

# New cross talk between ROS, ABA and auxin controlling seed maturation and germination unraveled in APX6 deficient *Arabidopsis* seeds

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**Keywords:** ascorbate peroxidase, APX, Seed, germination, ROS, redox, auxin, ABA, ABI3, ABI4, ABI5

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Submitted: 08/18/2014

Revised: 08/25/2014

Accepted: 08/26/2014

<http://dx.doi.org/10.4161/15592324.2014.976489>

Chen C, Letnik I, Hacham Y, Dobrev P, Ben-Danie, B H, Vankova R, Amir R & Miller G. Ascorbate peroxidase 6 protects *Arabidopsis thaliana* desiccating and germinating seeds from stress and mediates crosstalk between ROS, ABA and auxin. *Plant Physiol* 2014; <http://www.ncbi.nlm.nih.gov/pubmed/25049361>.

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Successful execution of germination program greatly depends on the seeds' oxidative homeostasis. We recently identified new roles for the H<sub>2</sub>O<sub>2</sub>-reducing enzyme ascorbate peroxidase 6 (APX6) in germination control and seeds' stress tolerance. APX6 replaces APX1 as the dominant APX in dry seeds, and its loss-of-function results in reduced germination due to over accumulation of ROS and oxidative damage. Metabolic analyses in dry *apx6* seeds, revealed altered homeostasis of primary metabolites including accumulation of TCA cycle metabolites, ABA and auxin, supporting a novel role for APX6 in regulating cellular metabolism. Increased sensitivity of *apx6* mutants to ABA or IAA in germination assays indicated impaired perception of these signals. Relative suppression of *ABI3* and *ABI5* expression, and induction of *ABI4*, suggested the activation of a signaling route inhibiting germination in *apx6* seeds that is independent of *ABI3*. Here we provide additional evidence linking *ABI4* with ABA- and auxin-controlled inhibition of germination and suggest a hypothetical model for the role of APX6 in the regulation of the crosstalk between these hormones and ROS.

Oxidative imbalances due to stress-induced perturbations during the seed's development can greatly reduce its potential for proper germination. Over-accumulation of reactive oxygen species (ROS) can cause oxidative damage to a wide range of cellular components, including DNA, proteins and lipids, and reduce seeds ability to

germinate.<sup>1-3</sup> An optimal range of ROS levels, termed the 'oxidative window of germination', is required for successful germination; <sup>2</sup> too little ROS will result in suppressed germination (e.g., in dormant seeds) and too much ROS will lead to delaying or inhibiting germination due to accumulation oxidative damage.<sup>2</sup> A tight regulation is therefore required to balance ROS production and scavenging and maintain the cellular redox poise.

Cytosolic ascorbate peroxidase 6 (APX6) play an important role in the regulation of the oxidative state in 'mature drying' seeds as well as during early stages of germination, and loss of function mutants show reduced germination rate compared to WT seeds.<sup>4</sup>

In addition to the role of APXs as major H<sub>2</sub>O<sub>2</sub>-reducing enzymes, they are important components of the Foyer-Halliwel-Asada pathway, also known as the ascorbic acid-glutathione (AA-GSH) cycle that function in the cytosol and plastids. As such, APX activity also contribute to maintaining cellular redox homeostasis by regulating the ratios of the redox couples AA-dehydroascorbate (DHA), GSH-GSSH and NAD(P)<sup>+</sup>-NAD(P)H.<sup>5</sup> Accumulation of tricarboxylic acid (TCA) cycle metabolites, altered levels of hormones and amino acids in *apx6* seeds, suggested that APX6 play important regulatory role in seed metabolism by modulating ROS and redox signals.<sup>4</sup>

## APX6 is Required for Seeds' Stress Tolerance

Proper execution of the desiccation program, accumulation of storage proteins

and transcription of genes that are translated during the imbibition phase will determine the seed's commitment for germination.<sup>6-10</sup> Therefore, 'seed vigor', i.e., the potential for rapid uniform emergence and development under a wide range of field conditions, greatly depends on proper execution of seed maturation and desiccation related processes.<sup>10</sup> Knockout *apx6* seeds showed increased sensitivity to hyperosmotic conditions (NaCl and Sorbitol) or heat stress (HS) during germination. In addition, *apx6* seeds collected following HS applied during the 'mature drying' phase showed severe retardation in germination under favorable conditions.<sup>4</sup> These observations suggested that APX6

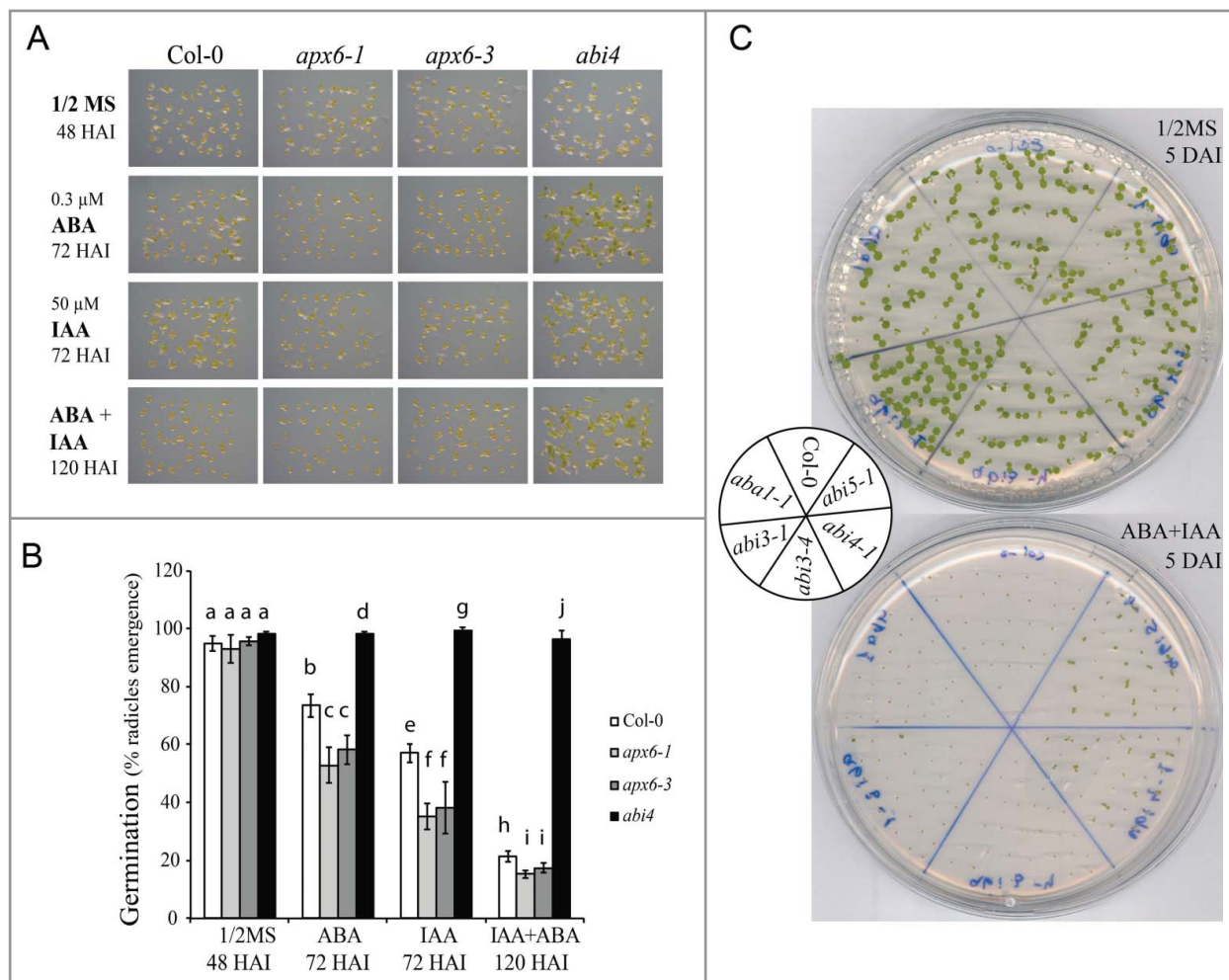
has an important role in protecting seeds and maintaining their vigor.

### APX6-Associated Crosstalk Between ROS, ABA and Auxin May Involve ABI4

Increased ROS level in *apx6* is associated with higher expression level of the transcription factor *ABI4* in dry and imbibed seeds.<sup>4</sup> Accumulated evidence from recent years suggest that *ABI4* is activated in response to redox signals involved in retrograde signaling, developmental processes as well as in response to stress.<sup>11-13</sup>

In addition *ABI4*-mediated pathways and AA-dependent processes in Arabidopsis were suggested to function interdependently.<sup>11</sup>

*ABI4* was also recently shown to positively regulate dormancy by promoting ABA synthesis.<sup>14</sup> Moreover, AA function as a co-factor in enzymatic activities including for nine-cis-epoxycarotenoid dioxygenase (NCED) enzyme that synthesizes the ABA precursor xanthoxin.<sup>15</sup> Furthermore, in the AA-deficient mutant *vtc1*, the level of ABA was 60% higher compared to the WT due to an increase in ABA synthesis transcripts including that of NCED.<sup>16</sup> Interestingly, a similar increase in ABA level observed in dry seeds



**Figure 1.** Germination response to ABA and IAA. Freshly harvested seeds were germinated on 1/2MS supplemented with ABA, IAA or their combination. Sample pictures of seed germination (A) and histograms (B) of germination were recorded at the indicated hours after imbibition (HAI). Standard deviations represent an average of 6 replicates of 50 seeds each. Different lowercase letters indicate values from the seedling lines significantly differ from each other within each treatment by one-way ANOVA and Bonferroni test ( $P < 0.05$ ). (C) Germination of ABA-related mutants on 1/2MS agar plates without (top) and with 0.3  $\mu$ M ABA+50  $\mu$ M IAA (bottom). Photograph was taken 5 d after imbibition (DAI).

of *apx6* was associated with changes in the AA pool.<sup>4</sup>

Hence, potentially ABI4 pathway could be activated in response to the ROS/redox signal generated in *apx6* seeds.

### Regulation of Germination by ABA and Auxin

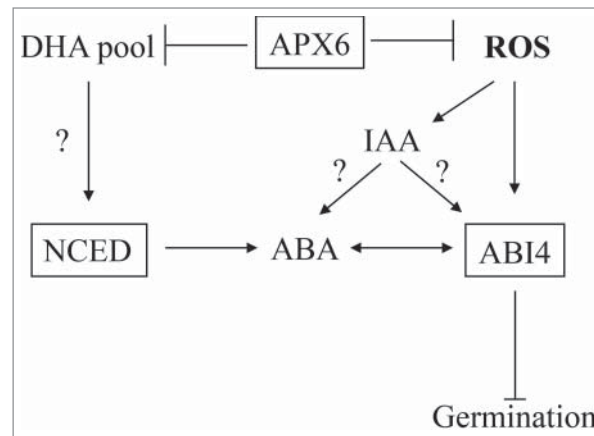
In addition to increased level of ABA, *apx6* seeds accumulated high level of auxin.<sup>4</sup>

Auxin action in seeds dormancy requires the ABA signaling pathway (and vice versa), indicating that the roles of auxin and ABA in seed dormancy are interdependent.<sup>17</sup>

High levels of auxin and activation of IAA signaling enhance ABA-mediated dormancy by supporting the persistence of the expression of ABI3, the major regulator of seed dormancy.<sup>17</sup> Increased sensitivity of *apx6* seeds to either ABA or IAA, suggested these signals might be involved in the germination inhibition phenotype of the mutants.<sup>4</sup> However, since ABI3 and ABI5 expression were relatively suppressed in *apx6* seeds, it is likely that the crosstalk between auxin and ABA does not involve activation of the ABI3 signaling route, but rather the ABI4 route.

Here we tested whether combination of both ABA and IAA would enhance the inhibition of germination of *apx6* and whether *abi4* is also insensitive to auxin (Fig. 1). Results show that combination of ABA and IAA enhance the inhibitory effect of germination and that both *apx6* lines are more sensitive compared to the WT (Fig. 1). This further suggests that over accumulation of ROS in seeds increase sensitivity to both ABA and auxin. In contrast, reduced ability to generate ROS in Arabidopsis mutant deficient in 2 major respiratory burst oxidase homologs *rbobD/F*, decreased sensitivity to ABA-mediated auxin response in roots.<sup>18</sup>

In striking contrast to *apx6*, *abi4* maintained close to 100% germination under all conditions, indicating that this mutant is also insensitive to auxin and resistant to its combination with ABA (Fig. 1). Additionally we tested whether other ABA-related mutants behave similarly to *abi4*



**Figure 2.** Hypothetical model for APX6-regulated ROS/redox signaling in seeds. APX6 function in seeds to reduce the levels of ROS and redox imbalances during stress, protecting seeds and maintain their vigor. ROS and redox signals generated in the absence of functional APX6 or during severe stress conditions lead to increased levels of ABA and auxin and promote their inhibitory effect on germination. APX6 effect the balance between the AA pool and DHA pool.<sup>5</sup> Decreased availability of AA result in activation of ABA biosynthesis genes including NECD leading accumulation of ABA.<sup>15</sup> ABI4 expression and pathway are activated in response to the increase in oxidative load and the level of ABA. It is not clear yet if ABI4 responds directly to auxin or indirectly by enhancing the ABA-mediate response.

on IAA containing media. Both *aba1* and the *abi3* lines showed sensitivity to auxin (Fig. 1C and S1), suggesting that nor ABA synthesis or ABI3 are required for auxin-mediated inactivation of germination. Surprisingly, *abi5* mutant line showed IAA-insensitivity similar to *abi4* (Fig. 1C and S1), supporting a role for *abi5* in the response to auxin.

These results suggest that ABI4 may be a convergence target point for ABA- and auxin-controlled inhibition of germination and that ABI5 is also involved in this response.

Crosstalk between ABA and auxin involving ABI4 has been shown in Arabidopsis lateral root development,<sup>19</sup> primary root growth and seeds germination,<sup>20</sup> and in time of flowering.<sup>21</sup> Furthermore, cooperation or interaction between ABI4 and ABI5 in gene regulation was previously reported in germination control and response to stress.<sup>22-25</sup> However, since ABI5 expression is relatively suppressed in *apx6* seeds,<sup>4</sup> it is likely that the mutant's sensitivity to auxin is not mediated by this transcription factor.

Therefore we suggest a hypothetical model in which APX6 is the modulator of the cytosolic ROS and redox signals that regulates germination in seeds. This regulation involves ABA and auxin

metabolism and signaling and is mediated by ABI4 (Fig. 2).

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Funding

This work was supported by the Marie Curie Actions-International Career. Integration Grant (grant no. 293999), the Israel Science Foundation (grant no. 938/11) and the Binational Agricultural Research & Development Fund (project IS-4652-13 R).

#### Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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