CADMIUM-INDUCED EFFECTS ON ENDOREDUPLICATION IN LEAVES OF *ARABIDOPSIS THALIANA*

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Introduction

Endoreduplication is an alternative cell cycle mode in which DNA replication takes place without intervening mitosis, resulting in endopolyploidy. This process occurs in many organisms and is particularly common in plants, playing an important role in plant growth and development. In addition to its regulation by developmental stimuli, endoreduplication is also affected by environmental stress conditions including UV-B irradiation, drought and cold stress (Scholes and Paige, 2015). In this project, the effect of cadmium (Cd) exposure on endoreduplication is investigated in leaves of *Arabidopsis thaliana* plants, as this metal is known to disturb plant growth and development.

Methods

Wild-type *A. thaliana* seeds (Columbia ecotype) were surface-sterilized and cultivated in hydroponics as described by Keunen et al. (2011). Eleven days after sowing, plants were exposed to 1, 2.5, 5 or 10 µM $CdSO_4$ supplied to the roots or further grown under control conditions. Rosette fresh weight was kinetically monitored for 10 days, starting from the day of exposure. In addition, the extent of endoreduplication was determined in the third leaf of plants exposed to Cd for 10 days. To this end, nuclei were extracted and stained with propidium iodide using the CyStain® PI Absolute P Kit (Sysmex-Partec). Subsequently, the nuclear DNA content of at least 5000 nuclei was measured using a FACSCalibur flow cytometer (BD Biosciences). Using FCS Express 4 (De Novo Software), the percentage of nuclei corresponding to each ploidy level (2C to 32C) was determined. The endoreduplication factor was calculated as follows:
[(0 x % 2C) + (1 x % 4C) + (2 x % 8C) + (3 x % 16C) + (4 x % 32C)] / 100.

Results

Results obtained in this study indicate that Cd significantly affects leaf growth and development of *A. thaliana* plants, as demonstrated by concentration-dependent decreases of rosette fresh weight (Fig. 1).

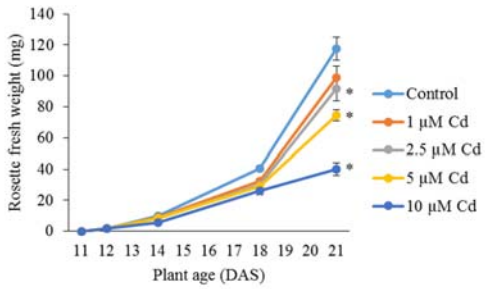

Figure 1. Rosette fresh weight (mg) of *A. thaliana* plants exposed to 0, 1, 2.5, 5 or 10 µM Cd, starting from day 11 after sowing. Data represent the average ± S.E. of 6 biological replicates. Significance levels at 21 DAS are indicated compared to the control (one-way ANOVA; $p < 0.05$).

Furthermore, Cd was shown to decrease the extent of endoreduplication in the third leaf of *A. thaliana* plants. As shown in Fig. 2, the percentage of nuclei with 4C or 8C ploidy levels was significantly increased in Cd-exposed as compared to control plants. This effect corresponded to significant decreases in the percentage of 16C and 32C nuclei, indicating that Cd exposure either delays or inhibits the progression of nuclei to higher ploidy levels. In addition, the endoreduplication factor (i.e. the average number of endocycles per cell) was significantly decreased in leaves of Cd-exposed plants as compared to the control.

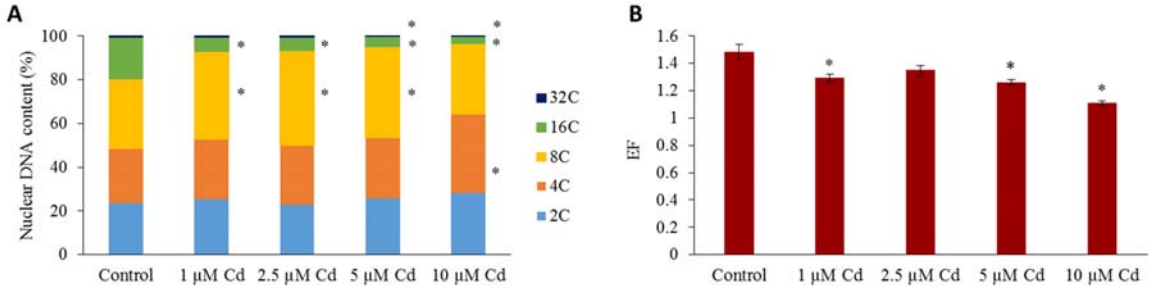

Figure 2. Nuclear DNA content in the third leaf of 21-days-old *A. thaliana* plants exposed to 0, 1, 2.5, 5 or 10 µM Cd for 10 days. (A) Percentage of nuclei corresponding to each ploidy level. Colors indicate the different ploidy levels (2C to 32C). (B) Endoreduplication factor (EF). Data represent the average ± S.E. of 4 biological replicates. Significance levels compared to the control are indicated for each ploidy level and the EF (one-way ANOVA; $p < 0.05$).

Conclusion

Taken together, the results of this study indicate that Cd exposure negatively affects leaf growth and development, an effect which is possibly related to Cd-induced decreases in nuclear DNA content. Future experiments aim to elucidate the molecular mechanisms underlying the effect of Cd on endoreduplication.

References

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