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Review

Nitric oxide as a secondary messenger during stomatal closure as a part of plant immunity response against pathogens

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ABSTRACT

Stomata facilitate the loss of water, as well as CO_2 uptake for photosynthesis. In addition, stomatal closure restricts the entry of pathogens into leaves and forms a part of plant defense response. Plants have evolved ways to modulate stomata by plant hormones as well as microbial elicitors, including pathogen/microbe associated molecular patterns. Stomatal closure initiated by signals of either abiotic or biotic factors results from the loss of guard cell turgor due mainly to K^+/anion efflux. Nitric oxide (NO) is a key element among the signaling elements leading to stomatal closure, hypersensitive response and programmed cell death. Due to the growing importance of NO as signaling molecule in plants, and the strong relation between stomata and pathogen resistance, we attempted to present a critical overview of plant innate immunity, in relation to stomatal closure. The parallel role of NO during plant innate immunity and stomatal closure is highlighted. The cross-talk between NO and other signaling components, such as reactive oxygen species (ROS) is discussed. The possible sources of NO and mechanisms of NO action, through post-translational modification of proteins are discussed. The mini-review is concluded with remarks on the existing gaps in our knowledge and suggestions for future research.

Contents

1. Introduction

Stomata are minute pores present on the surface of leaves of terrestrial plants, which facilitate transpiration and CO_2 uptake. Stomata also act as gateways for the entry of pathogens. When plants are exposed to drought/water stress, stomata are closed and this response is mediated by mobilization of plant hormones, such as abscisic acid (ABA). Similarly, whenever challenged by plant pathogens, stomatal closure restricts the entry of pathogenic microorganisms and helps in plant innate immunity [1–3]. In view of this emerging concept, several recent reviews summarized the crucial step of stomatal closure as one of the effective components of plant defense responses [4–6]. Many signaling components are common in stomatal closure or defense response, and one of such compound is nitric oxide (NO).

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NO, a reactive nitrogen species, plays an important physiological role as a signaling component during plant–pathogen interactions, plant resistance, hypersensitive response (HR) and expression of related genes [7–9]. During defense responses, NO interacts with various other signaling molecules upstream and downstream including mitogen activated protein kinase (MAPK's), reactive oxygen species (ROS), cyclic nucleotides and free Ca^{2+} [10]. Apart from its effective role in plant defense, NO also plays a major role in stomatal closure induced by ABA as well as the elicitors/pathogen associated molecular patterns (PAMPs) [11]. Studies using NO donors, NO-modulators and mutant plants confirm the role of NO in stomatal signaling cascade [12,13].

There are excellent reviews which appeared in the last 4 years, on the role of NO during stomatal closure [14–16] as well as the importance of NO during the innate immunity responses in plants [8,10,17,18]. However, it is not clear if the regulation by NO of defense responses and stomatal closure is a closely integrated process or NO exerts its effect parallely. Readers interested in the earlier work, may refer to some of the reviews, which appeared before 2009 [7,12,13,19–24].

This article is an overview of the importance of NO during stomatal closure in relation to defense responses against pathogens. The interdependence and interaction of NO and ROS are pointed out. The continuing ambiguity on the enzymatic sources of NO is discussed. The growing interest in molecular mechanisms (S-nitrosylation, tyrosine nitration and metal nitrosylation) of NO action is pointed out.

2. Significance of stomatal closure in plant defense response

The stomatal aperture is modulated due to dynamic changes in ionic status of guard cells. During stomatal opening, guard cells accumulate osmotically active molecules such as potassium, anions and malate leading to water uptake, increase in the turgor of guard cells and stretch the aperture to open [3]. The opposite events of stomatal closure, namely the efflux of potassium/anions and movement of H_2O from guard cells and flaccid guard cells, cause stomatal closure [25,26]. Several environmental signals, such as high CO_2, drought, light, humidity, internal signals such as phytohormones, for example ABA, methyl jasmonate (MJ), ethylene and even elicitors cause stomatal closure. Auxins and cytokinins induce stomatal opening [27–30]. Most of the pathogens, including fungi and bacteria try to enter the plants through natural openings like stomata or wounds. Stomatal closure restricts further entry of pathogens into leaves and is a typical component of plant immune response against pathogenic microbes. Cross-defense responses can also occur during plant–pathogen interactions. For example when Arabidopsis plants are challenged by *Pseudomonas syringae* DC 3000 (a virulent plant pathogen), stomatal closure is induced, as an initial response, to restrict the entry. After 3 hours of incubation, the pathogen *Pseudomonas syringae* DC 3000 causes re-opening of the stomata by producing a polyketide toxin, coronatine [1,2].

3. Elicitors/microbe associated molecular patterns (MAMPs) mediate plant defense responses and stomatal closure

Plants initiate basal defense response, soon after sensing the attack by pathogens. The early recognition of the microbial presence is often mediated by elicitors, which are either digested products from the microbial cell walls or produced by the plant cell. There is a cross-talk between host plants and pathogens, mediated by elicitors or molecular patterns. PAMPs are evolutionarily conserved molecular signatures present on both pathogen and non-pathogenic microorganisms, so these are later re-named as MAMPs [2,31]. Several MAMPs were discovered, such as, flg22 and lipopolysaccharides (LPS) from bacteria; xylanase, chitin, chitosan (a deacylated derivative of chitin) and ergosterol from fungi; and glucan, pep13, elicitin from oomycetes. Effector triggered defense response often culminate in the hypersensitive response and programmed cell death (PCD) (Table 1).

Most of these elicitors/PAMPs, e.g. flg22 or oligochitosan, induced stomatal closure in wide spectrum of plants like *L. esculentum, C. communis, P. sativum, A. thaliana, N. benthamiana, B. napus* (Table 2). Each MAMP is perceived by its cognate receptor present on plasma membrane, and when bound the complex initiates signaling cascade, leading to stomatal closure. For example flg22 is perceived by its cognate receptor FLS2, and chitin by chitin elicitor receptor kinase 1 [56]. Upon perception of the elicitors by their respective pattern-recognition receptors on stomatal guard cells, elicitors or PAMPs induce stomatal closure in plants. There is misconception that HR is equivalent to PCD but HR is a subset of PCD and may involve multiple components. For example, elicitor activation causes elevation of ROS, cytosolic free Ca^{2+} levels, phytoalexin accumulation, phenyalanine ammonia-lyase (PAL) gene expression and hypericine accumulation [32,34,38,40,48].

4. Role of nitric oxide in plant innate immunity and stomatal closure

Multiple approaches have been used to demonstrate the importance of NO during plant defense responses and stomatal closure. These include (i) modulation of NO by donors or scavengers or inhibitors of NO-synthesizing enzymes: (ii) monitoring NO by fluorescent probes, and finally (iii) validation of the NO role by suitable mutants deficient in up-stream and down-stream steps of NO action.

The levels of NO in plant tissues, can be increased by NO donors, such as sodium nitroprusside (SNP) or S-nitroso-N-acetylpenicillamine (SNAP) [57]. The levels of NO can be lowered by scavengers like 2-phenyl-4,4,5,5-tetramethyl imidazoline-1-oxyl 3-oxide (cPTIO). Examples of inhibitors of NO synthesizing enzymes are N-nitro-L-Arg-methyl ester (L-NAME, inhibitor of NOS like enzyme) and tungstate (NR inhibitor) [58]. These inhibitors decrease NO production, and restrict stomatal closure by ABA, MJ or elicitors [1,27,51,59–63]. The levels of NO can be monitored and related to the extent of defense responses or stomatal closure. Fluorescent dyes, like DAF-2DA are used for monitoring NO in plant cells, but are being questioned for their target-specificity [64]. Studies using DAF-2DA indicated that NO production occurs prior to the ROS [51]. High NO can in turn elevate other signaling components, such as PLDα1, PLD, PA, during stomatal closure [65–68].

Since the use of pharmacological compounds is only of limited use, the role of signaling elements is validated by using Arabidopsis mutants, deficient in a given signaling component. The ABA-insensitive mutants (ABI1 and ABI2) indicated that protein phosphatases could act up-stream of NO in the ABA signal-transduction cascade [22]. Impaired NO production and closure in *atrbohD/F, NtbrbohA* and *NtbrbohB* single and double silenced plants in response to ABA or elicitors, demonstrated that ROS production was essential for NO production and subsequent signaling steps [27,50]. Similarly, the use of Arabidopsis mutants (*nia1,nia2,nia1/2*) revealed the role of NR as a possible source of NO [59,69].

The first indications related to the role of NO in defense mechanism came from the studies on potato tuber tissues, treated with l-hydroxy-2-oxo-3,3-bis(2-aminoethyl)-1-triazene (NOC-18, a NO donor), during induction of rishitin (a phytoalexin) accumulation. Such accumulation was restricted by the addition of cPTIO. Pearl millet (*Pennisetum glaucum* L.) seeds, pre-treated with SNP (NO donor), were able to improve their resistance against downy mildew. Conversely, treatment with cPTIO rendered the plants susceptible for pathogen infection [70]. Lipopolysaccharide (LPS) treated Arabidopsis mesophyll cells showed enhanced NO production, which was restricted by incubating the protoplasts with a mammalian NOS