SHORT COMMUNICATION

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Jasmonic acid interacts with abscisic acid to regulate plant responses to water stress conditions

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Phytohormones are key players in signaling environmental stress conditions. Hormone profiling together with proline accumulation were studied in leaves and roots of different mutant lines of Arabidopsis. Regulation of proline accumulation in this system seems complex and JA-deficient (*jar1-1*) and JA-insensitive (*jai1*) lines accumulating high levels of proline despite their very low ABA levels seems to discard an ABA-dependent response. However, the pattern of proline accumulation in *jai1* seedlings parallels that of ABA. Under stress conditions, there is an opposite pattern of ABA accumulation in roots of *jar1-1*/*coi1-16* (in which ABA only slightly increase) and *jai1* (in which ABA increase is even higher than in WT plants). This also makes JA-ABA crosstalk complex and discards any lineal pathway that could explain this hormonal interaction.

Proline accumulation is commonly used as a marker of plant responses to stress conditions such as drought,[1] and salinity,[2] and a causal relationship between proline synthesis and hyperosmotic stress tolerance has been demonstrated.[3] It was postulated that proline might play a role as an organic osmotic regulator,[4] nevertheless, the contribution of proline accumulation to osmotic adjustment in some *Species* seems to be quite small. Besides its role in osmotic regulation, proline can function as a chaperonine, protecting proteins and membranes from degradation. There are also studies linking proline accumulation to ROS scavenging activity.[5]

Whereas proline accumulation under stress conditions is extensively documented, the molecular basis of the signaling underlying its regulation has not been clearly established. Proline accumulation seems to be mediated by both ABA-dependent and ABA-independent pathways. The dominant role of ABA in mediating plant responses to water stress has been questioned several times, basically due to a poor correlation between ABA concentrations and physiological and growth parameters such as stomatal conductance or inhibition of shoot growth.[6] This led to research focused on the ABA-independent pathways regulating water stress responses.[7]

In a previous publication it was shown that jasmonates and in particular Jasmonic acid Isoleucine (JA-Ile) accumulation and, hence JA-dependent signaling, was necessary for consistent ABA accumulation in roots of Arabidopsis.[8] Although plants defective in JA-Ile accumulation (*jar1-1*) and JA-Ile dependent signaling (*coi1-16*) were able to accumulate ABA in roots after dehydration, accumulation was significantly lower compared to their respective WT. Interestingly, in complementary experiments using the same system, JA transiently accumulates in roots of dehydrated plants but this increase is coupled with a decrease in the hormone concentration in shoots. It is also important to note that JA-dependent build-up of ABA seems restricted to the roots, at least in short-term responses. However, according to,[9] leaf ABA accumulation in Arabidopsis is also influenced by JA in long-term responses. Moreover, exogenous treatment with jasmonates increased ABA levels in different *Species* both under control conditions or exposed to drought.[10,11] The reciprocal treatment with exogenous ABA had no significant effect on endogenous JA levels in citrus plants.[11]

In order to understand the effect of JA on plant responses to desiccation, proline accumulation after dehydration was analyzed in different Arabidopsis seedlings: a line defective in JA-Ile biosynthesis (*jar1-1*), 2 JA insensitive lines (*jai*1 and *coi1-16*), and an ABA insensitive line (*abi*2).

Relative water content (RWC) was similar in well-watered plants of the different genotypes. After stress imposition, this parameter decreased a 25% in average with no significant differences among genotypes with the exception of *jar1-1*where lower values were recorded (38% of reduction with respect control plants).

After 300 min of desiccation all lines but *jar1-1* accumulated high concentrations of JA-Ile in roots (**Fig. 1, bottom**). In leaves, only *jai*1 and *coi1-16* accumulated JA-Ile after dehydration (**Fig. 1, top**). ABA increased in roots of all lines except in *jar1-1* and *coi1-16*; surprisingly, *jai*1 (defective in one of the 2 branches of JA signaling) not only accumulated ABA to the same extent

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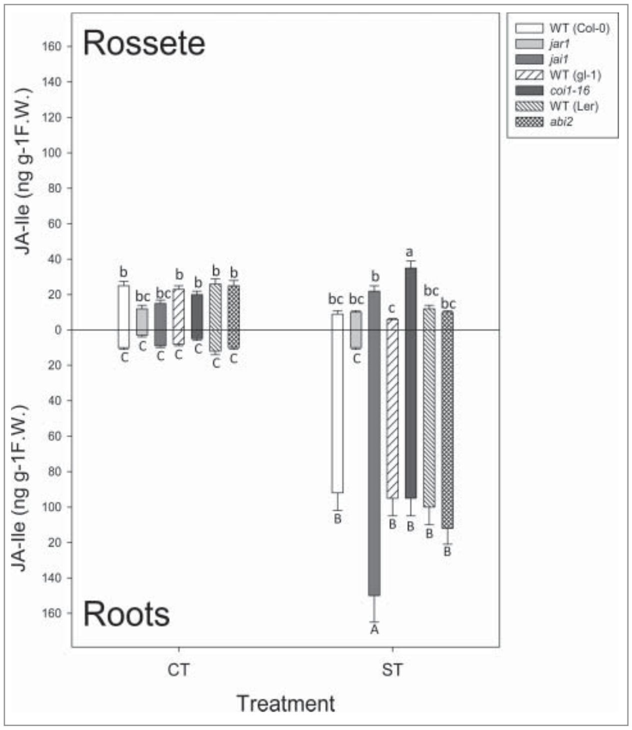

Figure 1. Jasmonoyl Isoleucine (JA-Ile) in leaves (**top**) and roots (**bottom**) of Arabidopsis under control conditions (CT), and after 300 min of desiccation (ST). Lines under treatment: Columbia-0 (Col-0), WT background for *jar1-1* and *jai1*, glabra (gl-1), WT background for *coi1-16*, Lansberg Erecta (Ler), WT background for *abi2*. Data are mean values ± standard deviation of 3 independent determinations. Letters denote statistical significance after Fisher's LSD ($p \leq 0.05$) between lines under the same conditions.

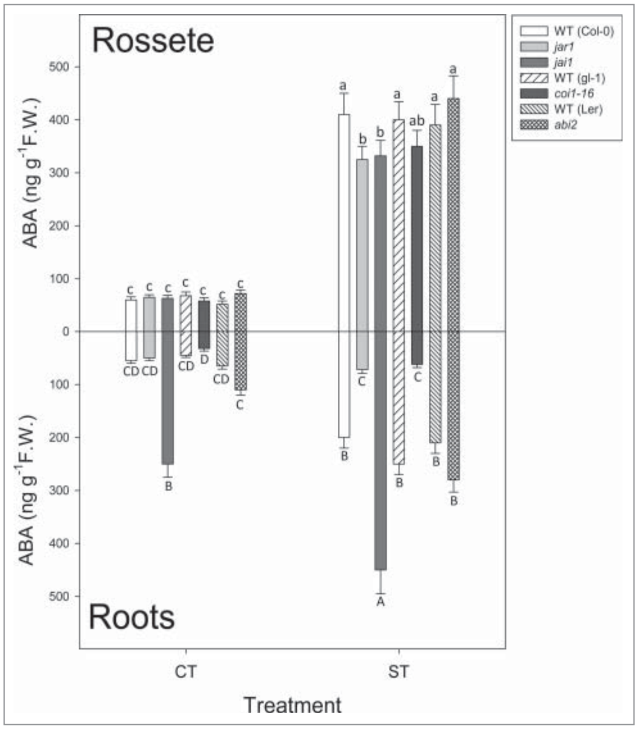

Figure 2. Abscisic acid (ABA) in leaves **(top)** and roots (**bottom**) under control conditions (CT), and after 300 min of desiccation (ST). Lines under treatment: Columbia-0 (Col-0), WT background for *jar1-1* and *jai1*, glabra (gl-1), WT background for *coi1-16*, Lansberg Erecta (Ler), WT background for *abi2*. Data are mean values ± standard deviation of 3 independent determinations. Letters denote statistical significance after Fisher's LSD ($p \leq 0.05$) between lines under the same conditions.

that WT plants but levels were the highest among lines. It is interesting that even under well-watered conditions *jai*1 seedlings had a significantly higher ABA content in roots (**Fig. 2, bottom**). On the other hand, ABA accumulation in leaves was similar among the different lines (**Fig. 2, top**). It is also important to point out that *abi2* mutants accumulated the same amount of JA-Ile than WT plants, indicating that ABA-dependent signaling has no influence on jasmonate biosynthesis under dehydration.

Proline accumulation was also different among the studied genotypes after dehydration; only the JA-deficient/insensitive lines had a significant proline accumulation in roots with respect to well-watered plants, and among these lines *jai1* seedlings accumulated more proline than the rest (**Fig. 3, bottom**). Similarly, in leaves, JA-deficient/insensitive lines accumulated more proline than other lines. It must be noted that, in the aerial part, proline concentration increased in WT lines (Col-0, gl-1 and Ler) to lower concentrations than in JA-deficient/insensitive lines. Proline levels in roots of *abi*2 seedlings did not significantly increase with respect to well-watered plants whereas in shoots, proline accumulation after desiccation increased to a similar extent than in *jai1* plants (around 25% increase). However, these levels were much lower than the accumulation found in *jar1* and *coi1-16* (186% and 144% increase, respectively, **Fig. 3, top**).

The pattern of proline accumulation is difficult to explain in terms of ABA accumulation/signaling: first, proline accumulation in *jai*1 seedlings parallels that of ABA but the fact that the JA-defective/insensitive seedlings are those that differentially accumulate more proline in roots and shoots despite the lower ABA accumulation in roots points to a non-ABA related phenotype.

Regarding the JA-ABA crosstalk in roots it is surprising that *jai*1 seedlings not only were able to accumulate ABA to the same extend than WT lines but levels of this hormone were even higher when exposed to the same dehydration conditions. The JA-insensitiveness in *jai*1 is caused by the loss of function of the MYC2 transcription factor (TF) that performs several functions such as controlling the activation of the PDF2.1/HEL branch of the JA-dependent signaling, antagonizing the VSP1/LOX2 branch, and organizing ABA dependent responses.[12] Therefore, the promiscuity of MYC2 makes very difficult to assess its role in JA/ABA crosstalk. Nonetheless, the completely opposite behavior in terms of ABA accumulation in roots of *jar1-1*/*coi1-16* (lower levels) and *jai*1 (higher levels) makes this crosstalk very interesting in terms of understanding the modulation of ABA synthesis and signaling in response to stress.

MYC2 has been defined as an ABA and drought responsive gene.[12] Constitutive expression of MYC2 and gene disruption by

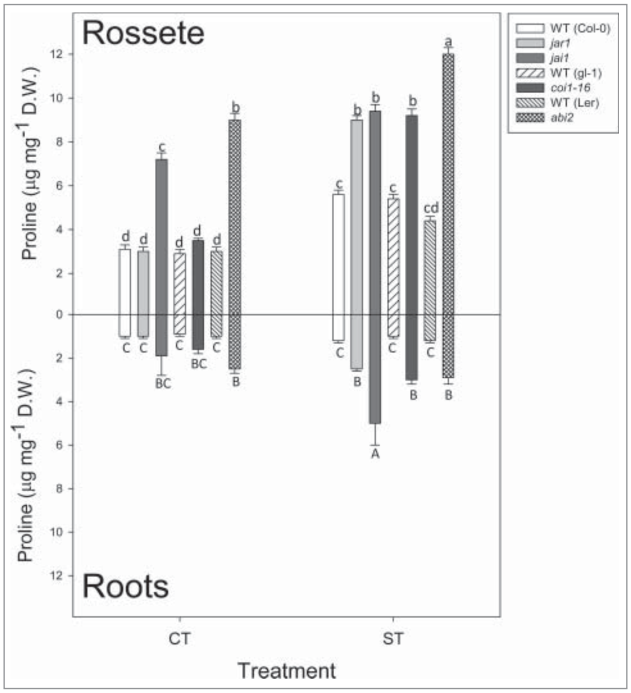

Figure 3. Proline in leaves **(top)** and roots (**bottom**) under control conditions (CT), and after 300 min of desiccation (ST). Lines under treatment: Columbia-0 (Col-0), WT background for *jar1-1* and *jai1*, glabra (gl-1), WT background for *coi1-16*, Lansberg Erecta (Ler), WT background for *abi2*. Data are mean values ± standard deviation of 3 independent determinations. Letters denote statistical significance after Fisher's LSD ($p \leq 0.05$) between lines under the same conditions.

knockout resulted in enhanced and reduced sensitivity to ABA,[13,14] respectively. In addition, MYC2 activates the expression of RD22 suggesting a positive role on ABA signaling.[13] However, according to,[15] Arabidopsis myc2/jai1 seedlings showed increased drought tolerance (based on biomass reduction data). Therefore, *myc2* phenotype under drought conditions is far from being completely characterized.

The fact that JA is involved in plant responses to drought also raises some interesting questions about the ABA-JA crosstalk. ABA seems to suppress the ERF1/PDF1.2 branch of the JA pathway but strongly induces the MYC2-VSP1 branch of JA signaling pathway. Interestingly, it has been demonstrated that TFs ANAC019 and ANAC055 (containing a NAC domain) also induce this pathway, being this effect reduced in myc2 mutants.[16] These TFs are also activated by JA which suggest that ANAC019 and ANAC055 act downstream of both JA and ABA. A recent study has shown that ANAC019, ANAC055 and the homologous ANAC072 play synergistic and antagonistic roles in ABA signaling and osmotic stress.[17]

Recently CML37, a Ca^{2+} sensor involved in the regulation of herbivore-induced plant defenses mediated by jasmonates was also identified as a positive regulator of ABA in drought stress.[18] The *cml37-1* mutant displays a lower expression of JA related genes in response to wounding and, interestingly, this mutant also has a reduced ABA accumulation in response to drought stress,[19] moreover, another member of this Ca^{2+} sensor family, CML42 has antagonistic effects and Arabidopsis mutant defective in this protein is able to accumulate ABA in response to drought.

Results here presented and recent literature point to a complex crosstalk between JA and ABA. Both signaling pathways seem to act together modulating each other responses and hence other metabolic and signaling pathways of responses to biotic and abiotic stress. Although challenging, this complex interplay offers the opportunity to modulate plants responses to various types of stress.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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