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Article Addendum

*At*PDCD5 Plays a Role during dark-senescence in Arabidopsis

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ABSTRACT

In this work, we investigated the role of an Arabidopsis protein, *At*PDCD5, during senescence after a 24h-dark period. Previously, we demonstrated that *At*PDCD5 participates in programmed cell death (PCD) after UV-B exposure and in age-induced senescence. The results presented here, together with previous data, demonstrate that *At*PDCD5 not only plays an important role during DNA damage responses induced by UV-B radiation, but also takes part in PCD programs such as dark-induced senescence in Arabidopsis.

Key words: darkness; PDCD5; programmed cell death; senescence; UV-B radiation.

Abbreviations: PCD: Programmed Cell Death; OE: Overexpressing.

Programmed cell death (PCD) is an essential process of life. In animals and plants, PCD is involved in different aspects of development, shaping structures or eliminating unwanted tissues. [1,2] In plants, PCD occurs as an essential part of development but also as a reaction to biotic and abiotic environmental challenges. [3] During stress conditions, abiotic stresses such as heat, salt, UV radiation or extended darkness; or biotic stresses such as pathogen attacks can lead to cell death. [4,5] On the other hand, differentiation induced PCD occurs as a final differentiation step in specific cell types, for instance, in xylem tracheary elements, the root cap, or the anther tapetum layer;[6-8] but also age-induced PCD takes place as the last step of organ senescence that occurs in all tissues of an organ or even the entire plant at the end of its life cycle. [9] Leaf senescence involves different physiological, biochemical, and molecular changes, including a decline in photosynthetic efficiency, decreases in chlorophyll and protein contents, and increases in membrane ion leakage and expression of senescence-associated genes. [10] Although senescence occurs in an age-dependent manner and it is controlled by an innate genetic program; unfavorable environmental stresses, such as darkness, can also trigger senescence during leaf development. Plant growth and development requires light, and plants require photoreceptors to adapt to ambient light conditions throughout development. Interestingly, plants that overexpress the photoreceptors phytochrome A or B show delayed leaf yellowing,[11,12] while the knockout *phyB* mutant is hyposensitive to a dark treatment,[13] suggesting that phytochromes participate in the regulation of leaf senescence. Photoactivated phytochromes move from the cytosol to the nucleus, where they interact with regulators of light signaling such as the transcription factors PIF4 and PIF5 are positive factors of dark-induced senescence in Arabidopsis.[14]

Previously, we reported that an Arabidopsis protein, Programmed Cell Death protein 5 (*At*PDCD5), which is highly similar to the human PDCD5 protein, is induced by UV-B radiation and participates in PCD in UV-B DNA damage response.[15] In

humans, PDCD5 binds to the histone acetyltransferase TIP60 to enhance its activity to repair DNA damage and it also regulates different types of PCD; for example the translocation of Bax, a pro-apototic factor, from the cytosol to the mitochondria, inducing cytochrome c release and an activation of caspase-3 activity, which are early events of the beginning of apoptosis.[16] On the other hand, *Hs*PDCD5 participates in the PCD pathway regulated by the Tumor Necrosis Factor Receptor TNFRSF19, a paraptosis-like cell death pathway.[17] In Arabidopsis, *At*PDCD5 transcripts are increased after UV-B exposure, and transgenic plants overexpressing this protein show increased cell death in roots after UV-B exposure, while mutants in this gene are less affected by the treatment than WT plants.[15] *pdcd5* mutants also have an altered antioxidant metabolism and accumulate higher levels of DNA damage after UV-B exposure; while plants overexpressing *At*PDCD5 show less DNA damage. Interestingly, *At*PDCD5 also participates in age-induced programmed cell death. Plants deficient in *AtPDCD5* expression exhibit a delayed leaf senescence characterized by higher chlorophyll content compared to WT plants, whereas *PDCD5* OE plants show lower levels of total chlorophylls in the leaves than WT plants.[15] Interestingly, while WT plants show a significant decrease in both chlorophyll a and b after a 24-h darkness period compared to levels in plants that were kept under a 16-h-light/8-h-dark photoperiod (**Fig. 1A and B**), *pdcd5* mutants have a lower although still significant decrease in chlorophyll content after an extended period of darkness. This lower decrease in chlorophyll content in *pdcd5* mutants was similar as that measured after UV-B exposure,[15] suggesting that PDCD5 may participate not only in PCD after UV-B exposure, but also in dark-induced senescence. Although *PDCD5* overexpressing plants (*PDCD5* OE) were more chlorotic than WT plants as already reported,[15] the decrease in chlorophylls after 24h of darkness was similar as that measured in WT plants (**Fig. 1A and B**).

In order to confirm a putative role of *At*PDCD5 in dark senescence, we evaluated the integrity of the cells by measuring the electrolyte leakage of leaves. Previously, we found that *PDCD5* OE lines exhibited a higher increase in electrolyte leakage than WT plants after a UV-B treatment, while the opposite was observed in *pdcd5* mutants.[15] A similar result was obtained when WT and plants with altered PDCD5 expression levels were kept in the darkness for 24h. **Fig. 1C** shows that, while electrolyte leakage was similar in the different set of plants kept under a 16-h-light/8-h-dark photoperiod; after 24h of darkness, the *PDCD5* OE line showed a significantly higher increase in electrolyte leakage than WT plants, whereas *pdcd5* plants showed a significantly lower increase than WT plants. In addition, fully expanded leaf #4 from WT, *pdcd5* and *PDCD5* OE plants were stained with trypan blue to visualize the presence of death cells after 24h of darkness. **Fig. 1D** shows that leaf #4 from both WT and *PDCD5* OE plants are considerably stained after extended darkness (mainly in veins and surrounding cells, **Fig 1D**, magnification), while leaves from *pdcd5* mutants in the dark display similar stain intensity as leaves from control plants under a 16-h-light/8-h-dark photoperiod. Taking together, our results suggest that, besides its role in cell death after UV-B exposure and age-induced senescence as described in Falcone Ferreyra et al. (2016),[15] *AtPDCD5* also participates in dark-induced senescence in Arabidopsis. Interestingly, Arabidopsis seedlings changed to darkness conditions at midday showed an increase in ribosome-bound *PDCD5* mRNAs, suggesting that *PDCD5* expression would be regulated at post-transcriptional and/or translational levels in response to light deprivation. [18,19] Moreover, *PDCD5* transcript levels are significantly higher in senescing leaves than in young and developed rosette leaves (**Fig 1E**), demonstrating that PDCD5 plays a role in senescence programs in Arabidopsis plants.

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Figure legend

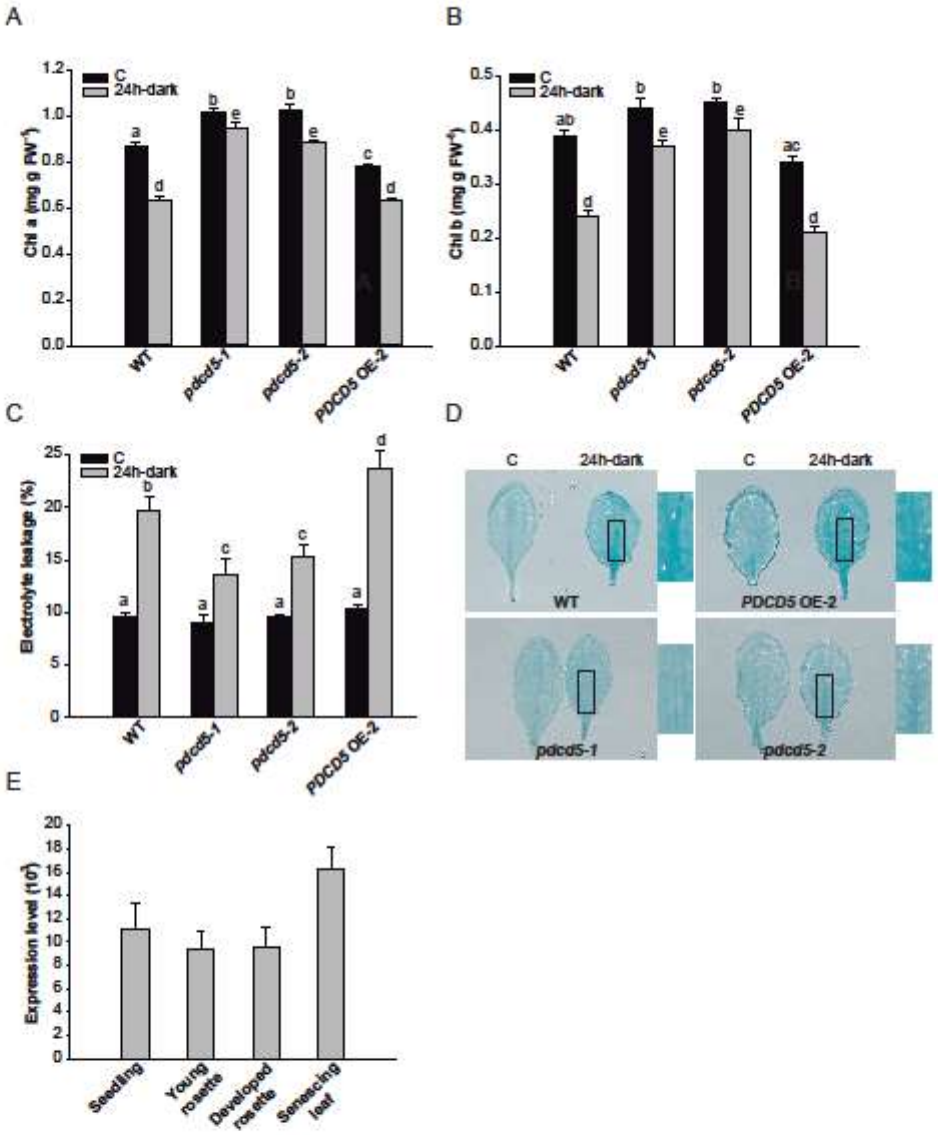

Figure 1. PDCD5 participates in dark-induced leaf senescence in Arabidopsis. Arabidopsis plants were grown in a growth chamber under a 16-h-light/8-h-dark photoperiod; after 3 weeks, a set of plants were kept under dark conditions for 24 h; while a control group of plants were maintained under a 16-h-light/8-h-dark photoperiod. (A, B) Chlorophyll content (Chl a and Chl b) and (C) electrolyte leakage (%) measured in WT (Col-0), *pdcd5* mutants (*pdcd5-1* and *pdcd5-2*) and *PDCD5* overexpressing plants (OE-2, line 2) under control conditions (C) and after a 24 h-dark period. Leaves #4 and #5 were harvested and used for these assays. Results represent the average ± S.E.M. of three independent biological replicates. Statistical significance was analyzed using two-way ANOVA test, differences with $P < 0.05$ are marked with different letters. FW, fresh weight. (D) Trypan blue staining of death cells in fully expanded leaf #4. Boxes indicate the enlarged area of each leaf showed on the right. (E) *PDCD5* transcript levels during plant development. Microarrays data were retrieved from Genevestigator. [18]