Investigating the Induction of the Flash Effect By Ultra-High Dose Rate (UHDR) Electron Beams through Differential Gene Expression in a Plant Species

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INTRODUCTION

- The FLASH effect produces normal tissue sparing, while maintaining equivalent tumor control in mammalian tissues, however the mechanism behind this effect is still unknown
- Plant species, including *Arabidopsis thaliana*, lack both vasculature and a human-like immune response, allowing for the evaluation of the FLASH effect on a physical and physiochemical level

METHODS AND MATERIALS

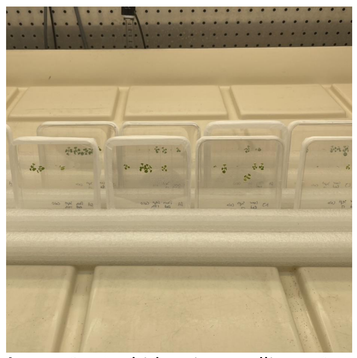

Figure 1. Arabidopsis seedlings 1.5 weeks after seeding.

Figure 2. EBTXD film alignment on blank Petri dish.

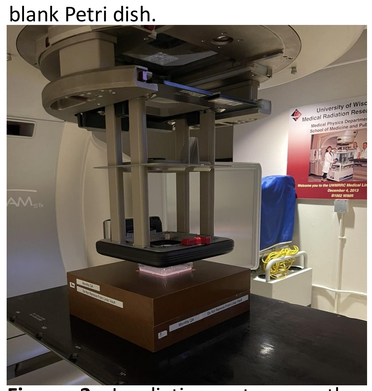

Figure 3. Irradiation setup on the Varian TrueBeam.

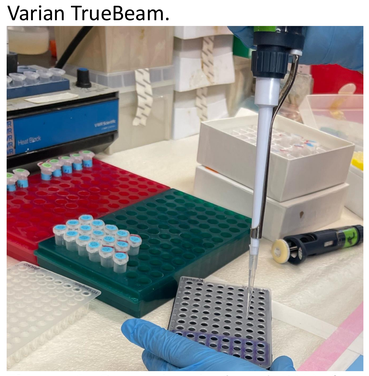

Figure 4. Preparing isolated RNA for shipment and sequencing.

Grew six *Arabidopsis thaliana* seeds on 12 plates to a two-week maturity

Planned prescription dose of 19.2 Gy using EBTXD film on and below a blank Petri dish on a Varian TrueBeam and an IntraOp Mobetron

Irradiated Petri dishes simultaneously on both linear accelerators using film and a surface-to-below ratio to calculate dose to the plants

Collected shoot and root samples one-hour post-irradiation and isolated the RNA using Sigma Spectrum Total RNA Kit

Quantified the FLASH effect by analyzing the differential gene expression of each replicate from RNA sequencing of the samples

RESULTS

Trial	UHDR Dose (Gy)	CONV Dose (Gy)
1	18.4 ± 0.3	19.34 ± 0.03
2	19.1 ± 0.3	19.31 ± 0.03
3	18.8 ± 0.3	19.37 ± 0.03

Table 1. Dosimetry results for the Petri dishes relative to prescription dose of 19.2 Gy. The UHDR rate was 93.79 Gy/s. The UHDR dish in trial 1 received a lower dose due to a misdelivery of the first pulse.

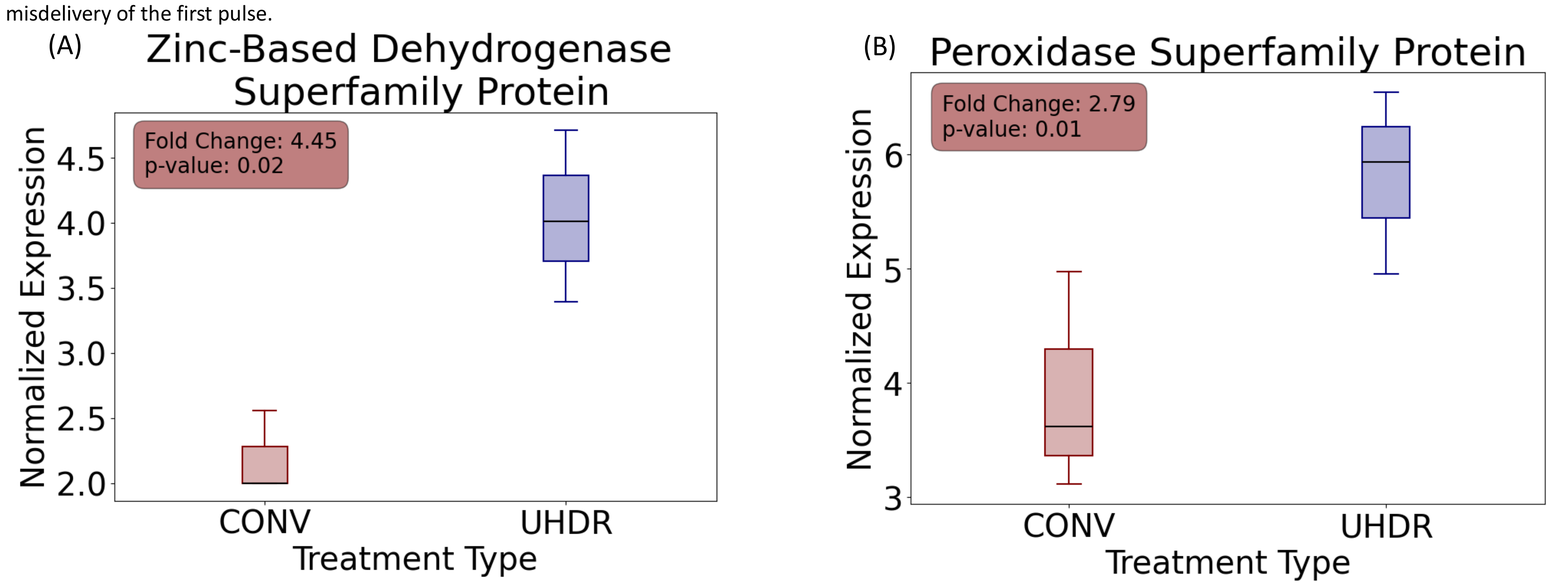

Figure 5. Differential transcriptional response of (A) AT5G38000 and (B) AT5G15180 in the shoots between UHDR and CONV dishes. Fold changes represent a log-base 2 change.

- AT5G3800 is an NADP-dependent oxidoreductase that belongs to the zinc-based dehydrogenase superfamily protein and responds to oxidative stress and reactive oxygen species[1]
- The peroxidase superfamily protein belongs to the reactive oxygen species (ROS) regulatory network, which controls the concentration of ROS and H_2O_2 throughout the plant[2]
- The peroxidase superfamily protein is also apart of the antioxidant system which is upregulated to reduce ROS concentrations within the cell[2,3]

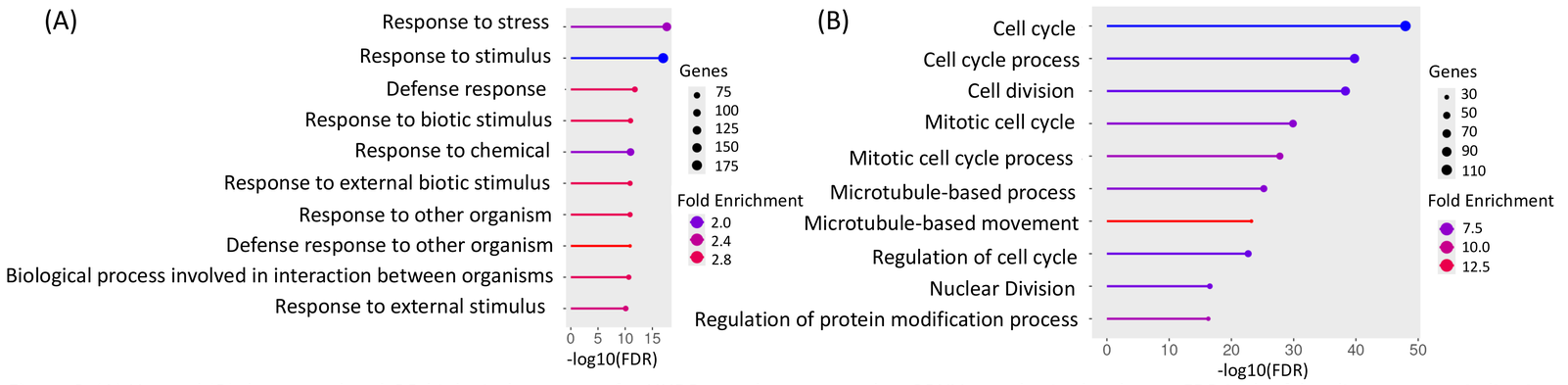

Figure 6. (A) Up- and (B) down-regulated GO biological processes for UHDR samples compared to CONV samples in the shoots. FDR is the false discovery rate indicating the significance of the result. There were 415 total up-regulated genes and 622 total down-regulated genes in the UHDR samples compared to the CONV samples for the shoots.

CONCLUSIONS

- There is strong evidence supporting the presence of the FLASH effect in *Arabidopsis thaliana*, specifically regarding oxidative stress
- Employing a prescription dose of 19.2 Gy to Arabidopsis thaliana resulted in a differential response between UHDR and CONV samples
- Further investigation is required to verify and expand upon these differential results and to determine how this further defines the mechanisms of the FLASH effect

Future Work

- Expanding upon the RNA sequencing analysis and developing a larger scale experiment

ACKNOWLEDGEMENTS

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