A mechanism of rapid ABA signaling inactivation through tyrosine nitration of PYR/PYL/RCAR receptors

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ABA induces nitric oxide (NO) production in plants, and NO has been considered to be a required intermediate in ABA triggered responses such as stomata closure. However, we found that the triple *nia1nia2noa1-2* mutant plants, with low endogenous NO content, were hypersensitive to ABA in seed germination, stomata closure or tolerance to water deficit, thus suggesting NO acts somehow as a negative regulator of ABA signaling. We identified a mechanism by which NO exerts a rapid and negative regulation on ABA signaling that is based on the nitration of key Tyr residues of PYR/PYL/RCAR receptors. *In vitro* nitration receptors led to their inability in inhibiting phosphatase activity thus suggesting nitration of Tyr residues made receptors inactivated. Inactivation was specifically due to Tyr nitration because the same receptors can be also S-nitrosylated *in vitro* but S-nitrosylated forms were fully active in binding ABA and further inhibiting phosphatase activity. The use of recombinant proteins and *in vitro* induced NO-related PTMs was further complemented by an *in planta* protemic approach based on the use of transgenic plants expressing tagged versions of PYR/PYL/RCAR receptors, immunopurification procedures based on anti- tag-coated magnetic beads and LC-MS/MS techniques. We found that PYR/PYL/RCAR receptors are nitrated in Tyr, S-nitrosylated in Cys and also ubiquitylated in Lys residues *in vivo.* We also identified the modification sites for PYR1, PYL4 and PYL8 receptors *in planta*. Nitration of Tyr residues and polyubiquitylation of the nitrated population of receptor molecules seems to be linked, and prepare the modified protein for further proteasomal degradation, which is on the basis of the mechanism allowing the desensitation to ABA and the subsequent resetting. This mechanism of rapid inactivation of the ABA receptors through Tyr nitration events could be especially useful for those conditions requiring faster responses than those based on the catabolism of the hormone.