenic related gene (PR) (*Tornero et al., 1997*).
184 In the present study, *PR-4* and *PR-10* were selected to assess the effects of FLG22 and
185 chitohexose. *PR-4* is associated with chitinase and has antifungal activity, *PR-10* gene family
186 encodes small proteins with cytoplasmic localization, which play roles in RNA enzymes and
187 post-translational modifications and respond to both non-biological and biological factors (*Kaku*
188 *et al., 2006*). Chitohexose belongs to chitohexose fragments, and chitohexose oligosaccharide is
189 a highly effective plant defense stimulator. Both FLG22 and chitohexose significantly promoted
190 the expression of *PR-4* (Fig. 1a, 1c) and *PR-10* genes (Fig. 1b, 1c), and the increase in their
191 expression level was more evident at the presence of Ca^{2+} (Fig. 1) suggesting that peanut can
192 respond to plant immune regulation positively, and Ca^{2+} signal transduction pathway is involved
193 into the immune response caused by FLG22 and chitohexose.
194

195 **Effects of FLG22 and chitohexose on the activity of photosystem reaction center**

196 $PI_{(abs)}$ was an important parameter which could comprehensively reflect the density, absorption
197 and electron transfer of PSⅡ optical reaction center. When treated with FLG22, $PI_{(abs)}$ of CA
198 group decreased and then remained unchanged, while that of NC group increased slightly and
199 then kept unchanged, compared with that of untreated samples, the $PI_{(abs)}$ of CA group was lower
200 while that of NC group was higher (Table 1). $PI_{(abs)}$ of NC group did not change when treated
201 with chitohexose, and $PI_{(abs)}$ of CA group decreased at first and almost returned to the initial
202 level after 4 hours of treatment (Table 1). It seems that the immune response triggered by FLG22
203 and chitohexose can reduce the number of reaction centers and the performance index of leaf
204 light absorption. And the decrease of the number of reaction centers would cause the decrease of
205 the maximum light conversion efficiency and photosynthetic performance index, and then reduce

the photosynthesis of plants, while the effects of chitohexose were not obvious. Since Ca^{2+} has a positive response to FLG22 but chitohexose triggered immune response and can mobilize resources for the immune pathway more quickly, the density of response centers with Ca^{2+} involvement is significantly lower than that without Ca^{2+}, and the Ca^{2+} signal transduction pathway reduces the reducing power of PSⅡ reaction centers, thus improving the protective role of plants.

FLG22 and chitohexose induced CP12 and PsbS gene expression

PsbS encodes one of PSⅡ protein component which is related to NPQ. Under FLG22 treatment, the expression of *PsbS* gene showed an up-regulated trend in NC group, however, it showed a down-regulated trend in CA group, even it had a short increase at first 1h treatment, and the expression level of CA group was significantly lower than that in NC group (Fig. 2a). When treated with chitohexose, the expression trend of *PsbS* showed down-regulated trends in both CA group and NC group with no obvious difference between CA group and NC group (Fig. 2c).

CP12 participated in Calvin cycle which was localized in chloroplastid. The expressions of *CP12* were detected when treated with FLG22 and chitohexose, respectively. The expression of *CP12* showed a down-regulated trend in CA group, even it slowly up-regulated at the end of the treatment which was not reach the initial level. The expression showed an up-regulated trend in NC group, and its expression level is obviously higher than that in CA group (Fig. 2b). When treated with chitohexose, the expression of *CP12* showed a down-regulated trend in both NC group and CA group, even it had a short up-regulation in first 2 h in CA group. During the whole treatment, its expression level showed higher in CA group than that in NC group (Fig. 2d). These results suggested that FLG22 induced the expression of *CP12*, while Ca^{2+} prevented this progress and become less effective. After treated with chitohexose, the expression of *CP12* was restrained, although Ca^{2+} can only slow this process.

Influence of immune response triggered by FLG22 and chitohexose on energy dissipation

During the process of plant immunity, the immune response was more efficient when the plants

activated the inhibited photosynthesis through MAPK cascade signal. The captured light energy was mainly consumed through three pathways including photochemical electron transfer, chlorophyll fluorescence emission and heat dissipation during photosynthesis. Among them, photochemical electron transport was related to the synthesis of photosynthate, and chlorophyll fluorescence emission was only a small part of light energy consumption, and the excess energy would cause damage to PSⅡ through the photooxidation induced by the accumulation of ROS. Therefore, heat dissipation was an important way to consume excess light energy and prevent photodamage. To excess excitation energy as heat dissipation in a harmless way was called NPQ. Violaxanthin de-epoxidase (*VDE*) encodes Violaxanthin de-epoxidase, which interacted with *PsbS* to participate the composition of NPQ. The expression of *VDE* was continued down-regulated till 2 h after treated with FLG22, and slightly up-regulated at 4 h in both NC and CA groups, and the expression of *VDE* showed similar in CA group and NC group, while at 4 h, the *VDE* expression was significantly lower in CA group comparing to NC group (Fig. 3a). Meanwhile, the *VDE* expressed up-regulated at 1 h and 2 h but down-regulated at 4 h after treaded with chitohexose in CA group while only up-regulated at 1 h and down-regulated at both 2 h and 4 h in NC group (Fig. 3c). These results suggested that chitohexose could induced up-regulation of *VDE* comparing with FLG22, and Ca^{2+} promoted the regulation of the expression of *VDE*.

NPQ can reflect excess energy dissipation in plants and is related to PsbS protein and the xanthophyll cycle. As shown in Fig. 3, when treated with FLG22, the NPQ of both CA group and NC group significantly decreased, while the NPQ of NC group was higher than that in CA group. And, when treated with chitohexose, NPQ of both CA and NC groups showed a downward trend, and NPQ in CA group was significantly lower than that in NC group (Fig. 3b, 3d). These results suggested that both FLG22 and chitohexose induced immune responses can reduce energy dissipation in peanut leaves, and Ca^{2+} is involved in reducing non-photochemical energy dissipation in the immune response process stimulated by both of them.

Changes in the content of ROS induced by FLG22 and chitohexose

ROS plays an important role in various physiological processes such as immune response,

development, cell elongation and hormone signaling in plants (*Wan et al., 2008*). When exposure to Ca^{2+}, plant immune regulation is often accompanied by the rise of ROS, which act as signal molecules in the immune pathway and enhance the ability of cells to resist pathogenic microorganisms (*Bautista-Baños and Hernández-López, 2004*). The rise of H_2O_2 and O^{2-} is one of the characteristics of early defense response after PAMPs recognition by plants (*Li et al., 2016*). There was no obvious difference of the H_2O_2 content between the NC group and the CA group under both FLG22 and chitohexose treatment (Fig. 4a, 4c) with the increase of the early stage of treatment. The contents of O_2^{-} in the cytoplasm of peanut leaves began to increase during the first 1 h under both FLG22 and chitohexose treatment, while the NC group was slightly higher than the CA group within following 3 h (Fig. 4b, 4d), which may be related to the Ca^{2+} signaling pathway improving the ROS scavenging capacity of plants (*Katiyar et al., 2015*). These results indicated that the rise of ROS in peanut leaves is an important process of plant immune regulation. The rise of ROS in peanut leaves is an important process of plant immune regulation, but the role of Ca^{2+} regulation only has slight effects on the accumulation of O^{2-} (Fig. 4b, 4d).

Discussion

Peanut (*Arachis Hypogaea*) is an important oil crop in China, and its yield and quality are of great significance to ensure the safety of grain and oil in China. In the late growth period, peanut leaves often suffer from disease infection, which seriously affects the yield and quality of peanut. When leaves are threatened by disease, the plant will devote a lot of energy to resist the disease threat. In this process, photosynthesis and other physiological and biochemical indicators will inevitably be reduced. The main purpose of this study was to study the effects of FLG22 and chitohexose on the immune response process and the regulation of photosynthesis in peanut. Since Ca^{2+} is an essential element in plant growth and plays a regulatory role in plant photosynthesis (*Yang et al., 2015b; Yang et al., 2013*), this study further analyzed the synergistic effect of Ca^{2+} with plant immune modulator FLG22 and chitohexose in photosynthesis regulation.

Being the motor organ that enables bacteria to respond to stimuli (*Hajam et al., 2017*), flagellin is derived from the conserved N-terminal or C-terminal of the flagella of various

bacteria and belongs to the protein PAMPs. Because of these characteristics, animal and plant cells can recognize flagellin. Additionally, chitohexose is a natural, biodegradable polymer material, which is widely used in beauty, food, biology, medicine, agriculture and other fields (*Bautista-Baños and Hernández-López, 2004*). Chitohexose, a fragment of chitosan belonging to sugar PAMPs, has the same biological effects, although it is a high polymer (*Li et al., 2016*).

As a marker gene that responds to immune-related plant hormones such as salicylic acid, jasmonic acid and ethylene, and also an important component of plant defense system, *PR-4* and *PR-10*, ~~which~~ belong to *PR* gene family, can be induced by biological or abiotic stress (*Liu et al., 2014; Du et al., 2017*), ~~both of their~~ expression increased when treated with FLG22 and Chitohexose (Fig. 1), and the high expression level of *PR-4* and *PR-10* companied by the high content of ROS in peanut leaves (Fig. 4) suggested that FLG22 and Chitohexose could trigger an immune response to improve the plant's ability to environmental stress. ROS play an important role in promoting tissue repair and resistance to pathogenic microorganisms in plants (*Asada, 2006*). Plants often respond to stress by forming ROS (*Vera-Jimenez and Nielsen, 2013*) to improve the resistance. As a second messenger, Ca^{2+} participates in the regulation of most cell physiological metabolism. A large number of experimental studies have shown that Ca^{2+} signal is also involved in the signal transduction process of plant-pathogen interaction (*Ma et al., 2009*). The existence of Ca^{2+} inhibits the accumulation of $O_2^{\cdot-}$ in plant immune processes during response to FLG22 and chitohexose (Fig. 4b, 4d). In addition, it seems that Ca^{2+} signal transduction pathway can not only stabilize the immune response, but also improve the disease resistance of plants since Ca^{2+} synergistically inducing the expression of *PR-4* and *PR-10* genes in peanut leaves, and promoting the regulation of FLG22 and chitohexose on the expression of pathogen-related genes in peanut leaves (Fig. 1).

When the plant defense signal reaches the cytoplasm, it is further transmitted to each organelle. There are calcium-related channels in the chloroplast membrane that transmit signals to chloroplast bodies. The performance index $PI_{(abs)}$ based on the absorption of light energy showed that Ca^{2+} was involved in the activity of PSⅡ reaction center in the process of down-regulating the immune response induced by FLG22, while NC group relatively moderated the effect of immune regulation on PSⅡ activity (Table 1). Gao et al., treated tobacco leaves infected with pathogenic microorganisms with Ca^{2+} channel inhibitor $LaCl_3$ and obtained similar results (*Gao et al., 2012*). In FLG22-induced immune pathway, Ca^{2+} can actively respond to the immune

pathway with causing lower $PI_{(abs)}$. It seems that Ca^{2+} and chitohexose has different role on PSⅡ reaction center activity since chitohexose has not significant effects on $PI_{(abs)}$ (Table 1).

CP12 is a chloroplast and photosynthesis-related protein that participates in the Calvin cycle of photosynthesis by interacting with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and Phosphoribulokinase (PRK) (*Rocha and Vothknecht, 2013*). Although the different responses of *CP12* after treated with FLG22 and chitohexose, chitohexose inhibited the expression of *CP12* gene compared with FLG22, and then inhibited the Calvin cycle in plants, thus reducing plant photosynthesis. At the same time, Ca^{2+} participation can still inhibit *CP12* gene expression, although it slows down the effects (Fig. 2).

The expression level of *PsbS* and *VDE* were identified in this study which participate in the stabilization of PSⅡ. When treated with FLG22 and chitohexose, the relative expression of PsbS and VDE suggested that compared with FLG22, the immune pathway induced by chitohexose induced *PsbS* gene down-regulation and *VDE* gene up-regulation, while in the case of chitohexose treatment, Ca^{2+} plays a more significant role in regulating the expression of *VDE* gene. Since *PsbS* gene expression can improve photosynthetic capacity, and *VDE* gene expression can protect photosynthetic organs from damage excess light energy, then explain, chitohexose can reduce the photosynthesis of plants, but it can make the photosynthetic organs from the destruction of excess light energy, improve the plant of photosynthetic organ protection (*Sylak-Glassman et al., 2014*).

Heat dissipation is an important pathway to protect plant photosynthetic reaction centers. NPQ can be used to reflect the ability of plants to dissipate excess light energy. The larger NPQ is under strong light, the stronger the plant's photoprotection ability is. It has been clear that NPQ is closely related to calcium ions (*Yang et al., 2013*). FLG22 and chitohexose could reduce the heat dissipation, while Ca^{2+} further inhibited the heat dissipation treated with FLG22 and chitohexose (Fig. 3b, 3d). Besides NPQ, the reaction center inactivation also helps plant to dissipate excess energy when treated with Ca^{2+}, because of the decreased expression of *CP12* (Fig. 2b, 2d). The Ca^{2+}- free treatment may dissipate heat mainly by inactivation of the reaction center. The decrease of heat dissipation may be attributed to the fact that plants can increase the energy utilization rate of plants by reducing energy dissipation, which is used to rapidly cause the eruption of reactive oxygen species (Fig. 4), thereby accelerating the triggering of immune regulation (*Göhre et al., 2012*).

FLG22 and chitohexose can enhance the immune response of plants by reducing photosynthesis, thus improving the disease resistance of plants. inhibited photosynthesis by inducing down-regulation of *PsbS* and *CP12* genes (Fig. 2), promoting up-regulation of *VDE* genes, and decreasing heat dissipation and non-photochemical energy dissipation of peanut leaves (Fig. 3). Therefore, it seems that FLG22 and chitohexose can enhance the immune response with different pathway to increase the resistance of peanuts, and FLG22 is focused on downregulating activation of photosynthetic reaction centers, while chitohexose mainly regulating the accumulation of ROS, especially $O_2^{\cdot-}$.

Conclusions

Hypersensitivity reaction is a common mechanism of plant immune response. On the surface of plant cells, there are recognition receptors that stimulate the immune response. The conserved components of pathogens that can be recognized by receptors, such as flagellin ~~flg22~~ and chitosan. At present, there are many studies on the regulation of photosynthesis under adverse stress, but there are relatively few studies on the regulation of photosynthesis by immune regulation, while that regulation of peanut photosynthesis by immune pathway triggered by PAMPs has not been reported. ~~In this study, "Huayu 25" peanut seedlings were used as experimental materials, and peanut leaves were treated with flg22 and Chitohexaose respectively. Since calcium is the initial signal of immune regulation, peanut seedlings were watered with common nutrient solution (with calcium: CA) and non-calcium nutrient solution (without calcium: NC). Finally, the photosynthesis and other parameters of the treated leaves were measured.~~ The results indicated that flg22 and chitosaccharides triggered peanut immune pathways through Ca^{2+} signaling. flg22 triggered the immune pathway mediated by Ca^{2+} can decrease the activity of photosynthetic reaction center. However, the lack of Ca^{2+} can alleviate the damage caused by the immune pathway to PSⅡ, but the activity of PSⅠ will be affected. Compared with flg22, the immune pathway triggered by chitosaccharides has less influence on photosynthetic electron transport, and both flg22 and chitosaccharides cause reduced energy

dissipation, while the down-regulation of NPQ requires Ca^{2+} participation.

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List of abbreviations

FLG22, flagellin 22; H_2O_2, Hydrogen peroxide; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MAPK, Mitogen-activated protein kinase; NPQ, Non-photochemical quenching; PAMP, Athogen-associated molecular patterns; PFD, Photon flux density; PR, pathogenic related gene; PRK, Phosphoribulokinase; PRR, Pattern recognition receptors; PSI, Photosystem I; PSII, Photosystem II; ROS, Reactive oxygen species; VDE, Violaxanthin de-epoxidase.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

SW and XL designed the study, QW and YZ carried out most of the experiments and data analysis and wrote part of the manuscript. SY wrote part of the manuscript and finalized the figures and tables. LC and JM performed part of the experiments. All authors have read and approved the manuscript.

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Figure legends

Fig. 1 Effects of FLG22 and chitohexose treatments on expression level of pathogen related genes in peanut leaves. (a) Expression of *PR-4* gene in peanut leaves treated with 1μM FLG22; (b) Expression of *PR-10* gene in peanut leaves treated with 1μM FLG22; (c) Expression of *PR-4* gene in peanut leaves treated with 200μg/ml chitohexose; (d) Expression of *PR-10* gene in peanut leaves treated with 200μg/ml chitohexose. Means ± SD.

Fig. 2 Effects of FLG22 and chitohexose treatments on expression level of photosynthesis-related genes *CP12* and *PsbS* in peanut leaves. (a) Expression of *PsbS* gene in peanut leaves treated with 1μM FLG22; (b) Expression of *CP12* gene in peanut leaves treated with 1μM FLG22; (c) Expression of *PsbS* gene in peanut leaves treated with 200μg/ml chitohexose; (d) Expression of *CP12* gene in peanut leaves treated with 200μg/ml chitohexose. Means ± SD.

Fig. 3 Effects of FLG22 and chitohexose treatments on expression level *VDE* gene and NPQ in peanut leaves. (a) Expression of *VDE* gene in peanut leaves treated with 1μM FLG22; (b) NPQ in peanut leaves treated with 1μM FLG22; (c) Expression of *VDE* gene in peanut leaves treated with 200μg/ml chitohexose; (d) NPQ in peanut leaves treated with 200μg/ml chitohexose. Means ± SD.

Fig. 4 Effects of FLG22 and chitohexose treatments on content of H_2O_2 and $O_2^{\cdot-}$ in peanut leaves. (a) Changes of content of H_2O_2 in peanut leaves treated with 1μM FLG22; (b) Changes of content of $O_2^{\cdot-}$ in peanut leaves treated with 1μM FLG22; (c) Changes of content of H_2O_2 in peanut leaves treated with 200μg/ml chitohexose; (d) Changes of content of $O_2^{\cdot-}$ in peanut leaves treated with 200μg/ml chitohexose. Means ± SD.

Table. 1 Effects of FLG22 and chitohexose treatments on the activity of photosystem reaction center indicated by $PI_{(abs)}$.

Table 1(on next page)

Effects of FLG22 and chitohexose treatments on the activity of photosystem reaction center indicated by $PI_{(abs)}$.

Table 1. Effects of FLG22 and chitohexose treatments on the activity of photosystem reaction center indicated by $PI_{(abs)}$

Time (h)	FLG 22 (1μM)		Chitohexose (200μg/ml)	
	NC (Means ± SD)	CA (Means ± SD)	NC (Means ± SD)	CA (Means ± SD)
1	8.7832 ± 0.2625	9.1767 ± 0.372	9.5630 ± 1.02834	10.0273 ± 1.0144
2	9.2564 ± 0.02548	7.8374 ± 0.3453	9.6554 ± 1.16634	9.6740 ± 1.53454
3	9.0753 ± 0.3731	7.9554 ± 0.3327	9.8453 ± 0.82384	9.0673 ± 0.74309
4	8.8453 ± 0.5834	7.9443 ± 0.1899	9.8026 ± 0.5864	9.8632 ± 1.18847

Figure 1

Effects of FLG22 and chitohexose treatments on expression level of pathogen related genes in peanut leaves.

(a) Expression of *PR-4* gene in peanut leaves treated with 1μM FLG22; (b) Expression of *PR-10* gene in peanut leaves treated with 1μM FLG22; (c) Expression of *PR-4* gene in peanut leaves treated with 200μg/ml chitohexose; (d) Expression of *PR-10* gene in peanut leaves treated with 200μg/ml chitohexose. Means ± SD.

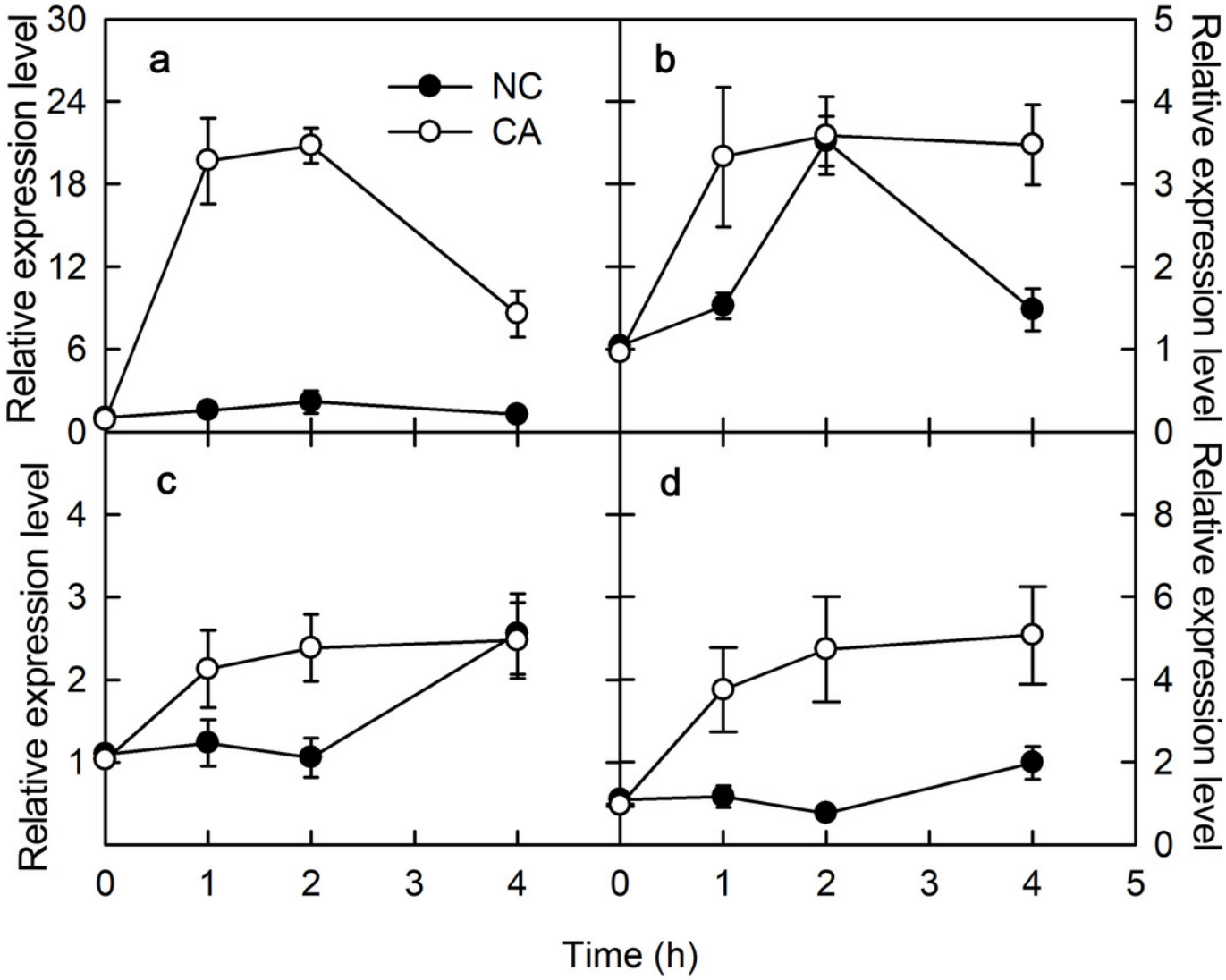

Figure 2

Effects of FLG22 and chitohexose treatments on expression level of photosynthesis-related genes *CP12* and *PsbS* in peanut leaves.

(a) Expression of *PR-4* gene in peanut leaves treated with 1μM FLG22; (b) Expression of *PR-10* gene in peanut leaves treated with 1μM FLG22; (c) Expression of *PR-4* gene in peanut leaves treated with 200μg/ml chitohexose; (d) Expression of *PR-10* gene in peanut leaves treated with 200μg/ml chitohexose. Means ± SD.

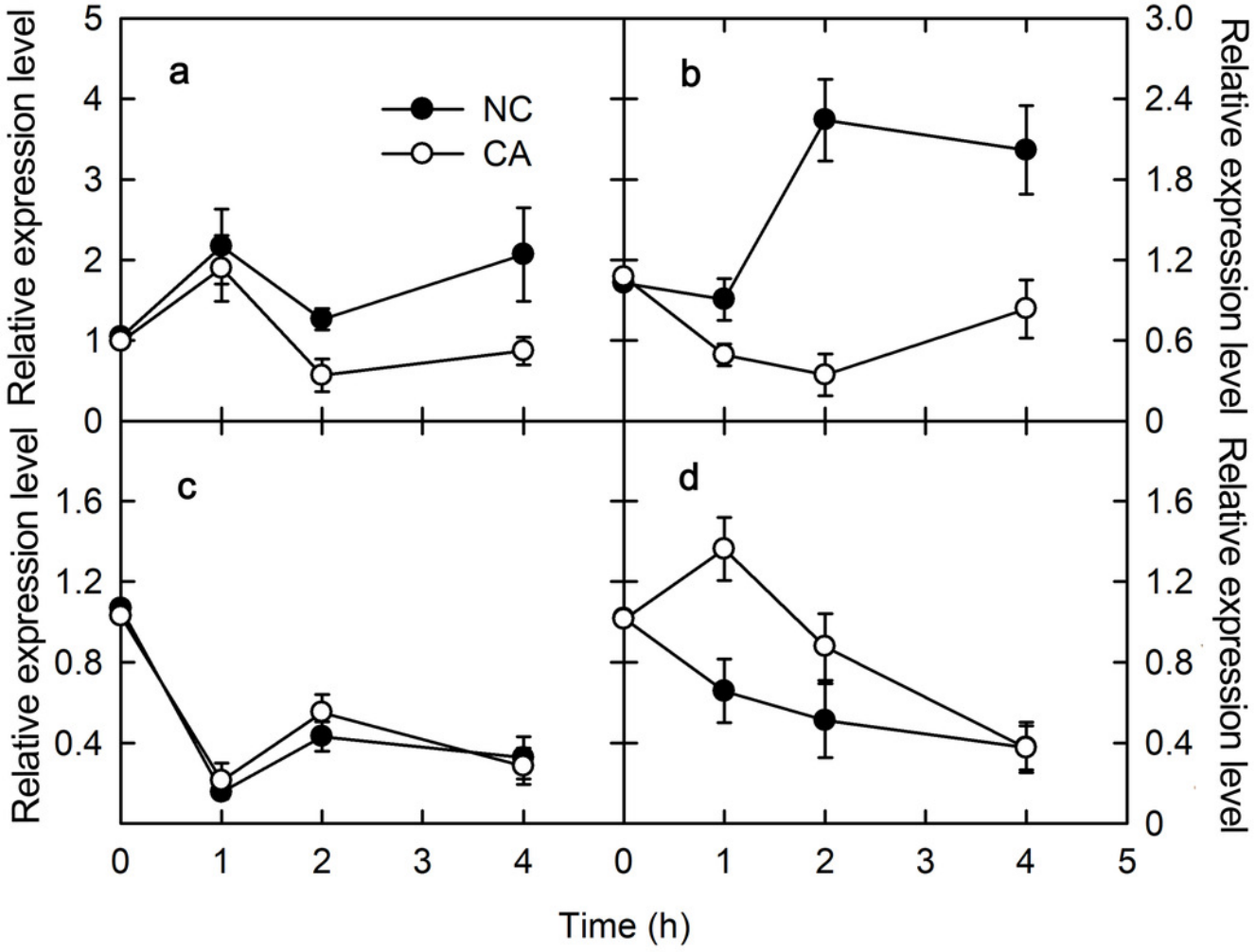

Figure 3

Effects of FLG22 and chitohexose treatments on expression level *VDE* gene and NPQ in peanut leaves.

(a) Expression of *VDE* gene in peanut leaves treated with 1μM FLG22; (b) NPQ in peanut leaves treated with 1μM FLG22; (c) Expression of *VDE* gene in peanut leaves treated with 200μg/ml chitohexose; (d) NPQ in peanut leaves treated with 200μg/ml chitohexose. Means ± SD.

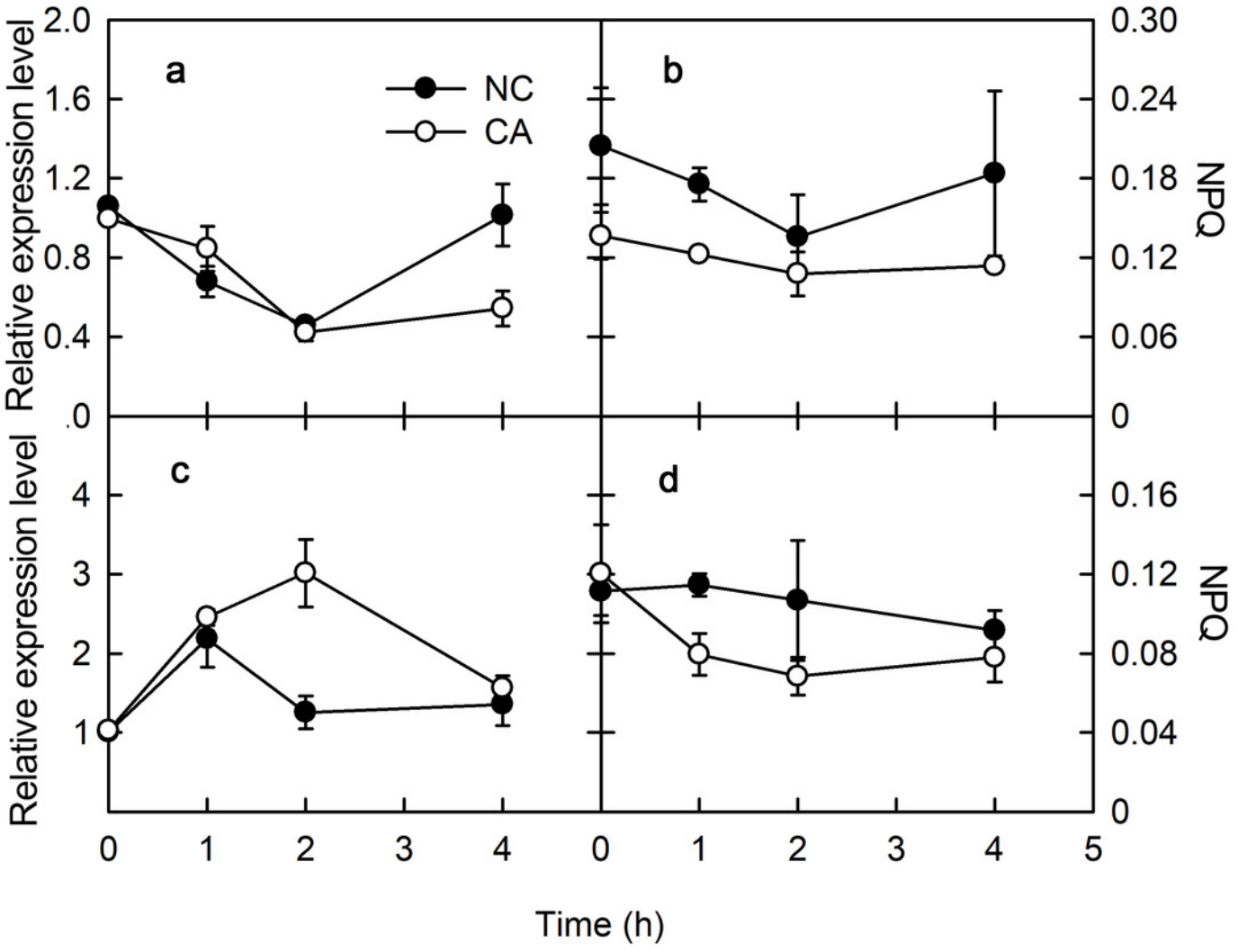

Figure 4

Effects of FLG22 and chitohexose treatments on content of H_2O_2 and $O_2^{\cdot-}$ in peanut leaves.

(a) Changes of content of H_2O_2 in peanut leaves treated with 1μM FLG22; (b) Changes of content of $O_2^{\cdot-}$ in peanut leaves treated with 1μM FLG22; (c) Changes of content of H_2O_2 in peanut leaves treated with 200μg/ml chitohexose; (d) Changes of content of $O_2^{\cdot-}$ in peanut leaves treated with 200μg/ml chitohexose. Means ± SD.