Nitric Oxide(NO) and Plant Stress

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Abstract: In depth study of NO reveals that NO is a bioactive molecule that exerts a number of roles in many physiological and pathological process. NO is produced in response to drought, salinity, temperature shock and pathogen attack. NO rapidly reacts with ROS, ABA and other hormones and directly or indirectly regulate ethylene biosynthesis. The authors review the response of between plant NO and kinds of stresses, and possible mechanism was discussed.

Key words: nitric oxide (NO); plant stress; ethylene; ROS

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NO is an important bioactive agent that mediates a wide variety of physiological and pathological events in plants as in animal. Recent study shows that it plays an important role in improving the plant's resistance to a variety of stresses.

1 Properties of NO

Nitric oxide is a diatomic gaseous free radical. It has an unpaired electron, but remains uncharged. It can change to a more favorable electron structure through gaining or losing an electron, so that NO can exist as three interchangeable species: the radical(NO); the nitrosonium cation(NO^+); and the nitroxyl radical(NO^-).[1] NO is a small water and lipid soluble gas; it not only is able to move by diffusion in aqueous parts of the cell, but also to move freely through the lipid phase of membranes. It can move from one cell to another or within a cell. As a reactive free radical, it has a relatively short half-life (5～12 s). NO rapidly reacts with oxygen to produce a variety of nitrogen oxides. The stability and decay of NO are dependent on its concentration, the redox status of the system, and the concentration of its target molecules and metals.[2]

Since its discovery as an endogenous free radical, NO has been proposed to be either cytotoxic or cytoprotective.[3] NO as a reactive molecule, can damage the host plant. The cytoprotection is based on NO's ability to regulate the level and toxicity of ROS. The complex redox chemistry of NO, which is related to changes in the ambient redox milieu, is hypothesized to provide a general mechanism for cell redox homeostasis regulation.[4] Thus, NO can exert a protective action against oxidative stress provoked by an increased concentration of superoxide, hydrogen peroxide, and alkyl peroxides. In addition, the NO molecule itself possesses antioxidant effect.[5]

2 NO and drought stress

Water deficit is associated with the accumulation of ABA and the induction of ABA-regulated genes.[6] ABA can control stomatal movement to regulate the water loss through the transpiration stream while balancing the requirement of gas exchange for photosynthesis.[7] ABA accumulates in leaf tissue, generating a net loss of guard cell turgor that leads to stomatal closure, thus reducing transpiration water loss.[8]

Recent research has indicated that NO and its synthesis is a prerequisite for ABA signal transduction in Arabidopsis and Vicia guard cells. The experiment showed that NO selectively regulates Ca^{2+} sensitive ion channels of vicia guard cells by releasing Ca^{2+} from intracellular stores to raise cytosolic-free (Ca^{2+}). NO scavenger failed to block the activation of the K^+ channels evoked by ABA. These results showed that NO functions within one branch of the Ca^{2+} – signaling pathways was engaged by ABA.[9]

Both detached wheat leaves and wheat seedlings which were subjected to drought stress condition were pretreated with exogenously NO and retained up to 15% more water compare to the pretreated with water or NO_2^- / NO_3.[10]

Recently, it was showed that exogenous addition of NO to both monocot and dicotyledonous epidermal strips was sufficient to induce stomatal closure through a Ca^{2+} – dependent process.[10] Moreover it was further reported that in Pisum stativum and Vicia fava, ABA induces an increase of endogenous NO levels. This bulk of ABA induced NO production was reported to be sufficient and necessary for

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ABA induction of stomatal closure.[11,12]

Oxidative stress was proved to be generated as a consequence of drought in the plant. NO can alleviate several consequence of oxidative stress such as chlorosis, DNA fragmentation and apoptotic cell death.[3] One consequence of the oxidative stress is the ion leakage from the cell to intercalary compartment. The result [10]showed that the percentage of ion leakage was significantly lower in those leaves treated with NO after a drought period and subsequent rehydration. A number of water-deficit-induced gene products are predicted to protect cellular structures from the effect of water loss, which were named lea genes. Garcia Mata C et al (2001) found that leaves detached from wheat seedlings treated with the NO release SNP, there is an accumulation of the LEA3 transcript after different drought periods compared with control leaves, according to the accumulation of the LEA3 protein and the increase of the lea RNA. Those result suggested that NO can acting somewhere in the signal transduction pathway of the LEA expressed during the drought stress response.[10]

3 Salt, heat, metal stress and NO

Using NO donor and inhibitors, it was found that a close relation between NO accumulation and element ratio. The treatment of Dune reed with NaCl resulted in the generation of NO, which reduced the Na^+ percentage and increased the K^+ to Na^+ ratio. NO enhanced RWC (relative water content) and reduced MP (membrane permeability) under salt stress. NO induced salt resistance by influencing element ratios in the Dune reed.[13] Shen w-b et al found that the NO donor can effectively attenuated the wheat leaves oxidative damage caused by salt stress. NO can markedly activate the SOD and CAT activities, delay the accumulation of O_2^- and H_2O_2, but increase PAL level.[14]

Pretreating rice seedlings with low level of NO permitted the survival of more green leaf tissue, and of higher quantum yield for photosystem Ⅱ, than in non-treated controls, under salt and heat stress because pretreatment induces not only active oxygen scavenging enzymes activities, but also expression sucrose-phophate synthase.[15]

Kopyra M found that NO donor sodium nitroprusside (SNP) stimulates seed germination and root growth of lupin (*Lupinus luteus* L. cv Ventus). The promoting effect of NO on seed germination persisted even in the presence of heavy metals (Pb, Cd) and sodium chloride. The inhibitory effect of heavy metals on root growth was accompanied by increased activity of superoxide dismutase (SOD). So they conclude that the protective effect of NO in stressed lupin roots due to the stimulation of SOD activity and/or direct scavenging of the superoxide anion.[16]

NO can regulate the synthesize and activity of ROS. It can protect against cellular damage produced by methylviologens diquat and paraquat in potato leaves. Three NO donors were all able to prevent chlorophyll loss. However the special NO scavenger, carboxy-PTIO, inhibited NO-mediated chlorophyll protection. Methylviologen compounds can cause an overproduction of reactive oxygen species (ROS) within chloroplasts, subjecting the plant to a severe oxidative stress. During the oxidative stress, cell ion leakage to intercellular compartments occurs. NO proved to specifically decrease the extent of ion leakage originated by diquat. NO can strongly protect plants from methylviologen damage and strengthen the evidence in favor of NO as a potential antioxidant in some situations.[3]

But Malerba M's study showed that the Fusicoccin-induced accumulation of nitricoxide in sycamore cultured cells is not required for the toxin-stimulated stress-related responses. NO production induced by Fusicoccin result in an accumulation of NO in the culture medium and in the sycamore cells. Scavenging of NO by cPTIO does not prevent the stimulation effect of Fusicoccin on stress-related responses. So they thought that NO does not act as a signal for the other Fusicoccin-induced responses here considered, but rather its production can be regarded as another symptom of a stress condition brought about by the toxin.[17]

4 Nitric oxide and PCD

PCD is a genetically determined, metabolically directed cellular process resulting in cell suicide. NO induced cell death also possessed the characteristics of PCP, such as chromatin condensation, the requirement for gene expression and the activation of a caspase-like cascade.[18]

Delledone et al have shown that the interaction between NO and ROS can determine whether or not PCD occur. NO by itself does not induce PCD in soybean cell culture, but it may be that the NO to superoxide ratio determines PCD.[19] A correlation among H_2O_2, NO and antioxidant levels has been demonstrated recently by de Pinto et al. In tobacco BY-2 cells, neither NO nor H_2O_2 alone at low concentrations had any effect on PCD or on the activity of PAL. However, treatment with both H_2O_2 and NO together induced a substantial increase in cell death with characteristics of PCD, as well as PAL activity. This treatment also caused an increase in the activities of enzymes reducing ascorbate and glutathione.[20] Beligni et al (2002) have provided data indicating an antioxidant role for NO acting during developmental PCD induced by hormones. In barley aleurone layers, GA-induced PCD was delayed in the presence of NO, which correlated with a delayed loss of activity of the antioxidants catalase (CAT) and superoxide dismutase (SOD).[21]

5 NO Ethylene and Senescence

Ethylene plays an active role in many responses to

environmental stresses and to endogenous signals transformation. Ethylene influences many stages of plant growth and development, from germination and cell expansion to stress responses and fruit ripening.[22]

Some reports indicate that NO may have anti-senescence properties. Evidence of the interplay between NO and ethylene in the maturation and senescence of plant tissue suggests an antagonistic effect of both gases during the plant's development. Leshem & Harumaty found that application of an NO donor to pea leaves under senescence-promoting conditions decreased generation of ethylene, an endogenous maturation hormone. That was shown to result from an inhibition of ethylene biosynthesis.[23] Recently, it was demonstrated by a noninvasive photoacoustic spectroscopic method, that endogenous NO and ethylene content maintains an inverse correlation during the ripening of strawberries and avocados. While unripe, green fruits contain high-NO and low-ethylene concentrations; the maturation process is accompanied by a marked decrease of NO concomitant with the increase of ethylene.[24]

The storage course of the horticultural product is an integrated stress for the horticulture production. Lesshem et al found that treatment with NO can markedly delay senescence and extend shelf of both climacteric and non-climacteric fruit, flowers, vegetable and legume sprout species.[25]

In our laboratory, we found that application of exogenous NO by direct fumigation in an O_2^- free atmosphere can extend the shelf life in tomato. NO treatment not only markedly delay the timing of the respiration and ethylene peaks, but also can attenuated the accumulation of membrane penetration and counteract the degradation of chlorophyll (Ren et al. unpublished). These results agree with Tu Jie's experiment.[26]

6 Nitric oxide and plant disease

Nitric oxide plays a key role in plant disease. Treatment of potato tuber tissues with the NO donor NOC-18(1～10 mM) provoked an accumulation of phytoalexin rishitin, an endogenous antibiotic compound. The effect of NOC-18 was counteracted by the specific NO-scavenger carboxy-PTIO and by the free radical scavenger Tiron.[27]

Nitric oxide has been proved to protect chlorophyll levels in potato leaves that were infected with the pathogen *Phytophthora infestans*.[28] The effect was achieved with low NO concentrations (10～100μM SNP). NO did not affect *P. infestans* growth and viability, suggesting that its protective effect inpotato was related to the plant's defense mechanisms. Beligni et al found with enough evidence that an increase in the mRNA levels of phenylalalanine-ammonialase (PAL), β-1, 3-glucanase and glyceraldehydes-3-phosphate dehydrogenase upon treatment with NO donors.[3]

NO donors can invoke a hypersensitive response (HR). HR is a response characterized by rapid host cell death at the pathogen penetration site. As a result, the growth and development of the fungus is restricted. Thus it helps protect fungus spread to other parts of the plant. The HR is triggered by a rapid and transient production of ROS, called the oxidative burst. In the resistant tobacco TMV inoculation led to an increase of NOS activity, but no infection.[29] Pretreatment of the tobacco plants or suspension cells with NO donors or recombinant NOS protein can trigger expression of pathogenesis-related protein (RP-1), PAL and increase total salicylic acid (SA) levels; SA is a plant messenger molecule involved in response to biotic stress.[30] The ability of NO to up-regulate PAL mRNA levels means that this gas may be involved in plant defense mechanisms by both SA-dependent and SA-independent pathways.[31]

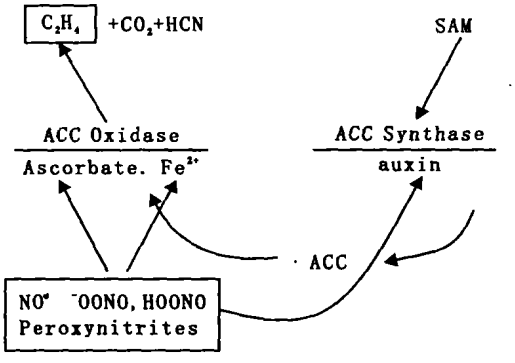

Fig1 Proposed mode NO and peroxynitrite down regulation of ethylene

7 Possible Mechanism

Ethylene is not only an kind of endogenous maturation hormone, but also is a kind of environmental stress cofactor. Since many environmental stress responses are associated with ethylene upsurge, this can stimulate senescence, abscission of casualty tissue, and induce special defense protein production. Experiments showed that application of exogenous NO can decrease the ethylene emission. Lesslem et al hypothesized that NO block the ethylene biosynthesis through the following mode, as show in Fig. 1. The cofactors for the ACC oxidase (ACO) are ascorbate and Fe^{2+}. ACC synthase (ACS), the penultimate enzyme in ethylene synthesis, is promoted by auxin. As presented in fig5, it is suggested that NO and/or the peroxynitrites may inhibit the ethylene biosynthesizing activity of both enzymes by oxidative inactivation of their co-factors: ascorbate and Fe^{2+} in the case of ACO; auxin the case of ACS. Thus the presence of the NO free radical may markedly reduce the rate of ethylene emission.[25]

Recently, nitric oxide together with reactive oxygen species (ROS) has been postulated to be required for the activation of the hypersensitive reaction, a defense response

induced in noncompatible plant-pathogen interaction.[29] NO also can interacts with ROS, which comes from drought stress,[10] salinity,[13,14] heat, chilling and other stresses,[15] in various ways and might function as an antioxidant.[32]

Although there is now more evidence that NO exerts an unprecedented diversity of biological effects, our knowledge about the real role of NO during the many stress conditions is very limited. The integration of NO functions with plant physiological and pathological processes, especially with plant stress will have necessarily to be examined in more detail in order to illuminate potential function in plant NO biology. Treatment with exogenous NO in horticulture production might provide an effective way to study the relationships among NO, ROS and ethylene in order to demonstrate the possible mechanism of NO and plant stress.

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操作, 智能化、交互性是该阶段的最大特点。专家系统、人工智能、神经网络等被融入到数字流域中, 使流域在数据获取、分析、管理、应用方面实现高度智能化, 自动化程度将大大提高。建立基于 Java 分布式服务系统, 通过 Java Applet 构建用户端请求图形元素方式的系统体系结构, 用户可以对所得到的图形元素或地图图像进行浏览、查询、并编辑生成新的图形, 实现信息的智能化获取与交互性操作。管理上, 分布式虚拟管理系统可以将平面管理的信息延伸至任意想象空间, 流域虚拟再现成为现实。

4.3 高级阶段

"数字流域"全面网络化、社会化服务阶段。建成以各种高新技术集成于一体为特征的先进、便捷、实用化系统。数据获取(包括空间信息的自动提取、基于特征的专题属性的知识挖掘技术、GPS、DOQ 对流域空间信息和动态更新、图形的智能化生成等)、数据分析处理(包括各类叠加分析、统计分析、专题模拟、缓冲区分析、DTM 模型分析等)、数据管理(包括基于纯面向对象数据库 OODB 的对象与底层表示分离、空间属性与非空间属性平等定位, 实现属性数据和空间数据一体化管理体制)、数据应用(实现网络虚拟地理环境, 让用户从不同应用角度的视点出发, 利用自然的技能和某些设备对这一生成的虚拟世界客体进行浏览和交互考察、提供优化实施方案和动态模拟等)实现一体化。真正实现定位需求、定位感知、最优决策的个性服务, 整个流域形成一个开放的、规范的、虚拟的、网络化的系统。

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