Cultivos Tropicales, 2015, vol. 36, special edition, pp. 51-58

ISSN impreso: 0258-5936
ISSN digital: 1819-4087

Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

Review

NITRIC OXIDE AND ITS ROLE IN PLANT RESPONSES TO WATER STRESS

Reseña bibliográfica
Óxido Nítrico y su papel en las respuestas de las plantas al estrés hídrico

Yamile Vidal Aguiar[1]✉, Akira Pérez Márquez[1] and Loiret Fernández García[2]

ABSTRACT. Water deficit is currently the abiotic stress with highest incidence on growth and crop productivity. Numerous reports affirm that nitric oxide (NO) is a signaling molecule involved in mechanisms of response to this stress condition. Through the application of NO donor compounds have been obtained experimental evidence in support these functions for NO, but it´s little known about the natural production of NO in response to drought stress and its implication for the plant. Likewise, the mechanism by which this molecule exerts its effects and the molecular targets of NO in plants under water stress have not yet been described. Additionally various data indicate that stomatal closure is effected through intracellular signaling in which NO is a component too. Thus, these data suggest an emerging model of drought stress response in which NO has been included.

Key words: growth, stoma, stress, productivity

RESUMEN. El déficit hídrico constituye en la actualidad el estrés abiótico de mayor incidencia en el crecimiento y productividad de los cultivos. Numerosos reportes afirman que el óxido nítrico (NO) es una molécula señal involucrada en los mecanismos de respuestas ante esta condición de estrés. Mediante la aplicación de compuestos donadores de NO se han obtenido evidencias experimentales que apoyan estas funciones para el NO, pero poco se sabe acerca de la producción natural de NO en respuesta al estrés por sequía y su implicación para la planta. Asímismo, el mecanismo por el cual dicha molécula ejerce sus efectos y las dianas moleculares del NO en plantas sometidas a déficit hídrico aún no han sido descritas. Adicionalmente varios datos indican que el cierre de los estomas se efectúa a través de la señalización intracelular, en cual el NO es un componente. Por lo tanto, estos datos sugieren un nuevo modelo en la respuesta de la planta al estrés hídrico en la cual el NO debe ser incluido.

Palabras clave: crecimiento, estoma, estrés, productividad

INTRODUCTION

Water shortage is the most prevalent abiotic stress on plant growth (1, 2). In recent years, global changes in weather conditions have made drought more intense and longer (1, 3).

This type of stress occurs in plants when water loss by transpiration exceeds root absorption capacity, resulting in cell dehydration and its consequent damage, leading to cell death.

At cell level, water deficit induces a reactive oxygen species (ROS) overproduction, which is responsible for the oxidative damage to biomolecules associated with this type of stress. Plants usually respond to this condition by modifying gene expression, related to key enzyme production in osmolyte synthesis, protective proteins, antioxidant enzymes, transcription factors and other proteins involved in responses to water stress (4). Most of these responses are regulated by abscisic acid (ABA), although some independent hormone regulation forms have also been described (5).

[1] Instituto Nacional Ciencias Agrícolas (INCA), gaveta postal 1, San José de las Lajas, Mayabeque, Cuba, CP 32700.

[2] Facultad Biología, Universidad de La Habana.

✉ yvidalaguiar@gmail.com

Under these conditions, one of the most important defense mechanisms is stomatal closure induced by ABA synthesis and redistribution. Among molecules involved in ABA-mediated signaling, nitric oxide (NO) is an essential mediator.

NO endogenous requirement induced by this hormone during stomatal closure has been shown by the use of genetic and biochemical tools (6).

Recent reports also involve that NO and other reactive nitrogen species take place as systemic plant response mechanisms to water deficit. However, available information about NO metabolism in plants subjected to water stress is limited and even though investigations on this molecular role in numerous physiological responses has increased in recent years, production sources and molecular mechanisms by which NO responds are still poorly known, so that it is being studied in many laboratories worldwide.

This paper was aimed at showing an updated report on NO action mechanisms as well as its production pathways involved and this molecular role in response to water deficit. This is a relevant topic to explain molecular, biochemical and physiological bases, where NO is a mediator that could act harmoniously with ROS in plant response mechanism to this condition and also to practical agriculture use.

REACTIVE NITROGEN SPECIES (RNS)

This term was introduced in literature to refer to NO and other molecules related to it, such as S-nitrosothiols (SNOs), S-nitrosoglutathione (GSNO), peroxynitrite and other compounds having a relevant role in many physiological processes of plant cells (7).

Experimental evidence in animals demonstrates NO interaction with various biomolecules, such as lipids (8), nucleic acids (9) and proteins, modifying their functions. However, the latter is the most deeply studied.

Numerous plant reports confirm that RNS have their signaling through specific protein posttranslational modifications. In this sense, NO may react with proteins in different pathways: transition metals present in the protein giving complexes called nitrosylated metals (10), sulfhydryl groups of cysteine residues by S-nitrosylation process (11) and by adding a nitro group to tyrosine residues mediated by a nitration process (12, 13).

Special attention has been given to S-nitrosylation of glutathione tripeptide (GSH) process to form S-nitrosoglutathione (GSNO), since this molecule may function as a mobile reserve of NO (14, 15) and can regulate the balance between GSNO and nitrosylated proteins by a trasnitrosylation process. In this sense, GSNO reductase enzyme appears to be a key element, since it catalyzes GSNO reduction depending on reduced nicotinamide adenine dinucleotide (NADH) to oxidized glutathione (GSSG) and NH_3. Therefore, this enzyme controls intracellular GSNO level and as a result NO effects in cells (16, 17). Available information regarding plant metabolism of SNOs and RNS is still limited compared with animal models.

NO SYNTHESIS IN PLANTS

In the past decade, NO has emerged as an important signaling molecule in plants. The synthesis of this compound is a well-established animal mechanism, enzymatically catalyzed by three nitric oxide synthase (NOS), which have different locations and functions. This enzyme converts L-arginine to L-citrulline and NO and it requires various enzyme cofactors for its catalysis (18).

However, NO generation in plants is more controversial. To date, several NO generating systems, both enzymatic and non-enzymatic ones, have been described.

In 1996, the presence of NOS activity in *Lupinus albus* roots and nodules was reported for the first time by using radioactively labeled arginine and a NOS activity inhibitor in animals, NG-monomethyl-L-arginine (NMMA) (19).

Additionally, this activity has been described in other species like *Nicotiana tabacum* (20) and *Zea mays* (21), also in different cellular compartments, such as *Glycine max* chloroplasts (22) and *Pisum sativum* peroxisomes (23).

In 2003, AtNOS1 gene was identified in *Arabidopsis*, which encoded a protein having NOS activity and such activity required the same cofactors described in

animals, but the gene showed no sequence homology with any isoforms described in mammals. According to these data, authors reported the discovery of different NOS from those described so far in literature, capable of regulating growth and signaling plant hormone (24). Nevertheless, later studies showed that recombinant protein AtNOS1 did not show NOS activity (25) and such protein was actually a GTPase that may be required for *in vivo* NO production (26).

Although several authors suggest the presence of such plant activity, the gene encoding this enzyme has not yet been characterized, which is one of the most controversial issues regarding this enzyme in plants (25, 27).

On the other hand, nitrate reductase (NR) is one of the reported enzymes, capable of producing NO in plants. This enzyme reduces nitrite to NO in a NAD(P)H dependent reaction (28, 29). In 2002, it was shown that NO generation mediated by this enzyme is stimulated under hypoxia conditions, and can be modulated by its phosphorylation status (30). These data suggest that a potential regulatory mechanism can exist *in vivo*.

A specific root protein bound to a plasma membrane: nitrite NO-reductase (NI-NOR) has been described as NO source. Such enzyme uses cytochrome c as *in vivo* electron donor and catalyzes NO production from nitrite. However, neither its physiological role nor the gene encoding this enzyme has been reported so far (31).

NO formation derived non-enzymatic reactions have been referred in *Nicotiana tabacum* plants after nitrite mitochondrial reduction to NO (32) and this reaction is favored at acid pH, where nitrite can dismutate to NO (33). Meanwhile, *in vivo* NO generation by H_2O_2 and L-Arginine reaction was reported in 1997 (34). Additionally, a light-mediated reaction, where nitrite is reduced by carotenoids has also been demonstrated as a source of NO production (35).

Recent studies demonstrated that nitrate-free reductase plant cells are capable of forming NO from hydroxylamine exogenous supplement (36), an already characterized pathway in bacteria and animals (37). Likewise, polyamines can induce NO production, but the mechanism under which this occurs has not yet been described (38). Further analysis in this sense might define new signaling pathways considering that polyamines are involved in response mechanisms to stress conditions (39).

NO ROLE IN WATER STRESS

Many authors point out that plants produce certain NO levels under their natural environment (40) as a generalized response to environmental stress (41). Likewise, NO protective role in plants subjected to water stress has been reported by several researchers. Two interrelated mechanisms by which NO can mitigate stress effects have been described. First, NO can function as antioxidant by directly removing ROS generated under these conditions, with the subsequent peroxynitrite formation or by antioxidant enzyme expression (42). Furthermore, NO can act as signaling molecule allowing the expression of genes involved in plant response to water deficit and modifying protein activity by posttranslational modifications (43). NO chemical properties (small molecule, half-short life time, no load and high diffusivity) suggest that NO could function as a signal molecule in response to cellular stress (44). Most previous research studies on NO effect and its role in drought stress use NO chemical donors and compounds removing this molecule or inhibitors of enzymes involved in NO production. However, just few publications have naturally monitored NO production rate in response to water stress.

Previous studies have shown that NO reduces water loss in wheat leaves and those results are in correspondence with 20% transpiration rate decrease. Besides, plant exposure to SNP (sodium nitroprusside, NO donor) decreases ion loss and cellular damage by 25 % and these results are attributed to this molecule because SNP pre-incubation with NO-removing compounds reverse these effects (45). These results suggest that NO exogenous application may confer increased plant tolerance to drought stress conditions.

Recent publications have shown that exogenous NO increases drought tolerance in wheat plants (46). Such treatment enhances seedling growth maintaining a high water content and reducing oxidative damage by antioxidant enzyme production (47). However, high NO doses (2 mM SNP) enhance stress effect as a result of ROS overproduction and an inefficient ability to control ROS by antioxidant system expression. These data indicate NO potential ability to eliminate hydrogen peroxide is at least partly due to antioxidant defense mechanism induction (46).

Other studies in corn plants subjected to water stress by applying 10 % polyethylene-glycol showed a rapid NO increase in mesophyll cells. NOS activity was induced under these conditions in cytosolic and microsomal fractions, it being higher in the last. Pre-treatments with NOS and NR activity inhibitors decreased NO production, suggesting that this molecule is produced by the synergistic action of these two enzymes in corn plants exposed to water deficit. Enzyme activity of superoxide dismutase, ascorbate peroxidase and glutathione reductase is additionally induced together with NO expression (48).

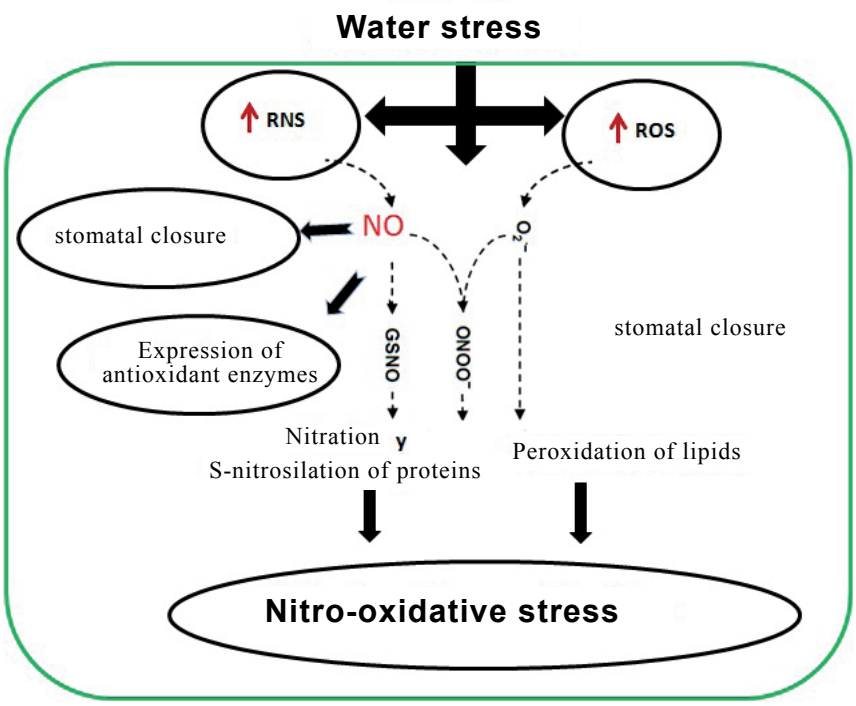
Meanwhile, a slight increase in NO synthesis at root tips and in the close region to elongation zone has been described in *Cucumis sativus* seedlings. This production was reduced by pre-treatment with NOS and NR inhibitors. Exogenous NO application (SNP and GSNO) indicate an adaptive root response to water stress, with a positive correlation between NO levels and plant water status (49). These results make evident that NO increase is closely linked to plant mechanisms by which it manages to decrease water deficit effects.

Despite this protective effect represents NO exogenous application to specific concentrations of this compound, recent studies show that such molecular overproduction as a plant physiological response to water stress along with increased ROS can mediate biomolecular damage, protein modifications being the most studied ones.

Recent studies prove that *Lotus japonicus* plants exposed to water stress presented significant changes at ROS and RNS levels. These changes were accompanied by increases in lipid peroxidation and protein nitration respectively, indicating that under these conditions, water stress caused oxidative and nitrosative stress (50). These results suggest that increased NO levels can have multiple roles in water stress-adapting mechanism and protein modifications may be involved in specific signaling mechanisms mediated by these molecules.

These two families of molecules (ROS and RNS) can be considered as endogenous signal molecules involved in plant response mechanism to drought conditions, but an overproduction of both can cause a nitro-oxidative stress with toxic consequences for the plant.

Figure 1 shows NO metabolism model in plant cells under water stress and its interaction with ROS. Under these conditions and depending on its concentration, NO can function as antioxidant by directly removing ROS or by antioxidant enzyme expression, or may be involved in stomatal closure by ABA-dependent signaling pathways. ROS and RNS overproduction can modify proteins and lipid peroxidation respectively, causing a nitro-oxidative stress in plants subjected to drought stress (51).



Taken from Francisco J Corpas and Juan B. Barroso, 2013 with some modifications
RNS: reactive nitrogen species
ROS: reactive oxygen species
NO: nitric oxide
GSNO: S-nitrosoglutathione;
ONOO-: peroxynitrite

NO metabolism model in plant cells subjected to water stress

STOMATAL CLOSURE DURING WATER STRESS. NO ROLE

Stomatal closure in response to ABA is mediated by a signaling network that envelops dependent and independent calcium pathways (52, 53), activated by a series of intermediaries including hydrogen peroxide (54, 55) and NO (56).

The finding that NO is a key molecule that mediates the response to ABA in pea plant guard cells (57) has been confirmed in *Vicia Faba* (58) and *Arabidopsis* (28). It is known that this response depends on time and NO concentration and it is mediated by a completely reversible process (57). The use of fluorescent probes such as 4,5 diaminofluorescein diacetate (DAF-2DA) has been widely reported in literature to detect NO production in real time. Increases in DAF-2DA fluorescence was observed in the cytoplasm and around the chloroplasts of ABA-treated guard cells. This fluorescence is specific for NO, and when using the inactive probe 4-diacetate diaminofluorescein (4AF-DA), no increases were observed in this signal. Exogenous application of 2-phenyl-5,5-tetrametilimidazoline-1-oxyl 3-oxide(PTIO) or 2-(4-carboxyphenyl)-4,4,5,5-tretametilimidazolina-1-oxyl-3-oxide(cPTIO), which are compounds removing NO, they inhibit stomatal closure induced by this hormone, showing the endogenous role of this molecule in this response (45, 57, 58). These studies showed that NO synthesis is essential for stomatal closure induced by ABA in several species (28, 45, 57).

To confirm NO role in stomatal closure, several studies have used NO exogenous donors, such as SNP. By using this compound, it was observed that it induces stomatal closure and reduces transpiration in three different plant species: *Tradescantia* sp (monocots) and two dicots (*Vicia faba* and *Salpichroa organifolia*) (45). These results have been confirmed by others in pea and *Arabidopsis* plants (28, 57).

In 2002, it was shown that stomatal closure is dependent on NO synthesis mediated by NR in guard cells and that treatment of these *Arabidopsis* cells with nitrite induces NO synthesis and consequently stomatal closure (28). The use of tungstate, NR activity inhibitor, inhibits NO synthesis induced by nitrite and ABA (59). Additionally, the use of N6-nitro-L-arginine methyl ester (L-NAME, NOS activity inhibitor) does not inhibit NO synthesis induced by ABA or stomatal closure in *Arabidopsis* epidermis (28). All these data suggest that NR serves as NO source induced by ABA in *Arabidopsis* guard cells.

In epidermal cells from *nia1 nia2* double mutant (shows less than 0,5 % NR activity), neither ABA nor nitrite induce stomatal closure and NO synthesis (28). Interestingly, it was found that guard cells from *nia1/nia2* mutant do not respond to other stimuli related to stomatal closure, such as darkness, hydrogen peroxide and use of NO donors, demonstrating an important role of such cell function (28). These results provide genetic evidence about NR role as NO source during stomatal closure in *Arabidopsis* mediated by ABA.

Specifically, considering both NR isoforms encoded by *nia1 and nia2* genes, stomatal closure is highly dependent on isoform encoded by *nia1* gene (28).

This NO-mediated regulation may be by Ca^{2+} modulation in guard cells. It is reported that NO selectively activates intracellular Ca^{2+} channels in *Vicia faba* through dependent signaling pathways cGMP/cADPR, suggesting NO role as a signal molecule in ABA-induced stomatal closure (57, 60).

CONCLUSIONS

Despite significant advances in NO cellular metabolism of plants, it should be recognized that knowledge about this molecular function and other RNS is very limited, which may be involved in water stress. A limiting factor is the need to identify NO production sources in each plant organ, which are crucial to this stress condition. Further researches should be directed to study signal transduction mechanisms involved in NO response mechanism as well as the identification and characterization of their molecular targets. A complete understanding of water stress phenomena require studying not only ROS role but also RNS and elucidate how these two kinds of molecules can interact during water stress.

BIBLIOGRAPHY

1. Chaves, M. M.; Maroco, J. P. y Pereira, J. S. ‘‘Understanding plant responses to drought-from genes to the whole plant’’. *Functional Plant Biology*, vol. 30, no. 3, 2003, pp. 239-264, ISSN 1445-4408, DOI http://dx.doi.org/10.1071/FP02076.

2. Shao, H. B.; Chu, L. Y.; Jaleel, C. A.; Manivannan, P.; Panneerselvam, R. y Shao, M. A. ''Understanding water deficit stress-induced changes in the basic metabolism of higher plants – biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe''. *Critical Reviews in Biotechnology*, vol. 29, no. 2, 2009, pp. 131-151, ISSN 0738-8551, DOI 10.1080/07388550902869792.
3. Chaves, M. M. y Oliveira, M. M. ''Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture''. *Journal of Experimental Botany*, vol. 55, no. 407, 11 de enero de 2004, pp. 2365-2384, ISSN 0022-0957, 1460-2431, DOI 10.1093/jxb/erh269.
4. Bray, E. A. "Plant responses to water deficit''. *Trends in Plant Science*, vol. 2, no. 2, 1 de febrero de 1997, pp. 48-54, ISSN 1360-1385, DOI 10.1016/S1360-1385(97)82562-9.
5. Roychoudhury, A.; Paul, S. y Basu, S. ''Cross-talk between abscisic acid-dependent and abscisic acid-independent pathways during abiotic stress''. *Plant Cell Reports*, vol. 32, no. 7, 2013, pp. 985-1006, ISSN 0721-7714, 1432-203X, DOI 10.1007/s00299-013-1414-5.
6. Neill, S.; Barros, R.; Bright, J.; Desikan, R.; Hancock, J.; Harrison, J.; Morris, P.; Ribeiro, D. y Wilson, I. ''Nitric oxide, stomatal closure, and abiotic stress''. *Journal of Experimental Botany*, vol. 59, no. 2, 2008, pp. 165-176, ISSN 0022-0957, 1460-2431, DOI 10.1093/jxb/erm293.
7. Durzan, D. J. y Pedroso, M. C. "Nitric Oxide and Reactive Nitrogen Oxide Species in Plants''. *Biotechnology and Genetic Engineering Reviews*, vol. 19, no. 1, 2002, pp. 293-338, ISSN 0264-8725, DOI10.1080/02648725.2002.10648032.
8. Desel, C. y Krupinska, K. ''The impact of tocochromanols on early seedling development and NO release''. *Journal of Plant Physiology*, vol. 162, no. 7, 2005, pp. 771-776, ISSN 0176-1617, DOI 10.1016/j.jplph.2005.04.008.
9. Akuta, T.; Zaki, M. H.; Yoshitake, J.; Okamoto, T. y Akaike, T. ''Nitrative stress through formation of 8-nitroguanosine: Insights into microbial pathogenesis''. *Nitric Oxide*, vol. 14, no. 2, 2006, pp. 101-108, ISSN 1089-8603, DOI 10.1016/j.niox.2005.10.004.
10. Gupta, K. J.; Hebelstrup, K. H.; Mur, L. A. J. y Igamberdiev, A. U. ''Plant hemoglobins: Important players at the crossroads between oxygen and nitric oxide''. *FEBS Letters*, vol. 585, no. 24, 2011, pp. 3843-3849, ISSN 0014-5793, DOI 10.1016/j.febslet.2011.10.036.
11. Astier, J.; Rasul, S.; Koen, E.; Manzoor, H.; Besson-Bard, A.; Lamotte, O.; Jeandroz, S.; Durner, J.; Lindermayr, C. y Wendehenne, D. ''S-nitrosylation: An emerging post-translational protein modification in plants''. *Plant Science*, vol. 181, no. 5, 2011, pp. 527-533, ISSN 0168-9452, DOI 10.1016/j.plantsci.2011.02.011.
12. Besson, B. A.; Pugin, A. y Wendehenne, D. ''New Insights into Nitric Oxide Signaling in Plants''. *Annual Review of Plant Biology*, vol. 59, no. 1, 2008, pp. 21-39, ISSN 1543-5008, 1545-2123, DOI 10.1146/annurev.arplant.59.032607.092830.
13. Cecconi, D.; Orzetti, S.; Vandelle, E.; Rinalducci, S.; Zolla, L. y Delledonne, M. "Protein nitration during defense response in *Arabidopsis thaliana*''. *Electrophoresis*, vol. 30, no. 14, 1 de julio de 2009, pp. 2460-2468, ISSN 1522-2683, DOI 10.1002/elps.200800826.
14. Durner, J. y Klessig, D. F. ''Nitric oxide as a signal in plants''. *Current Opinion in Plant Biology*, vol. 2, no. 5, 1999, pp. 369-374, ISSN 1369-5266, DOI 10.1016/S1369-5266(99)00007-2.
15. Barroso, J. B.; Corpas, F. J.; Carreras, A.; Rodríguez, S. M.; Esteban, F. J.; Fernández, O. A.; Chaki, M.; Romero, P. M. C.; Valderrama, R.; Sandalio, L. M. y Río, L. A. del. ''Localization of S-nitrosoglutathione and expression of S-nitrosoglutathione reductase in pea plants under cadmium stress''. *Journal of Experimental Botany*, vol. 57, no. 8, 2006, pp. 1785-1793, ISSN 0022-0957, 1460-2431, DOI 10.1093/jxb/erj175.
16. Leterrier, M.; Chaki, M.; Airaki, M.; Valderrama, R.; Palma, J. M.; Barroso, J. B. y Corpas, F. J. ''Function of S-nitrosoglutathione reductase (GSNOR) in plant development and under biotic/abiotic stress''. *Plant Signaling & Behavior*, vol. 6, no. 6, 2011, pp. 789-793, ISSN 1559-2316, 1559-2324, DOI 10.4161/psb.6.6.15161.
17. Corpas, F. J.; Alché, J. de D. y Barroso, J. B. ''Current overview of S-nitrosoglutathione (GSNO) in higher plants''. *Plant Physiology*, vol. 4, 2013, p. 126, ISSN 0032-0889, 1532-2548, DOI 10.3389/fpls.2013.00126.
18. Mayer, B. y Hemmens, B. ''Biosynthesis and action of nitric oxide in mammalian cells''. *Trends in Biochemical Sciences*, vol. 22, no. 12, 1997, pp. 477-481, ISSN 0968-0004, DOI 10.1016/S0968-0004(97)01147-X.
19. Cueto, M.; Hernández, P. O.; Martín, R.; Bentura, M. L.; Rodrigo, J.; Lamas, S. y Golvano, M. P. ''Presence of nitric oxide synthase activity in roots and nodules of *Lupinus albus*''. *FEBS Letters*, vol. 398, no. 2, 1996, pp. 159-164, ISSN 0014-5793, DOI 10.1016/S0014-5793(96)01232-X.
20. Durner, J.; Wendehenne, D. y Klessig, D. F. ''Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose''. *Proceedings of the National Academy of Sciences*, vol. 95, no. 17, 18 de agosto de 1998, pp. 10328-10333, ISSN 0027-8424, 1091-6490, DOI 10.1073/pnas.95.17.10328.
21. Ribeiro, E. A.; Cunha, F. Q.; Tamashiro, W. M. S. C. y Martins, I. S. ''Growth phase-dependent subcellular localization of nitric oxide synthase in maize cells''. *FEBS Letters*, vol. 445, no. 2, 1999, pp. 283-286, ISSN 0014-5793, DOI 10.1016/S0014-5793(99)00138-6.
22. Jasid, S.; Simontacchi, M.; Bartoli, C. G. y Puntarulo, S. "Chloroplasts as a Nitric Oxide Cellular Source. Effect of Reactive Nitrogen Species on Chloroplastic Lipids and Proteins''. *Plant Physiology*, vol. 142, no. 3, 11 de enero de 2006, pp. 1246-1255, ISSN , 1532-2548, DOI 10.1104/pp.106.086918.

23. Barroso, J. B.; Corpas, F. J.; Carreras, A.; Sandalio, L. M.; Valderrama, R.; Palma, J.; Lupiáñez, J. A. y Río, L. A. del. ‘‘Localization of Nitric-oxide Synthase in Plant Peroxisomes’’. *Journal of Biological Chemistry*, vol. 274, no. 51, 1999, pp. 36729-36733, ISSN 0021-9258, 1083-351X, DOI 10.1074/jbc.274.51.36729.
24. Guo, F. Q.; Okamoto, M. y Crawford, N. M. ‘‘Identification of a Plant Nitric Oxide Synthase Gene Involved in Hormonal Signaling’’. *Science*, vol. 302, no. 5642, 2003, pp. 100-103, ISSN 0036-8075, 1095-9203, DOI 10.1126/science.1086770.
25. Zemojtel, T.; Fröhlich, A.; Palmieri, M. C.; Kolanczyk, M.; Mikula, I.; Wyrwicz, L. S.; Wanker, E. E.; Mundlos, S.; Vingron, M.; Martasek, P. y Durner, J. ‘‘Plant nitric oxide synthase: a never-ending story?’’. *Trends in Plant Science*, vol. 11, no. 11, 2006, pp. 524-525, ISSN 1360-1385, DOI 10.1016/j.tplants.2006.09.008.
26. Moreau, M.; Lee, G. I.; Wang, Y.; Crane, B. R. y Klessig, D. F. ‘‘AtNOS/AtNOA1 Is a Functional *Arabidopsis thaliana* cGTPase and Not a Nitric-oxide Synthase’’. *Journal of Biological Chemistry*, vol. 283, no. 47, 2008, pp. 32957-32967, ISSN 0021-9258, 1083-351X, DOI 10.1074/jbc.M804838200.
27. Gupta, K. J.; Fernie, A. R.; Kaiser, W. M. y van Dongen, J. T. ‘‘On the origins of nitric oxide’’. *Trends in Plant Science*, vol. 16, no. 3, 2011, pp. 160-168, ISSN 1360-1385, DOI 10.1016/j.tplants.2010.11.007.
28. Desikan, R.; Griffiths, R.; Hancock, J. y Neill, S. ‘‘A new role for an old enzyme: Nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*’’. *Proceedings of the National Academy of Sciences*, vol. 99, no. 25, 12 de octubre de 2002, pp. 16314-16318, ISSN 0027-8424, 1091-6490, DOI 10.1073/pnas.252461999.
29. Kaiser, W. M.; Weiner, H.; Kandlbinder, A.; Tsai, C.-B.; Rockel, P.; Sonoda, M. y Planchet, E. ‘‘Modulation of nitrate reductase: some new insights, an unusual case and a potentially important side reaction’’. *Journal of Experimental Botany*, vol. 53, no. 370, 2002, pp. 875-882, ISSN 0022-0957, 1460-2431, DOI 10.1093/jexbot/53.370.875.
30. Rockel, P.; Strube, F.; Rockel, A.; Wildt, J. y Kaiser, W. M. ‘‘Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and *in vitro*’’. *Journal of Experimental Botany*, vol. 53, no. 366, 1 de enero de 2002, pp. 103-110, ISSN 0022-0957, 1460-2431, DOI 10.1093/jexbot/53.366.103.
31. Stöhr, C. y Stremlau, S. ‘‘Formation and possible roles of nitric oxide in plant roots’’. *Journal of Experimental Botany*, vol. 57, no. 3, 2006, pp. 463-470, ISSN 0022-0957, 1460-2431, DOI 10.1093/jxb/erj058.
32. Planchet, E.; Jagadis, G. K.; Sonoda, M. y Kaiser, W. M. ‘‘Nitric oxide emission from tobacco leaves and cell suspensions: rate limiting factors and evidence for the involvement of mitochondrial electron transport’’. *The Plant Journal*, vol. 41, no. 5, 2005, pp. 732-743, ISSN 1365-313X, DOI 10.1111/j.1365-313X.2005.02335.x.
33. Bethke, P. C.; Badger, M. R. y Jones, R. L. ‘‘Apoplastic Synthesis of Nitric Oxide by Plant Tissues’’. *The Plant Cell*, vol. 16, no. 2, 2004, pp. 332-341, ISSN 1532-298X, DOI 10.1105/tpc.017822.
34. Nagase, S.; Takemura, K.; Ueda, A.; Hirayama, A.; Aoyagi, K.; Kondoh, M. y Koyama, A. ‘‘A Novel Nonenzymatic Pathway for the Generation of Nitric Oxide by the Reaction of Hydrogen Peroxide and D- or L-Arginine’’. *Biochemical and Biophysical Research Communications*, vol. 233, no. 1, 1997, pp. 150-153, ISSN 0006-291X, DOI 10.1006/bbrc.1997.6428.
35. Cooney, R. V.; Harwood, P. J.; Custer, L. J. y Franke, A. A. ‘‘Light-mediated conversion of nitrogen dioxide to nitric oxide by carotenoids’’. *Environmental Health Perspectives*, vol. 102, no. 5, 1994, pp. 460-462, ISSN 0091-6765.
36. Rümer, S.; Kapuganti, J. G. y Kaiser, W. M. ‘‘Oxidation of hydroxylamines to NO by plant cells’’. *Plant Signaling & Behavior*, vol. 4, no. 9, 2009, pp. 853-855, ISSN 1559-2316, 1559-2324, DOI 10.4161/psb.4.9.9378.
37. Vetrovsky, P.; Stoclet, J. C. y Entlicher, G. ‘‘Possible mechanism of nitric oxide production from NG-hydroxy-l-arginine or hydroxylamine by superoxide ion’’. *The International Journal of Biochemistry & Cell Biology*, vol. 28, no. 12, 1996, pp. 1311-1318, ISSN 1357-2725, DOI 10.1016/S1357-2725(96)00089-1.
38. Tun, N. N.; Santa, C.; Begum, T.; Silveira, V.; Handro, W.; Floh, E. I. S. y Scherer, G. F. E. ‘‘Polyamines Induce Rapid Biosynthesis of Nitric Oxide (NO) in *Arabidopsis thaliana* Seedlings’’. *Plant and Cell Physiology*, vol. 47, no. 3, 2006, pp. 346-354, ISSN 0032-0781, 1471-9053, DOI 10.1093/pcp/pci252.
39. Groppa, M. D. y Benavides, M. P. ‘‘Polyamines and abiotic stress: recent advances’’. *Amino Acids*, vol. 34, no. 1, 2007, pp. 35-45, ISSN 0939-4451, 1438-2199, DOI 10.1007/s00726-007-0501-8.
40. Wildt, J.; Kley, D.; Rockel, A.; Rockel, P. y Segschneider, H. J. ‘‘Emission of NO from several higher plant species’’. *Journal of Geophysical Research: Atmospheres*, vol. 102, no. D5, 1997, pp. 5919-5927, ISSN 2156-2202, DOI 10.1029/96JD02968.
41. Magalhaes, J. R.; Pedroso, M. C. y Durzan, D. ‘‘Nitric Oxide Apoptosis and Plant Stresses’’. *Physiology and Molecular Biology of Plants*, vol. 5, 1999, pp. 115–125, ISSN 0971-5894.
42. Radi, R.; Beckman, J. S.; Bush, K. M. y Freeman, B. A. ‘‘Peroxynitrite-induced membrane lipid peroxidation: The cytotoxic potential of superoxide and nitric oxide’’. *Archives of Biochemistry and Biophysics*, vol. 288, no. 2, 1991, pp. 481-487, ISSN 0003-9861, DOI 10.1016/0003-9861(91)90224-7.

43. Wendehenne, D.; Pugin, A.; Klessig, D. F. y Durner, J. ''Nitric oxide: comparative synthesis and signaling in animal and plant cells''. *Trends in Plant Science*, vol. 6, no. 4, 2001, pp. 177-183, ISSN 1360-1385, DOI 10.1016/S1360-1385(01)01893-3.
44. Foissner, I.; Wendehenne, D.; Langebartels, C. y Durner, J. ''In vivo imaging of an elicitor-induced nitric oxide burst in tobacco''. *The Plant Journal*, vol. 23, no. 6, 2000, pp. 817-824, ISSN 1365-313X, DOI 10.1046/j.1365-313X.2000.00835.x.
45. García, M. C. y Lamattina, L. ''Nitric Oxide Induces Stomatal Closure and Enhances the Adaptive Plant Responses against Drought Stress''. *Plant Physiology*, vol. 126, no. 3, 2001, pp. 1196-1204, ISSN 1532-2548, DOI 10.1104/pp.126.3.1196.
46. Tian, X. y Lei, Y. ''Nitric oxide treatment alleviates drought stress in wheat seedlings''. *Biologia Plantarum*, vol. 50, no. 4, 2006, pp. 775-778, ISSN 0006-3134, 1573-8264, DOI 10.1007/s10535-006-0129-7.
47. Hao, G. P. y Zhang, J. H. ''The Role of Nitric Oxide as a Bioactive Signaling Molecule in Plants under Abiotic Stress'' [en línea]. En: eds. Hayat S., Mori saki, Pichtel J., y Ahmad A., *Nitric Oxide in Plant Physiology*, edit. Wiley-VCH Verlag GmbH & Co. KGaA, 2009, pp. 115-138, ISBN 978-3-527-62913-8, [Consultado: 29 de noviembre de 2015], Disponible en: <http://onlinelibrary.wiley.com/doi/10.1002/9783527629138.ch9/summary>.
48. Sang, J.; Jiang, M.; Lin, F.; Xu, S.; Zhang, A. y Tan, M. ''Nitric Oxide Reduces Hydrogen Peroxide Accumulation Involved in Water Stress-induced Subcellular Anti-oxidant Defense in Maize Plants''. *Journal of Integrative Plant Biology*, vol. 50, no. 2, 2008, pp. 231-243, ISSN 1744-7909, DOI 10.1111/j.1744-7909.2007.00594.x.
49. Arasimowicz, J. M.; Floryszak, W. J. y Kubiś, J. ''Involvement of nitric oxide in water stress-induced responses of cucumber roots''. *Plant Science*, vol. 177, no. 6, 2009, pp. 682-690, ISSN 0168-9452, DOI 10.1016/j.plantsci.2009.09.007.
50. Signorelli, S.; Corpas, F. J.; Borsani, O.; Barroso, J. B. y Monza, J. ''Water stress induces a differential and spatially distributed nitro-oxidative stress response in roots and leaves of *Lotus japonicus*''. *Plant Science*, vol. 201-202, marzo de 2013, pp. 137-146, ISSN 0168-9452, DOI 10.1016/j.plantsci.2012.12.004.
51. Corpas, F. J. y Barroso, J. B. ''Nitro-oxidative stress vs oxidative or nitrosative stress in higher plants''. *New Phytologist*, vol. 199, no. 3, 2013, pp. 633-635, ISSN 1469-8137, DOI 10.1111/nph.12380.
52. Webb, A. A. R.; Larman, M. G.; Montgomery, L. T.; Taylor, J. E. y Hetherington, A. M. ''The role of calcium in ABA-induced gene expression and stomatal movements''. *The Plant Journal*, vol. 26, no. 3, 2001, pp. 351-362, ISSN 1365-313X, DOI 10.1046/j.1365-313X.2001.01032.x.
53. Kim, T. H.; Böhmer, M.; Hu, H.; Nishimura, N. y Schroeder, J. I. ''Guard Cell Signal Transduction Network: Advances in Understanding Abscisic Acid, CO_2, and Ca^{2+} Signaling''. *Annual Review of Plant Biology*, vol. 61, no. 1, 2010, pp. 561-591, ISSN 1543-5008, 1545-2123, DOI 10.1146/annurev-arplant-042809-112226.
54. Yuchen, M.; Chunpeng, S.; Facai, D. y Xuechen, W. ''ABA-induced hydrogen peroxide generation in guard cells of *Vicia faba*''. *Acta phytophysiologica Sinica / Zhongguo zhi wu sheng li xue hui zhu bian*, vol. 26, no. 1, 1999, pp. 53-58, ISSN 0257-4829.
55. Wang, P. y Song, C. P. ''Guard-cell signalling for hydrogen peroxide and abscisic acid''. *New Phytologist*, vol. 178, no. 4, 2008, pp. 703-718, ISSN 1469-8137, DOI 10.1111/j.1469-8137.2008.02431.x.
56. Desikan, R.; Cheung, M. K.; Bright, J.; Henson, D.; Hancock, J. T. y Neill, S. J. ''ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells''. *Journal of Experimental Botany*, vol. 55, no. 395, 2004, pp. 205-212, ISSN 0022-0957, 1460-2431, DOI 10.1093/jxb/erh033.
57. Neill, S. J.; Desikan, R.; Clarke, A. y Hancock, J. T. ''Nitric Oxide Is a Novel Component of Abscisic Acid Signaling in Stomatal Guard Cells''. *Plant Physiology*, vol. 128, no. 1, 2002, pp. 13-16, ISSN 1532-2548, DOI 10.1104/pp.010707.
58. García, M. C. y Lamattina, L. ''Nitric Oxide and Abscisic Acid Cross Talk in Guard Cells''. *Plant Physiology*, vol. 128, no. 3, 2002, pp. 790-792, ISSN 1532-2548, DOI 10.1104/pp.011020.
59. Bright, J.; Desikan, R.; Hancock, J. T.; Weir, I. S. y Neill, S. J. ''ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H_2O_2 synthesis''. *The Plant Journal*, vol. 45, no. 1, 2006, pp. 113-122, ISSN 1365-313X, DOI 10.1111/j.1365-313X.2005.02615.x.
60. García, M. C. y Lamattina, L. ''Abscisic acid, nitric oxide and stomatal closure – is nitrate reductase one of the missing links?''. *Trends in Plant Science*, vol. 8, no. 1, 2003, pp. 20-26, ISSN 1360-1385, DOI 10.1016/S1360-1385(02)00009-2.

Received: October 7th, 2014
Accepted: March 18th, 2015

Revista Cultivos Tropicales
Instituto Nacional de Ciencias Agrícolas