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BRIEF COMMUNICATION

Abscisic acid mediates hydrogen peroxide production in peanut induced by water stress

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Abstract

Peanut plants exposed to water stress induced by polyethylene glycol (PEG) accumulated abscisic acid (ABA) and hydrogen peroxide (H_2O_2), the increase being significant at 12 and 24 h after addition, respectively. To address the question whether the increase in H_2O_2 production was related to ABA accumulation, the peanut leaves were pretreated with ABA biosynthesis inhibitor (sodium tungstate) and then exposed to water stress. Under these conditions, a decrease of ABA and H_2O_2 content were found after 12 h. The addition of 100 μM ABA restored H_2O_2 content reaching values similar to those under water stress at 12 h. We concluded that ABA accumulation is the first signal that triggers the H_2O_2 generation in peanut during first 12 h but its subsequent production is partially ABA-independent.

Additional key words: *Arachis hypogaea,* polyethylene glycol, sodium tungstate.

Peanut (*Arachis hypogaea* L.) is grown as an important crop in a wide range of environments and its production is limited by water deficit periods almost every year (Collino *et al.* 2001). The plant responses to water stress include changes in stomatal conductance and growth, osmolyte accumulation, and expression of specific genes. The abscisic acid (ABA) is defined as the major stress hormone due to its rapid accumulation under drought and participation in physiological and biochemical processes that allow plants to survive to this challenge (Zhang *et al.* 2006). Signal molecules, such as hydrogen peroxide (H_2O_2) and nitric oxide (NO) are involved in the ABA-induced stomatal closure and the enhanced activities of antioxidant enzymes in maize, *Arabidopsis*, and *Stylosanthes guianensis* (Jiang and Zhang 2002, Desikan *et al.* 2004, Zhou *et al.* 2005, Bright *et al.* 2006, Zhang *et al.* 2006, 2007). New insight towards ABA induced stomatal closure comprehension has identified a phospholipase C-independent but calcium-mediated ABA signalling in response to drought in *Arabidopsis thaliana* (Cousson 2009). Additionally, a recent model proposes that ABA activates phospholipase D resulting in phosphatidic acid (PA) production. PA binds to the cytosolic region in the N-terminus of Rboh(D)-NADPH oxidase, resulting in the stimulation of enzymatic activity and reactive oxygen species (ROS) production in *Arabidopsis* guard cells (Zhang *et al.* 2009).

In peanut, our previous studies demonstrated that 30-d-old plants exposed to drought stress for 14 d reached an osmotic potential of -0.6 MPa (control plants -0.4 MPa) and accumulated ABA and H_2O_2. These changes were completely reversed upon rehydration (Furlan *et al.* 2012). To elucidate whether ABA accumulation triggers H_2O_2 production, *in vitro* assays were done using polyethylene glycol (PEG) as water stress inductor.

Seeds of peanut (*Arachis hypogaea* L.) cv. Granoleico (obtained from *Criadero El Carmen*, *General Cabrera*, Córdoba, Argentina) were surface sterilized (Vincent 1970) and pre-germinated in Petri dishes for 96 h. Pre-germinated seeds were transferred to pots filled with 200 g of sterile volcanic sand. Plants were irrigated twice a week alternately with distilled water and Hoagland and Arnon (1950) nutrient solution without nitrogen in order to keep the field capacity of 13 % which was determined

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Abbreviations: ABA - abscisic acid; LC - liquid chromatography; MS-MS - tandem mass spectrometry; PA - phosphatidic acid; PEG - polyethylene glycol; Rboh - respiratory burst oxidase homolog; ROS - reactive oxygen species.

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by pressure-plate method (Burk 1996). They were grown in a controlled growth chamber (a 16-h photoperiod, irradiance of 200 µmol m^{-2} s^{-1}, temperature of 28 ºC, and relative humidity of 50 %). Seven days after sowing, the plants were inoculated with *Bradyrhizobium* sp. SEMIA6144 (10^8 UFC cm^{-3}; *Mircen*, Porto Alegre, Brazil). Thirty days after sowing the plants were excised at the base of the stem, rinsed in distilled water, and the cut ends of the stems were placed in beakers wrapped with aluminium foil containing 100 cm^3 of distilled water (control) or 20 % PEG solution (osmotic potential of -0.6 MPa; water stress) for 48 h. The selected osmotic potential of PEG solution was in accordance with the value obtained in stressed plants by water withholding during 14 d. In order to study the effects of inhibitors, the detached plants were pretreated with sodium tungstate (5 mM) for 12 h and then exposed to water stress treatment (-0.6 MPa) for 12 and 24 h under the same conditions as described above. Besides, to test whether the effects of tungstate could be overcome by exogenous ABA, the detached plants were pretreated with 5 mM tungstate plus 100 µM ABA for 12 h and then exposed to water stress. Detached plants maintained in distilled water for the whole period served as controls. After 12 and 24 h of water stress treatment, the second leaves were sampled and imme-diately frozen in liquid N_2 and then stored at -80 °C for further analysis. Five replicate plants per treatment were sampled at each time point.

Abscisic acid content was measured according to Zhou *et al.* (2003). Briefly, 150 mg of plant material was homogenized with liquid nitrogen and hydrochloric acid solution (pH 2.8 - 3.0). As a standard, 5 ng of [2H6]-ABA (gift of Dr. J.D. Chen, USDA-ARS, Beltsville, Maryland, USA) was added. The aqueous phase was purified by adding an equal volume of ethyl acetate and organic phase was completely evaporated at 35 ºC. Extract was resuspended in 0.1 cm^3 of methanol (100 %), placed in specific vials, and 0.01 cm^3 of each sample was used to determine ABA content by liquid chromatography (LC) (*Waters*, New York, USA) tandem mass spectrometry (MS-MS) (*Micromas*, Manchester, UK) with a monitoring software (*Masslink 4.1*). Hydrogen peroxide was measured spectrophotometrically after reaction with KI (Alexieva *et al.* 2001). The reaction mixture consisted of 0.16 cm^3 of 0.1 % (m/v) trichloroacetic acid (TCA), the leaf extract, 0.16 cm^3 of 100 mM K-phosphate buffer, and 0.68 cm^3 of 1 M KI. The reaction was developed for 1 h in darkness and then absorbance measured at 390 nm. All data were analyzed using *InfoStat* (*v. 2011*) program. Two way *ANOVA* was used to determine the effect of the treatments at each time point. Duncan´s multiple range test was used as post-hoc analysis to determine differences between means. Differences were considered significant for $\alpha < 0.05$.

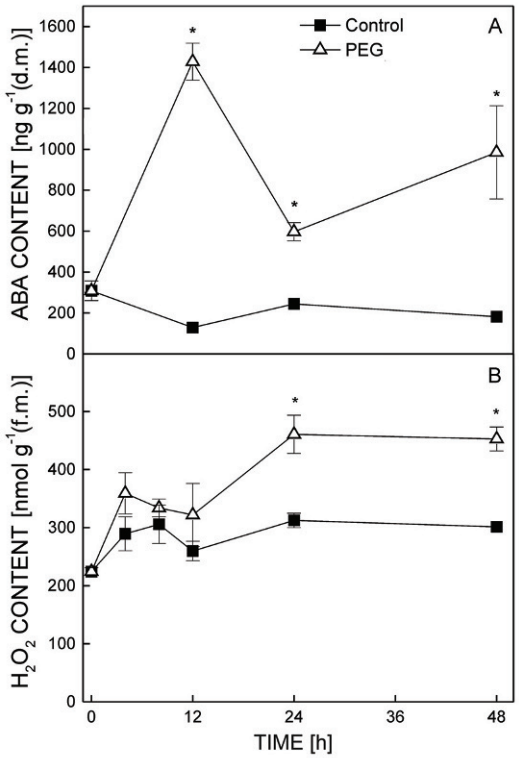

Fig. 1. Effects of water stress induced by PEG addition on ABA (*A*) and H_2O_2 (*B*) content in peanut leaves. Means ± SE of 5 replicates. *Asterisks* indicate significant differences between the treatment and the control for the same time point ($\alpha < 0.05$) according to Duncan´s multiple range test.

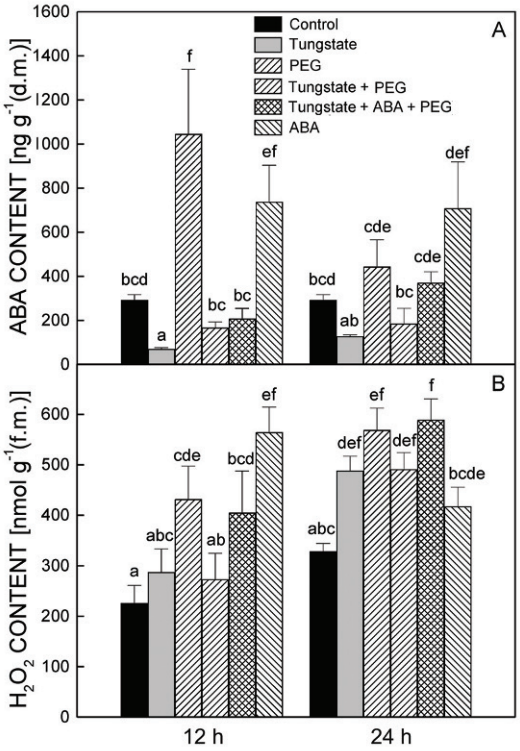

Fig. 2. ABA (*A*) and H_2O_2 (*B*) content in peanut leaves exposed to water stress after different pretreatments (see text for details). Means ± SE of 5 replicates. Different letters in columns indicate significant differences between the treatments for the same time point ($\alpha < 0.05$).

A significant ABA accumulation occurred upon 12 h in PEG-treated plants, whereas this increment was less pronounced at 24 and 48 h compared with the control plants (Fig. 1*A*). On the other hand, a significant increase in H_2O_2 content occurred after 24 h (Fig. 1*B*). Based upon these results, we determined sample dates at 12 and 24 h to address the question whether the increase in H_2O_2 production was related to ABA accumulation in peanut leaves exposed to water stress. For that, a second group of plants was used to investigate the effects of pretreatment with ABA biosynthesis inhibitor, tungstate, which impairs ABA-aldehyde oxidase, on H_2O_2 production. Moreover, a treatment with 100 μM ABA addition was carried out to test whether the effects of inhibitor could be overcome. After pretreatment with 5 mM tungstate, the increase in ABA content induced by water stress was inhibited (Fig. 2*A*). At the same time, this pretreatment suppressed the increase in H_2O_2 generation in leaves exposed to water stress at 12 h (Fig. 2*B*). The application of 100 μM ABA increased the content of ABA partially decreased by tungstate at 24 h and prevented the reduction in H_2O_2 generation.

ROS generation in response to ABA accumulation has been reported by several authors (Yan *et al.* 2007, Cho *et al.* 2009, Jammes *et al.* 2009, Lu *et al.* 2009). In this study, the time-course production of both compounds shows that the peak of ABA preceded that of H_2O_2 (Fig. 1). Similar results were found in maize plants (Jiang and Zhang 2002). Additional studies demonstrate that NADPH oxidases are involved in ABA-mediated ROS generation as well as in antioxidant enzyme induction in response to ROS accumulation in bermudagrass (Lu *et al.* 2009). Our findings show that stressed plants pretreated with the inhibitor tungstate did not accumulate either ABA or H_2O_2 at 12 h post-treatment, strongly suggesting that the ABA triggered ROS production. However, when tungstate pretreated plants were exposed to water stress for 24 h, H_2O_2 was accumulated whereas ABA content remained rather low. A possible explanation about this increase of H_2O_2 content could be that some of the ROS sources, which include an electron transport, many enzymatic and non-enzymatic reactions (Asada 1999, Foyer and Noctor 2000), photorespiration, and Mehler reaction (Cruz de Carvalho 2008), might be ABA independent.

Taking together, this study demonstrated that ABA accumulation under water stress was the first signal which trigger the increased H_2O_2 content, but during stress progression, H_2O_2 production was partially ABA independent. Similar results were found in *Stylosanthes guianensis* (Zhou *et al.* 2005). In conclusion, ABA mediates ROS generation in legumes under drought conditions, thus contributing to the understanding a general response model of agronomically important plants to this environmental constraint.

References

Alexieva, V., Sergiev, I., Mapelli S., Karanov, E.: The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. - Plant Cell Environ. **24**: 1337-1344, 2001.

Asada, K.: The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. - Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 601-639, 1999.

Bright, J., Desikan, R., Tancock, J.T., Weir, I.S., Neill, S.J.: ABA-induced NO generation and stomatal closure in *Arabidopsis* are dependent on H_2O_2 synthesis. - Plant J. **45**: 113-122, 2006.

Burk, R. (ed.): Soil Survey Laboratory Methods Manual. - National Soil Survey Center, Lincoln 1996.

Cho, D., Shin, D., Wook Jeon, B., Kwak, J.M.: ROS-mediated ABA signaling. - J. Plant Biol. **52**: 102-113, 2009.

Collino, D., Dardanelli, J., Sereno, R., Racca, R.: Physiological responses of argentine peanut varieties to water stress, light interception, radiation use efficiency and partitioning of assimilates. - Field Crops Res. **70**: 177-184, 2001.

Cousson, A.: Involvement of phospholipase C-independent calcium-mediated abscisic acid signalling during *Arabidopsis* response to drought. - Biol. Plant. **53**: 53-62, 2009.

Cruz de Carvalho, M.H.: Drought stress and reactive oxygen species. Production, scavenging and signaling. - Plant Signal. Behav. **3**: 156-165, 2008.

Desikan, R., Cheung, M.K., Bright, J., Henson, D., Hancock, J.T., Neill, S.J.: ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells. - J. exp. Bot. **55**: 205-212, 2004.

Foyer, C.H., Noctor, G.: Oxygen processing in photosynthesis: regulation and signalling. - New Phytol. **146**: 359-388, 2000.

Furlan, A., Llanes, A., Luna, V., Castro, S.: Physiological and biochemical responses to drought stress and subsequent rehydration in the symbiotic association peanut-*Bradyrhizobium* sp. - ISRN Agronomy. DOI: 10.5402/2012/318083.

Hoagland, D., Arnon, D.: The water culture method for growing plants without soil. - Calif AES Bull. **347**: 1-39, 1950.

Jammes, F., Songa, C., Shina, D., Munemasab, S., Takedaa, K., Gua, D., Choa, D., Leea, S., Giordoa, S., Sritubtimd, S., Leonhardte, N., Ellisd, B.E., Muratab, Y., Kwaka, J.M.: MAP kinases MPK9 and MPK12 are preferentially expressed in guard cells and positively regulate ROS-mediated ABA signaling. - PNAS **106**: 20520-20525, 2009.

Jiang, M., Zhang, J.: Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and upregulates the activities of antioxidant enzymes in maize leaves. - J. exp. Bot. **53**: 2401-2410, 2002.

Lu, S., Su, W., Li, H., Guo, Z.: Abscisic acid improves drought tolerance of triploid bermudagrass and involves H_2O_2- and NO-induced antioxidant enzyme activities. - Plant Physiol. Biochem. **47**: 132-138, 2009.

Vincent, J. (ed.): A manual for the Practical Study of Root Nodule Bacteria. - Blackwell Scientific Publication, Oxford 1970.

Xue, B., Zhang, A., Jiang, M.: Involvement of polyamine oxidase in abscisic acid induced cytosolic antioxidant

defense in leaves of maize. - J. Integr. Plant Biol. **51**: 225-234, 2009.

Yan, J., Tsuichihara, N., Etoh, T., Iwai, S.: Reactive oxygen species and nitric oxide are involved in ABA inhibition of stomatal opening. - Plant, Cell Environ. **30**: 1320-1325, 2007.

Zhang, A., Jiang, M., Zhang, J., Ding, H., Xu, S., Hu, X., Tan, M.: Nitric oxide induced by hydrogen peroxide mediates abscisic acid-induced activation of the mitogen-activated protein kinase cascade involved in antioxidant defense in maize leaves. - New Phytol. **175**: 36-50, 2007.

Zhang, A., Jiang, M., Zhang, J., Tan, M., Hu, X.: Mitogen-activated protein kinase is involved in abscisic acid-induced antioxidant defense and acts downstream of reactive oxygen species production in leaves of maize plants. - Plant Physiol. **141**: 475-487, 2006.

Zhang, Y., Zhu, H., Zhang, Q., Li, M., Yan, M., Wang, R., Wang, L., Welti, R., Zhang, W., Wangb, X.: Phospholipase Dα1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in *Arabidopsis*. - Plant Cell **21**: 2357-2377, 2009.

Zhou, B., Guo, Z., Xing, J., Huang, B.: Nitric oxide is involved in abscisic acid induced antioxidant activities in *Stylosanthes guianensis*. - J. exp. Bot. **56**: 3223-3228, 2005.

Zhou, R., Squires, T.M., Ambrose, S.J., Abrams, S.R., Ross, A.R., Cutler, A.J.: Rapid extraction of abscisic acid and its metabolites for liquid chromatography-tandem mass spectrometry. - J. Chromatogr. A **1010**: 75-85, 2003.