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An *Arabidopsis* WDR protein coordinates cellular networks involved in light, stress response and hormone signals

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ABSTRACT

The WD-40 repeat (WDR) protein acts as a scaffold for protein interactions in various cellular events. An *Arabidopsis* WDR protein exhibited sequence similarity with human *WDR26*, a scaffolding protein implicated in H₂O₂-induced cell death in neural cells. The *AtWDR26* transcript was induced by auxin, abscisic acid (ABA), ethylene (ET), osmostic stress and salinity. The expression of *AtWDR26* was regulated by light, and seed germination of the *AtWDR26* overexpression (OE) and seedling growth of the T-DNA knock-out (KO) exhibited altered sensitivity to light. Root growth of the OE seedlings increased tolerance to ZnSO₄ and NaCl stresses and were hypersensitive to inhibition of osmotic stress. Seedlings of OE and KO altered sensitivities to multiple hormones. Transcriptome analysis of the transgenic plants overexpressing *AtWDR26* showed that genes involved in the chloroplast-related metabolism constituted the largest group of the up-regulated genes. *AtWDR26* overexpression up-regulated a large number of genes related to defense cellular events including biotic and abiotic stress response. Furthermore, several members of genes functioning in the regulation of Zn homeostasis, and hormone synthesis and perception of auxin and JA were strongly up-regulated in the transgenic plants. Our data provide physiological and transcriptional evidence for *AtWDR26* role in hormone, light and abiotic stress cellular events.

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1. Introduction

The WD (Trp-Asp)-40 repeat (WDR) was first discovered in the β -subunit of heterotrimeric GTP-binding proteins (G proteins) [1]. G proteins are implicated in diverse cellular events through G protein-coupled receptor (GPCR) signaling to downstream targets, which may ultimately result in transcriptional regulation [2]. The WD-40 repeat motif consists of repeating amino acid sequences separated by approximately 40 residues and contains amino acids, tryptophan and aspartate (WD) at the end of the repeat. WDR proteins commonly contain four to eight WD-40 repeats; however, the separating distance and actual amino acid sequence within individual repeats is highly variable. The WD repeat domains form a platform for the assembly of multiple proteins and therefore play key roles in the formation of protein-protein complexes [3]. It is now believed that the WD-40 motifs may contribute to the formation of a stable protein complex that participates in various signaling pathways. In the human genome, the function of the WDR

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http://dx.doi.org/10.1016/j.plantsci.2015.09.024 0168-9452/© 2015 Elsevier Ireland Ltd. All rights reserved. protein shows a high degree of diversity, including signal transduction, RNA processing, cell cycle control, transcriptional regulation, vesicular trafficking, regulation of cytoskeleton assembly, nuclear export, RNA processing, and chromatin modification [4].

In the Arabidopsis genome, more than 200 putative WD-40 domain-containing proteins have been predicted [5]. A number of the WDR proteins have been functionally characterized to play a role in light signaling, photomorphogenesis, and flowering, such as Constitutively photomorphogenic 1 (COP1), Suppressor of Phy-A-105 (SPA1), and Anthocyanin11 (AN11) [6,7]. Several WDR proteins play a role in gametogenesis [8], abiotic stress [9], auxinregulated embryogenesis [10], meristem maintenance [11], floral development [12], and metal ion binding [13]. Understanding of the molecular mechanism indicates that WDR proteins may alter the cellular homeostasis of reactive oxygen species (ROS) through protein interactions with components such as histones, ribosome biogenesis proteins, or chromatin assembly factors to participate in developmental regulation [9,11,14-16]. ROS molecules are crucial for plant development, and an increased ROS level is necessary for plants to initiate proper signals for acclimation responses to environmental stress [17]. For example, light-induced auxin biosynthesis occurs through the control of ROS production [18].





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Fig. 1. Homologous genes of AtWDR26. Sequence alignment between AtWDR26 and human WDR26 (A). Letters in black and grey shadow indicate the identical and conserved amino acid residues, respectively. The deduced amino acid sequence of AtWDR26 contained the LisH, CTLH, and WD (WD-40) domains (B). Homologous genes of ATWDR26 identified in diverse plant species. ^a indicates the amino acid (a.a) residues of the coding region, ^b indicates % of positive a.a residues, ^c indicates % of identical a.a residues (C).



Fig. 2. Expression of AtWDR26. RT-PCR using primers specific to the coding sequence of AtWDR26 was conducted to detect the AtWDR26 expression. RNA isolated from different parts of tissues of 4-week-old *Arabidopsis* plants was used for RT-PCR analysis for detecting tissue specific expression of AtWDR26 (A). RNAs isolated from 7-day-old whole seedlings treated with different hormones including 10 µM IAA, 150 µM ABA and 50 µM ACC, and abiotic stresses including 150 mM NaCl and 300 mM mannitol (man) were used for RT-PCR analysis to detect the AtWDR26 inducible expression (B). RNAs isolated from the 7-day-old whole seedlings grown under the light (100 µmol s⁻¹ m⁻²) and dark conditions at 23 °C were used for RT-PCR analysis to demonstrate the light inducible expression (C). *Actin 2 (ACT2)* expression was used as an internal control. The gene expression levels of silique in (A), water in (B) and dark in (C) were used as the bases for comparative expression.

Ethylene (ET), jasmonic acid (JA) and ABA function in the control of stomatal closure through the modulation of ROS levels in the guard cells [19–21]. ET and salicylic acid (SA) are implicated in the crosstalk between high light acclimation and disease resistance through mediation of ROS molecules [22,23]. Plant acclimation to high light stress results in reduced stomatal conductance, increased temperature, and increased activity of heat shock transcription factors [24]. In addition, higher levels of ABA and auxin accumulate in plant tissues subjected to high light stress [25,26].

Human *WDR26*, a novel member of the WD-40 gene family, functions in the regulation of H_2O_2 homeostasis, cell movement, and apoptosis [27]. *Arabidopsis* homolog of human *WDR26*, designated as *AtWDR26* (At5G08560), has been identified as an

interacting protein of the *Arabidopsis* RanBPM (Ran-binding protein in the microtubule-organizing center), a component of the C-terminal to the LisH motif (CTLH) complexes [28]. In yeast, the CTLH complex of Gid/Vid proteins plays a role in vacuole and proteasome-dependent fructose-1,6-bisphosphatase degradation [29]. The mammalian CTLH complexes also exhibit a similar function in lysosome and proteasome-dependent proteolysis [30]. The AtRanBPM complex has been identified as a cytosolic protein complex; however, its cellular function remains unknown [28]. The function of *AtWDR26* was determined in the current study. The physiological and transcriptional evidence suggests that *AtWDR26* is a component implicated in the crosstalk regulation between light, hormone and abiotic stress response. Download English Version:

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