Volume 58(1):21-26, 2014
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

The effect of xanthan gum as an elicitor on guard cell function and photosynthesis in *Vicia faba*

Attila Ördög*, Dóra Bernula, Barnabás Wodala

Department of Plant Biology, University of Szeged, Szeged, Hungary

ABSTRACT Many plant species respond to pathogen attacks by closing stomata in a process called basal resistance. Pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRRs). This interaction can lead to the activation of different signaling cascades, which can lead to stomatal closure. The immune-active behavior of the bacterial elicitor xanthan gum has been demonstrated in barley where a xanthan treatment lead to the accumulation of the enzyme ß-1,3 glucanase. However, its short-term physiological effects have not been investigated yet. In this study we investigated the effect of xanthan gum on the guard cell function. Xanthan gum applied at dawn can induce stomatal closure, reduce stomatal conductance and the rate of CO_2 assimilation, and it can also reduce PSII photochemistry in guard cells.

Acta Biol Szeged 58(1):21-26 (2014)

KEY WORDS

xanthan gum
biotic stress
guard cell
photosynthesis

Stomata are formed by guard cells – two specialized cells in the epidermis, which are morphologically and functionally different from general epidermal cells. Guard cells are multisensory hydraulic valves responsible for regulating the aperture of stomata (Franks and Farqhuar 2007) in order to control gas exchange between the inside of the leaf and the environment and also to control transpirational water loss (Lawson 2009). In addition, stomata are responsible for leaf cooling, metabolite fluxes, long distance signaling and they also serve as the first line of defence against pathogens. As natural openings, stomata are ideal points of entry for pathogenic microorganisms. Guard cells recognize and respond to the presence of microbes by perception of PAMPs, such as xantan gum (Nicaise et al. 2009). The first response is the induction of immediate stomatal closure or the inhibition of the stomatal opening (Rong et al. 2010). In wheat, it has been shown that a pre-treatment with 0.5 mg ml^{-1} xanthan gum (48 h before inoculation) provides protection against an infection by *Bipolaris bicolor*, *Bipolaris sorokiniana*, and *Drechslera tritici-repentis* (Bach et al. 2003). The efficiency of the xanthan gum treatment was attributed to biochemical changes, such as an increase in the concentration of proteins and the enzyme ß-1,3-glucanase, and a decrease in phenol concentration. Castro and Bach (2004) obtained similar results in the induction of local and systemic resistance using xanthan gum as an elicitor in barley plants, with protection levels over 90%, and attributed the efficiency to the increased synthesis and accumulation of the enzyme ß-1,3 glucanase. However, the short-term physiological effects of xanthan gum have not been investigated yet.

Accepted Aug 11, 2014
*Corresponding author. E-mail: aordog@bio.u-szeged.hu

In the present work, we explore the short-term effects of xanthan gum on guard cell function. We examine whether it affects stomatal opening and closure in the model plant *Vicia faba*. We also study whether the action of xanthan involves a reduction in the photosynthetic activity of mesophyll and guard cells. Fluorescent probes were used to determine the levels and cellular localization of signaling components H_2O_2 and NO following the xanthan gum treatment.

Materials and Methods

Plant material and experimental solutions

All experiments, except leaf photosynthetic measurements, were carried out on abaxial epidermal strips peeled from the third to fourth completely unfolded leaves of 2-3 week-old broad bean (*Vicia faba* L. cv. Mirna) plants. Plant were grown hydroponically in a solution containing: 2 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 0.5 mM KCl, 0.5 mM KH_2PO_4, 0.5 mM Na_2HPO_4, 0.001 mM $MnSO_4$, 0.005 mM $ZnSO_4$, 0.0001 mM $(NH_4)_6Mo_7O_{24}$, 0.01 mM H_3BO_4 and 0.02 mM Fe(III)-EDTA, pH 6 in a controlled environmental chamber (Fitoclima S 600 PLH, Aralab, Portugal) under 12h light (6:00 am – 18:00 pm) and 12h dark (18:00 pm – 6:00 am) cycles at 25 °C (day) and 20 °C (night).

Xanthan gum (Sigma-Aldrich, Budapest, Hungary) was dissolved in the experimental solution (10 mM MES, 100 µM $CaCl_2$, 10 mM KCl, pH 6.15 with TRIS) to a final concentration of 1 mg ml^{-1}. The experimental solution without xanthan gum was used as control in all experiments.

Stomatal aperture measurements

The width of stomatal apertures was measured in digital

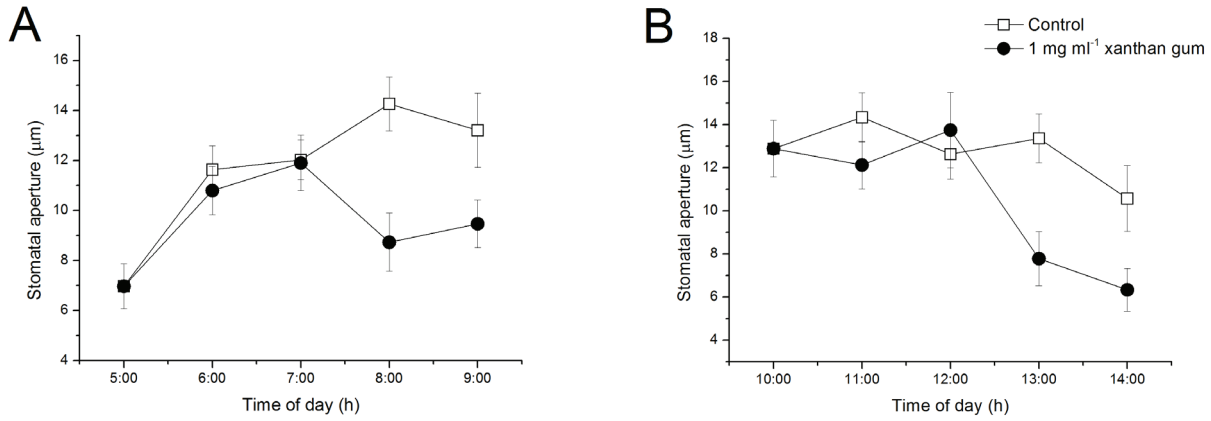

Figure 1. Stomatal aperture sizes of control and 1 mg ml^{-1} xanthan gum treated *Vicia faba* leaves in the course of a day. Treatments were applied 60 min before the start of illumination at 5:00 am (A) and by day at 10:00 am (B). The data presented are the average values (± SE) of 3 independent experiments, each yielding the average of at least 90 aperture widths.

images taken from freshly peeled epidermal strips using the Image-Pro Plus 5.1 image analyzer software. The experiments were repeated on three different days, each yielding the average of at least 90 aperture widths. The data presented are the average values (± SE) of these results.

Epidermal strip bioassay

Experiments in this study were carried out using epidermal strips, which eliminated the influence of mesophyll cells on stomatal function. Prior to each experiment, the abaxial epidermis was peeled carefully from the third to fourth completely unfolded leaf submerged in the experimental solution. The strips contained only small regions contaminated with mesophyll cells – mainly around the major veins – and these regions were excised with a razor blade. The strips were transferred and washed for 5 minutes in the hypoosmotic control solution in order to remove any remaining mesophyll cell debris and mesophyll chloroplasts by severe osmotic shock.

Cell viability tests carried out following the preparation of epidermal strips showed that guard cells remained intact after floating in the incubating medium. Microscopy PAM measurements confirmed that this treatment did not influence the chlorophyll fluorescence parameters in guard cells. In addition, no correlation was found in the photosynthetic performance of epidermal strips and the age of the plant (2 to 3 weeks), or the position of the leaf (third or fourth) they were collected from (Ördög et al. 2013).

Photosynthesis measurements

Chlorophyll a fluorescence measurements

Chlorophyll a fluorescence of 4-5 stomata from a mesophyll-free abaxial epidermis patch was monitored with a MYCROSCOPY-PAM chlorophyll fluorometer (Heinz Walz GmbH, Germany) mounted on an inverted epifluorescence microscope (Zeiss Axiovert 40, Zeiss GmbH, Germany). Light intensities were measured with a Micro Quantum Sensor (MC-MQS, Heinz Walz GmbH, Germany).

Rapid light-response curves were obtained using a light-curve protocol, consisting of 8 consecutive 30-s steps with increasing actinic light intensity. Due to the short time of each step, the photosynthetic activity of guard cells did not achieve steady state at any of the light intensities. At the end of each step the steady state fluorescence was measured, which was followed by a 0.8 s width saturation pulse to obtain the maximal fluorescence yield of the light-adapted sample. Prior to the light response curve the stomata were dark-adapted and the minimum as well as the maximum fluorescence in the dark-adapted state were recorded. Based on these fluorescence values the following fluorescence parameters were calculated: maximum quantum efficiency of dark-adapted PS II (F_v/F_m according to Butler 1978), maximum quantum efficiency of light-adapted PSII photochemistry (F_v'/F_m'), the coefficient of photochemical fluorescence quenching (qL according to Kramer et al. 2004), the apparent relative linear electron transport rate (ETR) and the Stern-Volmer type non-photochemical quenching (NPQ according to Bilger and Björkman 1990).

Determination of assimilation rate and stomatal conductance

Photosynthetic activity of broad bean leaves were assayed by measuring assimilation and stomatal conductance using an LI-6400 Portable Photosynthesis and Fluorescence System (LI-COR Environmental, Lincoln, Nebraska, USA) equipped with a 6400-40 leaf chamber fluorometer (LI-COR Environmental). Measurements were carried out at room temperature, ambient CO_2 concentration and humidity values using a fixed chamber illumination of 150 µmol m^{-2} s^{-1} provided by the in-

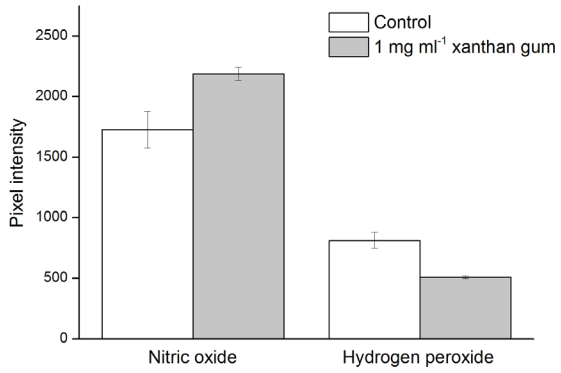

Figure 2. Average fluorescence intensities of specific DAF-FM (NO) and AR (H_2O_2) fluorophores in *Vicia faba* guard cells after 3 hours of treatment with experimental solution or 1 mg ml^{-1} xanthan gum. The data presented are the average values (± SE) of 3 independent experiments, each yielding the average of at least 20 stomata.

ternal light source of the leaf chamber fluorometer. Stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) and assimilation rate (P_n, µmol CO_2 m^{-2} s^{-1}) values of each leaves were collected in every five minutes over 4 hours (Poór et al. 2011).

Localization of fluorescent probes using confocal microscopy

Localization of the fluorescent probes in the abaxial epidermis of intact leaves was visualized using confocal laser scanning microscope (Olympus FV1000 LSM, Olympus Life Science Europa GmbH, Hamburg, Germany). H_2O_2 was detected by a specific probe 10-acetyl-3,7-dihydroxyphenoxazine (Amplex Red, Molecular Probes Invitrogen, Carlsbad, CA). NO was localized by the frequently used cell-permeable 4-amino-5-methylamino-2 ,7-difluorofluorescein diacetate (DAF-FM DA, Sigma-Aldrich, Budapest, Hungary) .

Results and Discussion

Stomatal opening and closure requires diverse signalization pathways and transporters (Pandey et al. 2007). As xanthan gum may act differently on the opening and closure of stomata, leaves were treated with xanthan gum at dawn when most stomata were closed and also by day when they were already fully open.

Xanthan gum slightly inhibits stomatal opening at dawn and induces stomatal closure applied by day

In the first experiment the broad bean leaves were sprayed with the experimental solution with or without 1 mg ml^{-1} xanthan gum. After 30 minutes the leaves were illuminated and leaves from control and treated plants were peeled hourly and digital images were taken immediately to determine the actual stomatal aperture sizes. At 5:00 am stomata were almost entirely closed with an average size of 6.97 ± 0.89 µm. After 3 hours of illumination stomatal apertures of control plants reached their maximal value with an average size of 14.26 ± 1.08 µm, while the stomata of treated plants showed a slight decrease in the opening with an average aperture size of 8.73 ± 1.16 µm (Fig. 1A).

When the plants were treated at 10:00 am – when stomata were almost fully opened (12.88 ± 1.31 µm) – the aperture sizes of control plants did not change during the 4 hours of the experiment while the stomata treated with 1 mg m^{-1} xanthan gum showed a significant decrease (7.78 ± 1.25 µm) after 3 hours (Fig. 1B).

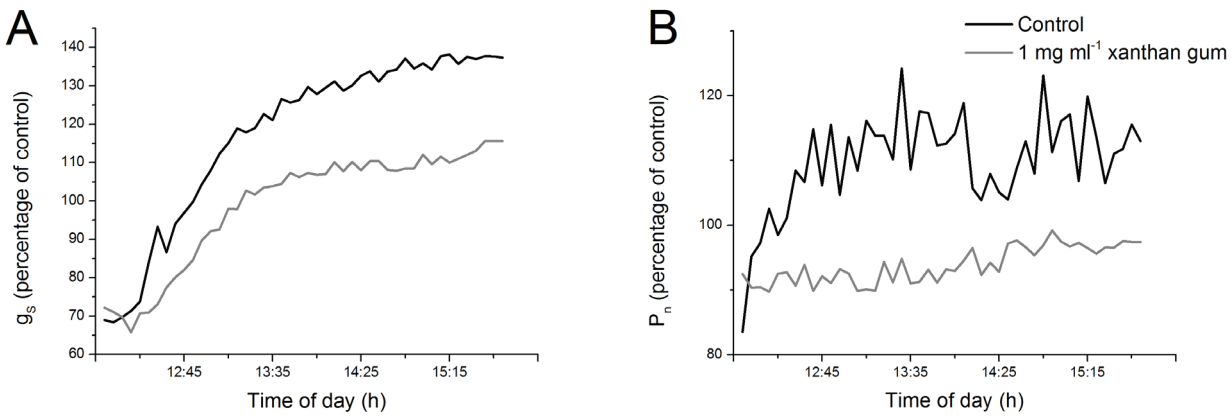

Figure 3. Stomatal conductance (A) and assimilation rate (B) after 1 mg ml^{-1} xanthan treatment. Both parameters are shown as percentage of control, which was measured prior to each treatment. It can clearly be seen that the treated leaves showed decreased stomatal conductance and assimilation rate during the whole experiment. Sampling was made every five minutes. The data presented are the average values of 3 independent experiments.

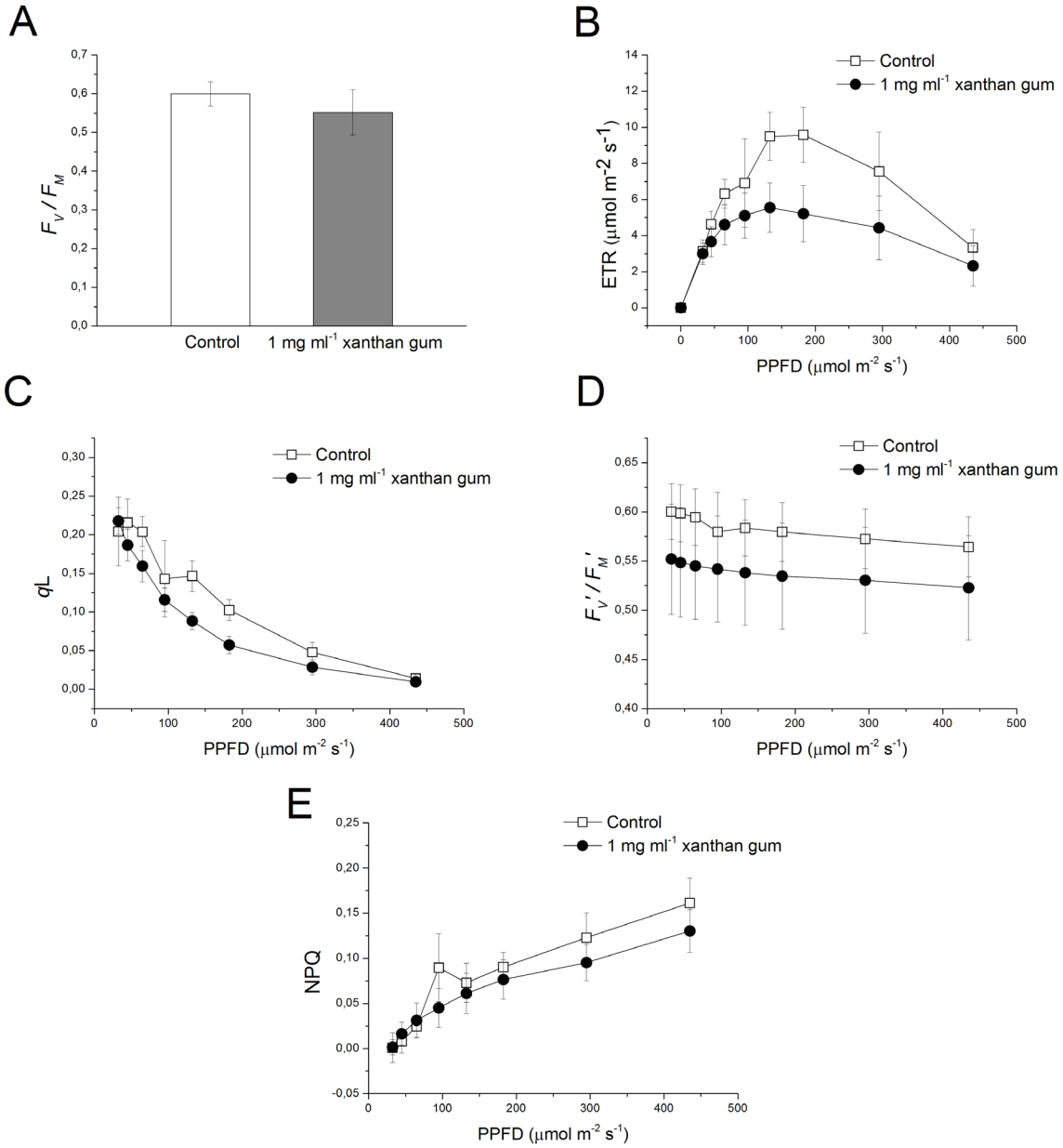

Figure 4. Photosynthetic parameters obtained from rapid light-response curves of dark-adapted abaxial guard cells in control and xanthan gum-treated leaves. Treatments were applied before the light period at 5:00 am and measurements were taken between 7:00 and 9:00 am. The data are displayed as averages (± SE) of 3 independent experiments, each measuring the fluorescence of 4-5 stomata.

These results clearly show that xanthan gum is recognized by guard cells of broad beans and that it requires 3 hours to induce stomatal closure both at dawn and during the daytime, which prompted us to investigate its effects on guard cell signaling and photosynthesis.

Xanthan gum induced NO accumulation but decreased the level of H_2O_2 in the guard cells of *Vicia faba*

Both H_2O_2 and NO are crucial components of the signaling of stomatal movements, especially stomatal closure (Srivastava et al. 2009). In order to investigate the production of H_2O_2 following the xanthan gum treatment, we applied a specific fluorescent sensor AR (Snyrychova et al. 2009). Abaxial epidermal peels from control and xanthan-treated leaves were incubated in experimental solution containing 50 μM AR for 30 min in dark. The peels were washed twice. AR reacts with H_2O_2 producing the highly fluorescent resorufin, whose emission was detected between 585 and 610 nm using 543 nm HeNe laser excitation. We found that the treatment

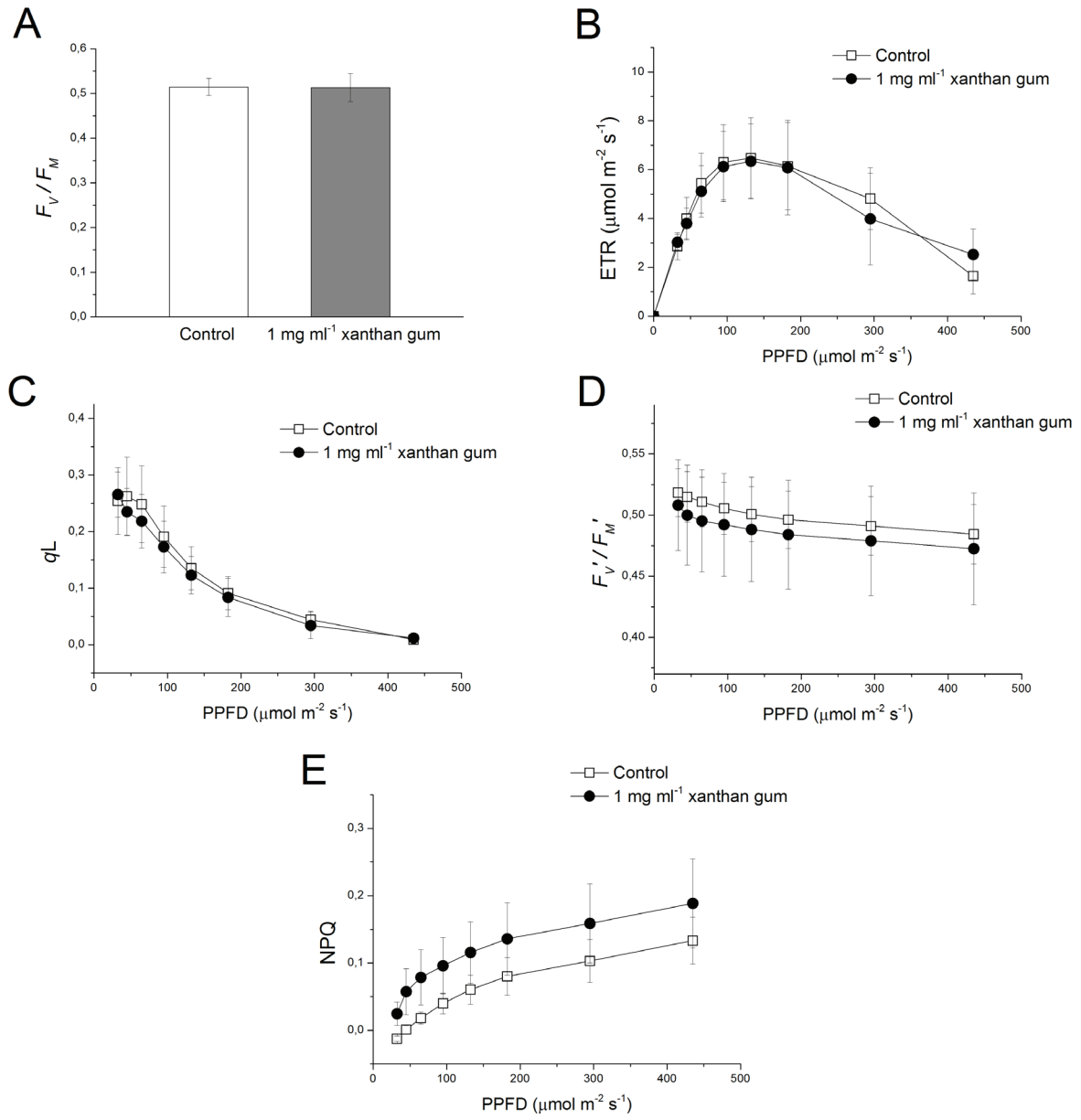

Figure 5. Photosynthetic parameters obtained from rapid light-response curves of abaxial guard cells in control and xanthan gum-treated leaves. Treatments were applied before 10:00 am and measurements were taken between 13:00 and 14:00 am. The data are displayed as averages (± SE) of 3 independent experiments, each measuring the fluorescence of 4-5 stomata.

with xanthan gum decreased the detectable level of resorufin fluorescence compared to control (Fig. 2), which was quite surprising as it is well established that stomatal closure is induced by an increase in the level of H_2O_2 (Desikan et al. 2004).

The level of NO was monitored by the fluorophore DAF-FM DA. In these experiments abaxial epidermal peels from control and 1 mg ml^{-1} xanthan gum treated leaves were incubated in experimental solution containing 10 μM DAF-FM DA for 15 min in dark. We found that xanthan gum only slightly induced the production of NO, which was mainly localized in the chloroplasts (Fig. 2).

Xanthan gum decreased the stomatal conductance and also the CO_2 assimilation

To continue the investigation on the background of xanthan-induced stomatal closure, stomatal conductance and CO_2 assimilation rate of leaves were monitored for 4 hours after the xanthan treatment with a leaf chamber fluorometer.

The xanthan gum treatment led to a decrease in stomatal conductance (Fig 3A). Dropping g_s values result in reduced CO_2 availability inside the leaves, so it is not surprising that xanthan gum treatment led to a moderate, but marked decline in CO_2 assimilation as well (Fig 3B).

Xanthan gum applied at dawn slows down the linear electron flow in guard cells of *Vicia faba*

As xanthan can reduce the rate of CO_2 fixation, we also investigated the photosynthetic activity in guard cells of *Vicia faba*. Since we found a difference in the effect of xanthan gum on stomatal movements at dawn and during the daytime, the photosynthetic measurements were also examined in these periods.

Figure 4 (A–C) shows that xanthan gum applied at dawn (5:00 am, 1 hour before the onset of illumination) slightly decreased the maximal quantum efficiency of dark-adapted PSII (*Fv/Fm*), the coefficient of photochemical fluorescence quenching (*q*L) and the apparent relative linear electron transport rate (ETR), while the values of the maximal quantum efficiency of light-adapted PSII (*Fv'/Fm'*) and the Stern-Volmer type non-photochesmical quenching (NPQ) did not change significantly. These results suggest that the xanthan-induced decrease in photochemistry might result in a lower level of ATP and NADPH in guard cells, which can lead to altered stomatal function.

When leaves were treated at day (10:00 am) there was no difference in the photosynthetic activity of guard cells from control and xanthan-treated leaves (Fig. 5A–E).

Conclusions

Many plant species respond to pathogen attack by closing stomata in a process called basal resistance, or PAMP-triggered immunity (Boller and Felix 2009). PAMPs can be recognized by PRRs, which can lead to the activation of different signaling cascades including the secondary transducer H_2O_2 and NO. We have shown that xanthan gum causes a moderate decrease in the level of H_2O_2 and increases the level of NO after 3 hours of treatment. We have previously shown that NO decreases the photosynthetic activity of guard cells (Ördög et al. 2013) so we decided to investigate whether xanthan gum can also influence the photosynthetic activity of guard cells. Xanthan gum applied at dawn slightly reduces the linear electron flow in the PSII reaction centers, which can lead to a reduced amount of ATP and NADPH, which in turn may prevent stomatal opening and induce stomatal closure.

In summary, our work shows that xanthan gum may influence the plant immune response not only through the accumulation of the enzyme ß-1,3 glucanase, but it can also induce stomatal closure. However, the underlying process of xanthan-induced stomatal closure is still unknown and awaits elucidation.

Acknowledgements

This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program'.

References

Bach EE, Barros BC, Kimati H (2003) Induced resistance against *Bipolaris bicolor*, *Bipolaris sorokiniana* and *Drechslera tritici-repentis* in wheat leaves by xanthan gum and heat-inactivated conidial suspension. J Phytopatol 151(7-8):411-418.

Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Phot Res 25(3):173-185.

Boller T, Felix G (2009) A Renaissance of Elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol 60:379-406.

Butler WL (1978) Energy-distribution in photochemical apparatus of photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 29:345-378.

Castro OL, Bach EE (2004) Increased production of beta-1,3 glucanase and proteins in *Bipolaris sorokiniana* pathosystem treated using commercial xanthan gum. Plant Physiol Biochem 42(2):165-169.

Desikan R, Cheung MK, Bright J, Henson D, Hancock JT, Neill SJ (2004) ABA, hydrogen peroxide and nitric oxide signaling in stomatal guard cells. J Exp Bot 55(395):205-212.

Franks P, Farqhuar GD (2007) The mechanical diversity of stomata and its significance in gas-exchange control. Plant Phys 143(1): 78–87.

Kramer DM, Johnson G, Kiirats O, Edwards G E (2004) New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. Phot Res 79(2):209-218.

Lawson T (2009) Guard cell photosynthesis and stomatal function. New Phytol 181(1):13-34.

Nicaise V, Roux M, Zipfel C (2009) Recent advances in PAMP-triggered immunity against bacteria: Pattern recognition receptors watch over and raise the alarm. Plant Phys 150(4): 1638-1647.

Ördög A, Wodala B, Rózsavölgyi T, Tari I, Horváth F (2013) Regulation of guard cell photosynthetic electron transport by nitric oxide. J Exp Bot 64(5):1357-1366.

Pandey S, Zhang W, Assmann SM (2007) Roles of ion channels and transporters in guard cell signal transduction FEBS Letters 581(12):2325-2336.

Poór P, Gémes K, Horváth F, Szepesi Á, Simon ML, Tari I (2011) Salicylic acid treatment via the rooting medium interferes with stomatal response, CO_2 fixation rate and carbohydrate metabolism in tomato, and decreases harmful effects of subsequent salt stress. Plant Biol 13(1):105-114.

Rong W, Feng F, Zhou JM, He CZ (2010) Effector-triggered innate immunity contributes *Arabidopsis* resistance to *Xanthomonas campestris*. Mol Plant Pathol 11(6):783-793.

Snyrychova I, Ayaydin F, Hideg E (2009) Detecting hydrogen peroxide in leaves *in vivo* - a comparison of methods. Phys Plantarum 135(1):1-18.

Srivastava N, Gonugunta VK, Puli MR, Raghavendra AS (2009) Nitric oxide production occurs downstream of reactive oxygen species in guard cells during stomatal closure induced by chitosan in abaxial epidermis of *Pisum sativum*. Planta 229(4):757-765.