Effects of Exogenous ABA and Ethylene Application and Waterlogging on Photosynthesis in Mungbean (Vigna radiata (L.) Wilczak)

Selina AHMED, Hirokazu HIGUCHI, Eiji NAWATA and Tetsuo SAKURATANI

Laboratory of Tropical Agriculture, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

Correspondence: Selina Ahmed, Laboratory of Tropical Agriculture, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, 606-8502, Japan. Tel. 001-81-75-6353, Fax. 001-81-75-6352, E-mail: papri@kais.kyoto-u.ac.jp

Abstract The role of abscisic acid (ABA) and ethylene in the responses to waterlogging were studied in mungbean plants under greenhouse conditions. Photosynthetic rate (P), transpiration rate (Tr), stomatal conductance (gs), chlorophyll fluorescence yields (Fv/Fm) and leaf water potential (ΨL) were determined at vegetative and reproductive stages during the 8-day period of waterlogging and 8-day period after the removal of waterlogging in treated (ABA and ethylene) and un-treated plants. None of the treatments affected ΨL, indicating that the decrease in P, Tr and gs was not associated with the plant water status. In response to soil waterlogging and ethylene treatment, P was reduced within one day after the beginning of the treatments before any substantial changes in Tr and gs were detected, indicating that early reduction of P might be due to a mechanism independent from stomatal closure. However, ABA inhibited P concomitantly with the reduction of Tr and gs. Since there was only a slight decrease in the ratio of Fv/Fm, the early reduction in P was not attributed to a depression in photosystem II (PSII) in the ABA treated plants, but to stomatal closure. Although at the later stages of the experiments exogenous phytohormones and waterlogging showed various interactions, the results suggest that the suppression of photosynthesis by waterlogging was due to both stomatal and non-stomatal factors, both of which are related to ethylene and ABA that are considered to be biosynthesized in association with waterlogging. In particular, the early reduction of photosynthesis in the waterlogged plants may be caused by the inactivation of PSII in response to ethylene.

Key words ABA, Chlorophyll fluorescence, Ethylene, Mungbean, Photosynthetic rate, Waterlogging

Introduction

Mungbean is a popular pulse and forage crop that is cultivated throughout tropical and subtropical Asia. This grain legume is important because of the high level of vegetable dietary protein and wide adaptability to cultivation. Due to the rapid growth and early maturity, this legume is adapted to various cropping systems, resulting in the increase of small land holders' income and improvement of soil fertility. This crop is considered to be generally unsuited to the wet tropics, where annual rainfall is above 1,000 mm. In Bangladesh, during or after the rainy season when sudden flood occurs, mungbean is considerably damaged by excess soil moisture as well as waterlogging.

Soil waterlogging is one of major problems for natural vegetation and agricultural crops, limiting the growth of many plants in the humid
tropics\(^8\). As a serious water-related constraint, waterlogging has been found to injure plants due to the rapid reduction in the rate of photosynthesis and stomatal conductance\(^9\). Meyer and Genty\(^18\) observed that the decrease of photosynthesis was mainly mediated through stomatal closure induced by dehydration of leaves, although this may not correspond to the actual mechanism of stomatal control. Conversely, our previous results showed that the early depression of photosynthesis, following waterlogging, was independent of stomatal closure, while the leaf water potential remained unchanged\(^1,3\). Therefore, the factors responsible for this photosynthetic depression have not yet been elucidated. Beyschlag et al.\(^5\) indicated that during the period of depressed photosynthesis, biochemical/photochemical factors may be involved in addition to stomatal constraints. Although there are evidences that the capacity of leaves for active oxygen generation increases and often results in oxidative damages under environmental stresses\(^27\), our previous studies suggested that oxidative damage may not be directly involved in the damages to the photosynthetic machinery in waterlogged mungbean plants\(^3\).

The depression of various activities including photosynthesis by waterlogging is known to be highly dependent on two phytohormones, i.e. ABA and ethylene\(^21,28\). In waterlogged plants, ABA bio-synthesis is accelerated as in the case of the plants subjected to other environmental stresses\(^10\). It has been reported that the direct application of ABA inhibits photosynthetic processes and stomatal conductance\(^25\). ABA inhibited the photosynthetic process as evidenced by the lower values of the chlorophyll fluorescence kinetics parameters \(F_{v}/F_{m}\) (reflecting a low photosystem II activity) compared with the plants not treated with ABA\(^20\). On the other hand, Ivanov et al.\(^12\) reported that exogenous application of ABA to barley seedlings for 7 days partially protected the photosystem II (PSII) complex against the damaging effects of high-intensity light at 5\(^\circ\)C.

Ethylene has been known to hasten maturity and senescence in plant tissues, but its precise role on photosynthesis has not been fully elucidated\(^1\). Exogenous ethylene application reduced PSI and PSII activities during 72 hours under both light and dark conditions\(^17\). On the other hand, many studies have focused on the relationship between ethylene production and some morphological changes (i.e. aerenchyma\(^9\) and adventitious root formation\(^17\), etc.) in waterlogged plants, which may reflect their ability to tolerate waterlogging stress. However, there is no direct evidence of the effect of ethylene on photosynthesis in waterlogged plants.

It is generally recognized that chlorophyll fluorescence together with \(CO_2\) gas exchange is a suitable tool for detecting PSII activity\(^19\). In our previous study, early reduction of the value of the \(F_{v}/F_{m}\) ratio of chlorophyll fluorescence by waterlogging may indicate that the early reduction of the photosynthetic rate was correlated with the photoinhibition of photosystem II\(^3\). There is, however, no information available concerning the involvement of ABA and ethylene in PSII during waterlogging in mungbean plants.

Our present study aimed to determine whether ABA or ethylene plays a significant role in the waterlogging-induced photosynthetic reduction of mungbean plants. This study was also designed to investigate whether these hormones affect the photoinhibition of the photosystem II in waterlogged plants.

**Materials and methods**

1. **Plant cultivation**

Seeds of the mungbean cultivar, KPS1, collected from Thailand, were sown in vermiculite on July 15, 2001. This cultivar was selected, because yield reduction had been found to be low under waterlogging conditions in our preliminary experiments. The seedlings were transplanted to clay pots 18 cm tall and 23 cm in diameter containing sterilized sand on July 22. Sterilized sand was used to minimize the effect of soil microorganisms on plant responses to waterlogging\(^9\). Plant spacing was 70 cm between rows and 30 cm between plants. Watering was normally applied twice a day. Proper amounts of “Enshi” solution (containing 16, 4, 8 milliequivalents/l N, P, K respectively)\(^26\) for macro-elements and “Arnon” solution\(^4\) for micro-elements were applied three times a week. Pesticides were also applied when they were needed. Plants were grown in a plastic greenhouse of Kyoto University, Japan, without precise control of the temperature. Mid-day weather conditions during the experimental periods were characterized by a relatively clear sky (i.e., photosynthetic photon
flux density > 1000 μmol m⁻² sec⁻¹), maximum air temperature of 28-34 °C and 73-75% relative humidity.

2. Experimental treatments

The experimental treatments consisting of ABA and ethylene application, and waterlogging were arranged in a randomized complete block design with eight replications. For the ABA treatments, whole leaves of well-irrigated plants were sprayed with about 50 ml (for the vegetative stage: W)V and 80 ml (for the reproductive stage: W) of an aqueous solution of (±)-ABA (99% purity, Sigma Co. Ltd.) at a concentration of 2 mM as described by Li et al.⁰¹°. For the ethylene treatments, an aqueous solution of ethephon (ISK Co. Ltd., Japan) at a concentration of 70 mM was applied by the same procedure as that for the ABA treatments. These concentrations of ABA and ethylene were selected, because the reduction of photosynthesis in the ABA and ethylene-treated plants was almost similar to that of waterlogged plants in our preliminary experiment. For the waterlogging treatment, each pot was put in a plastic bucket 28 cm in diameter and the outer container was filled with water containing nutrients, up to 2 cm above the soil surface. After 8 days of the waterlogging treatment, outer containers were removed. Thus the experiment comprised 8 treatments as follows: control (C), waterlogging (W), control + ABA (C+A), waterlogging + ABA (W+A), control + ethylene (C+E), waterlogging + ethylene (W+E), control + ABA + ethylene (C+A+E) and waterlogging + ABA + ethylene (W+A+E). The last 2 treatments were imposed to analyze the interaction of ABA and ethylene on mungbean plants with or without waterlogging. All the treatments were imposed at three weeks after sowing (vegetative stage) and when the first flower appeared (reproductive stage) in plants. Different plants were used for each waterlogging period to prevent possible compounding of repeated waterlogging effects. Control and waterlogged-treated pots were fully irrigated at the beginning of each waterlogging period to maintain similar initial soil-water contents in the pots.

3. Physiological measurements

Photosynthetic rate (P), transpiration rate (T) and stomatal conductance (g) were measured using a Leaf Chamber Analyser (LCA-4, ADC Bio Scientific Ltd. Hoddesdon, UK). P, T, and g, were measured on uppermost, fully expanded, terminal trifoliolate leaflets (abaxial surface) between 10:00 and 12:00 in the temperature range of 29-32 °C. Chlorophyll fluorescence yields (Fv/Fm) were measured using a portable chlorophyll fluorometer (MINI-PAM, WALZ, Germany). The measurements were carried out on the same leaf as that used for the gas exchange determination, after a dark adaptation time of 30 minutes. The same leaflet of each plant was used for the leaf water potential (ψw) measurement using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). All the physiological measurements were carried out using eight (or more) plants for each treatment at 0, 1, 2, 4 and 8 days after the beginning of the waterlogging treatment (DAW) and at 1, 2, 4 and 8 days after the end of waterlogging (DAE).

Results

1. Photosynthetic rate (P)

P remained approximately constant in the untreated control (C) plants throughout the experiment (Fig. 1a and 1b), while it decreased by the ABA, ethylene and all kinds of waterlogging treatments from 1DAW, at both growth stages. In response to the W treatment, P decreased by around 26 and 32% at the vegetative and reproductive stages, respectively, at 1DAW, and there after, it gradually decreased with the increase of the duration of the treatment. P in the C+A and C+E-treated plants followed almost the same pattern as that of the W-treated plants during the first 8-day period of the experiment. There was no significant difference among the P values of the C+A, C+E and W-treated plants at 1 DAE. The reduction percentages of P in those plants reached the highest value at 1DAE. The P value of the plants in these 3 treatments increased thereafter and that of the W-treated plants reached the control value at 8DAE at both stages, whereas the P value of the C+E and C+A-treated plants was still lower than that of the control. P in the C+A-treated plants was higher than in the C+E plants during the final 8 days of the experiment. P of W+A, W+E, C+A+E and W+A+E treated plants was lower than the other treatments on all the measurement days during the first 8 days of the experiment, and reached
the lowest value at 8DAW or 1DAE. After that, P of those plants increased, but even at 8DAE P was much lower than that of control at both stages. Among them, P of W+A-treated plants increased rapidly after the removal of waterlogging and showed values near those of C+A-treated plants. P of W+A+E-treated plants showed lowest values at all measurement days. There were little differences in changing patterns of all plots between growth stages.

2. Transpiration rate (Tr)

As with P, changing patterns of Tr in each treatment plot did not differ markedly between both stages. Tr was approximately constant in control plants throughout the experiment (Fig. 1c and 1d). Tr of waterlogged and ethylene treated plants (C+E, W and W+E) was not reduced at 1DAW, whereas in all the ABA treated plants (C+A, W+A, C+A+E and W+A+E) declined markedly on the same day. After that Tr of all treated plants declined sharply as the duration of the treatment prolonged except in C+E-treated plants. The reduction percentage of Tr in C+E-treated plants compared to the control was about 30% at 8DAW, whereas all the other treated plants showed more than 50% reduction percentage at both stages. Tr of all treated plants started to increase gradually from 1DAE, except in C+E, where very low recovery rate was observed. Although Tr of waterlogged (W) plants reached the control value at 8DAW, the other ABA, ethylene and/or waterlogging treated plants did not completely recover. As was observed in P, at 8DAW, Tr in W+A-E-treated plants showed lowest of all, whereas in C+E and W+E-treated plants the reduction of Tr was relatively small.

3. Stomatal conductance (gs)

gs followed a tendency almost similar to that of Tr (Fig. 1e and 1f). gs of W, C+E and W+E-treated plants remained static at 1DAW and later declined sharply except in C+E-treated plants. Reduction of gs of all the ABA treated plants (C+A, W+A, C+E+A and W+E+A) was much bigger than plants subjected to the other treatments, at both stages. Similar to those of P and Tr, W+A+E-treated plants showed the highest reduction percentage of gs at 8DAW. After the removal of waterlogging or during the second 8-day period, gs of all treated plants showed almost the similar patterns to the results of Tr. Similar trend was observed in changing patterns of gs of all treated plants at the reproductive stage to that at the vegetative stage.

4. Chlorophyll fluorescence (Fv/Fm)

Fig. 2a and 2b show the effects of the treatments on the PSII activity, measured in terms of Fv/Fm. The Fv/Fm value was almost static on control (C) plants, throughout the experiment at both stages, while it decreased by the ABA, ethylene and waterlogging treatments at 1DAW. The reduction of Fv/Fm in C+E-treated plants was small, whereas the other treatments, including ethylene or waterlogging, showed more than four fold reduction at 1DAW in both stages. After that Fv/Fm declined slowly up to 1DAE in all the treated plants. Fv/Fm values showed their lowest value at 8 DAW or 1DAE in all the treated plants at both stages. Later on, Fv/Fm value increased, and that of W-treated plants reached their control level, whereas all the other treated plants showed some reduction even at 8DAW. Both ABA treated plants (C+A and W+A) showed relatively small reduction (around 4% and 7% respectively) at 8DAE, whereas all ethylene treated plants (C+E, W+E, C+A+E and W+A+E) showed much lower values of Fv/Fm than control. There were little differences in changing patterns of all plots between growth stages.

5. Leaf water potential (ψl)

ψl of all the treated plants was not significantly different from that of the control plants during whole experimental period at both stages of growth (Fig. 2c and 2d).

Discussion

All the treatments used in this study gave influences on photosynthesis and transpiration of the mungbean plants variously (Fig. 1, 2a and 2b), but neither waterlogging nor exogenous application of phytohormones affected plant water status, as indicated by the changing patterns of leaf water potential (Fig. 2c and 2d). This may suggest that physiological changes induced by both waterlogging and exogenous phytohormones were not directly related to plant water status. In addition, effects of exogenous phytohormones and waterlogging on mungbean plants did not differ between plant growth stages. It
Fig. 1. Mean photosynthetic rate ($P$), transpiration rate ($T$) and stomatal conductance ($g_s$) in mungbean plants during the 8-day period of waterlogging and 8-day period after the end of waterlogging treatment at vegetative (WV) and reproductive (WF) stages. Dashed lines show the waterlogged plants (W, W+A, W+E, W+A+E) and solid lines the control plants (C, C+A, C+E, C+A+E). DAW and DAE indicate the days after the beginning of the waterlogging treatment and days after the end of the waterlogging treatment, respectively, and arrows when waterlogging was removed. Means are shown ± SE, n=8 (SE smaller than symbols are not shown).
Exogenous application of ABA and ethylene induced a decrease in photosynthetic rates irrespective of waterlogging in our study. However, \( T_r \) and \( g_s \) were not affected by ethylene and waterlogging treatments (C+E and W+E, W) within one day after the initiation of the treatments. During the same period, \( P \) decreased sharply, indicating that the early reduction of \( P \) by ethylene and waterlogging might be through a mechanism independent of stomatal closure, although ethylene may be involved in stomatal closure through a function of its soluble precursor, 1-aminocyclopropane-1-carboxilic acid (ACC), biosynthesized in roots under anaerobic soil conditions\(^{13}\). In our study, after 1 DAW, \( P \), \( T_r \) and \( g_s \) decreased gradually with the duration of treatments (C+E and W+E, W) prolonged during the first 8-day period, suggesting that the reduction of \( P \) was partly caused by stomatal closure. Although \( T_r \) and \( g_s \) of ethylene treated (C+E) plants showed mild depression during the first 8-day period, the lower value of W+E
treated plants indicated some additive effects of waterlogging on depressed photosynthetic rate. Furthermore, the lower value of all measured parameters of C+E and W+E-treated plants than those of waterlogged (W) plants at 8 DAE have shown a residual and additive effect of ethylene on mungbean plants. Non-stomatal reduction of \( P \) by ethylene was evident during the whole experimental period, because during the second 8-day period of ethylene treatment, \( P \) of C+E-treated plants was still lower than C+A-treated ones, whose \( T_i \) and \( g_s \) were lower than those of C+E-treated ones. A group of researchers suggested that possibly, some metabolic factors such as a down-regulation of PSII could be involved in \( P \) reduction\(^6\). In our study, the chlorophyll fluorescence measurement showed a decrease in \( F_{v}/F_{m} \) ratio by ethylene and waterlogging treatment (C+E, W+E and W). Since \( F_{v}/F_{m} \) was affected before \( g_s \) and \( T_i \), it is probable that initial reduction in \( P \) by waterlogging and ethylene treatment is due to the damages to photosystem II. The lower value of \( F_{v}/F_{m} \) in W+E-treated plants than those of C+E and W-treated plants during whole experimental periods may indicate effects of increased concentration of ethylene, which may be produced by waterlogging, on depressed photosynthetic rates.

\( P, T_i \) and \( g_s \) declined within one day after the initiation of ABA treatments (C+A and W+A) and later declined progressively with duration of the treatments prolonged during the first 8-day period. It indicated that the reduction of \( P \) might have been caused by \( CO_2 \) deficiency through the stomatal closure. This finding are in agreement with Downton et al. stating that stomatal closure fully accounts for the inhibition of photosynthesis by ABA\(^7\). In our study, \( P \) of W+A-treated plants was a little bit smaller than those of C+A-treated ones with a similar changing pattern, showing some additional effects of ABA on depressed photosynthetic rate. Moreover, lower value of \( T_i \) and \( g_s \) in W+A-treated than W-treated plants also indicated the additive effects of ABA concentrations on stomatal limitations of photosynthetic rates in waterlogged plants. On the other hand, mild depression of \( F_{v}/F_{m} \) in C+A-treated plants during whole experimental periods had shown some non-stomatal effects of ABA on the reduction of \( P \) in mungbean plants. This non-stomatal effect was masked when ABA was treated to waterlogged plants, indicated by small or no decrease of \( P \) and \( F_{v}/F_{m} \) in W+A-treated plants in comparison to W-treated plants, suggesting the interaction between ABA and ethylene, which may be produced by waterlogging.

In this study, the fact that \( P \) of C+A+E-treated plants was depressed slightly than C+A and C+E-treated ones also indicated some interaction between ABA and ethylene. A group of researchers observed that treatment with ABA partially protected the PSII complex against subsequent photo-induced damages at a low temperature in leaves of barley seedlings\(^8\). From the observation of \( F_{v}/F_{m} \) ratio in C+A and C+E-treated plants, we could state that ethylene damaged PSII complex more pronouncedly than ABA. Hyung and Whang also observed that PSII activities were reduced by ethylene\(^9\). It seems that \( F_{v}/F_{m} \) ratio in W+A+E-treated plants was affected more conspicuously by ethylene than ABA, because the additional effect of ethylene on \( F_{v}/F_{m} \) (shown by W+A vs. W+A+E) was bigger than that of ABA (shown by W+E vs. W+A+E). This may also suggest that waterlogging-induced photosynthetic reduction was partly due to inactivation of PSII by ethylene. In W+A+E-treated plants, \( P \) was much lower than those in C+A+E-treated plants. This may be caused by additional effects of both ABA and ethylene produced by waterlogged plants in addition to exogenous applications, indicated by additional decrease of \( T_i \) and \( g_s \) in comparison to W-A-treated plants and that of \( F_{v}/F_{m} \) in comparison to W+E-treated plants.

In this paper we have addressed the question whether ABA or ethylene plays a relevant role in the waterlogging-induced photosynthetic reduction in mungbean plants. The results presented here show that waterlogging-induced \( P \) reduction was mediated by both stomatal and non-stomatal factors. The effect of ABA on stomata was rapid and quite large, whereas the effect of ethylene on stomata was slow and relatively smaller than that of ABA and waterlogging. In a recent work with tomato plants, Hussain \textit{et al.} proposed that ethylene was not involved in determining stomatal conductance and that ABA dominated stomatal responses when the roots encountered water stress condition in compacted soil\(^10\). These may suggest that effects of ethylene on stomatal closure may be indirect and probably through some unknown mechanisms. On the other
hand, the effect of ethylene on non-stomatal factor, including damages to PSII systems was more pronounced than that of the effect of ABA, and seemed similar to that of waterlogging effect. In addition, although the effect of ABA on PSII was mild, the effect of ABA seemed to interact with ethylene in mungbean plants. These results suggest that early reduction of P is mainly caused by ethylene in waterlogged plants. In later stages, both phytohormones play their roles to suppress photosynthesis through both stomatal and non-stomatal factors. Our observations indicate that the causes of photosynthetic depression induced by waterlogging need to be better understood to assist in the development of mungbean with improved waterlogging stress tolerance through breeding program. Further studies on dynamics of endogenous plant hormones during waterlogging are necessary.

References


