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Original Paper

Evaluation the response of maize to osmotic stress using acoustic emission

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The present study deals with examining the response of maize to osmotic stress condition. In the experiment, two investigated plants which are maize were chosen to be tested in the controllable environment. One plant was controlled to be in well water condition, whereas another one was treated by PEG-6000 for being encountered the osmotic stress condition throughout the experimental period. The main measuring technique used to monitor the tested plants in the experiment consisted of acoustic emission techniques. The experimental results indicated that the distinct reactions between a control plant and an influenced plant according to osmotic stress condition could be differentiated by acoustic emission method.

Keywords: acoustic emission, maize, plant stress, sap flow, stomatal conductance

1 Introduction

Transport of water from the root system to the aerial parts of the plant occurs in specialized conducting cells of the xylem. In these cells water is transported under highly negative pressure, typically of -1 to -2 MPa, in some cases the pressure may be even as low as -10 MPa (Tyree and Sperry, 1989). Under these conditions, cavitation occurs i.e. embolism due to suction of air bubbles into the tracheal elements through the pits in the cell wall (Jarbeau et al., 1995; Hacke and Sperry, 2001). Drought stress results in decrease of xylem pressure and increased frequency of cavitation (Barigah et al., 2013). Interruption of the water column in xylem vessels is accompanied by a characteristic, measurable acoustic signal (Milburn and Johnson, 1966; Milburn, 1973). This event often causes loss of hydraulic conductivity of affected vessel (Hölttä et al., 2009). As a response to reduced water availability stomatal closure occurs (Assmann and Shimazaki, 1999). Stomatal opening or closure can be quantified by measuring stomatal conductance by a porometer (Yunusa et al., 2008).

Under experimental conditions, drought stress can be simulated by cultivation of the plants in a hydroponic culture with application of osmoticum to the nutrient solution, because lack of water in the soil usually also causes a reduction in soil water potential (Dasgupta et al., 2015) and consequently reduces water availability for the plant root system. The effects of water and osmotic stress on plants are very similar in many aspects, although not the same in all respects (Chen and Kao, 1993). Advantage of this method is well defined value of nutrient solution osmotic potential in rhizosphere. For this purpose various compounds have been used e.g. mannitol, sorbitol, sodium chloride or polyethylene glycols (PEGs) (Soheilikhah et al., 2013; Zhao and Schaller, 2004; Mohammadkhani and Heidari, 2008). Disadvantage of use of sugar alcohols like mannitol is the fact that these compounds penetrate into the apoplast and also into the symplast which results in an increase in osmotic pressure of cells (Hohl and Schopfer, 1991). Sodium chloride is also taken up by root cells and besides osmotic stress, toxic effects of Na^+ and Cl^- ions occurs (Zhao et al., 2010). In contrast, very low uptake of high molecular weight PEGs about 1 mg g^{-1} fresh weight per week occurs in plants with undamaged roots (Lawlor, 1970), which makes use of this compounds more convenient method to induce drought stress.

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Acoustic emission testing (AET) is a non-destructive testing (NDT) technique that detects and monitors the release of ultrasonic stress waves from localized sources when a material deforms under stress. Many specific applications have been developed using acoustic emission because it is an extremely powerful technology that can be deployed within a wide range of usable applications of non-destructive testing. The AET is associated with the detection and conversion of high frequency elastic waves to electrical signals. This is accomplished by directly coupling piezoelectric transducer (AE sensor), which can be either resonant and bandwidth types. Sensor is coupled to the structure and the output of piezoelectric sensor is amplified through a low-noise preamplifier. Then, the signals pass through a filter to remove any extraneous noise (Mistras, 2016). After that, the signals are amplified by the main amplifier again before being sent to the signal conditioner. Finally, the AE signals are subtracted and stored in a computer for further analysis. During investigations, other parameters, such as, load, deformation, pressure, and temperature, can also be recorded as other parametric inputs. The majority of acoustic emission testing is based on the processing of signals with frequency content in the range from 30 kHz to about 1 MHz. In special applications, detection of acoustic emission at frequencies below 20 kHz or near audio frequencies can improve testing and conventional microphones or accelerometers are sometimes used. Acoustic emission is based on measuring acoustic sound waves that are usually emitted during the growth of microscopic defects, such as stress cracks or pitting. It is the flaw growth or even plastic deformation that generates the sound waves that are detected by the acoustic sensors (Pierre, 2012).

Thus, the objective of present study was to justify the characteristics of detected AE signals from investigated plants under controllable environment in order to find more information of using AE signals for indicating the water stress condition on plant and monitoring the impact on transpiration and photosynthesis.

2 Materials and methods

2.1 Plant material, growth conditions and treatments

Plants of maize (*Zea mays* L.) cultivar ‚Vitras' were cultivated in the controllable greenhouse in Richter´s nutrient solution (Richter, 1926) for 1 month before experiment. Then healthy plants at the same developmental stage (7 fully developed leaves – 17th phenological stage according to BBCH scale for maize – Meier, 2001) has been chosen for experiment. In stressed treatment, the nutrient solution was replaced by nutrient solution supplemented by addition of 200 g l^{-1} PEG 6000 (Rotipuran, Carl Roth, Germany) to generate osmotic pressure of -1.09 MPa (Hellal et al., 2018). There was one stress period applied to stressed plant for a period of 28 hrs in total. Control plants were cultivated in Richter´s nutrient solution only (there was no stress period). The greenhouse was shielded during the experiment. Illumination of the plants was provided by the Philips Master Agro 400 W high-pressure sodium lamps (Philips, NL) and light intensity was measured using a sensor (units – lumens).

2.2 Stomatal conductance

Stomatal conductance of selected leaves was measured with a cyclic porometer AP4 (Delta – T Devices, Cambridge, UK) during seven hours period, two hours before and five hours after PEG application into nutrient solution, regularly at intervals of 30 min. Before the measurement, the device was calibrated according to the manufacturer's recommendations. For detection of stomatal conductance, fourth, fifth and sixth leaves from base of the stem were chosen, in text referred to as first, second and third leaves respectively.

2.3 Acoustic emission device

Acoustic emission (AE) method was also used to acquire the AE signals of investigated plants during stomatal conductance measurement. The used AE device as shown in Figure 1 consisted of two AE transducers connected with a metal waveguide, preamplifers, an environmental monitoring sensor (EMS), and an acquisition device. The used transducers were a broadband IDK-09 type (piezoelectric sensor) from Dakel (Prague, Czech Republic). To magnify AE signals, the AE preamplifiers of 35 dB were utilized to magnify the detected AE signals before converted from analog signals to digital signals by

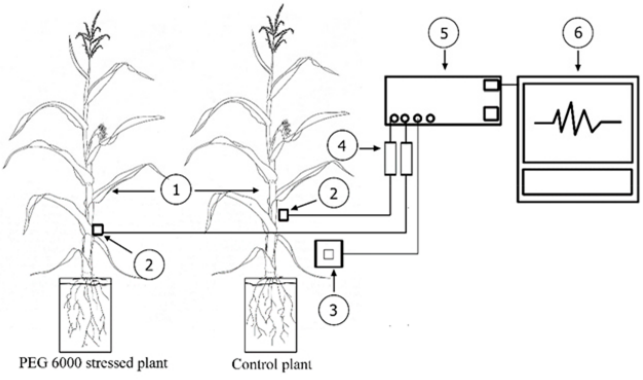

Figure 1 Schematic diagram of acoustic emission device monitoring the investigated plants
1 – investigated plants, 2 – AE sensors, 3 – environmental sensor, 4 –AE preamplifiers, 5 – AE acquistion system, 6 – software and computer
AE – acoustic emission

an AE acquisition unit. The acquisition unit was completely computer controlled. The waveforms and the classical acoustic parameters (AE accumulative event number, AE count number, etc.) were saved on a hard disk as soon as detected, and also EMS would simultaneously record the environmental parameters data such as air temperature, relative humidity, and light intensity. The all experimental results from AE system would be analyzed and displayed by Daemon and Deashow software (Dakel, Prague, Czech Republic). The position where AE transducers were mounted on each investigated plant was at the low part of stem of test plant as shown in Figure 1.

3 Results and discussion

2.5 hours after PEG treatment, the stomatal conductance of selected leaves of an influenced plant started to decrease gradually and during the whole period of measurement was substantially lower compared to a control plant. Even 24 hours after treatment, stomatal conductance in an influenced plant remained below

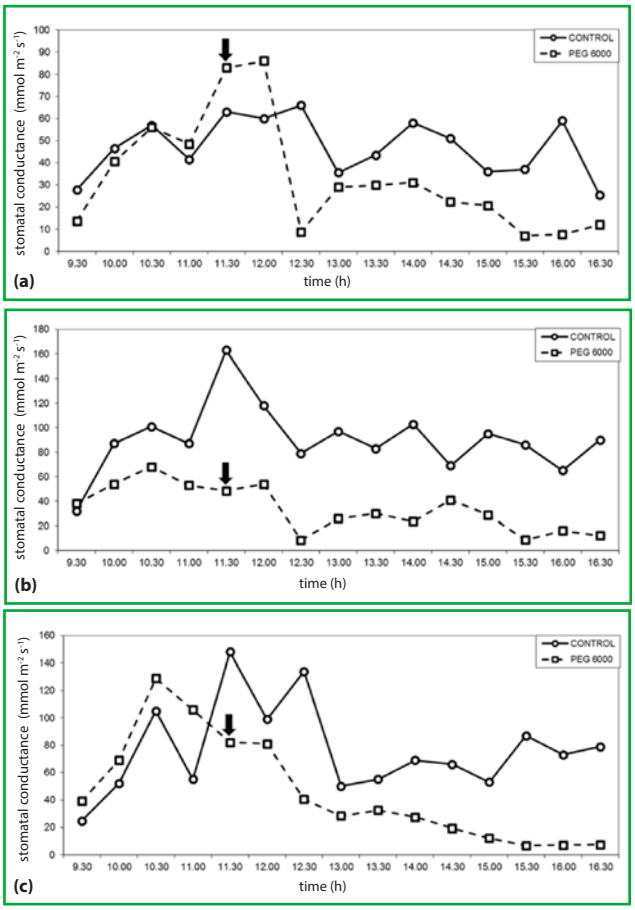

Figure 2 The effect of PEG 6000 induced osmotic stress on stomatal conductance
Trial: first (a), second (b) and third leaves (c). Open circles represent control plant, open squares represent plant treated with 200 g l^{-1} PEG. Arrows indicate the time of PEG application into nutrient solution

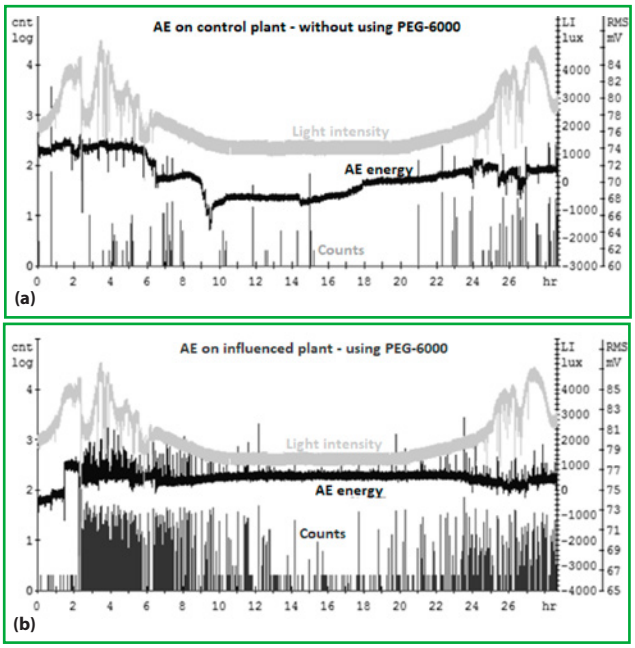

Figure 3 Charts of recorded AE signals and light intensity during experiment
Trial: a control plant (3a), and the chart from that of an influenced plant (3b) against experimental period

20 mmol m^{-2} s^{-1} as depicted in Figure 2 which illustrates the stomatal conductance values of both an influenced plant and a control plant measured at first, second, and third leaves.

The acoustic emission (AE) technique was used to examine the AE signals generated from both an influenced plant (PEG 6000) and a control plant over the whole period of experiment. In order to observe the relations between AE signals and environmental parameters, the light intensity (LI) was also recorded during this study. From the experimental results of AE testing, detected AE signals from both plants as illustrated in Figure 3 interestingly indicated the three significant events as following: Firstly, AE signals were generated much stronger during daytime than AE signals were generated at night time. Secondly, in comparison of AE signals in each plant, an influenced plant intentionally simulated to be under water stress condition was likely to form the number of AE signals during test more than AE signals generated from a control plant. Lastly, the response of AE signals of both investigated plants to environmental conditions as displayed in Figures 3 show that light intensity value have the strong effect to the change of value of AE signals parameters which are AE energy – root mean square (RMS) and AE count number values (counts).

According to this result, two parameters of AE signal which were the AE count number and the AE accumulative event number values could differentiate the AE signal

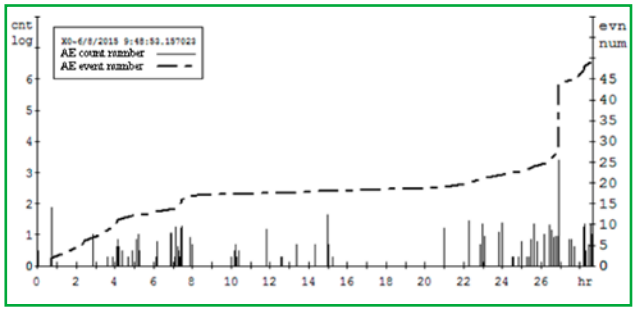

Figure 4 Relation between the number of AE count number and the number of AE accumulative event number detected from the control plant versus experimental period
AE – acoustic emission

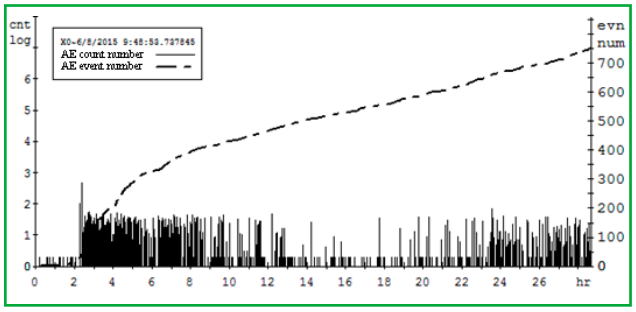

Figure 5 Relation between the number of AE count number and the number of AE accumulative event number detected from the PEG 6000 stressed plant versus experimental period
AE – acoustic emission

characteristics of each investigated plants as depicted in Figures 4 and 5. Figure 4 illustrates the value of AE accumulative event number and AE count number value throughout experimental period of a control plant, and also Figure 5 shows the value of that of an influenced plant. As can be seen from these figures, the maximum value of AE accumulative event number of an influenced plant is equal to 750 approximately, whereas a chart of control plant shows a maximum value of AE accumulative event number of 49 only. Furthermore, considering the AE count number value of an influenced plant with that of a control plant, it was obvious that the value of AE count number of an influenced plant was more intensive and higher than that of a control plant throughout whole experimental period, particularly in the day.

The results of this work are completely unique and it is difficult to discuss them with other authors. This type of research is original. From this result of AE measurement, it was found that AE accumulative event number and AE count number could be considered the suitable AE parameters to differentiate the water stress condition of plant in this study since the values of these AE parameters between a control plant and an influenced plant were obviously different as illustrated in Figures 4 and 5.

Measuring the values of stomatal conductance of both investigated plants indicated the stomatal conductance value was changeable, when plant was under water stress condition. As can be seen from experimental results, the value of stomatal conductance of an influenced plant gradually dropped over experimental period because of its water stress condition. On the other hand, a control plant did not obviously show decrease of its stomatal conductance throughout period of experiment. For AE measurement in both plants, it was clearly observed that an influenced plant generated more AE signals than a plant in normal condition. This is because an influenced plant was controlled to be under water stress condition by adding PEG 6000 into nutrient solution of an influenced plant, and this solution induced the occurrence of a lot of AE signals inside the stem of an investigated plant due to its cavitation activity (Ryu et al., 2016; Gleason et al., 2017).

From this result of AE measurement, we found that AE accumulative event number and AE count number could be considered the suitable AE parameters to differentiate the water stress condition of plant in this study since the values of these AE parameters between a control plant and an influenced plant were obviously different. Moreover, comparing the result of stomatal conductance measurements and the outcome of AE measurement in this experiment confirmed that AE technique can estimate the condition of plant when it encounters water stress condition or be under well water condition.

They comprise also general responses of plants to osmotic stress such as changed water and osmotic potentials (Nepomucenko et al., 1998) and activation of protective mechanisms including e.g. cellular osmotic regulatory processes with altered proline and ABA contents (Wilson et al., 2014), altered Rubisco functioning (Demirevska et al., 2009), decreased content of chlorophyllase (García et al., 1987), enhanced synthesis of antioxidative enzymes (Grzesiak et al., 2013). Therefore, future studies focused on biophysical detection and evaluation of embolism in plants would address also the above aspects of plant physiological responses.

4 Conclusions

Application of acoustic emission (AE) technique for estimating the water stress condition of plant is possibly a great potential technique for controlling and managing the irrigation system of plants. Two plants of maize which were an influenced plant and a control plant were studied under the controllable environment. The experimental achievement of this study clarify that AE signals will

be generated from a plant very strong, when a plant is under water stress condition. Thus, from this experiment, it present that the response of plant due to water stress condition of plant can be recognized by using the AE method.

Application of acoustic emission (AE) technique for estimating the water stress condition of plant is possibly a great potential technique for controlling and managing the irrigation system of plants. Therefore, in this experiment, study of osmotic stress of plants by using the cyclic porometer for measuring the stomatal conductance of leaves and AE device for monitoring AE signals of plant was implemented to validate that evaluation of the osmotic stress condition of plant might be conducted by AE method. Two plants of maize which were an influenced plant and a control plant were studied under the controllable environment. The experimental achievement of this study clarify that AE signals will be generated from a plant very strong, when a plant is under water stress condition, and this result is consistent with the outcome of stomatal conductance analysis. Thus, from this experiment, it present that the respond of plant due to water stress condition of plant can be recognized by using the AE method.

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