PRIMING FOR RESISTANCE AGAINST PATHOGENS: CELLULAR RESPONSES OF ARABIDOPSIS TO UV-C RADIATION

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Background

Plants face biotic and abiotic challenges, including pathogen attack and damage from ultraviolet light. When *Arabidopsis thaliana* is irradiated with non lethal doses of UV-C radiation, leaves show increasing resistance against a virulent isolate of the oomycete *Hyaloperonospora arabidopsis* in a dose-dependant manner (Figs 1 & 2). Previous research suggests this priming response is linked to DNA damage and repair, invoked after UV-C irradiation (Fig 3). The events that regulate this induced defence response and how they may relate to DNA damage/repair is unclear.

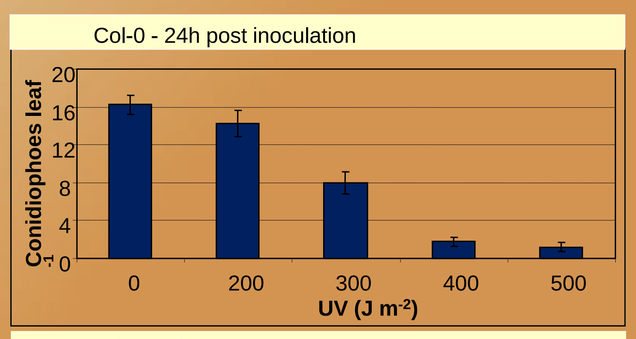
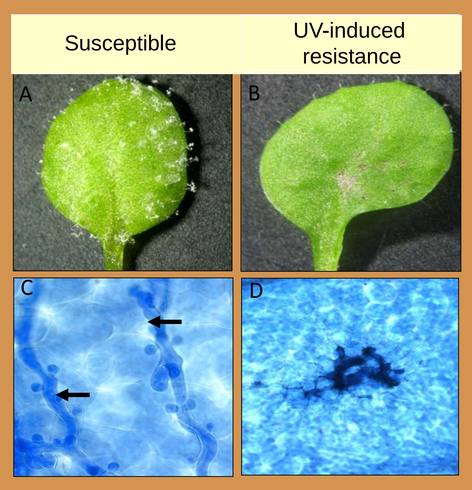


Figure 1 (above): *H. arabidopsis* conidiophores per leaf at varying UV-C dosage. Figure 2 (right): *H. arabidopsis* (Noks1) infected tissue, A and C: typical compatible interaction. B and D: irradiated leaf tissue infected with *H. arabidopsis* showing resistance response



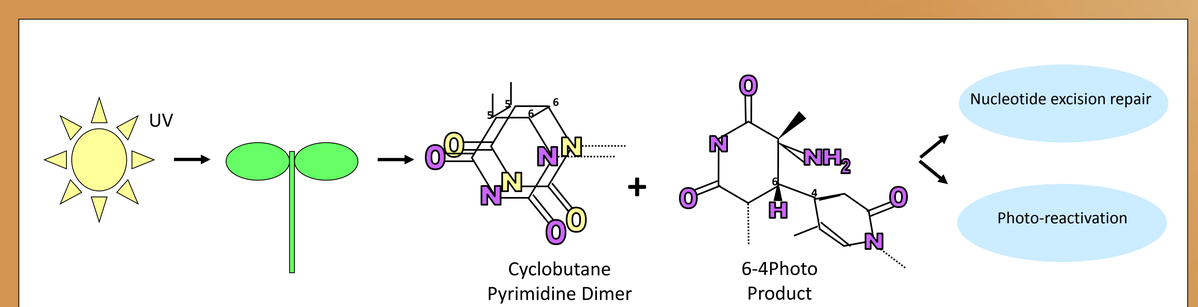


Figure 3: Types of DNA damage and Repair mechanisms. Energetic wavelengths of light cause lesions in DNA such as cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts, these lesions are repaired by DNA repair processes such as photo reactivation and nucleotide excision repair.

Aim

We aim to measure the effects of UV-C irradiation on leaf cells of Arabidopsis and to examine their roles in priming cells for resistance to *H. arabidopsis*. We have examined, following irradiation with UV-C, cell death, production of reactive oxygen species and callose synthesis. We have also undertaken microarray analysis of the interaction of *H. arabidopsis* at 12 h after UV irradiation and inoculation.

Methods

UV-C treatments

Arabidopsis Col-0 plants were irradiated with doses of UV-C at 250, 500 and 1000 J m^{-2}. Plants were returned to normal growth conditions before harvesting at 0, 24 and 48 hours post irradiation.

Visualisation of callose, cell death and H_2O_2

Irradiated leaves were stained with aniline blue (0.5%) to visualise callose deposition and 2' 7'-dichlorofluorescein diacetate (H2DCF DA). to visualise H_2O_2 production using fluorescence microscopy. Leaves were stained with Lactophenol trypan blue (LTB) in order to visualise cell death .

Callose assay

Callose was extracted and fluorescence was measured with aniline blue (as in Kohler *et al.,* 2000).

Microarray analysis

Arabidopsis was irradiated with 350 J m^{-2} UV-C and inoculated with *H. arabidopsis.* Comparisons were made with untreated incompatible reaction and UV treated only, 12 h after inoculation.

Results

Callose deposition in irradiated tissue

Staining for the beta-1,3-glucan callose demonstrated that induction occurred in UV-treated leaf tissue in a dose-dependant manner at 24 and 48 hours. No change occurred in the negative control 0 J m^{-2} (Fig 4).

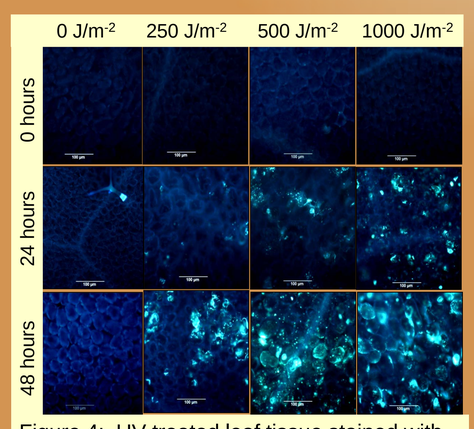


Figure 4: UV treated leaf tissue stained with aniline blue. Bars represent 100µm.

Callose fluorescence quantification

Increased concentrations of callose were found in the 500 and 1000 J m^{-2} treatment groups at 24 and 48 hours (Fig 5).

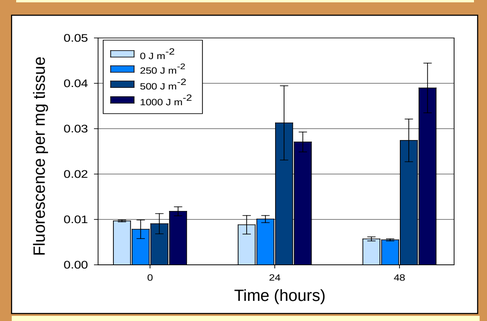


Figure 5: Callose fluorescence

Hydrogen peroxide localisation in irradiated leaf tissue

UV treated groups showed an increase in H_2O_2 accumulation directly after UV exposure (Fig 6).

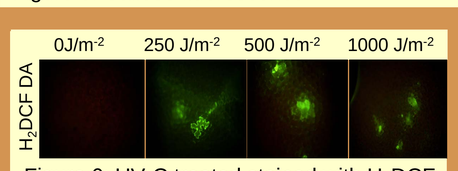


Figure 6: UV-C treated stained with H_2DCF DA directly after irradiation.

Cell death after UV irradiation

LTB staining showed no cell death in the 0 J m^{-2} control or the 250 J m^{-2} treatment groups (Fig 7). Cell death was observed at 24 and 48 hours, in increasing numbers, post irradiation in the 500 and 1000 J m^{-2} treatments.

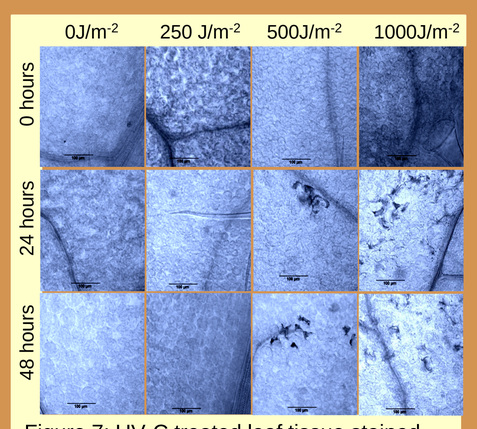
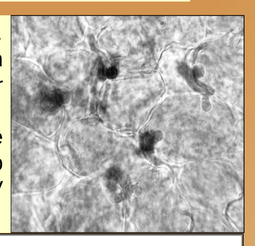


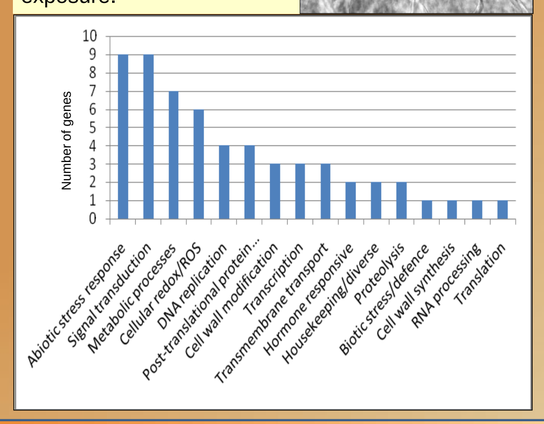
Figure 7: UV-C treated leaf tissue stained with LTB. Bars represent 100µm

Microarray analysis of UV-C priming in infected plants

We examined the interaction with *H. arabidopsis* (Noks1 compatible) at a time point where the pathogen is just beginning to penetrate leaf cells (Fig 8). UV-C exposure prior to inoculation stimulated genes that are associated with a number of general stress responses. Figure 9 shows the number of genes expressed in groups that are also expressed in a normal incompatible interaction.

Figure 8: (right) *H. arabidopsis* ingression (arrow) 12h after inoculation.
Figure 9 (below): gene groups that were up regulated after UV exposure.





Conclusions and Future work:

- UV-C irradiation induces cellular responses in leaves of Arabidopsis at relatively high dose levels but not at lower dose levels
- The increased resistance found following exposure of leaves to UV-C treatment observed previously may be due in part to UV-C induced cellular changes
- Microarray analysis of the interaction of Arabidopsis with *H. arabidopsis* show that many genes that are induced during normal incompatible responses are up regulated following UV treatment.
- We need to now demonstrate that the UV induced resistance for the biotrophic pathogen holds for other biotrophs and necrotrophs

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