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A possible mechanism for the apocynin-induced nitric oxide accumulation in plants

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Nitric oxide (NO) is a small, ubiquitous bioactive molecule, postulated as a broad spectrum anti-stress compound. The NADPH oxidase inhibitor apocynin induces the accumulation of endogenous NO in leaves of maize seedlings through a nitric oxide synthase (NOS)-like activity, and confers an augmented tolerance to UV-B-induced oxidative damage. Here we propose a mechanism for the apocynin-induced NO increase in plants. NOS catalyzes the oxidation of arginine to citrulline and NO. It is suggested that apocynin inhibit arginase, the enzyme that hydrolyzes L-arginine to urea and L-ornithine, increasing the arginine availability for arginine-dependent NO synthesis. Superoxide (O_2^-) is a strong NO scavenger due to its high reactivity with NO to give peroxynitrite ($ONOO^-$). Superoxide is mainly produced by plant NADPH oxidase (pNOX). Inhibition of pNOX by apocynin at relatively high NO concentration, could reduces the formation of O_2^- and $ONOO^-$, increasing the availability of a huge amount of NO. We consider apocynin as a very attractive compound for studying NO-regulated processes in plants since it can replace the use of NO donors and overcome the subsequent technical problems.

Key words: apocynin, nitric oxide, NO, UV-B, oxidative stress, nitric oxide synthase, NOS

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NO is a small, highly diffusible atmospheric gas and a ubiquitous bioactive molecule, proposed as a broad spectrum anti-stress compound.[1] Because NO is a reactive gas with a short halflife in air, the vast majority of NO research in living organisms has involved application of NO donors. Floryszak and co workers[2] argue that although treating plant tissue with NO donors is a simple methodological approach, it has yielded some technical problems because the process of donor decomposition depends on numerous external factors. For instance, the mostly used NO donor, sodium nitropruside (SNP), is extremely photosensitive and its degradation is promoted also by oxygen and temperature.[3] Nonreductive decomposition of S-nitrosothiols as S-nitrosoglutathione (GSNO) releases NO, but it is dependent on light, temperature and pH.[4]

It was reported that the steady-state level of NO was increased by apocynin in human endothelial cells.[5,6] Apocynin (4-hydroxy-3-methoxyacetophenone, acetovanillone, CAS 498-02-2) is a methoxy-substituted catechol originally extracted from the roots of *Picrorrhiza kurroa*, a small perennial herb that grows in the Himalayas. Extracts of *P. kurroa* are used in traditional medicine for treating diseases associated with chronic inflammation.[7,8]

We have recently demonstrated that apocynin induces the dose-dependent accumulation of NO in leaves of maize seedlings through a nitric oxide synthase (NOS)-like activity. This NO production is bioactive and antioxidant since it confers an augmented tolerance to UV-B induced oxidative stress. Therefore, the use of apocynin as an alternative approach to study NO functionality in plants has been proposed.[9] Here it is postulated that the apocynin-induced NO increase in plants is due to the confluence of at least two effects: the inhibition of arginase and NADPH oxidase.

Apocynin and Arginase Blockage

The nitric oxide synthase (NOS) is one of the enzymatic sources of NO in plants.[10]

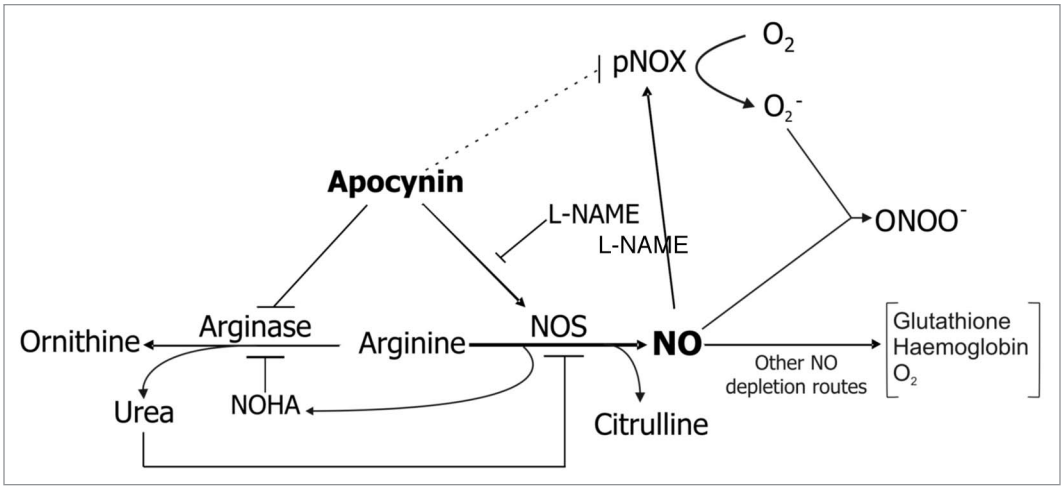

Figure 1. Schematic model of the proposed mechanism for apocynin-mediated NO induction in plants. The model integrate available data from plant and animal systems (see the text for explanation). Apocynin inhibits the arginase activity, increasing the pool of arginine available for the NOS-derived arginine-dependent NO synthesis. NOHA, an intermediate of the NOS-catalyzed reaction is also an inhibitor of arginase. As a negative feedback, high NO concentration activates pNOX, resulting in O_2^- production. Superoxide reacts rapidly with NO to give $ONOO^-$. It is suggested that apocynin inhibits pNOX at high NO concentration promoting a NO burst. Other NO detoxification systems include oxygen, glutathione and haemoglobin. L-NAME: N^G-nitro-l-arginine methyl ester; NO: nitric oxide; NOHA: N^G-hydroxy-L-arginine; NOS: nitric oxide synthase; O_2^-: superoxide; $ONOO^-$: peroxynitrite; pNOX: plant NADPH oxidase. Dashed line indicates hypothetical (non reported) pNOX inhibition.

NOS catalyzes the oxidation of arginine to citrulline and NO. The production of NOS-derived arginine-dependent endogenous NO has been demonstrated in animals, plants and bacteria.[10] Arginase is the enzyme that hydrolyzes L-arginine to urea and L-ornithine. Thus, arginine is a substrate for both arginase and NOS. At first sight, it can be hypothesized that a high arginase activity results in a lowered arginine pool available for NOS-dependent NO production. In fact, in animals was demonstrated that the crossroad between the arginase and NOS pathways is more than a simple common substrate usage. Arginase inhibits the production of NO by several mechanisms (reviewed by Durante et al.[11]): (i) competing with NOS for L-arginine, (ii) affecting the translation and stability of inducible NOS (iNOS) protein, (iii) inhibiting iNOS activity via the generation of urea, and (iv) sensitizing NOS to its endogenous inhibitor, asymmetric dimethyl-L-arginine.

It has been observed that apocynin is able to prevent the upregulation of arginase in mammals.[12] Arginase inhibition by apocynin could also increase arginine-dependent NO synthesis in plants. This is supported by recent data showing an increase of NO production in *argah1-1* and *argah2-1*, two Arabidopsis mutants defective in the two arginase genes ARGAH1 and ARGAH2.[13,14] Moreover, it was also reported that N^G-hydroxy-L-arginine (NOHA), the intermediate in the NOS-catalyzed production of NO, is a strong inhibitor of arginase in tomato.[15]

Apocynin and pNOX Inhibition

Superoxide has been reported as a strong NO scavenger because it reacts rapidly with NO to produce peroxynitrite ($ONOO^-$).[10] Superoxide is mainly generated by NADPHoxidase (NOX). In animal cells, NOX activity is inhibited by apocynin, through the blockage of the enzyme assembly to its p47phox regulator.[7,16] Although there is no report of apocynin as a plant NOX (pNOX) inhibitor, we can not exclude this possibility. High NO concentration was reported as pNOX activator, increasing the O_2^- production and consequently the $ONOO^-$ concentration.[17] In this scenario, pNOX inhibition by apocynin may reduce the formation of $ONOO^-$, increasing the NO concentration. In addition to superoxide, plant cells hold other NO detoxification systems that include oxygen, glutathione and haemoglobin.[10]

Altogether, the available data suggest at least two ways in which apocynin may induce the endogenous NO production in plants. Thus, apocynin may become a very attractive compound for studying NO regulated process, replacing the use of NO donors and overcoming the subsequent technical problems.

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