16th WORLD FERTILIZER CONGRESS OF CIEC

TECHNOLOGICAL INNOVATION FOR A SUSTAINABLE TROPICAL AGRICULTURE

PROCEEDINGS

INTERNATIONAL SCIENTIFIC CENTRE OF FERTILIZERS

NPK

C.I.E.C.

International Scientific Centre of Fertilizers (CIEC)

16th WORLD FERTILIZER CONGRESS OF CIEC

TECHNOLOGICAL INNOVATION FOR A SUSTAINABLE TROPICAL AGRICULTURE

October 20-24, 2014
Rio de Janeiro, RJ - Brazil

PROCEEDINGS

Vinicius de Melo Benites
Adilson de Oliveira Junior
Paulo Sergio Pavinato
Paulo César Teixeira
Milton Ferreira Moraes
Regina Maria Villas Bôas de Campos Leite
Ronaldo Pereira de Oliveira
Editors

Rio de Janeiro, RJ
2014

34

MITIGATION OF SALT STRESS OF TOMATO USING PRE-TREATMENT WITH HUMIC ACIDS

DANIEL BASÍLIO ZANDONADI[1], MIRELLA PUPO SANTOS[2]; LEONARDO OLIVEIRA MEDICI[3]

[1] Laboratório de Biologia Celular, Rodovia BR-060, Km 09 (Brasília/Anápolis), Fazenda Tamanduá Caixa Postal: 218, Brasília, 70359-970, BRAZIL (daniel.zandonadi@embrapa.br); [2] Laboratório de Transformação Genética, Parque Estação Biológica - PqEB - Av. W5 Norte Caixa Postal 02372, Brasília, 70770-917, BRAZIL (mirellapupo@yahoo.com.br); [3] Departamento de Ciências Fisiológicas, BR 465 km 7, Seropédica, 23890-000, BRAZIL (lmedici@gmail.com)

Introduction

Increasing environmental constraints, such as salinity are threatening the productivity of crops worldwide. Near to 77 million hectares land is affected by this problem (FAO, 2007). The intensive use of soluble fertilizers is considered one of the causes of salinization. Either natural soil organic matter or humic substances has been reported as important to ameliorate both drought and salinity of soils due their indirect and direct effects (Bot and Benites, 2005; Mora et al. 2014). The effects of salt stress may alter photosynthesis and cause oxidative stress. Humic Acids (HAs) isolated from vermicompost appear to restore water capacity of ABA-deficient tomato *sitiens*, thus it is not surprising that HAs could affect plant physiology during abiotic stress. In this work we evaluated vermicompost HAs as potential alleviator of salt stress in tomato plants. Pre-treatment with this synthetic organic material helped plants to cope with salt stress by a mechanism related to photosynthesis maintenance, membranes stability and the activity of nutrient uptake and salt stress related enzymes. It is postulated that HAs decreases the harmful effects of salt stress. To the best of our knowledge this is the first work showing that plasma membrane H^+-ATPase is related to Na exclusion in NaCl stressed plants pre-treated with HAs and that humic substances can directly affect photosynthesis processes during stress.

Methods

Growth and salinization of plant material was performed as described by Binzel (1995) with some modifications. Briefly, after 30 days under half strength Hoagland's solution (HSHS), tomato (*Solanum Lycopersicum* cv Rheinlands Ruhm, RR) plants were pre-treated with half strength Hoagland's solution only (con) or HSHS plus HAs for 4 days (HAs). Subsequently, plants were transferred to HSHS plus 200mM NaCl for additional 3 days (con>NaCl or HAs>NaCl). Plants were analyzed afterwards. Each treatment consisted of 10 plants. Experiments were repeated three times.

Results and discussion

Humic substances have been used to improve fertilizers efficiency, since it could enhance mineral nutrients uptake and reduce nutrient fixation in soil (Erro et al., 2008; Jannin et al., 2012). Humic substances isolated from different sources are able to increase proton pumps activity, apparently due its auxin-like effect and nitric oxide production (Zandonadi et al., 2010), but little is known about the role of these organic substances during plant stress. Tomato plants (cv. Rheinlands Ruhm), exposed to increasing salt concentration until 300 mM NaCl presented a decline of its initial indole 3-acetic acid (IAA) levels in the roots by nearly 75% (Dunlap and Binzel, 1996). The presence of IAA in the HAs structure was detected (Canellas et al., 2002) and here we show that the pre-treatment of tomato plants with humic acids (HAs) resulted in an alleviation of saline deleterious effects (Figs. 1 and 2). The mean photosynthetic rate, transpiration rate, the stomatal conductance and chlorophyll a content of tomato plants were reduced by exogenous application of 200mM NaCl (Fig. 1). Membrane integrity and oxidative stress as measured by means of malondialdehyde (MDA) content were affected by NaCl and recovery due HAs pre-treatment was also observed (Fig. 1). Mitigation of salt stress by humic substances appears to be related to both direct and indirect features of photosynthesis. To date the only work showing effects of HAs on plant photosynthesis during salt stress concluded that there is no improvement in salt tolerance due HAs treatments (Liu and Cooper, 2002). It is hard to compare the results from this study with the present work, since here we did a pre-treatement instead an addition of HAs during salt stress. In addition, plant metabolisms are very different in each case.

The plant membrane transporters are crucial in increasing both nutrient content and resistance salinity stress. Binzel (1995) reported a stimulation of both plasma membrane and tonoplast ATPase proton pumps transcripts in tomato roots during 150-400 mM NaCl stress. In agreement, here HAs were able to enhance proton pumps activity prior to salt exposition, an important step for detoxifying Na^+ ions from cytosol (Fig. 1). Auxin levels declines when plants are challenged with NaCl (Dunlap and Binzel, 1995). The Has sample used here has IAA in its structure, thus IAA could be associated to either a direct effect on plant proton pumps as endogenous hormonal balance favoring the salt tolerance observed. It is worthy to note that HAs are able to induce nitric oxide production (Zandonadi et al., 2010), a very important signal during salt stress. In line with this results leaf nitrate reductase activity, also related to enzymatic nitric oxide production (Yamasaki and Sakihama, 2000) and assimilatory nitrogen metabolism, was affected by both NaCl and HAs (Fig 1).

Conclusions

Tomato plants previously treated with HAs and then challenged with NaCl exhibited increased sodium tolerance, by a mechanism related to auxin-like effects on proton pumps, holding nitrate reductase activity and photosynthesis in the same level as unstressed plants. We cannot exclude the possible interaction of HAs with mineral nutrients during NaCl stress. It remains to be elucidated whether mitigating mechanisms are due auxin-like molecules of HAs structure or modification of endogenous plant hormone balance by HAs. Despite the promising results presented here, the role of HAs as an addictive for fertilizers need more research mainly regarding its positive effects in field conditions.

Keywords: organic matter, photosynthesis, biostimulant, proton pumps.

Acknowledgements

We gratefully acknowledge the financial support of the National Counsel of Technological and Scientific Development (CNPq).

References

Mora, V., M. Olaetxea, E. Bacaicoa, R. Baigorri, M. Fuentes, A. M. Zamarreño, and J. M. Garcia-Mina. “Abiotic Stress Tolerance in Plants: Exploring the Role of Nitric Oxide and Humic Substances.” In Nitric Oxide in Plants: Metabolism and Role in Stress Physiology, pp. 243-264. Springer International Publishing, 2014.

Baigorri, R., Urrutia, O., Erro, J., Mandado, M., Pérez-Juste, I., & Garcia-Mina, J. M. (2013). Structural Characterization of Anion–Calcium–Humate Complexes in Phosphate-based Fertilizers. ChemSusChem, 6(7), 1245-1251.

Binzel, M. L. (1995). NaCl-induced accumulation of tonoplast and plasma membrane H^+-ATPase message in tomato. Physiologia Plantarum, 94(4), 722-728.

Bot A, Benites J (2005) The importance of soil organic matter. Key to drought-resistant soil and sustained food production. FAO, Rome.

Dunlap, J. R., & Binzel, M. L. (1996). NaCl reduces indole-3-acetic acid levels in the roots of tomato plants independent of stress-induced abscisic acid. Plant physiology, 112(1), 379-384.

FAO, 2007. Extent and Causes of Salt-affected Soils in Participating Countries. AGL: Global Network on Integrated Soil Management for Sustainable use of Salt-affected Soils. <http://www.fao.org/ag/agl/agll/spush/topic2.htm>

Jannin, L., Arkoun, M., Ourry, A., Laîné, P., Goux, D., Garnica, M., ... & Etienne, P. (2012). Microarray analysis of humic acid effects on Brassica napus growth: Involvement of N, C and S metabolisms. Plant and soil, 359(1-2), 297-319.

Liu, C., & Cooper, R. J. (2002). Humic acid application does not improve salt tolerance of hydroponically grown creeping bentgrass. Journal of the American Society for Horticultural Science, 127(2), 219-223.

Yamasaki, H., & Sakihama, Y. (2000). Simultaneous production of nitric oxide and peroxynitrite by plant nitrate reductase: in vitro evidence for the NR-dependent formation of active nitrogen species. Febs Letters, 468(1), 89-92.

Zandonadi, D. B., Santos, M. P., Dobbss, L. B., Olivares, F. L., Canellas, L. P., Binzel, M. L., ... & Façanha, A. R. (2010). Nitric oxide mediates humic acids-induced root development and plasma membrane H+-ATPase activation. Planta, 231(5), 1025-1036.

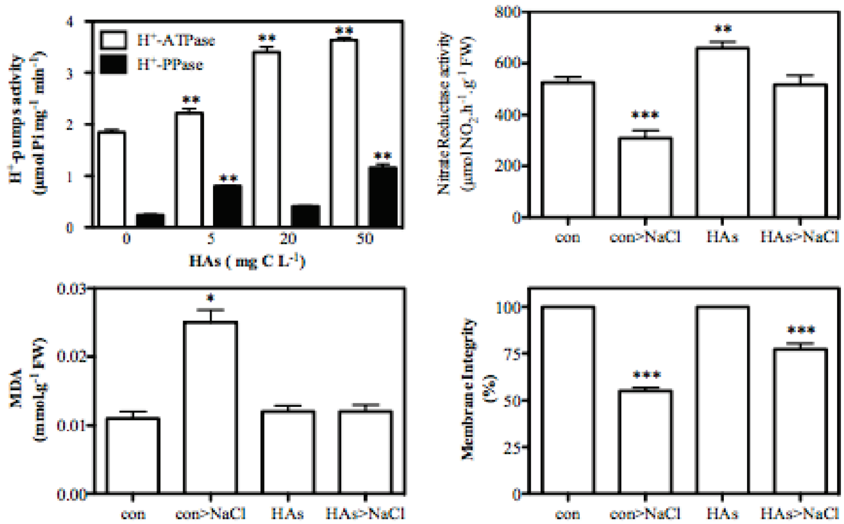

Figure 1. Root plasma membrane H+-ATPase and tonoplast H+-PPase activities, leaf nitrate reductase activity, root malondialdehyde (MDA) content and root membrane integrity

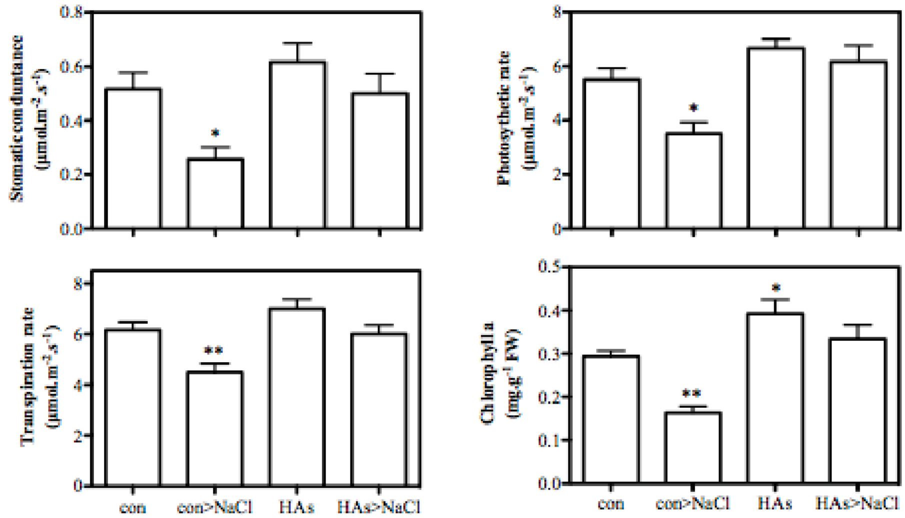

Figure 2. Stomatic condutance, photosynthetic rate, transpiration rate and chlorophyll a content of tomato plants