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Roles of YUCCAs in auxin biosynthesis and drought stress responses in plants

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Auxin, a plant hormone, plays crucial roles in diverse aspects of plant growth and development reacting to and integrating environmental stimuli. Indole-3-acetic acid (IAA) is the major plant auxin that is synthesized by members of the YUCCA (YUC) family of flavin monooxygenases that catalyse a rate-limiting step. Although the paths to IAA biosynthesis are characterized in *Arabidopsis*, little is known about the corresponding components in potato. Recently, we isolated eight putative *StYUC* (*Solanum tuberosum YUCCA*) genes and five putative tryptophan aminotransferase genes in comparison to those found in *Arabidopsis*.[1] The specific domains of YUC proteins were well conserved in all StYUC amino acid sequences. Transgenic potato (*Solanum tuberosum* cv. Jowon) overexpressing *AtYUC6* showed high-auxin and enhanced drought tolerance phenotypes. The transgenic potatoes also exhibited reduced levels of ROS (reactive oxygen species) compared to control plants. We therefore propose that YUCCA and TAA families in potato would function in the auxin biosynthesis. The overexpression of *AtYUC6* in potato establishes enhanced drought tolerance through regulated ROS homeostasis.

Keywords: *Arabidopsis* YUCCA6, auxin, drought, potato, reactive oxygen species

Abbreviations: IAOx, the indole-3-acetaldoxime; IAM, indole-3-acetamide; TAM, tryptamine; IPA, indole-3-pyruvic acid; Trp, tryptophan; TAA, tryptophan aminotransferase of *Arabidopsis*; IAA, indole-3-acetic acid; ROS, reactive oxygen species

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Plants produce various phytohormones, including auxins, gibberellins, cytokinins, ethylene, abscisic acid and brassinosteroids. Their synthesis is delicately regulated to orchestrate normal cell, organ and plant growth and development. In addition, the phytohormones co-adjust agonistically or antagonistically to demands originating from environmental cues. Among the phytohormones, auxin is essential as a regulator of growth and development, involved in diverse processes, such as cell division, expansion and differentiation, and also in lateral root formation, flowering, tropic responses, and senescence.[2-5] Recent studies also provided evidence for the function of auxin in responses to environmental stresses, including drought, salinity and pathogen attack.[6-8]

Indole-3-acetic acid (IAA) is the main plant auxin synthesized by both tryptophan (Trp)-dependent and -independent pathways.[9] Although molecular components and physiological functions of the Trp-independent pathway are unknown, the Trp-dependent pathway is well defined as multiple pathways that proceed through four metabolic intermediates.[5] These can be divided into the indole-3-acetaldoxime (IAOx), indole-3-acetamide (IAM), tryptamine (TAM) and indole-3-pyruvic acid (IPA) pathways. Genetic and biochemical studies in *Arabidopsis* have shown the preponderance of the Trp-dependent pathway in de novo auxin biosynthesis. Evidently the Trp-dependent pathway is involved in embryogenesis, seedling growth, flower development, vascular patterning, while it affects other developmental processes as well.[10-13]

Significantly, the IPA pathway constitutes a simple two-step pathway in *Arabidopsis*.[14,15] The first step is the conversion of tryptophan to indole-3-pyruvic acid (IPA) by a family of tryptophan aminotransferase of *Arabidopsis* (TAA). The TAA family consists of three closely related genes in *Arabidopsis* (*TAA1*, *TAR1* and *TAR2*). Mutations of these genes

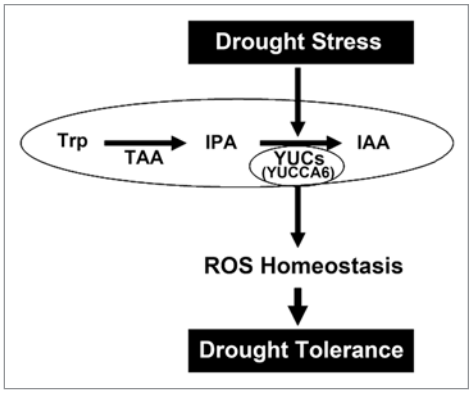

Figure 1. Model proposed for the function of YUCCA6 in drought stress. YUCCA6 protein is involved in auxin biosynthesis as a rate-limiting step converting IPA to IAA. It also functions in a drought stress signaling pathway through effects of ROS equilibrium in plants. Trp, tryptophan; TAA, tryptophan aminotransferase of *Arabidopsis*; IPA, indole-3-phyruvic acid; IAA, indole-3-acetic acid; ROS, reactive oxygen species.

resulted in partial auxin deficiency phenotypically revealed by altered responses to shade avoidance, ethylene and auxin transport inhibitors, respectively.[12,13,16] The defects of *taa* mutants could be partially rescued by auxin supplemented to growth media.[12,13] These results strongly suggest that the *TAA* family genes constitute essential components for auxin biosynthesis. This fact suggested detailed studies on functions of *TAA* family genes in crop plants. We focused *Solanum tuberosum*, potato, in an attempt to define and isolate putative *TAA* gene family members. Based on the potato genome database (solanaceae.plantbiology.msu.edu/pgsc_download.shtml), we isolated five putative *TAA* family genes, termed *Solanum tuberosum TRYPTOPHAN AMINOTRANSFERASE RELATED*1 to 5 (*StTAR*1 to 5). The StTARs showed approximately 50% deduced amino acid sequence identity with *Arabidopsis* TAA/TAR1/TAR2. Also present in the potato sequences are the characteristic alliinase C and aromatic aminotransferase domains of TAA proteins.[1]

In addition, the rate-limiting second step in Trp-dependent auxin biosynthesis is conversion of IPA to IAA by members of the YUCCA family of flavin monooxygenases (FMOs). Previously, the involvement of YUCCA proteins in the TAM pathway, a different Trp-dependent auxin biosynthesis pathway was reported because of the ability of YUCCA proteins to use TAM as a substrate.[5] This view has been replaced by recent studies in *Arabidopsis* that revealed the function of YUCCA proteins in the IPA pathway with TAA/TARs.[14,15] In *Arabidopsis*, the YUCCA family consists of 11 genes. Lines overexpressing the proteins and activation-tagged mutants of individual genes showed auxin overproduction phenotypes, such as elongated hypocotyl, epinastic cotyledons, curled-down rosette leaves and strong apical dominance.[8,17-20] However, single *YUC* gene mutations fail to show a particular phenotype, suggesting overlapping functions for members of the family. For example, a quadruple mutant line, including *yuc1yuc4yuc10yuc11*, showed the classical developmental defects of auxin deficiency.[10,11] *YUC* genes have been identified as auxin-related genes in petunia, rice, corn and tomato.[21-25] We now isolated eight putative *YUC* genes (*StYUCs*) from potato with 50% to 70% amino acid identity with their *Arabidopsis* counterpart YUCCA proteins and including canonical, conserved YUCCA sequence domains. In addition, *YUCCA6* overexpressed in *Arabidopsis* and potato plants led to auxin overproduction and drought tolerance phenotypes.[1] We surmise that the curled leaf structure observed in *YUCCA6*-overexpressing plants may cause a decrease in transpiration that could confer or support drought tolerance, but direct evidence is yet to be provided.

Several phytohormones are involved in stress responses. Examples are the GA repressor DELLA in salt stress and SA repressor JAZ in pathogen infections.[26,27] Networks of ROS signaling in the chloroplast and mitochondria play essential roles in response to abiotic stresses in plants.[28] In addition, the crosstalk between auxin regulatory networks and ROS regulates the integration of environmental stress signals.[29] Auxin and ROS homeostasis would be interconnected by a redox balance, which could control the environmental stresses. However, an effect for auxin has not yet been clearly identified. *yuc6-1D* showed lower accumulation of hydrogen peroxide, a reactive oxygen species (ROS), compared to wild-type plants, while other antioxidant genes are not enhanced by *YUCCA6* overexpression. In plants subjected to environmental stresses, ROS and Ca^{2+} accumulate significantly in plant cells. Thus, low concentrations of ROS in *yuc6-1D* could be at the basis of the observed drought tolerance. Recently, *YUCCA7* transcript was observed to increase under drought stress, but *YUCCA7* overexpression failed to correlate with drought tolerance in an ABA dependent manner.[8] This could imply that drought tolerance by *YUCCA6* overexpression may include post-transcriptional control or the involvement of an unknown ROS scavenging system induced by *YUCCA6* overexpression to maintain the ROS homeostasis (**Fig.1**). One recent observation could point in this direction as well. Disruption of thioredoxin and glutathione systems resulted in the inhibition of auxin transport displaying the inflorescence stem, pin-like phenotypes, indicating that auxin signaling has some interplay with thioredoxin and glutathione systems.[30,31] In addition, it is reported that auxin and ABA interplay through the reduced auxin levels caused by mitochondrial ROS overexpression.[32] These may correlate with unknown functions of YUCCA6 in conferring drought tolerance. Further investigations into the stress signaling responses related to auxin's action will be necessary. This will provide better understanding of the roles of YUCCAs in the cross-talks between auxin dynamics and drought stress responses of plants.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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