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Cyclic nucleotide gated channel and Ca^{2+}-mediated signal transduction during plant senescence signaling

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Abbreviations: ABA, abscisic acid, *AtNOA1*, Arabidopsis *NO ASSOCIATED PROTEIN1*; CaM, calmodulin; CAT, catalase; CNGC, cyclic nucleotide gated channel; GA, gibberellin; MeJA, methyl jasmonate; NO, nitric oxide; NOD, nitric oxide dioxygenase; NOS, nitric oxide synthase; PCD, programmed cell death; PM, plasma membrane; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase; WT, wild type; W7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide

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Previous studies reveal that both Ca^{2+} and nitric oxide (NO) play pivotal roles in the plant senescence signaling cascade. However, not much is known about the molecular identity of the Ca^{2+} entry during senescence programming and its relationship to the downstream NO signal. Our recent study shows that Arabidopsis cyclic nucleotide gated channel2 (CNGC2) contributes to Ca^{2+} uptake and senescence signaling. The CNGC2 loss-of-function mutant *dnd1* displays reduced Ca^{2+} accumulation in leaves and a series of early senescence phenotypes compared to wild type (WT). Notably, endogenous NO content in *dnd1* leaves is lower than leaves of WT. Application of an NO donor can effectively rescue a number of early senescence phenotypes found in the *dnd1* plants. Current evidence supports the notion that NO functions as a negative regulator in senescence signaling and our model supports this point. In this article, we expand our discussion of CNGC2 mediated Ca^{2+} uptake and other related signaling components involved in the plant senescence signaling cascade.

Both Ca^{2+} and nitric oxide (NO) have important functions in plant senescence signaling. Ca^{2+} can defer the senescence initiated in detached leaves.[1] Methyl jasmonate (MeJA) induced leaf senescence can also be postponed by Ca^{2+}.[2] Overexpression of a bacterial NO dioxygenase (NOD) (scavenging NO in transgenic Arabidopsis leaves) results in the occurrence of early senescence phenotypes.[3] Application of NO gas can rescue these early senescence phenoytpes occurring in transgenic NOD plants.[3] Arabidopsis *NO ASSOCIATED PROTEIN1* (*AtNOA1*; formerly named *AtNOS1*) loss-of-function mutant *Atnoa1* displays impaired NO production in response to many stimuli[4] and displays an early senescence phenotype.[5] Application of an NO donor can rescue the early senescence phenotypes of the *Atnoa1* mutant.[5] Moreover, early senescence induced by other hormones (abscisic acid (ABA)/MeJA;[6,7] gibberellin (GA),[8]) can be rescued by NO donor application.

Current evidence suggests that Ca^{2+} (and the Ca^{2+} sensor camodulin [CaM]) can regulate NO production through NO synthase (NOS) activity. NO is synthesized from L-arginine by a NOS-type enzyme in plant cells.[9-16] However, a plant NOS gene has not been identified to date.[4,17,18]

There are a total of 57 genes encoding cation channels in Arabidopsis genome. Twenty of them are cyclic nucleotide gated channels (CNGCs).[19] It has been suggested that plant CNGCs are a family whose products contribute to uptake of extracellular Ca^{2+} into the plant cell.[20-26]

Recent work by Ma et al. has demonstrated that a plant cyclic nucleotide gated channel (CNGC2) is involved in senescence signaling.[27] Previous study showed that transcripts of *CNGC2* were increased during the early stage of the senescence process.[28] Here, Ma et al. reported the discovery of reduced Ca^{2+} accumulation in the *dnd1* (*cngc2* loss-of-function) mutant.[27] Previous work has shown that CNGC2 is a plasma membrane (PM)

localized ion channel and its mutation (loss-of-function) has led to the loss of inward Ca^{2+} current activated by cyclic nucleotide as well as a number of pivotal events occurring downstream from influx of Ca^{2+} during plant innate immune responses.[21,22] *dnd1* plants display a number of early senescence phenotypes, which can be successfully rescued by the application of an NO donor.[27] Importantly, endogenous NO content in *dnd1* plants is lower that wild type (WT) plants, which connects the early senescence phenotype with lower NO level in *dnd1* plants. Our work supports the concept that NO functions as a negative regulator in plant senescence signaling cascade as described by previous studies.[3,5]

CaM Involvement in the Senescence Signaling Cascade and Ca^{2+}/CaM Mediated NO Synthesis

There is evidence indicating the involvement of CaM in leaf senescence signal transduction. A study showed enhanced transcript accumulation of late-response senescence genes (*din2* and *din9*) in detached leaves treated with a CaM antagonist *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W7).[29] These results suggest that Ca^{2+}/CaM signaling controls the regulation that delays the expression of late-response senescence genes (*din2* and *din9*). As a result, these changes could attenuate leaf senescence.

Evidence in our work[27] depicts a model linking Ca^{2+} signaling and the Ca^{2+} sensor CaM with downstream NO production (through regulation of NOS activity) in the plant cell. NO mediates leaf senescence initiation as a negative regulator. Therefore, this work provides new information about the mechanism of plant senescence signaling from a molecular perspective. Recent studies have shown that some CNGCs other than CNGC2 contribute to Ca^{2+} uptake into the cytosol, which is associated with pivotal physiological processes.[20-25] However, there is little evidence connecting PM localized cation channels with leaf Ca^{2+} accumulation at a nutritional level. Furthermore, previous studies have shown that Ca^{2+} and CaM affect or somehow regulate NOS activity in plants.[10-16] Therefore, we hypothesized that reduced NO content in *dnd1* leaves during leaf senescence is due to the absence or impairment of a Ca^{2+}-conducting pathway (formed at least in part by the *CNGC2* translation product) in this mutant. Perhaps, senescence signaling is repressed in the presence of a functional Ca^{2+} influx pathway due to the positive effect of cytosolic Ca^{2+} presence on NOS activity. Nonetheless, our hypothesis is still speculative until a plant NOS is well characterized.

NO Function: An Antioxidant or a Prooxidant in Plant Cells?

NO has been shown to function as either an antioxidant or a prooxidant under different physiological conditions. Reactive oxygen species (ROS) scavenging enzymes can be inhibited by NO donor application.[30,31] Zeier et al. also reported that the pathogen induced oxidative burst in NO deficient transgenic Arabidopsis also was severely impaired.[32] NO can work with ROS synergistically in ABA or pathogen responses.[16,33-35] The ratio of NO to H_2O_2 is an important factor to initiate programmed cell death (PCD).[36] On the other hand, there is also some evidence that supports the idea that NO acts as an antioxidant in multiple physiological processes. For example, the Arabidopsis NO deficient mutant *Atnoa1* has an early senescence phenotype, elevated H_2O_2 levels and oxidative damages in leaves.[5] NO donor application has been also found to complement the H_2O_2 increase in rice induced by ABA or MeJA.[6,7] Addition of an NO donor can delay gibberellin (GA)-elicited PCD in barley (*Hordeum vulgare*) aleurone layers (regulated by ROS).[8] Beligni et al. found that NO can not only act as an antioxidant but also defer the loss of catalase (CAT) and superoxide dismutase (SOD) (treated with GA).[8] Furthermore, herbicide application can also result in oxidative stresses in plants. Studies in potato show that NO donor treatment can effectively protect the plant from the damage caused by herbicide induced oxidative stresses.[37] Our work[27] showed many early senescence associated oxidative stress phenotypes [such as H_2O_2 generation and lipid peroxidation (monitored as MDA elevation)] in *dnd1* plants can be reduced by adding an NO donor, which supports NO function as an antioxidant. Previous studies have indicated that H_2O_2 and salicylic acid (SA) treatments can induce each other.[38,39] Mateo et al. suggested that SA levels are paralleled by H_2O_2.[40] They also found that enhanced activity of SODs in mutants with enhanced SA levels, which could convert reactive superoxide anions to the more stable H_2O_2, resulting in the increase of H_2O_2 content.[40] Here, our study showed that NO donor treatment lowered high SA levels in *dnd1* plants in addition to reducing the high endogenous H_2O_2 levels.[27] Thus, our results are consistent with NO function as an antioxidant molecule in rescuing early senescence in *dnd1* plants.

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