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Article Addendum

The ascorbate peroxidase regulated by H_2O_2 and ethylene is involved in cotton fiber cell elongation by modulating ROS homeostasis

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Ascorbate peroxidase (APX) is a reactive oxygen species (ROSs) scavenging enzyme involved in regulation of intracellular ROS levels by reduction of H_2O_2 to water using ascorbate as an electron donor. In *New Phytologist* 2007; 175:462–71, we identified a cotton cytosolic APX1 (Gh*APX1*) that was significantly accumulated during the fast fiber-cell elongation period, through a proteomics approach. Both the transcript levels of Gh*APX1* and the total APX activity were highly induced in response to in vitro applied H_2O_2 or ethylene. Further analysis showed that ethylene promoted H_2O_2 production 1 day after it was included in the culture medium, suggesting that H_2O_2 induced cell elongation processes may be placed downstream of the ethylene signal transduction pathway. In this addendum, quantitative real-time RT-PCR showed that only cytosolic *APX1*, not other cotton *APX* genes including a second cytosolic *APX2*, a glyoxysomal and a stromal *APXs*, was up-regulated during fiber cell elongating. Exogenous H_2O_2 was found to induce ethylene production if wild-type cotton ovules were cultured for a longer period of time, implying that there was a feedback regulatory mechanism from H_2O_2 to ethylene biosynthesis in modulating cotton fiber development.

Reactive oxygen species (ROSs) including superoxide radicals, hydrogen peroxide, and hydroxyl radicals are formed by successive one-electron reductions of molecular oxygen. Interestingly, it has been proposed that a cross-talk between various ROSs might contribute to stabilize plants under different stress conditions.[1] ROS produced in plant is mainly hydrogen peroxide (H_2O_2) that is relatively stable and electron-neutral. H_2O_2 usually acts as signaling molecules in programmed cell death, in regulation of photosynthesis and perception of environmental stresses as well as in response to pathogen invasions.[2] Excess amounts of H_2O_2 are known to cause oxidative damages to the host cells. ROS was involved in regulation of plant cell expansion since an *Arabidopsis* mutant deficient in NADPH oxidase activity showed significantly stunted root hair growth.[3] ROS may exert its multi-facet effects via a complex network.[2,4]

Cotton is the most prevalent natural fiber used in textile industry and is one of the mainstays of Chinese as well as global economy. Cotton lint, or commonly known as cotton fiber, are single-celled trichomes evolved from the ovule epidermis and are perhaps the longest single cells in higher plants. Upland cotton (*Gossypium hirsutum* L.) generally grows up to 30–40 mm in length, about 15 μm in thickness at full maturity and accounts for more than 90% of the production in the world.[5-8] Ascorbate peroxidase (APX, EC, 1.11.1.11), one of the most important antioxidant enzymes in higher plants, utilizes ascorbate as electron donors to reduce H_2O_2 into water. APX, comprising a family of isozymes in different subcellular compartments, has a high affinity towards H_2O_2. Cytosolic, chloroplastic, mitochondrial and microsomal (glyoxysomal/peroxisomal) APX isoforms have been characterized in Arabidopsis.[9] Cytosolic APX1 was found to play an essential role for cross-compartment protection and maintenance of the cellular reactive oxygen network whereas APX2 is activated by variable stresses.[3,10,11] Chloroplastic APXs protect the photosynthetic apparatus against oxidation while microsomal APXs are involved in detoxifying H_2O_2 produced by fatty acid β-oxidation, especially during seed germination and photorespiration.[12,13] In addition to peroxisomal APX, peroxisomal membrane-bound monodehydroascorbate reductase 4 was important for scavenging H_2O_2 molecules that escaped the peroxisomes.[14]

Although many APX isozymes have been identified from many higher plants, their functions in regulation of plant growth and development remain elusive. We recently identified and characterized the cotton cytosolic *APX1* (*APX1*) gene that played an important role in modulating fiber cell elongating. Here, we obtained and submitted three additional putative full-length cDNAs encoding cotton *APXs* to GenBank, including a cytosolic *APX2* (Genebank accession no. EU244476), a glyoxysomal *APX* (Genebank accession no. EU244478) and a stromal *APX* (Genebank accession no. EU244477). We examined their expression profiles using QRT-PCR (Fig. 1) and found that only *APX1* exhibited a strong upregulation at 5 day post-anthesis (dpa) in comparison with its transcript level at 0 dpa ovules. Since the cytosol is an important location for cellular communication among different subcelluar compartments, our data, therefore, seems to suggest that cytosolic GhAPX1 may have a regulatory function in controlling the overall H_2O_2 level inside a plant cell. The current finding agrees with previous report that an *Arabidopsis*

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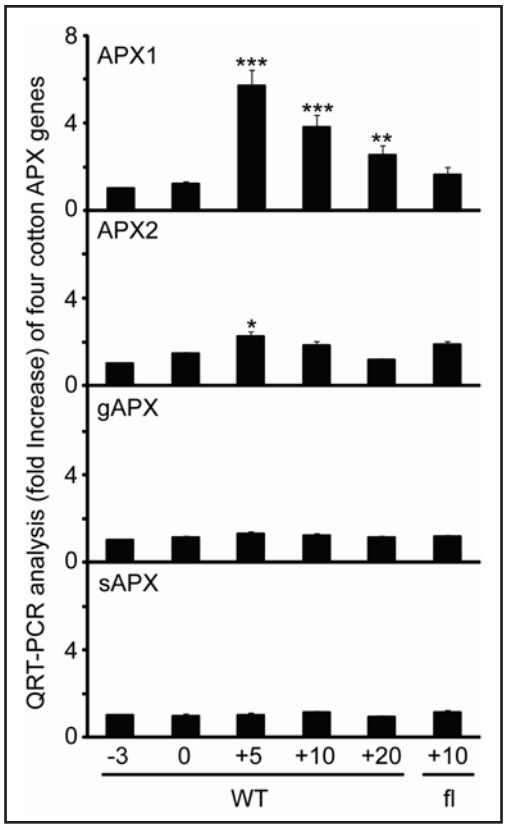

Figure 1. Analysis of transcript levels of various GhAPX genes during different developmental stages. QRT-PCR was performed using RNA samples prepared from triplicate cotton materials harvested from indicated growth stages. The cotton ubiquitin gene, *UBQ7* (Genebank accession no. AY189972) was included as a loading control. *APX1*, cytosolic *APX1* (Genebank accession no. EF432582); *APX2*, cytosolic *APX2* (Genebank accession no. EU244476); *gAPX*, glyoxysomal *APX* (Genebank accession no. EU244478); *sAPX*, stromal *APX* (Genebank accession no. EU244477).

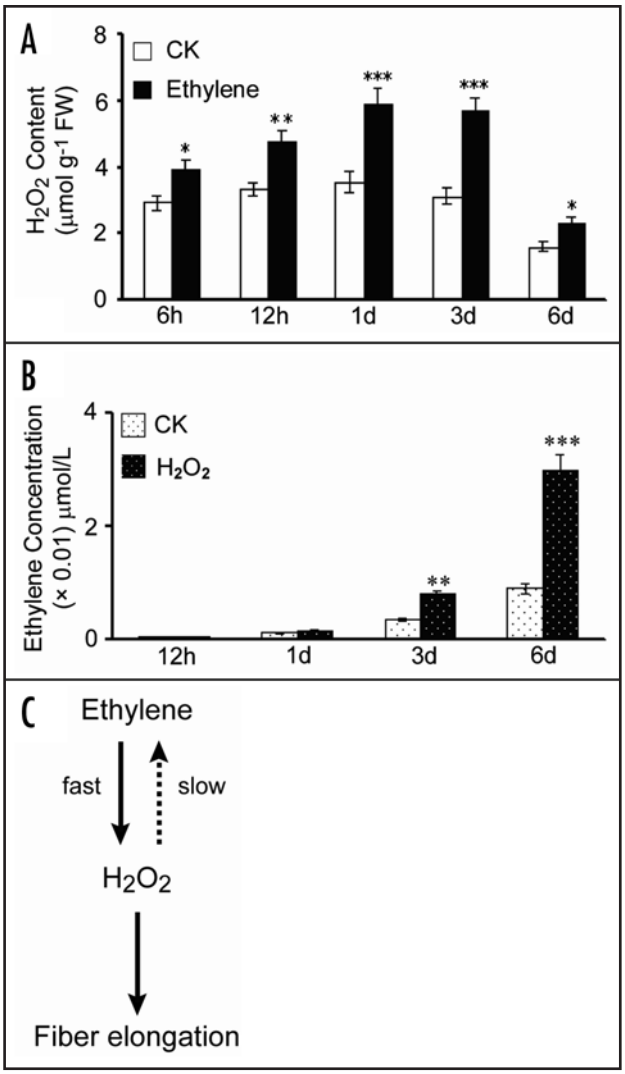

Figure 2. Ethylene and H_2O_2 interact to regulated cotton fiber elongation. (A) Exogenous ethylene induced H_2O_2 production starting from an early stage. (B) Exogenous H_2O_2 slowly stimulated ethylene production. (C) A proposed model for a regulatory mechanism between H_2O_2 and ethylene signaling in controlling fiber elongation. Wide-type ovules were harvested at 1 dpa and in vitro cultured in the medium (CK), in presence of 50 μM H_2O_2 or 0.1 μM ethylene for the time (h or d) indicated. H_2O_2 content was measured using titanium oxidation method (23). gFW, gram fresh weight. Ethylene production from cultured wild-type ovules was determined according to the protocol described (ref. 17). Statistical significances were determined using one-way ANOVA software combined with Tukey's test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

mutant deficient in cytosolic *APX1* displayed a stunted growth phenotype,[10,15] although a different study found that both the cytosolic and thylakoidal APXs were involved in subcellular communications from different compartments during abiotic stresses.[16]

Ethylene was shown to significantly promote fiber growth.[17] In vitro applied ethylene enhanced significant H_2O_2 production as early as 6 h until 1 d when reached a peak value (Fig. 2A), whereas, exogenous H_2O_2 was as well able to stimulate a significant ethylene production but after 1 d (Fig. 2B). Ethylene or hydrogen peroxide was found to regulate the expression of a soybean ascorbate peroxidase gene while abscisic acid was active in inducing H_2O_2 production.[18,19] H_2O_2-activated Ca^{2+} channels were important for stomatal movement.[19] Similar studies revealed that ethylene-induced stomatal closure in *Arabidopsis* depended on H_2O_2 production. Both ethylene and H_2O_2 signaling in guard cells were mediated by ethylene receptors.[20] Auxin was found to stimulate the biosynthesis of H_2O_2 during root gravity responses.[21] H_2O_2 generated through NADPH oxidase and superoxide dismutase was shown necessarily for regurgitant-induced increase of ethylene production.[22] Taken together, we propose the existence of a regulatory mechanism between H_2O_2 and ethylene to modulate cotton fiber and may be other related types of cell elongation (Fig. 2C). How plant hormones and ROS interact to regulate the expression of various APX isoenzymes during plant growth and development requires further investigation.

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