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Changes of endogenous ABA and ACC, and their correlations to photosynthesis and water relations in mungbean (*Vigna radiata* (L.) Wilczak cv. KPS1) during waterlogging

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Abstract

The effects of waterlogging on the dynamics of leaf abscisic acid (ABA) and root 1-aminocyclopropane-1-carboxilic acid (ACC, a precursor of ethylene) contents together with those on photosynthetic rate, leaf water potential and chlorophyll fluorescence were studied in mungbean (*Vigna radiata* (L.) Wilczak cv. KPS1) plants under greenhouse conditions. Waterlogging reduced the photosynthetic rate and water use efficiency rapidly without any changes of stomatal conductance, transpiration rate and ABA concentrations. Rapid reduction of photosynthetic rate and Fv/Fm ratio of chlorophyll fluorescence without increase of ABA indicates that early reduction of photosynthetic rate may not be related to ABA. In addition, the slower recovery of P, P/T_r and Fv/Fm values than ABA implies that ABA is not completely involved in photosynthetic reduction. Increased concentration of ACC during the waterlogging period and after the end of waterlogging may indicate the involvement of ethylene in photosynthetic reduction through the reduction of PSII activities, although early reduction of photosynthesis could not be explained by ethylene. After 2 days of waterlogging, ABA was increased concomitantly with the rapid reduction of P, T_r and g_s. It may suggest that ABA reduces photosynthesis through some ABA-related reactions, such as stomatal closure.

Keywords: ABA; Chlorophyll fluorescence; Ethylene; Mungbean; Photosynthesis; Waterlogging

1. Introduction

Decrease of crop growth and yield as a result of waterlogging results from the disturbance of many physiological functions of the plants. One of the main physiological effects of waterloggging is an inhibition of photosynthesis (Save and Scrran, 1986; Olivella et al., 2000). Recently, there has been growing evidence that changes in the root environment can influence shoot physiology as well as photosynthesis through the transmission of chemical signals. Davies and Zhang (1991) suggested that many of these physiological changes are associated with alterations in plant hormone concentrations. Zhang and Zhang (1994) have shown that a substantial abscisic acid (ABA) accumulation in leaves was mainly responsible for the severe stomatal closure following flooding in pea plants. Like other plant hormones, ABA has multiple effects during the life cycle of a plant. ABA has been reported to induce stomatal closure (Zhang and Zhang, 1994; Castonguay et al., 1993) and to enhance plant stress tolerance by reducing transpirational water loss (Wu et al., 1997). Although ABA is likely to play important regulatory roles, the involvement of this hormone in photosynthetic depression has not been elucidated. Recent studies have revealed that an important role of endogenous ABA is to limit ethylene production and that as a result of this interaction ABA is involved to maintain rather than inhibit shoot and root growth under water stress conditions (Sharp, 2002). However, such observations do not fully explain the function of endogenous ABA accumulation on photosynthesis under waterlogging conditions.

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As an alternative to the accumulation of ABA, ethylene may act as a root sourced chemical signal transported as its soluble precursor, 1-aminocyclopropane-1-carboxilic acid (ACC), under anaerobic conditions (Jackson, 1997). In anaerobic tomato plants ACC is synthesized and then transported in the xylem to the shoots where it is rapidly oxidized to ethylene and subsequently induces epinastic leaf curvature (Bradford et al., 1982; Banga et al., 1997). Based on these results, it is possible that ethylene may also be involved in mediating shoot responses as well as photosynthetic rates of waterlogged mungbean plants. In general, the synthesis of ethylene and the response of plants to ethylene differ among tissues and plant species (Abeles et al., 1992), and can be affected by different internal and environmental factors (Sharp et al., 2000; Grichko and Glick, 2001). For instance, ethylene stimulates growth in some aquatic and amphibious species, whereas it inhibits shoot elongation in most terrestrial plants (Abeles et al., 1992). As this hormone exerts profound effects on such diverse aspects during plant growth, its physiological action mechanism related to photosynthetic damage in waterlogged plants has attracted much interest.

To understand the role of ABA and ACC in the depression of photosynthesis during waterlogging, we studied the chlorophyll fluorescence yield (Fv/Fm) of photosystem II (PSII). The Fv/Fm measurements have been widely used as a tool for elucidating the mechanism of photoinhibition and the adaptational mechanisms for protection against photodamage (Schreiber, 1998). Although PSII is believed to play a key role in the response of photosynthesis to environmental perturbation (Baker, 1991), the correlation of waterlogging with PSII have not been thoroughly examined.

This study was undertaken to determine if ABA or ethylene accumulation play a relevant role in the waterlogging-induced photosynthetic reduction of mungbean plants. This study was also designed to investigate whether those hormones are related to photoinhibition of photosystem II in waterlogged plants.

2. Materials and methods

2.1. Plant culture

Mungbean seeds (cv. KPS1) from Thailand were sown in small pots with vermiculite on June 14, 2001. Six-day-old and approximately 5 cm tall seedlings were transferred into 18 cm tall and 23 cm diameter clay pots containing sterilized sand on June 22. Each pot contained one mungbean plant and pot spacing was 70 cm between rows and 30 cm between plants. Sterilized fine sand was used to minimize the effect of soil microorganisms on plant responses to waterlogging (Nawata et al., 1991). The plants were normally watered twice a day. A proper amount of "Enshi" solution (Yamasaki, 1982) for macro elements and "Arnon" solution (Arnon and Hoagland, 1940) for micro elements were applied three times a week. The solution of "Oltran" (0.1%, Takeda Pharmaceutical Co. Ltd.) was also applied on 12-day-old seedlings when aphid attacked them. Plants were grown in a plastic greenhouse at Kyoto University, Japan, under natural sunlight and photoperiod. Mid-day weather conditions during the experimental periods were characterized by relatively clear sky (i.e., photosynthetic photon flux density > 900 μmol m^{-2} s^{-1}), maximum air temperature of 27–32 °C and 72% relative humidity.

2.2. Experimental treatments

The experimental treatments consisting of different growth stages and waterlogging were arranged in a randomized complete block design with eight replications. Just before the start of the treatment, each pot was moved into a 28 cm diameter plastic bucket. Waterlogging treatments were given by filling the outer container with water containing nutrient solution, up to 2 cm above the soil surface. After 8 days of the waterlogging treatment, outer containers were removed. Waterlogging treatments were imposed three weeks after sowing (WV: vegetative stage) and when the first flower appeared (WF: reproductive stage) in plants. Separate plants were used for each waterlogging period to prevent possible compounding of consecutive waterlogging effects. Control and waterlogged treatment pots were fully irrigated just before the beginning of each waterlogging period to maintain similar initial soil–water contents in the pots.

2.3. Physiological measurements

Photosynthetic rate (P), transpiration rate (T_r) and stomatal conductance (g_s) were measured using a Leaf Chamber Analyser (LCA-4, ADC Bio Scientific Ltd. Hoddesdon, UK). P, T_r and g_s were measured on uppermost, fully expanded, trifoliolate leaflets (abaxial surface) between 10:00 am and 12:00 noon at the temperature range of 28–32 °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 used for the gas exchange determination, after a dark adaptation time of 30 min. All physiological measurements were replicated eight times (or more) from eight different leaves of eight different plants for each treatment on 0, 1, 2, 4 and 8 days after the beginning of the waterlogging treatment (DAW) and on 1, 2, 4 and 8 days after the end of waterlogging (DAE). Before each destructive harvest (used for hormonal analysis, described later), the uppermost, fully expanded, trifoliolate leaflets of each plant was used for measuring leaf water potential (Ψ_l), with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) on 0, 1, 2, 4, 8 DAW and on 1, 2, 4 and 8 DAE.

2.4. ABA determination

Leaf samples (usually 10 g) for both control (C) and waterlogged (W) plants were taken for ABA assay, just after phys-