

This is an Accepted Manuscript of the following article:

Dajana Blagojevica, YeonKyeong Lee, Li Xie, Dag A Brede, Line Nybakken, Ole Christian Lind, Knut Erik Tollefsen, Brit Salbu, Knut Asbjørn Solhaug, Jorunn E. Olsen. 2019. No evidence of a protective or cumulative negative effect of UV-B on growth inhibition induced by gamma radiation in Scots pine (*Pinus sylvestris*) seedlings.
Photochemical and Photobiological Sciences.
18 (8): 1945-1962.

The article has been published in final form at
<https://doi.org/10.1039/C8PP00491A>
by Royal Society of Chemistry.

It is recommended to use the published version for citation.

No evidence of a protective or cumulative negative effect of UV-B on growth inhibition induced by gamma radiation in Scots pine (*Pinus sylvestris*) seedlings

Dajana Blagojevic^{a,b†}, YeonKyeong Lee^{a,b†}, Li Xie^{b,c}, Dag A Brede^{b,d}, Line Nybakken^{b,d}, Ole Christian Lind^{b,d}, Knut Erik Tollefsen^{b,c}, Brit Salbu^{b,d}, Knut Asbjørn Solhaug^{b,d}, Jorunn E. Olsen^{*a,b}

^aDepartment of Plant Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway

^bCentre of Environmental Radioactivity (CERAD), Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway

^bNorwegian Institute for Water Research (NIVA), Section of Ecotoxicology and Risk Assessment, Gaustadalléen 21, N-0349, Oslo, Norway

^dFaculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway

*Corresponding author`s email: jorunn.olsen@nmbu.no

†These authors contributed similarly to the experimental data.

Exposure to ambient UV-B radiation may prime protective responses towards various stressors in plants, though information about interactive effects of UV-B and potentially stressful gamma radiation levels is scarce. Here, we aimed to test whether UV-B exposure could prime acclimatisation mechanisms contributing to tolerance to low-moderate gamma radiation levels in Scots pine seedlings, and concurrently whether simultaneous UV-B and gamma exposure may have a cumulative negative effect on seedlings that had previously not encountered either of these stressors. Responses to simultaneous UV-B (0.35 W m^{-2}) and gamma radiation ($10.2\text{-}125 \text{ mGy h}^{-1}$) for 6 days with or without UV-B pre-exposure (0.35 W m^{-2} , 4 days) were studied across various levels of organisation, as compared to effects of either radiation type. In contrast to UV-B, and regardless of UV-B presence, gamma radiation at $\geq 42.9 \text{ mGy h}^{-1}$ caused increased formation of reactive oxygen species and reduced shoot length, and reduced root length at 125 mGy h^{-1} . In all experiments there was a gamma dose rate-dependent increase in DNA damage at $\geq 10.8 \text{ mGy h}^{-1}$, generally with additional UV-B-induced damage. Gamma-induced growth inhibition and gamma- and UV-B-induced DNA damage were still visible 44 days post-irradiation, even at 20.7 mGy h^{-1} , probably due to genomic instability, but this was reversed after 8 months. In conclusion, there was no evidence of a protective effect of UV-B on gamma-induced growth inhibition and DNA damage in Scots pine, and no cumulative negative effect of gamma and UV-B radiation on growth in spite of the additional UV-B-induced DNA damage.

1 Introduction

As sessile organisms, plants need to cope with a range of changing environmental conditions and stressors, including high energy radiation such as ionising radiation (IR) and UV-B (280-315 nm). In natural environments IR is ubiquitously present and includes cosmic radiation and radiation from radionuclide-containing bedrock, soils and sediments. Since the origin of life the average global IR level from geological sources has decreased by a factor of approximately 8, and the current global mean background dose rate is $2.5 \text{ mGy year}^{-1}$, which corresponds to about $0.29 \text{ } \mu\text{Gy h}^{-1}$.¹ However, as in the past, many areas have naturally elevated IR due to high radionuclide content in the bedrock, such as Ihla Grande in Brazil, Ramsar in Iran and the Fen field in Norway, where dose rates have been measured at $14\text{-}15 \text{ } \mu\text{Gy h}^{-1}$, $4.4 \text{ } \mu\text{Gy h}^{-1}$ and $4 \text{ } \mu\text{Gy h}^{-1}$ respectively.¹⁻³ Furthermore, elevated levels of IR in nature are also due to radionuclide release from anthropogenic activities, such as waste and accidental releases from nuclear power plants, nuclear weapons and medical use.^{1,4}

Of the different types of electromagnetic radiation, gamma radiation has the highest energy, and accordingly high penetration potential. Low doses and dose rates of IR, such as gamma radiation, are currently defined as $\leq 100 \text{ mGy}$ and $\leq 6 \text{ mGy h}^{-1}$, respectively.^{5,6} It should be noted that these threshold values are largely related to health effects. On the basis of results from experiments and accidents, it has been generally noted that acute high doses of IR between $10\text{-}1000 \text{ Gy}$ can be lethal to plants, but that $<10 \text{ mGy day}^{-1}$ (about 0.42 mGy h^{-1}) probably have no detrimental effect on terrestrial plants in the field.^{1,7} From this it follows that plants may tolerate much higher IR levels than the natural background levels. However, different organisms, including various plant species, may respond differently to specific IR or gamma radiation dose rates or total doses. This may be particularly true for low-moderate doses or dose rates, which are less well studied than higher doses and dose rates.⁸⁻¹¹ Significant variation

in the effects of IR on plant morphology and physiology has been observed, with e.g. conifers being considered radiosensitive and *Arabidopsis thaliana* more radioresistant.^{1, 12-15} After the Chernobyl nuclear power plant accident in 1986, Scots pine (*Pinus sylvestris*) in particular showed high mortality close to the power plant zone.¹⁶⁻¹⁸ During the first two weeks after the accident conifers in this area received a dose of 3.7 Gy.¹⁹ Furthermore, under chronic IR exposure, loss of the apical dominance was observed in young populations of Scots pine in the Chernobyl exclusion zone as well as in Japanese red pine (*Pinus densiflora*) and Japanese fir (*Abies firma*) in the contaminated Fukushima power plant zone after the 2011 accident.^{20, 21}

UV-B has the highest energy of the solar UV radiation reaching the ground, and the levels vary with time of the day and year, latitude, altitude and cloud cover. To illustrate this, in a coastal area at 60°N (Helsinki, Finland) the UV-B in early June (2011) was measured at 1.2 W m⁻² under photosynthetically active radiation (PAR) of ~1600 μmol m⁻² s⁻¹.²² A considerable number of studies have described harmful effects of UV-B in a wide range of living organisms, including plants.^{23, 24} However, many earlier plant studies were performed with high UV-B levels under low light conditions, which has been shown to aggravate many UV effects.²⁵ In recent years, more realistic UV-B exposure conditions have been shown to rarely result in accumulation of UV-B-related damage.^{26, 27} It appears that UV-B radiation stress only becomes significant when plants are either challenged by other stressors, exposed to a high ratio of UV-B to PAR or exposed to very high UV-B levels in general.²⁶ Drought, nutrient deficiency and extreme climatic conditions are examples of stressors shown to result in aggravated UV stress.²⁸⁻³⁰

On the other hand, there is substantial evidence that UV-B at a moderate level acts as an important signal for induction of stress protection, as well as a morphogenic signal.³¹⁻³³ UV radiation has also been shown to induce cross-tolerance to stressors such as drought, cold, salt stress, wounding and pathogens.³⁴⁻⁴² Although such a relationship is not always clear, it has

been shown that the negative effects of simultaneous exposure to UV-B and Cadmium on photosynthesis were minimized by pre-exposure to either of these.⁴³ Thus, cross-tolerance is apparently due to acclimation.

Exposure to elevated IR results in production of reactive oxygen species (ROS) due to the radiolysis of water.¹ UV-B may also induce ROS formation, with the degree of ROS accumulation depending on the UV-B level.⁴⁴ ROS may interact rapidly with proteins, lipids and nucleic acids, resulting in damage and genotoxicity.⁴⁵ In addition, IR can cause direct ionisation of biomolecules, causing additional damage.¹ DNA damage may cause persistent mutations, which in turn can reduce plant genome stability and growth.^{46, 47} However, depending on the duration and level of irradiation, signals that activate DNA repair mechanisms may be triggered, in which cell cycle regulatory proteins and antioxidant genes also play a major role.^{1, 48-50}

To counteract ROS-induced oxidative stress, plants can modulate their antioxidative defence systems, which include ROS scavenging enzymes and non-enzymatic antioxidant metabolites. This enables plants to avoid cellular damage while still allowing ROS-dependent signalling that is known to be an integrated part of defence responses.^{47, 51, 52} Induction of antioxidants and genes encoding antioxidant enzymes in gamma-irradiated plants has been reported for a number of plant species.^{47, 48, 53, 54} UV-B is also well known to induce production of a range of phenolic compounds, including flavonoids, which protect against damage through their UV-B screening ability and by serving as ROS scavengers, thereby neutralizing free radicals before they damage the cells.^{31, 55} Information about whether gamma radiation may also induce such phenolic compounds is limited. Furthermore, although UV-priming of plant defence systems may afford the plant protection against different stressors, information about whether this may apply for low to moderate levels of gamma radiation is not available. It may be suggested that the ubiquitous presence of IR in nature, and the higher IR levels in the past,

may have helped to drive the evolution of DNA repair and protection towards oxidative stress as well as regulatory responses.¹ If so, cross-protection against oxidative stress generated by UV-B and low-moderate levels of gamma radiation may well be possible.

The overall aim of this work was to study interactive effects of UV-B and gamma radiation across various levels of organisation in Scots pine seedlings, using gamma doses realistic to those at different distances to accidental releases like in Chernobyl. The specific aims were to test 1) whether UV-B has the potential to prime stress acclimatisation mechanisms, thereby conferring some tolerance to low-moderate gamma radiation levels and producing Scots pine seedlings with better physiological sufficiency and growth than they would otherwise have had without UV-B radiation; 2) whether UV-B radiation exposure applied simultaneously with gamma radiation will have a cumulative negative effect on plants that have not previously encountered either of these two radiation types; and 3) whether there is a dose-dependent response of Scots pine to gamma radiation with an interactive effect of this response with exposure to UV-B radiation.

2 Materials and Methods

2.1 Plant materials and pre-growing conditions

Seeds of the Scots pine (*Pinus sylvestris* L.) provenance CØ1 from Halden, Norway (59°N latitude, 0-149 m altitude, seed lot 5632, Skogfrøverket, Hamar, Norway), were surface sterilized in 1% sodium hypochlorite for 5 min, rinsed five times in sterile, distilled water and dried on a sterile filter paper. The seeds were evenly sown on ½ strength MS medium⁵⁶ (Duchefa Biochemie, Harleem, Netherland) with 0.8% agar (Plant agar, Sigma-Aldrich, St. Louis, Mo, USA) in petri dishes of 5 cm diameter with 15-20 seeds per dish (germination rate of approximately 50-60%). The seeds were germinated for 6 days in a growth chamber at 20°C

under a photon irradiance of $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 400-700 nm (TL-D 58W/840 lamps Phillips, Eindhoven, The Netherlands) and a 16 h photoperiod. As the plastic lids of the petri dishes were not UV-B-transparent, all lids were replaced with UV-B-transparent cling film at the start of the experimental treatments.

2.2 Experimental growing conditions, gamma and UV-B radiation sources and dosimetry

During the experiments (the treatments are described in the chapter below), which started when the seedlings were six days old, the petri dishes with seedlings were kept in two identical growth chambers (without metal in the front and end walls; manufactured by the Norwegian University of Life Sciences (NMBU), Ås, Norway). The chambers were maintained at 20°C , with a 12 h photoperiod and a photon irradiance of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 400-700 nm. Light was provided by white light emitting diode panels (PCB1E 5000K, Evolys, Oslo, Norway) and incandescent lamps (Osram, Munchen, Germany). The irradiance was measured at the top of the petri dishes with a quantum sensor (Model LI-190 LI-COR, Lincoln, NE, USA). The red:far red (R:FR) ratio was 1.9, as measured by a 660/730 nm sensor (Skye Instruments, Powys, Wales, UK). The relative air humidity (RH) of the chambers was adjusted to 78%, corresponding to a water vapour deficit of 0.5 kPa.

The plants in one of the growth chambers were exposed to constant gamma radiation for six days (144 h), with the exception of 10-15 min in the middle of each experiment when the petri dishes were rotated. Gamma radiation was provided using the FIGARO UV and low dose rate gamma (^{60}Co ; 1173.2 and 1332.5 keV γ -rays) irradiation facility at the Norwegian University of Life Sciences (NMBU), Norway.⁵⁷ The growth chamber was placed in front of the collimator containing the ^{60}Co source, while the other growth chamber was kept outside the irradiation sector behind gamma radiation-shielding lead walls.

The gamma dosimetry of the exposed plants followed an established protocol.⁵⁸ Petri dishes with plants were positioned at different distances from the gamma source to obtain the intended average air kerma rates (Table 1). For each air kerma rate there were eight petri dishes with plants, four side by side, with four others immediately behind these. To obtain similar gamma exposure, the front and back petri dishes were interchanged, and all dishes rotated 180° in the middle of the experiment. Each average air kerma rate was calculated from measurements of the dose rates in front of and behind the two petri dish rows per kerma rate, using four nanodot dosimeter measurements in each case (microStar, Landauer Inc. Greenwood, IL, USA), and taking the rotation of the petri dishes into account. On the basis of the air kerma rates, the average, minimum and maximum dose rates to water were estimated according to Hansen et al. (2019)⁵⁸, and the average was used as a proxy for the dose rates provided to the plants (Table 1). The total doses and dose intervals were calculated from the estimated absorbed dose rates to water (mGy h^{-1}), multiplied by total exposure time (h).

In each growth chamber UV-B was provided for 10 h daily from two UV-B fluorescent tubes (UVB-313, Q-Panel Co., Cleveland, OH, USA) mounted in the ceiling of the growth chamber. The UV-B radiation started 1 h after the light was turned on and ended 1 h before the light was turned off. To block UV-wavelengths below 290 nm in the UV-B treatments, cellulose diacetate foil (0.13 mm, Jürgen Rachow, Hamburg, Germany) was placed on top of half of the petri dishes in each growth chamber. UV-blocking polycarbonate filters were placed on top of the rest of the petri dishes to provide non-UV-B-exposed controls.

The UV-B irradiance was measured at the top of the petri dishes (under the filters) with a broadband UV-B sensor (SKU340, Skye Instruments, Powys, UK). Based on a calibration factor obtained from simultaneous measurement of UV-B with an Optronic model 756 spectroradiometer (Optronic laboratories, Orlando, FL, USA) and the broadband UV-B sensor, the absolute UV-B irradiation was calculated to 0.35 Wm^{-2} (corresponding to $0.9 \mu\text{mol m}^{-2} \text{ s}^{-1}$

¹; calculated according to Aphalo et al. (2012)²²). Using the Green weighting function⁵⁹, which is based on relating the DNA damage at different wavelengths to the DNA damage at 300 nm (set to 1), the biologically effective UV-B (UV-B_{BE}) was calculated to 0.18 W m⁻² (corresponding to 0.45 μmol m⁻² s⁻¹).

2.3 The specific UV-B and gamma radiation treatments and experiments

To test whether UV-B combined with different dose rates of gamma radiation would have a cumulative negative effect on plants that have not previously encountered either of these radiation types, six repeated experiments including simultaneous UV-B and gamma exposure (without UV-B pre-treatment) were performed (Table 2). In each of these, eight subsets of plants (four petri dishes per subset) were exposed to different treatments as follows: In one growth chamber six subsets of plants (four petri dishes per subset) were exposed to gamma radiation at dose rates of either 20.7, 42.9 or 125 mGy h⁻¹ for six days (144 h), either in the presence (denoted “UV-B+gamma”) or absence (denoted “gamma”) of UV-B at 0.35 W m⁻² for 10 h daily (as described in the chapter above). The reason for selecting relatively high UV-B to PAR ratios was that conifers like Scots pine are highly tolerant to UV-B due to efficient screening in the epidermis.⁶⁰⁻⁶² In the growth chamber outside the gamma radiation sector, another subset of plants was exposed to UV-B only (denoted “UV-B” (no gamma)), and still another plant subset was not exposed to UV-B (denoted “control” (no UV-B, no gamma)).

To test whether UV-B has the potential to prime stress acclimatisation mechanisms contributing to tolerance to low-moderate gamma radiation levels, three further experiments including UV-B pre-treatment prior to irradiation with different gamma dose rates and UV-B were conducted (Table 2). In these experiments, eight subsets of plants were exposed to the different treatments described above (UV-B+gamma, gamma, UV-B and control), but prior to the UV-B+gamma and UV-B treatments, these plants were pre-treated with UV-B at 0.35 W

m^{-2} for 10 h daily for four days (with cellulose acetate on top of the petri dishes) in the growth chamber outside the gamma radiation sector. To study the effect of a lower gamma dose rate, 10.8, 20.7 and 42.9 mGy h^{-1} were used. Due to the limited length of the growth chamber, 125 mGy h^{-1} could not be included in these experiments. The UV-B pre-treatments started when the plants were six days old, and the plants not receiving UV-B were kept in the same growth chamber under UV-B blocking polycarbonate filters.

In an additional UV-B pre-treatment experiment the plants received the same treatments, except that 0.52 W m^{-2} UV-B (1.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$), corresponding to a UV-B_{BE} 0.26 W m^{-2} (0.65 $\mu\text{mol m}^{-2} \text{s}^{-1}$), was provided during the UV-B pre-treatment. This was done to test whether a higher UV-B level would make any difference since no significant UV-B effect on growth was observed at 0.35 W m^{-2} .

2.4 Growth parameter recordings at the end of the UV-B and gamma irradiation

At the end of the treatments, seedlings were placed between two transparent plastic sheaths with mm paper on top and scanned. The shoot and root lengths of the scanned seedlings were measured using the ImageJ software (US National Institutes of Health, Bethesda, MD, USA; <http://imagej.nih.gov/ij/>). Eight-15 seedlings per treatment were measured in each of the six repeated experiments with simultaneous UV-B+gamma radiation without UV-B pre-treatment, as well as 9-17 seedlings per treatment in each of the three repeated UV-B pre-treatment experiments (Table 2). In the experiment with 0.52 W m^{-2} UV-B pre-treatment, 10 seedlings were measured per treatment.

2.5 Post-irradiation growing conditions

Since the negative effects of gamma-related stress may take some time to be fully manifested, and the potential recovery from growth-inhibition and DNA damage may also take time,^{63, 64}

the after-effects of the UV-B and gamma treatments on the growth parameters and DNA damage were investigated. For this purpose, seedlings (number of experiments and plants per treatment described below and in Table 2) were transferred to pots (5 cm diameter and 5 cm height) filled with S-soil (45% low moist peat, 25% high moist peat, 25% perlite and 5% sand; Hasselfors Garden AS, Örebro, Sweden) with one plant per pot. The seedlings were then grown in growth chambers (manufactured by NMBU; different from those under the UV-B-gamma exposure) at temperature and RH the same as during the UV-B-gamma irradiation. A 24 h photoperiod was given with the 12 h main light period at a photon irradiance of $180 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a R:FR ratio of 1.7, using metal halide lamps (HPI-T Plus 250W, Phillips, Eindhoven, Netherlands) and incandescent lamps (Osram). This was followed by 12 h day extension with low-intensity light from the incandescence lamps only ($8\text{-}10 \mu\text{mol m}^{-2} \text{s}^{-1}$). Since the sensitivity to photoperiod in woody species increases after the first period following germination, and the length of the photoperiod sustaining growth increases with increasing northern origin, such long days were used to ensure growth of the northern ecotype used in the experiments.⁶⁵

To assess more long-term effects of the irradiation treatments on growth and DNA-damage, plants from one UV-B pre-treatment experiment with 0.35 W m^{-2} and one with 0.52 W m^{-2} UV-B were transferred to a greenhouse compartment at NMBU, Ås, Norway ($59^{\circ}39' \text{N}$. $10^{\circ}47' \text{E}$). The plants were transferred in the end of March and April 2018 after two months in the post-irradiation growth conditions described above and were grown for an additional six or five months, respectively. A greenhouse compartment was used as the growth chambers were no longer available. The greenhouse had UV-blocking acrylic plastic walls and UV-B-blocking glass roof. In addition to the natural light, supplementary light at $165 \mu\text{mol m}^{-2} \text{s}^{-1}$ was provided 16 h daily from HQI (Powerstar HQI-T 400 W, Osram) and high-pressure sodium (HPS 400 W Master PIA, Phillips) lamps (1:1 ratio). The temperature was set to 21°C and RH to 75%.

2.6 Post-irradiation growth parameter recordings

After the transfer of seedlings to pots in the growth chambers, plant height, number of needles and plant diameter of 10-15 plants per treatment were recorded over time, from 9-44 days post-irradiation. This was performed in two experiments with simultaneous UV-B+gamma irradiation without UV-B pre-treatment, and two experiments including also UV-B pre-treatment (0.35 W m^{-2} ; Table 2). Plant height was measured from the rim of the pot to the shoot apical meristem (SAM), and the cumulative growth was calculated. The average shoot diameter from needle tip to needle tip across the plant at the shoot apex was calculated from two perpendicular measurements per plant. Plant height and shoot diameter were also measured eight months post-irradiation in 6-10 plants per treatment in one UV-B pre-treatment experiment (0.35 W m^{-2}) (Table 2).

2.7 Plant tissue preparation and histological studies by microscopy

Histological studies of shoot and root tips were performed according to Lee et al. (2017)⁶⁶ in experiments with simultaneous UV-B-gamma exposure without UV-B pre-treatment (Table 2). Three millimetres of shoot tips were harvested at the end of the irradiation, and 3 mm of shoot tips, 3 mm of root tips and 3 mm of the middle part of expanded needles were harvested 44 days post-irradiation from each of 5 plants per treatment. The samples were immediately fixed in 4% formaldehyde and 0.025% glutaraldehyde in sodium phosphate buffer (PBS, pH 7.0), vacuum infiltrated at room temperature for 1 h and thereafter kept at 4°C overnight. The fixed samples were then washed with PBS, dehydrated in a graded ethanol series, infiltrated in a progressively increasing ratio of LR White resin (London Resin Company, London, UK) to ethanol and finally embedded in the resin. Thereafter 1 µm thick sections of the embedded plant materials were made using an Ultracut Leica EM UC6 microtome (Leica, Mannheim,

Germany), stained with toluidine blue O for visualisation of the cells ⁶⁷ (Sigma-Aldrich) and examined using a Leica DM6B light microscope (Leica).

2.8 COMET assay for analysis of DNA damage

To quantify the DNA damage (single and double strand breaks) in response to the gamma and UV-B treatments a COMET assay was performed according to the method described in Gichner et al. (2003) ⁶⁸ (with some modifications). The assay is based on the principle that damaged DNA moves out of the cell nucleus during electrophoresis of lysed cells/cell nuclei in an agarose gel, and visualisation of this is possible by fluorescence microscopy. DNA breaks are quantified on the basis of the intensity and length of the elongated cell nucleus (“COMET”) due to damaged DNA, relative to the head. The COMET analyses were performed at the end of the treatments (on shoot tips; two repeated experiments without and two with 0.35 W m^{-2} UV-B pre-treatment; Table 2). To test for persistence of the DNA damage, such analyses were also performed 44 days (on shoot and root tips; two repeated experiments without UV-B pre-treatment), and eight and seven months post-irradiation (on shoot tips; experiments with UV-B-pre-treatment with 0.35 W m^{-2} (Table 2) and 0.52 W m^{-2} , respectively). Three replicate biological samples per treatment (per experiment), each consisting of 3-4 mm of shoot tips or root tips from three plants, were investigated individually for DNA damage. For each sample, three technical replicates (gels) were analysed with 500-100 nuclei scored in each. As recommended by Koppen et al. (2017) ⁶⁹, the median value for each biological sample was calculated, followed by calculation of the average of these values for the three biological replicates.

To avoid light-induced ROS formation resulting in DNA damage, the COMET assay was performed under inactinic red light. The plant materials were placed in 400 μl cold extraction buffer (PBS, pH 7.0 and 200 mM EDTA) in a 9 cm petri dish. Cells/cell nuclei were isolated

by chopping the plant materials vigorously for 30 s with a razor blade and the nuclei solution without plant debris was collected. The nuclear suspension (75 μ l) and 1% low melting point agarose (50 μ l) (NuSieve GTG Agarose, Lonza, Basel, Switzerland) prepared in distilled water at 40°C, were gently mixed and 10 μ l aliquots placed on microscope slides pre-coated with 1% low melting point agarose. To unwind DNA prior to electrophoresis, the slides (gels) were placed on ice for 1 min, followed by 10 min in a horizontal gel electrophoresis tank containing freshly prepared cold electrophoresis buffer (1 mM Na₂EDTA and 300 mM NaOH, pH 13). Electrophoresis was performed at 20 V (300 mA) for 5 min at 4°C, and after electrophoresis, the slides were washed with distilled water and neutralised in PBS buffer for 10 min. The slides were then washed with distilled water, fixed in 95% ethanol and dried overnight before staining with Syber Gold (Life Technologies Ltd, Paisley, UK; dilution 1:5000) for 20 min and washing in distilled water three times for 5 min each. “COMETS” were scored using Comet IV (Perceptive Instruments Ltd, Bury St. Edmunds, UK) and an Olympus BX51 fluorescence microscope with a CCD camera (Olympus, Tokyo, Japan).

2.9 Analyses of the reactive oxygen species H₂O₂

The level of the reactive oxygen species H₂O₂ was quantified using 2',7'-dichlorofluorescein diacetate (H₂DCFDA) (Molecular Probes Inc., Eugene, OR, USA), which upon oxidation is de-esterified to the highly fluorescent 2',7-dichlorofluorescein (H₂DCFDA). A 50 mM stock solution of H₂DCFDA was prepared in DMSO and stored at -18 °C until use. After UV-B and gamma treatments in two repeated experiments without, and one experiment with UV-B pre-treatment (0.35 W m⁻²) (Table 2), four plants per treatment were randomly selected and washed with PBS (PBS tablet, Thermo Fisher Scientific Inc, Waltham, MA, USA) to remove any remaining agarose gel. After gentle drying with tissue paper, the plants were weighed with a microbalance, chopped individually into small pieces (0.5 cm) with a razor blade, and

immersed in 100 μ M H₂DCFDA in PBS for 3 h. The materials were then rinsed with PBS to remove excess probe, transferred to a 24-well microplate with 2 ml PBS per well, and the H₂DCFDA fluorescent signal for each of the four samples was measured by a microplate reader (Fluoroskan Ascent FL, Thermo, Vantaa, Finland) with excitation and emission wavelengths of 480 nm and 530 nm, respectively.^{70, 71} The background fluorescence (without presence of plant materials) was also analysed and the resulting fluorescence subtracted from the values for the samples. The relative fluorescence obtained was normalised by weight, and the results were presented as fold difference relative to the unexposed control (no gamma, no UV-B).

2.10 Analyses of total antioxidant capacity

After UV-B and gamma exposure (without UV-B pre-exposure; Table 2), total antioxidant capacity was determined using the OxiSelect Ferric Reducing Antioxidant Power (FRAP) Assay Kit (Cell Biolabs, San Diego, USA). In one experiment, four samples of individual, entire seedlings were analysed, while in another experiment, analyses were performed on four samples consisting of pooled shoots from three plants per sample (Table 2). The analysis was done according to the manufacturer's protocol (<https://www.cellbiolabs.com/sites/default/files/STA-859-frap-assay-kit.pdf>). Approximately 10 mg plant tissue was homogenised in 1 ml cold 1X Assay buffer and centrifuged at 12000 rpm for 15 min at 4°C and the supernatant collected. Thereafter, a 1 mM iron (II) standard solution, diluted from a freshly made 36 mM stock solution, was used to prepare a series of standards according to the manufacturer's recommendations. For the assay, in each well of a 96 well microplate, 100 μ l of the reaction reagent was added to 100 μ l sample or standard solution, mixed by pipetting and incubated for 10 min at room temperature. Three technical replicates were used per sample. Immediately after the incubation, the absorbance was detected in a microplate reader (Biochrom Asys UVM 340 with KIM, UK) at a wavelength of 540 nm.

The average absorbance values were determined for each sample and standard, and the net absorbance calculated by subtracting the zero-standard value. The sample results were determined on basis of the standard curve and normalised by weight.

2.11 Analyses of phenolic compounds

After the UV-B and gamma exposure in two experiments including UV-B-pre-treatment, plant materials were collected, divided into shoots and roots and freeze dried for 24 h to examine phenolic compounds. In each experiment, eight shoot samples per treatment were analysed. In one experiment (0.35 W m^{-2} UV-B pre-treatment), each sample consisted of shoots from 7-8 plants (Table 2), whereas in another experiment (0.52 W m^{-2} UV-B pre-treatment) shoots from 15-20 plants were pooled per sample.

The samples were transferred to 2 ml vials, each containing 600 μl MeOH and a stainless-steel bead (5 mm in diameter) and homogenised for 30 s in a centrifuge at 6500 rpm (Retsch, Haan, Germany). The vials were placed in an ice bath for 15 min and thereafter centrifuged for 3 min at 15000 rpm, followed by transfer of the supernatant from each sample to a 10 ml vial. The procedure was then repeated four times (without ice bath incubation), leaving the pellet colourless. The MeOH was evaporated using a SpeedVac (SAVANT SC210A, Thermo Scientific, Weaverville NC, USA) vacuum centrifuge, and the dried extracts were re-dissolved in 200 μl MeOH using an ultrasound bath and diluted with 200 μl Millipore-water. The extracts were then transferred to a 1.5 ml Eppendorf vial and centrifuged, followed by transfer to HPLC vials and analysis by HPLC (Agilent, Series 1100, Germany). The different metabolites were separated by use of a 50 x 4.6 mm ODS Hypersil column (Thermo Fisher Scientific Inc, Waltham, MA, USA). The samples were eluted (flow rate 2 ml min^{-1}) using a MeOH: water gradient according to Nybakken et al. (2012)⁷². The injection volume was 20 μl , and the column temperature was 30°C. The identification of the phenolic compounds was based on

their retention times and UV spectra as compared with those of commercial standards. The chromatogram peaks were used to quantify phenolic acids and flavonoid compounds.

2.12 Statistical analyses

In experiments with and without UV-B pre-treatment (summarised in Table 2), the effects of UV-B and gamma radiation on growth parameters (shoot and root length, post-irradiation cumulative shoot elongation, plant diameter and number of needles), DNA damage, H₂O₂ levels, antioxidant capacity and content of phenolic compounds were assessed by two-way analyses of variance (ANOVA) in the general linear model mode and by regression analysis using the Minitab statistical software (Minitab 18, Minitab Inc, PA, USA) ($p \leq 0.05$). For the post-irradiation growth parameters, the results from the final time point when the differences between the treatments were the largest were analysed. To test for differences between means, Tukey's post hoc test was used. When results from repeated experiments were available, the final statistical analyses included all these results. These individual experiments were first analysed separately to confirm equal responses.

3 Results

3.1 Effect of gamma radiation but no effect of UV-B on elongation growth

Exposure to UV-B radiation (0.35 W m^{-2} ; provided separately or in combination with gamma radiation, with or without UV-B pre-treatment) did not affect the shoot or root length or the SAM histology (Fig. 1 and Fig. S1). On the other hand, exposure to gamma radiation decreased the shoot length from 16 mm in the control plants to 12 mm at 42.9 mGy h^{-1} and 7 mm at 125 mGy h^{-1} : a reduction of 25% and 56% respectively. Likewise, our gamma-radiation treatments reduced root length from 16 mm in the controls to 9 mm at 125 mGy h^{-1} : a reduction of 44%

(Fig. 1A, B and Fig. S1A, B). Histological analysis revealed slightly impaired SAM development at 125 mGy h⁻¹ (Fig. 1C). However, in another series of experiments (including UV-B pre-treatment (0.35 W m⁻²)), there was no effect of exposure to gamma radiation at 42.9 mGy h⁻¹ (the highest tested gamma dose rate) on shoot and root length (Fig. 1D, E and Fig. S1C, D). Pre-treatment with a higher UV-B level (0.52 W m⁻²) was also tested but no effect on shoot or root length was observed (Fig. S1E-H).

3.2 Gamma and UV-B radiation-induced DNA damage

All our gamma radiation and UV-B treatments increased DNA damage in both experiments with and without the UV-B pre-treatments (Fig. 2 and Fig. S2). Exposure to UV-B radiation (0.35 W m⁻², without UV-B pre-treatment) resulted in 10% DNA in the COMET tail: a 10 fold increase compared with the treatment without UV-B which had only 1% tail DNA in the COMET tail (Fig. 2A and Fig. S2A). This compares with 5% tail DNA with UV-B exposure and 0.08% tail-DNA without UV-B (a 63% change) in the equivalent treatments for plants that had received a 0.35 W m⁻² UV-B pre-treatment (Fig. 2B and Fig. S2B). Seedlings exposed only to gamma radiation had DNA tails of 9% (20.7 mGy h⁻¹), 14% (42.9 mGy h⁻¹) and 19% (125 mGy h⁻¹); whereas after the gamma plus UV-B irradiation (without UV-B pre-treatment) the DNA tails were 17% (42.9 mGy h⁻¹) and 26% (125 mGy h⁻¹). This represents an increase for each of these dose rates of 21% and 37% additional DNA damage when the two types of radiation were given together (Fig. 2A and Fig. S2A). This compares with DNA tails of 7% (10.8 mGy h⁻¹), 11% (20.7 mGy h⁻¹) and 17% (42.9 mGy h⁻¹) in the gamma only treatments in the experiments including 0.35 W m⁻² UV-B pre-treatment (Fig. 2B and Fig. S2B). Here the UV-B plus gamma treatments produced DNA tails of 11% (10.8 mGy h⁻¹) and 14% (20.7 mGy h⁻¹): an increase of 57 and 27%, respectively, as compared to the gamma only treatment (Fig. 2B and Fig. S2B).

The higher-dosage UV-B pre-treatment of 0.52 W m^{-2} produced similar results to the lower-dosage UV-B pre-treatment described above. Here, the UV-B exposed plants had a 10% tail-DNA compared with 0.3% in the no UV-B controls: corresponding to a 33-fold increase (Fig. S2C and D). The plants exposed to gamma radiation had DNA-tails of 11% (10.8 mGy h^{-1}), 14% (20.7 mGy h^{-1}) and 17% (42.9 mGy h^{-1}), whereas those exposed to UV-B and gamma radiation had 14%, 17% and 26% DNA-tails at the same gamma dose rates, respectively. This represents an increase in DNA damage of 27%, 21% and 53% for the respective gamma radiation dose rates when combined with the UV-B treatment (Fig. S2C and D).

3.3 Effect of gamma radiation but no effect of UV-B on level of H_2O_2

There was no significant effect of UV-B on the H_2O_2 levels (Fig. 2C and D). On the other hand, compared to the unexposed control, significantly increased levels of H_2O_2 were observed in response to gamma radiation at 42.9 (an average of 96% and 48% in experiments without and with UV-B pre-treatment, respectively) and 125 mGy h^{-1} (154% increase; analysed in experiments without UV-B pre-treatment); Fig. 2C and D).

Analyses of total antioxidant capacity using the FRAP assay (analysed in experiments without UV-B pre-treatment) revealed no significant effect of UV-B or gamma radiation when analysing entire seedlings or shoots only (Fig. S3).

3.4 UV-B-induction of phenolic compounds but no effect of gamma radiation

There was no significant effect of gamma radiation on the levels of any of the phenolic compounds analysed in shoot tissue (analysed in experiments with 0.35 W m^{-2} UV-B pre-treatment; Fig. 3). On the contrary, UV-B-induction of some components was observed. Chlorogenic acid derivatives showed significant increase in shoots in response to UV-B in the no gamma control and at 20.7 mGy h^{-1} , whereas for the other gamma treatments there were no

significant differences (Fig. 3A). Quercetins showed no significant difference between UV-B-gamma and gamma only-treated plants in shoots, except at 20.7 mGy h⁻¹ (Fig. 3B). However, the levels of kaempferols increased significantly in response to UV-B as compared to the no gamma radiation (no UV-B) control and all gamma only-treated plants (Fig. 3C). Whereas stilbenes were not affected by UV-B, MeOH-soluble condensed tannins showed significant increases in response to UV-B in the no gamma control and at 10.8 mGy h⁻¹ gamma only (Fig. 3D and E).

3.5 Post-irradiation effect of gamma radiation on growth but no such effect of UV-B

There was no post-irradiation effect of the UV-B treatments on the cumulative shoot elongation, shoot diameter (from needle tip to needle tip) or number of needles (Fig. 4 and Fig. S4). However, compared to the unexposed control plants (no gamma, no UV-B), the cumulative elongation growth was significantly reduced by exposure to gamma radiation (Fig. 4 and Fig. S4). In the first series of experiments (without UV-B pre-treatment) the cumulative elongation growth was reduced by an average of 67%, 78% and 93%, respectively, 44 days after exposure to 20.7, 42.9 and 125 mGy h⁻¹ gamma radiation (from 0.9 cm growth in the control to 0.3, 0.2 and 0.06 cm at 20.7, 42.9 and 125 mGy h⁻¹) (Fig. 4A and Fig. S4A). Shoot diameter showed a significant reduction by 34%, 39% and 44% after exposure to 20.7, 42.9 and 125 mGy h⁻¹ (from 6.1 cm shoot diameter in the control to 4, 3.7 and 3.4 cm at 20.7, 42.9 and 125 mGy h⁻¹; Fig. 4B and Fig. S4B). The number of needles was also reduced by approximately 21% and 46% after exposure to 20.7 and 42.9 mGy h⁻¹, respectively (from 24 needles in the control to 19 and 13 needles at 20.7 and 42.9 mGy h⁻¹; Fig. 4C and Fig. S4C). After the highest dose rate (125 mGy h⁻¹) no or only very few new needles had developed (Fig. 4C, D and Fig. S4C). In the second series of experiments (with UV-B pre-treatment), the cumulative elongation growth was significantly reduced by 50% and 75%, respectively, 30 days after exposure to 20.7 and

42.9 mGy h⁻¹ (from 0.8 cm growth in the control to 0.4 and 0.2 cm at 20.7 and 42.9 mGy h⁻¹). Shoot diameter was reduced by 18% and 37% after 20.7 and 42.9 mGy h⁻¹ (from 5.7 cm shoot diameter in the control to 4.7 and 3.6 cm at 20.7 and 42.9 mGy h⁻¹), and the number of needles was reduced by 42% after 42.9 mGy h⁻¹ (from 26 needles in the control to 15 needles at 42.9 mGy h⁻¹; Fig. 4E-G, Fig. S4D-F).

Histological studies of shoot apical meristems and needles 44 days post-irradiation (experiment without UV-B pre-treatment), showed no visible cellular changes in any of the irradiation treatments compared to the unexposed controls (Fig. 5).

3.6 Persistent UV-B and gamma radiation-induced DNA damage 44 days post-irradiation

In shoot and root tips, a significant dose rate-dependent increase in % tail DNA values was observed with increased gamma dose rate, as recorded 44 days post-irradiation (Fig. 6 and Figure S5). In shoots there was 3%, 11% and 15% tail DNA after 20.7, 42.9 and 125 mGy h⁻¹, and in roots the corresponding values were 2%, 3%, 8% (Fig. 6A and Fig. S5A). No significant effect of the UV-B only-exposure on DNA damage in shoot and root tips was observed at this time point. However, as compared to the gamma radiation only, seedlings co-exposed to UV-B and 42.9 mGy h⁻¹ (16% tail DNA) or 125 mGy h⁻¹ (22% tail DNA) showed 45% and 47% additional increase in DNA damage in shoot tissue, respectively. In root tissue, co-exposure with UV-B resulted in 100% and 38% additional increase in DNA damage for 20.7 (4% tail DNA) or 125 mGy h⁻¹ (11% tail DNA), respectively (Fig. 6B and Fig. S5B).

3.7 Long-term growth post-irradiation eliminates DNA damage and normalises the phenotype

At eight months post-irradiation a normal phenotype was more or less restored (Fig 7). Although some plants at the highest dose rate (42.9 mGy h⁻¹) were still slightly shorter

compared to the controls (Fig. 7A), there were no overall significant differences in shoot diameter (needle tip to needle tip) or plant height (Fig 7B and C). Moreover, the COMET assay analysis revealed that there was no longer any significant DNA damage in the gamma and UV-B-exposed plants as compared to the unexposed controls (Fig 7D and Fig. S6A; analysed in experiments with 0.35 W m^{-2} UV-B pre-treatment).

Although some gamma-exposed plants were still smaller (plant height) than the unexposed controls in the experiment with 0.52 W m^{-2} UV-B-pre-treatment seven months post-irradiation, growth generally appeared rather normal (Fig. S6B). Analyses of DNA damage showed that there was a slight, but significantly higher degree of DNA damage in the UV-B only-exposed plants compared to the unexposed controls (no gamma-no UV-B) as well as in plants co-exposed to UV-B and 10.8 mGy h^{-1} compared to 10.8 mGy h^{-1} only (Fig. S6C and D). Furthermore, in this experiment all gamma-irradiated plants except the ones exposed to 10.8 mGy h^{-1} without UV-B, still had slightly, but significantly more DNA damage than the no gamma-no UV-B control plants.

4 Discussion

In their natural environments plants are normally exposed to low, non-damaging background levels of IR such as gamma radiation, but some areas have elevated, potentially harmful levels particularly due to releases from anthropogenic activities and accidents.¹ Although high levels of UV-B radiation may be stressful to plants, ambient UV-B levels have been suggested to prime defence mechanisms towards different stressors.³⁴⁻⁴² However, information about interactive effects of UV-B and gamma radiation is scarce. In experiments with or without UV-B pre-treatment prior to simultaneous UV-B and gamma irradiation, we tested whether UV-B can prime mechanisms contributing to tolerance to low-moderate gamma radiation levels in

seedlings of Scots pine, and whether simultaneous UV-B and gamma radiation may have a cumulative negative effect on plants not previously exposed to either of these radiation types.

Our results revealed no cumulative negative effect of six days of simultaneous gamma and UV-B irradiation on shoot or root elongation, only growth inhibition in response to the gamma dose rates of 42.9 (25%) and 125 mGy h⁻¹ (56%) in shoots and 125 mGy h⁻¹ in roots (44%) (Fig. 1A, B and Fig. S1). On the other hand, in experiments including UV-B pre-treatment for four days prior to the six days of simultaneous UV-B-gamma exposure, no growth inhibition was observed after 42.9 mGy h⁻¹ (highest dose rate tested). However, this applied also to the gamma only treatment and was accordingly not due to priming by UV-B pre-treatment. The reason for the difference in effect of 42.9 mGy h⁻¹ between the experimental series remains elusive. Growth inhibition in response to elevated levels of ionising radiation is well known in plants, although the sensitivity may vary with species and developmental stage, with Scots pine considered relatively sensitive.¹ Nevertheless, it could be noted that elongation growth was not affected by exposure to 20.7 and 10.8 mGy h⁻¹, which are far higher dose rates than the natural background levels.¹ This demonstrates that even this species is resistant to gamma radiation levels far higher than the background levels currently found in the natural environment.

Although UV-B has been shown to reduce shoot elongation and leaf expansion in a wide range of experiments with different plant species^{31, 32, 73-76}, we did not detect any significant effect of UV-B on shoot and root elongation after the 6 or 10 days of UV-B exposure (Fig. 1 and Fig. S1). This lack of effect of UV-B on elongation growth even at the relatively high UV-B to PAR ratio(s) used (0.35 W m⁻² UV-B for 6 or 10 days or 0.52 W m⁻² for 4 days followed by 0.35 W m⁻² for 6 days, all under a PAR of 200 μmol m⁻² s⁻¹), may be due to the efficient UV-screening in the epidermis of such evergreen conifers.⁶⁰⁻⁶²

Gamma radiation is well known to induce production of ROS, including H₂O₂, which in high amounts results in damage to macromolecules like lipids, proteins and DNA.^{46,47} Indeed,

the negative effect of 42.9 and 125 mGy h⁻¹ gamma radiation on elongation growth in the experiments without UV-B pre-treatment, correlated with significantly increased H₂O₂ levels compared to the unexposed control and lower gamma dose rates (Fig. 2C). The lack of growth inhibition after 42.9 mGy h⁻¹ in the experiments including UV-B pre-treatment may be at least partially explained by the overall lower increase in H₂O₂ (on average 48%) in these experiments compared to the same dose rate in the experiments without UV-B pre-treatment (an average of 96% increase) (Fig. 2D). UV-B has an energy level that may induce ROS formation⁷⁷, but consistent with the lack of effect of UV-B on elongation growth and efficient UV-B screening in the epidermis of conifers like Scots pine⁶⁰⁻⁶², no significant effect of UV-B on H₂O₂ levels was detected (Fig. 2C and D).

Furthermore, consistent with the increasing ROS levels with increasing gamma dose rate, the gamma irradiation resulted in a dose rate-dependent increase in DNA damage (Fig. 2A-B and Fig. S2A-B), as expected.^{1, 46, 47} This was the case in the experiments without, as well as with, UV-B pre-treatment in spite of effect on elongation growth in the first type of experiment only. Additionally, UV-B exposure, which did not affect H₂O₂ levels or elongation growth, resulted in increased DNA damage. Thus, although a relationship between DNA damage and growth inhibition may be expected, the results demonstrate tolerance to some degree of DNA damage since DNA damage also occurs in other conditions than those affecting growth. It should be noted that although the DNA damage levels in the controls were always low, there was some variation between experiments (ranging from 0.08% to 1% tail DNA). This made the difference in DNA damage between the exposed and control seedlings appear larger in experiments with the lowest control values. Nevertheless, the DNA damage levels (% tail DNA) were generally relatively similar for specific gamma dose rates and increased as expected with increasing dose rate. The reason for the variation in the controls remains elusive since action

was taken during the sample processing to avoid light-induced ROS production which may induce DNA damage.

UV-inducible phenolic compounds including flavonoids, which act as antioxidants, are important in protection against ROS generated by exposure to UV-B.^{31, 55} It may be hypothesised that their antioxidant activity may also protect against ROS formed in response to gamma radiation, and that they may be induced by low-moderate gamma radiation levels. The results demonstrate that gamma radiation does not induce production of any of the phenolic compounds analysed in the Scots pine seedlings (Fig. 3). In contrast, consistent with previous studies⁷⁸⁻⁸¹, general UV-B-induction of specific flavonoids was observed, *i.e.* glycosides of the flavonoid kaempferol. A significant increase in chlorogenic acid and methanol-soluble tannins in response to UV-B was also observed, but only when UV-B was provided separately or in combination with gamma dose rates not affecting elongation growth. However, the induction of phenolic compounds by UV-B did not protect against a negative effect of gamma radiation on growth, since gamma-induced growth inhibition at the highest dose rates was similar in the presence and absence of UV-B. Surprisingly, although both gamma radiation and UV-B are well known to induce the formation of different groups of antioxidants^{31, 47, 51, 52, 82}, no significant effect of the irradiation treatments on total antioxidant capacity could be detected when entire seedlings or shoots only were analysed (Fig. S3). The reason for this remains elusive.

To test whether damage resulting from the irradiation treatments may possibly take some time to be recovered from, or even fully manifested as shown in some other studies,^{63, 64} growth parameters and DNA damage were also assessed post-irradiation. Indeed, although the shoot apical meristems and needle anatomy appeared normal at all dose rates 44 days post-irradiation (Fig. 5), gamma-induced growth inhibition was generally visible at lower dose rates post-irradiation than at the end of the gamma exposure (Fig. 4 and Fig. S4). In contrast to findings

from the end of the gamma exposure, growth parameters were negatively affected post-irradiation by 20.7 mGy h⁻¹ in all experiments, and by 42.9 mGy h⁻¹ in the experiments with UV-B pre-treatment. On the other hand, consistent with lack of significant effect of UV-B at the end of the irradiation treatments, there were no after-effects of UV-B on growth parameters.

At day 44 post-irradiation, the gamma dose rate-dependent and UV-B-induced DNA damage was quite similar to what was found at the end of the six days of gamma exposure (Fig. 6 and Fig. S5). The growth-inhibition at even lower dose rates post-irradiation than that found at the end of the gamma exposure may suggest that the effect of DNA damage on growth may take some time to be fully realised. The post-irradiation DNA damage even in the UV-B-exposed plants may be due to the type of damage induced, e.g. possibly double strand breaks rather than photo-repairable UV-B-induced lesions,^{83, 84} or because of damage generated post-irradiation. Consistent with the latter, (at least for the gamma-exposed plants), genomic instability induced by IR has been shown in other organisms.^{63, 64, 85} This may involve mechanisms such as DNA repair defects due to mutations and programmed cell death. Genomic instability may also be related to epigenetic mechanisms such as changes in DNA methylation and deficiency in the histone variant H2AX, which is important for proper DNA repair.^{86, 87} However, seven and eight months post-irradiation, the DNA-damage was either fully or nearly recovered, consistent with a normalised phenotype with formation of long needles like in the unexposed control plants, and no significant overall difference in plant height or shoot diameter between the treatments (Fig. 7 and Fig. S6).

5 Conclusions

In conclusion, our results showed no evidence of a protective effect of UV-B on growth inhibition and DNA damage induced by low doses of gamma radiation (given as moderate to

high dose rates) in Scots pine seedlings. There was also no additive adverse effect of UV-B and gamma radiation on growth. Gamma radiation negatively affected growth parameters and resulted in increased ROS-production and DNA damage in a dose rate-dependent manner. In spite of additional DNA damage in response to UV-B, UV-B did not affect ROS production or the growth of shoots and roots. The DNA damage after the gamma and UV-B irradiation was long-lasting and may have been due to induction of genomic instability. Nevertheless, growth inhibition post-irradiation was observed only in response to gamma radiation, in a dose rate-dependent manner, suggesting tolerance to low levels of DNA damage.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

The Norwegian Research Council through its Centre of Excellence funding scheme (Grant 223268/F50) and the Norwegian University of Life Sciences are acknowledged for financial support. Thanks to Camilo Chiang, Marit Siira and Ida K Hagen for technical assistance in the plant growth experiments and to Sheona Innes for language revision.

References

1. N. Caplin and N. Willey, Ionizing Radiation, Higher Plants, and Radioprotection: From Acute High Doses to Chronic Low Doses, *Frontiers in Plant Science*, 2018, **9**.
2. A. C. Freitas and A. S. Alencar, Gamma dose rates and distribution of natural radionuclides in sand beaches—Ilha Grande, Southeastern Brazil, *Journal of Environmental Radioactivity*, 2004, **75**, 211-223.

3. J. Mrdakovic Popic, C. Raj Bhatt, B. Salbu and L. Skipperud, Outdoor ²²⁰Rn, ²²²Rn and terrestrial gamma radiation levels: investigation study in the thorium rich Fen Complex, Norway, *Journal of Environmental Monitoring*, 2012, **14**, 193-201.
4. U. N. S. C. o. t. E. o. A. Radiation, *Summary of low-dose radiation effects on health*, New York, 2010.
5. D. Averbeck, S. Salomaa, S. Bouffler, A. Ottolenghi, V. Smyth and L. Sabatier, Progress in low dose health risk research: Novel effects and new concepts in low dose radiobiology, *Mutation Research/Reviews in Mutation Research*, 2018, **776**, 46-69.
6. U. N. S. C. o. t. E. o. A. Radiation, *Sources, effects and risks of ionizing radiation*, 2017.
7. U. N. S. C. o. t. E. o. A. Radiation, Sources and effects of ionizing radiation, *New York*, 1996, 85.
8. G. M. Woodwell and A. L. Rebeck, Effects of Chronic Gamma Radiation on the Structure and Diversity of an Oak-Pine Forest, *Ecological Monographs*, 1967, **37**, 53-69.
9. C. L. Ballare, A. L. Scopel, A. E. Stapleton and M. J. Yanovsky, Solar Ultraviolet-B Radiation Affects Seedling Emergence, DNA Integrity, Plant Morphology, Growth Rate, and Attractiveness to Herbivore Insects in *Datura ferox*, *Plant Physiology*, 1996, **112**, 161-170.
10. S. A.-H.-. Mackerness, Plant responses to ultraviolet-B (UV-B: 280–320 nm) stress: What are the key regulators?, *Plant Growth Regulation*, 2000, **32**, 27-39.
11. H. Frohnmeyer and D. Staiger, Ultraviolet-B Radiation-Mediated Responses in Plants. Balancing Damage and Protection, *Plant Physiology*, 2003, **133**, 1420-1428.
12. G. M. Woodwell, Effects of Ionizing Radiation on Terrestrial Ecosystems, *Experiments show how ionizing radiation may alter normally stable patterns of ecosystem behavior*, 1962, **138**, 572-577.

13. T. Kawai and T. Inoshita, Effects of gamma ray irradiation on growing rice plants—I: Irradiations at four main developmental stages, *Radiation Botany*, 1965, **5**, 233-255.
14. D. D. Killion and M. J. Constantin, Gamma irradiation of corn plants: Effects of exposure, exposure rate, and developmental stage on survival, height, and grain yield of two cultivars, *Radiation Botany*, 1972, **12**, 159-164.
15. S. G. Wi, B. Y. Chung, J.-S. Kim, J.-H. Kim, M.-H. Baek, J.-W. Lee and Y. S. Kim, Effects of gamma irradiation on morphological changes and biological responses in plants, *Micron*, 2007, **38**, 553-564.
16. M. Tulik, Cambial history of Scots pine trees (*Pinus Sylvestris*) prior and after the Chernobyl accident as encoded in the xylem, *Environmental and Experimental Botany*, 2001, **46**, 1-10.
17. L. Zelena, B. Sorochinsky, S. von Arnold, L. van Zyl and D. H. Clapham, Indications of limited altered gene expression in *Pinus sylvestris* trees from the Chernobyl region, *Journal of Environmental Radioactivity*, 2005, **84**, 363-373.
18. V. Yoschenko, T. Ohkubo and V. Kashparov, Radioactive contaminated forests in Fukushima and Chernobyl, *Journal of Forest Research*, 2018, **23**, 3-14.
19. S. V. Fesenko, R. M. Alexakhin, S. A. Geras'kin, N. I. Sanzharova, Y. V. Spirin, S. I. Spiridonov, I. A. Gontarenko and P. Strand, Comparative radiation impact on biota and man in the area affected by the accident at the Chernobyl nuclear power plant, *Journal of Environmental Radioactivity*, 2005, **80**, 1-25.
20. Y. Watanabe, S. e. Ichikawa, M. Kubota, J. Hoshino, Y. Kubota, K. Maruyama, S. Fuma, I. Kawaguchi, V. I. Yoschenko and S. Yoshida, Morphological defects in native Japanese fir trees around the Fukushima Daiichi Nuclear Power Plant, *Scientific Reports*, 2015, **5**, 1-7.

21. N. Goltsova, Y. Abaturov, A. Abaturov, P. Melankholin, A. Girbasova and N. Rostova, Chernobyl radionuclide accident: Effects on the shoot structure of *Pinus sylvestris*, *Annales Botanici Fennici*, 1991, **28**, 1-13.
22. P. J. Aphalo, A. Albert, L. O. Björn, A. R. McLeod, T. M. Robson and E. Rosenqvist, *Beyond the visible: A handbook of best practice in plant UV photobiology*, Division of Plant Biology, Helsinki, 2012.
23. J. Rozema, J. van de Staaij, L. O. Björn and M. Caldwell, UV-B as an environmental factor in plant life: stress and regulation, *Trends in Ecology & Evolution*, 1997, **12**, 22-28.
24. M. A. K. Jansen, V. Gaba and B. M. Greenberg, Higher plants and UV-B radiation: balancing damage, repair and acclimation, *Trends in Plant Science*, 1998, **3**, 131-135.
25. L. O. Björn, On the history of phyto-photo UV science (not to be left in skoto toto and silence), *Plant Physiology and Biochemistry*, 2015, **93**, 3-8.
26. A. Coffey and M. A. K. Jansen, Effects of natural solar UV-B radiation on three *Arabidopsis* accessions are strongly affected by seasonal weather conditions, *Plant Physiology and Biochemistry*, 2019, **134**, 64-72.
27. M. A. K. Jansen, *Ultraviolet-B Radiation: Stressor and Regulatory Signal*, Pondicherry, India, 2017.
28. T. S. L. Lau, E. Eno, G. Goldstein, C. Smith and D. A. Christopher, Ambient levels of UV-B in Hawaii combined with nutrient deficiency decrease photosynthesis in near-isogenic maize lines varying in leaf flavonoids: Flavonoids decrease photoinhibition in plants exposed to UV-B, *Photosynthetica*, 2006, **44**, 394-403.
29. J. Belnap, S. L. Phillips, S. Flint, J. Money and M. Caldwell, Global change and biological soil crusts: effects of ultraviolet augmentation under altered precipitation regimes and nitrogen additions, 2008, **14**, 670-686.

30. K. R. Albert, T. N. Mikkelsen, H. Ro-Poulsen, M. F. Arndal and A. Michelsen, Ambient UV-B radiation reduces PSII performance and net photosynthesis in high Arctic *Salix arctica*, *Environmental and Experimental Botany*, 2011, **73**, 10-18.
31. M. A. K. Jansen and J. F. Bornman, UV-B radiation: from generic stressor to specific regulator, *Physiologia Plantarum*, 2012, **145**, 501-504.
32. T. M. Robson, K. Klem, O. Urban and M. A. K. Jansen, Re-interpreting plant morphological responses to UV-B radiation, *Plant, Cell & Environment*, 2015, **38**, 856-866.
33. M. Dotto and P. Casati, Developmental reprogramming by UV-B radiation in plants, *Plant Science*, 2017, **264**, 96-101.
34. S. Nogués and N. R. Baker, Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV-B radiation, *Journal of Experimental Botany*, 2000, **51**, 1309-1317.
35. V. Alexieva, I. Sergiev, S. Mapelli and E. Karanov, The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat, *Plant, Cell & Environment*, 2001, **24**, 1337-1344.
36. J. Stratmann, Ultraviolet-B radiation co-opts defense signaling pathways, *Trends in Plant Science*, 2003, **8**, 526-533.
37. C. Ouhibi, H. Attia, F. Rebah, N. Msilini, M. Chebbi, J. Aarouf, L. Urban and M. Lachaal, Salt stress mitigation by seed priming with UV-C in lettuce plants: Growth, antioxidant activity and phenolic compounds, *Plant Physiology and Biochemistry*, 2014, **83**, 126-133.
38. Y. Manetas, Y. Petropoulou, K. Stamatakis, D. Nikolopoulos, E. Levizou, G. Psaras and G. Karabourniotis, in *UV-B and Biosphere*, eds. J. Rozema, W. W. C. Gieskes, S.

- C. Van De Geijn, C. Nolan and H. De Boois, Springer Netherlands, Dordrecht, 1997, DOI: 10.1007/978-94-011-5718-6_9, pp. 100-108.
39. A.-M. Schmidt, D. P. Ormrod, N. J. Livingston and S. Misra, The Interaction of Ultraviolet-B Radiation and Water Deficit in Two *Arabidopsis thaliana* Genotypes, *Annals of Botany*, 2000, **85**, 571-575.
 40. L. Chalker-Scott and J. D. Scott, Elevated Ultraviolet-B Radiation Induces Cross-protection to Cold in Leaves of *Rhododendron* Under Field Conditions, 2004, **79**, 199-204.
 41. M. E. Poulson, M. R. T. Boeger and R. A. Donahue, Response of photosynthesis to high light and drought for *Arabidopsis thaliana* grown under a UV-B enhanced light regime, *Photosynthesis Research*, 2006, **90**, 79.
 42. T. M. Robson, S. M. Hartikainen and P. J. Aphalo, How does solar ultraviolet-B radiation improve drought tolerance of silver birch (*Betula pendula* Roth.) seedlings?, 2015, **38**, 953-967.
 43. X. Li, L. Zhang, Y. Li, L. Ma, N. Bu and C. Ma, Changes in photosynthesis, antioxidant enzymes and lipid peroxidation in soybean seedlings exposed to UV-B radiation and/or Cd, *Plant and Soil*, 2012, **352**, 377-387.
 44. É. Hideg, M. A. K. Jansen and Å. Strid, UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates?, *Trends in Plant Science*, 2013, **18**, 107-115.
 45. J. A. Reisz, N. Bansal, J. Qian, W. Zhao and C. M. Furdui, Effects of Ionizing Radiation on Biological Molecules—Mechanisms of Damage and Emerging Methods of Detection, *Antioxidants & Redox Signaling*, 2014, **21**, 260-292.
 46. S. Biedermann, S. Mooney and H. Hellmann, *Recognition and Repair Pathways of Damaged DNA in Higher Plants*, 2011.

47. S. S. Gill and N. Tuteja, Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants, *Plant Physiology and Biochemistry*, 2010, **48**, 909-930.
48. J. van de Walle, N. Horemans, E. Saenen, M. Van Hees, J. Wannijn, R. Nauts, A. van Gompel, J. Vangronsveld, H. Vandenhove and A. Cuypers, Arabidopsis plants exposed to gamma radiation in two successive generations show a different oxidative stress response, *Journal of Environmental Radioactivity*, 2016, **165**, 270-279.
49. K. O. Yoshiyama, K. Sakaguchi and S. Kimura, DNA Damage Response in Plants: Conserved and Variable Response Compared to Animals, *Biology*, 2013, **2**, 1338-1356.
50. A. Van Hoeck, N. Horemans, R. Nauts, M. Van Hees, H. Vandenhove and R. Blust, Lemna minor plants chronically exposed to ionising radiation: RNA-seq analysis indicates a dose rate dependent shift from acclimation to survival strategies, *Plant Science*, 2017, **257**, 84-95.
51. R. Mittler, S. Vanderauwera, M. Gollery and F. Van Breusegem, Reactive oxygen gene network of plants, *Trends in Plant Science*, 2004, **9**, 490-498.
52. P. Ahmad, C. A. Jaleel, M. A. Salem, G. Nabi and S. Sharma, Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress, *Critical Reviews in Biotechnology*, 2010, **30**, 161-175.
53. H. Vandenhove, N. Vanhoudt, A. Cuypers, M. van Hees, J. Wannijn and N. Horemans, Life-cycle chronic gamma exposure of Arabidopsis thaliana induces growth effects but no discernable effects on oxidative stress pathways, *Plant Physiology and Biochemistry*, 2010, **48**, 778-786.
54. N. Vanhoudt, N. Horemans, J. Wannijn, R. Nauts, M. Van Hees and H. Vandenhove, Primary stress responses in Arabidopsis thaliana exposed to gamma radiation, *Journal of Environmental Radioactivity*, 2014, **129**, 1-6.

55. T. Løvdal, K. M. Olsen, R. Slimestad, M. Verheul and C. Lillo, Synergetic effects of nitrogen depletion, temperature, and light on the content of phenolic compounds and gene expression in leaves of tomato, *Phytochemistry*, 2010, **71**, 605-613.
56. T. Murashige and F. Skoog, A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures, *Physiologia Plantarum*, 1962, **15**, 473-497.
57. O. C. Lind, D. Helen Oughton and B. Salbu, The NMBU FIGARO low dose irradiation facility, *International Journal of Radiation Biology*, 2019, **95**, 76-81.
58. E. L. Hansen, O. C. Lind, D. H. Oughton and B. Salbu, A framework for exposure characterization and gamma dosimetry at the NMBU FIGARO irradiation facility, *International Journal of Radiation Biology*, 2019, **95**, 82-89.
59. A. E. S. Green, T. Sawada and E. P. Shettle, THE MIDDLE ULTRAVIOLET REACHING THE GROUND*, *Photochemistry and Photobiology*, 1974, **19**, 251-259.
60. T. A. Day, T. C. Vogelmann and E. H. DeLucia, Are some plant life forms more effective than others in screening out ultraviolet-B radiation?, *Oecologia*, 1992, **92**, 513-519.
61. T. A. Day, Relating UV-B radiation screening effectiveness of foliage to absorbing-compound concentration and anatomical characteristics in a diverse group of plants, *Oecologia*, 1993, **95**, 542-550.
62. R. J. Fischbaher, B. Kossman, H. Panten, R. Steinbrecher, W. Heller, H. K. Seidlitz, H. Sandermann, N. Hertkorn and J.-P. Schnitzler, Seasonal accumulation of ultraviolet-B screening pigments in needles of Norway spruce (*Picea abies* (L.) Karst.), *Plant, Cell & Environment*, 1999, **22**, 27-37.
63. W. F. Morgan, J. P. Day, M. I. Kaplan, E. M. McGhee and C. L. Limoli, Genomic Instability Induced by Ionizing Radiation, *Radiation Research*, 1996, **146**, 247-258.

64. C. Mothersill and C. B. Seymour, Mechanisms and implications of genomic instability and other delayed effects of ionizing radiation exposure, *Mutagenesis*, 1998, **13**, 421-426.
65. J. E. Olsen, Light and temperature sensing and signaling in induction of bud dormancy in woody plants, *Plant Molecular Biology*, 2010, **73**, 37-47.
66. Y. Lee, C. Karunakaran, R. Lahlali, X. Liu, K. K. Tanino and J. E. Olsen, Photoperiodic Regulation of Growth-Dormancy Cycling through Induction of Multiple Bud–Shoot Barriers Preventing Water Transport into the Winter Buds of Norway Spruce, *Frontiers in Plant Science*, 2017, **8**, 2109.
67. R. Arora, L. Rowland and K. Tanino, *Induction and Release of Bud Dormancy in Woody Perennials: A Science Comes of Age*, 1953.
68. T. Gichner, Z. Patková and J. K. Kim, DNA Damage Measured by the Comet Assay in Eight Agronomic Plants, *Biologia Plantarum*, 2003, **47**, 185-188.
69. G. Koppen, A. Azqueta, B. Pourrut, G. Brunborg, A. R. Collins and S. A. S. Langie, The next three decades of the comet assay: a report of the 11th International Comet Assay Workshop, *Mutagenesis*, 2017, **32**, 397-408.
70. L. Xie, T. Gomes, K. A. Solhaug, Y. Song and K. E. Tollefsen, Linking mode of action of the model respiratory and photosynthesis uncoupler 3,5-dichlorophenol to adverse outcomes in *Lemna minor*, *Aquatic Toxicology*, 2018, **197**, 98-108.
71. J. Razinger, L. Drinovec and A. Zrimec, Real-time visualization of oxidative stress in a floating macrophyte *Lemna minor* L. exposed to cadmium, copper, menadione, and AAPH, *Environmental Toxicology*, 2010, **25**, 573-580.
72. L. Nybakken, R. Hörkkä and R. Julkunen-Tiitto, Combined enhancements of temperature and UVB influence growth and phenolics in clones of the sexually dimorphic *Salix myrsinifolia*, 2012, **145**, 551-564.

73. M. M. Caldwell, J. F. Bornman, C. L. Ballaré, S. D. Flint and G. Kulandaivelu, Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors, *Photochemical & Photobiological Sciences*, 2007, **6**, 252-266.
74. K. Hectors, E. Prinsen, W. De Coen, M. A. K. Jansen and Y. Guisez, Arabidopsis thaliana plants acclimated to low dose rates of ultraviolet B radiation show specific changes in morphology and gene expression in the absence of stress symptoms, *New Phytologist*, 2007, **175**, 255-270.
75. A. G. Roro, S. A. F. Dukker, T. I. Melby, K. A. Solhaug, S. Torre and J. E. Olsen, UV-B-induced Inhibition of Stem Elongation and Leaf Expansion in Pea Depends on Modulation of Gibberellin Metabolism and Intact Gibberellin Signalling, *Journal of Plant Growth Regulation*, 2017, **36**, 680-690.
76. M. A. K. Jansen, Ultraviolet-B radiation effects on plants: induction of morphogenic responses, *Physiologia Plantarum*, 2002, **116**, 423-429.
77. G. I. Jenkins, Signal Transduction in Responses to UV-B Radiation, *Annual Review of Plant Biology*, 2009, **60**, 407-431.
78. Y.-P. Cen and J. F. Bornman, The effect of exposure to enhanced UV-B radiation on the penetration of monochromatic and polychromatic UV-B radiation in leaves of Brassica napus, *Physiologia Plantarum*, 1993, **87**, 249-255.
79. S. Reuber, J. F. Bornman and G. Weissenböck, A flavonoid mutant of barley (*Hordeum vulgare* L.) exhibits increased sensitivity to UV-B radiation in the primary leaf, *Plant, Cell & Environment*, 1996, **19**, 593-601.
80. L. C. Olsson, M. Veit, G. Weissenböck and J. F. Bornman, Differential flavonoid response to enhanced uv-b radiation in brassica napus, *Phytochemistry*, 1998, **49**, 1021-1028.

81. J.-P. Schnitzler, Tim P. Jungblut, Carmen Feicht, Matthias Köfferlein, Christian Langebartels, W. Heller and H. S. Jr., UV-B induction of flavonoid biosynthesis in Scots pine (*Pinus sylvestris* L.) seedlings, *Trees*, 1997, **11**, 162-168.
82. a. K Hahlbrock and D. Scheel, Physiology and Molecular Biology of Phenylpropanoid Metabolism, *Annual Review of Plant Physiology and Plant Molecular Biology*, 1989, **40**, 347-369.
83. A. B. Britt, Repair of DNA Damage Induced by Solar UV, *Photosynthesis Research*, 2004, **81**, 105-112.
84. R. P. Rastogi, Richa, A. Kumar, M. B. Tyagi and R. P. Sinha, Molecular Mechanisms of Ultraviolet Radiation-Induced DNA Damage and Repair, *Journal of Nucleic Acids*, 2010, **2010**.
85. S. Hurem, T. Gomes, D. A. Brede, E. Lindbo Hansen, S. Mutoloki, C. Fernandez, C. Mothersill, B. Salbu, Y. A. Kassaye, A.-K. Olsen, D. Oughton, P. Aleström and J. L. Lyche, Parental gamma irradiation induces reprotoxic effects accompanied by genomic instability in zebrafish (*Danio rerio*) embryos, *Environmental Research*, 2017, **159**, 564-578.
86. U. Aypar, W. F. Morgan and J. E. Baulch, Radiation-induced genomic instability: Are epigenetic mechanisms the missing link?, *International Journal of Radiation Biology*, 2011, **87**, 179-191.
87. C. H. Bassing, K. F. Chua, J. Sekiguchi, H. Suh, S. R. Whitlow, J. C. Fleming, B. C. Monroe, D. N. Ciccone, C. Yan, K. Vlasakova, D. M. Livingston, D. O. Ferguson, R. Scully and F. W. Alt, Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX, *Proceedings of the National Academy of Sciences of the United States of America*, 2002, **99**, 8173-8178.

Table legends

Table 1 The gamma radiation dose rates and total doses applied in the experiments with 6 days gamma exposure of young seedlings of Scots pine using a ^{60}Co source. The minimum and maximum values represent the dose rates and total doses behind and in front of the petri dishes with the seedlings. Dose rates to water, which were used as proxies for the dose rates received by the seedlings, were calculated from the measured dose rate air kerma values.

Table 2 Overview of the treatments and recorded parameters/analyses in the gamma- and UV-B irradiation experiments without (totally six repeated experiments) or with UV-B pre-treatment (totally three repeated experiments). For growth measurements the number of plants per treatment is shown. For other parameters the number of samples per treatment is shown with the number of plants per sample in brackets. For DNA damage analysis the second number in the brackets refers to the number of technical replicates (gels) with 50-100 nuclei scored in each.

Figure legends

Fig. 1 Effect of 6 days of gamma irradiation without (UV-) or with (UV+) UV-B (0.35 W m^{-2}) in Scots pine seedlings; A) shoot (regression analysis value (R^2): UV- and UV+: 0.98) and B) root length (R^2 : UV- and UV+: 0.99). Mean \pm SE of 48-90 plants per treatment. C) Histology of shoot apical meristems. 5 plants analysed per treatment. Scale bars: $100 \mu\text{m}$. D) Shoot (R^2 : UV-: 0.12; UV+: 0.40) and E) root length (R^2 : UV-: 0.08; UV+: 0.23) in experiments including also 4 days UV-B (0.35 W m^{-2}) pre-treatment of the UV-B exposed plants. Mean \pm SE of 27-51 plants per treatment. The treatments started when plants were 6 days old. Different letters

within a plant part indicate significant differences ($p \leq 0.05$) based on analysis of variance followed by Tukey's test.

Fig. 2 Effect of 6 days of gamma irradiation without (UV-) or with (UV+) UV-B (0.35 W m^{-2}) in Scots pine seedlings; A) DNA damage (COMET assay) in shoot tips (regression analysis value (R^2): 0.92). B) DNA damage in shoot tips (R^2 : 0.91) in experiments including also 4 days UV-B (0.35 W m^{-2}) pre-treatment of the UV-B exposed plants. The line in each box = mean of median values for 6 samples per treatment with 3 technical replicates (gels) per sample with 50-100 nuclei scored per gel. Lower and upper box boundaries = 25 and 75% percentiles, error bars = 10 and 90% percentiles with data points outside shown as dots. C) Reactive oxygen species (ROS; i.e. H_2O_2) in experiments without UV-B pre-treatment (R^2 : UV-: 0.84; UV+: 0.94). Mean \pm SE of 8 samples per treatment. D) ROS in experiment with UV-B pre-treatment (R^2 : UV-: 0.97; UV+: 0.87). Mean \pm SE of 4 samples per treatment. The treatments started when plants were 6 days old. Different letters within a parameter indicate significant differences ($p \leq 0.05$) based on analysis of variance followed by Tukey's test.

Fig. 3 Effect of 6 days of gamma irradiation without (UV-) or with (UV+) UV-B (0.35 W m^{-2}), including also 4 days UV-B (0.35 W m^{-2}) pre-treatment of UV-B exposed plants, on levels of phenolic compounds in shoots of Scots pine seedlings; A) chlorogenic acid derivatives, B) quercetins, C) kaempferols, D) stilbenes and E) MeOH-soluble tannins. Mean \pm SE of 8 samples per treatment (shoots from 7-8 plants per sample). UV-B pre-treatment started when plants were 6 days old. Different letters within a parameter indicate significant differences ($p \leq 0.05$) based on analysis of variance followed by Tukey's test.

Fig. 4 Post-irradiation effects of 6 days of gamma irradiation without (UV-) or with (UV+) UV-B (0.35 W m^{-2}) in Scots pine seedlings; A) cumulative shoot elongation, B) shoot diameter (needle tip to needle tip), C) number of needles and D) phenotype 44 days post-irradiation. E) Cumulative shoot elongation, F) shoot diameter and G) number of needles in experiments including also 4 days UV-B (0.35 W m^{-2}) pre-treatment of the UV-B exposed plants. The irradiation treatments started when the seedlings were 6 days old, and time 0 corresponds to the day the irradiation treatments ended. The results are mean \pm SE of 24 plants per treatment. Different letters within a parameter indicate significant differences ($p \leq 0.05$) based on analysis of variance followed by Tukey's test. Regression analysis values (R^2): Without UV-B-pre-treatment for 0, 20.7, 42.9, 125 mGy h^{-1} ; cumulative shoot elongation UV-: 0.83, 0.93, 0.94, 0.77 and UV+: 0.89, 0.94, 0.84, 0.83, shoot diameter UV-: 0.99, 0.96, 0.90, 0.17 and UV+: 0.99, 0.93, 0.95, 0.15, number of needles UV-: 0.97, 0.70, 0.84, 0.78 and UV+: 0.96, 0.72, 0.60, 0.84. With UV-B pre-treatment: for 0, 10.8, 20.7, 42.9, mGy h^{-1} ; cumulative shoot elongation UV-: 0.98, 0.99, 0.97, 0.89 and UV+: 0.98, 0.97, 0.97, 0.94, shoot diameter UV-: 0.99, 0.99, 0.98, 0.99 and UV+: 0.99, 0.97, 0.96, 0.95, number of needles UV-: 0.99, 0.99, 0.98, 0.97 and UV+: 0.97, 0.99, 0.60, 0.99.

Fig. 5 Post-irradiation effect 44 days after 6 days of gamma irradiation without (UV-) or with (UV+) UV-B (0.35 W m^{-2}) on histology of A) shoot apical meristems and B) needles in seedlings of Scots pine. The irradiation treatments started when the seedlings were 6 days old. Five plants were analysed per treatment. Scale bar: 25 μm .

Fig. 6 Post-irradiation effect 44 days after 6 days of gamma irradiation without (UV-) or with (UV+) UV-B (0.35 W m^{-2}) on DNA damage (COMET assay) in A) shoot (regression analysis value (R^2): 0.90) and B) root tips (R^2 : 0.87) of Scots pine seedlings. The irradiation treatments

started when plants were 6 days old. The line in each box = the mean of median values for 6 samples per treatment with 3 technical replicates (gels) per sample with 50-100 nuclei scored per gel. Lower and upper box boundaries = 25 and 75% percentiles, error bars = 10 and 90% percentiles with data points outside shown as dots. Different letters within a plant part indicate significant differences ($p \leq 0.05$) based on analysis of variance followed by Tukey's test.

Fig. 7 Post-irradiation effect 8 months after 6 days of gamma irradiation without (UV-) or with (UV+) UV-B (0.35 W m^{-2}) in Scots pine, including also 4 days UV-B (0.35 W m^{-2}) pre-treatment of UV-B exposed plants. The irradiation treatments started when the seedlings were 6 days old. A) Phenotype. B) Plant height and C) shoot diameter (needle tip to needle tip). Mean \pm SE of 6-10 plants. D) DNA damage (COMET assay) in shoot tips (R^2 : 0.27). The line in each box = the mean of the median values for 3 repeated samples per treatment with 3 technical replicates (gels) per sample with 50-100 nuclei scored per gel. Lower and upper box boundaries = 25 and 75% percentiles, error bars = 10 and 90% percentiles with data points outside these shown as dots. Different letters within each parameter indicate significant differences ($p \leq 0.05$) based on analysis of variance followed by Tukey's test.

Supplementary Figure Legends

Fig. S1 Effect of 6 days of gamma irradiation without (UV-) or with (UV+) UV-B (0.35 W m^{-2}) in Scots pine seedlings; A) Shoot and B) root length relative to the unexposed control. Mean of 48-90 plants per treatment. C) Shoot and D) root length relative to the unexposed control in experiments including also 4 days UV-B at 0.35 W m^{-2} pre-treatment of the UV-B exposed plants. Mean of 27-51 plants per treatment. (The actual shoot and root lengths shown in Fig. 1). Relative E) shoot and F) root length and actual G) shoot (regression analysis values (R^2): UV-: 0.84; UV+: 0.20).and H) root length (R^2 : UV-: 0.91; UV+: 0.05) in an experiment

including 4 days UV-B pre-treatment at 0.52 W m^{-2} Mean \pm SE of 10 plants per treatment. The treatments started when plants were 6 days old. Different letters within a plant part indicate significant differences ($p \leq 0.05$) based on analysis of variance followed by Tukey's test.

Fig. S2 Effect of 6 days of gamma irradiation without (UV-) or with (UV+) UV-B (0.35 W m^{-2}) in Scots pine seedlings; A) DNA damage (COMET assay) in shoot tips relative to the unexposed control. B) DNA damage in shoot tips relative to the unexposed control in experiments including also 4 days UV-B (0.35 W m^{-2}) pre-treatment of the UV-B exposed plants. (The actual DNA damage values shown in Fig. 2). C) Relative and D) actual DNA damage (COMET assay) (regression analysis value (R^2): 0.87) in shoot tips in an experiment including 4 days UV-B pre-treatment at 0.52 W m^{-2} . Mean of 6 (A, B) or 3 (C, D) samples per treatment with 3 technical replicates (gels) per sample with 50-100 nuclei scored per gel. The treatments started when plants were 6 days old. Different letters indicate significant differences ($p \leq 0.05$) based on analysis of variance followed by Tukey's test.

Fig. S3 Effect of 6 days gamma irradiation with (UV+) or without (UV-) UV-B (0.35 W m^{-2}) on total antioxidant capacity (Ferric reducing antioxidant power (FRAP) assay) in A) entire Scots pine seedlings (mean \pm SE of 4 samples) or B) shoots only (mean \pm SE of 3 samples). Three technical replicates were analysed per sample. The treatments started when the seedlings were 6 days old. Different letters within a diagram indicate significant differences ($p \leq 0.05$) based on analysis of variance followed by Tukey's test.

Fig. S4 Post-irradiation effects of 6 days of gamma irradiation without (UV-) or with (UV+) UV-B (0.35 W m^{-2}) in Scots pine seedlings; A) Cumulative shoot elongation, B) shoot diameter (needle tip to needle tip) and C) number of needles relative to the unexposed control. D)

Cumulative shoot elongation, E) shoot diameter and F) number of needles relative to the unexposed control in experiments including also 4 days UV-B (0.35 W m^{-2}) pre-treatment of the UV-B exposed plants. (The actual values are shown in Fig. 4). The irradiation treatments started when the seedlings were 6 days old, and time 0 corresponds to the day the irradiation treatments ended. The results are mean \pm SE of 24 plants per treatment.

Fig. S5 Post-irradiation effect 44 days after 6 days of gamma irradiation without (UV-) or with (UV+) UV-B (0.35 W m^{-2}) on DNA damage (COMET assay) relative to the unexposed control in A) shoot and B) root tips of Scots pine seedlings. The irradiation treatments started when plants were 6 days old. The results are mean of 6 samples per treatment with 3 technical replicates (gels) per sample with 50-100 nuclei scored per gel.

Fig. S6 Post-irradiation effect 7 months after 6 days of gamma irradiation without (UV-) or with (UV+) UV-B (0.35 W m^{-2}) in Scots pine seedlings, including also 4 days UV-B (0.52 W m^{-2}) pre-treatment of UV-B exposed seedlings. A) Plant phenotype. DNA damage in shoot tips B) relative to the unexposed control and C) actual DNA damage values (regression analysis value R^2 : 0.33). The line in each box = the mean of the median values for 3 repeated samples per treatment with 3 technical replicates (gels) per sample with 50-100 nuclei scored per gel. Lower and upper box boundaries = 25 and 75% percentiles, error bars = 10 and 90% percentiles with data points outside these shown as dots. Different letters indicate significant differences ($p \leq 0.05$) based on analysis of variance followed by Tukey's test.

Table 1

Average dose rate air kerma ^a (mGy h ⁻¹)	Dose rate air kerma interval (mGy h ⁻¹)		Average dose rate to water (mGy h ⁻¹)	Dose rate to water interval (mGy h ⁻¹)		Average total dose (Gy) 144 h (6 days) exposure	Total dose interval ^b 144 h (6 days) exposure (Gy)	
	Minimum	Maximum		Minimum	Maximum		Minimum	Maximum
112 ^c	102.3	131.7	125	113.7	146.5	18.01	17.29	18.73
38.5	35.6	41.5	42.9	39.6	46.1	6.17	5.93	6.41
18.6	17.6	19.6	20.7	19.6	21.8	2.98	2.86	3.10
9.7	9.2	10.2	10.8	10.2	11.4	1.55	1.49	1.61
0.004 ^d	0.005	0.003	0.005	0.006	0.004	0.00072	0.0009	0.0006

^a Air kerma rates represent the averages of four nanodot measurements per treatment. ^b The interval represents the weighted minimum and maximum dose rates taking into account rotation of the samples. ^c Measured between the two rows of petri dishes. ^d Dose rate in lead-shielded control zone.

Table 2

		UV-B+gamma			UV-B pre-treatment→UV-B+gamma		
		Experimental factors			Experimental factors		
Chamber	Treatment	UV-B pre-treatment	Gamma	UV-B	UV-B pre-treatment	Gamma	UV-B
1	Control	-	-	-	-	-	-
	UV-B	-	-	+	+	-	+
2	Gamma	-	+	-	-	+	-
	UV-B+gamma	-	+	+	+	+	+
Treatment levels		-	0, 20.7, 42.9, 125 mGy h ⁻¹	0.35 W m ⁻²	0.35 W m ⁻²	0, 10.8, 20.7, 42.9 mGy h ⁻¹	0.35 W m ⁻²
Treatment duration (days)		-	6	6	4	6	6
		UV-B+gamma			UV-B pre-treatment→UV-B+gamma		
Time point	Parameter	Number of Experiments	Number of replicates treatment ⁻¹ experiment ⁻¹	Total number of replicates treatment ⁻¹ (total all exp.)	Number of Experiments	Number of replicates treatment ⁻¹ experiment ⁻¹	Total number replicates treatment ⁻¹ (total all exp.)
At the end of irradiation	Shoot and root length	6	8-15	48-90	3	9-17	27-51
	Histology	1	5	5	-	-	-
	DNA damage	2	3 (3:3)	6	2	3 (3:3)	6
	ROS	2	4	8	1	4	4
	Antioxidant capacity	1 (seedlings) 1 (shoots only)	4 (1: 3) 3 (3: 3)	4 3	-	-	-
	Phenolic compounds	-	-	-	1	8 (7-8)	8
Post-irradiation (44 days if otherwise not mentioned)	Growth parameters	2	12	24	2 1 (8 months)	10-15 6-10	20-30 6-10
	Histology	1	5	5	-	-	-
	DNA damage	2	3 (3: 3)	6	1 1 (8 months)	3 (3: 3) 3 (3: 3)	3 3

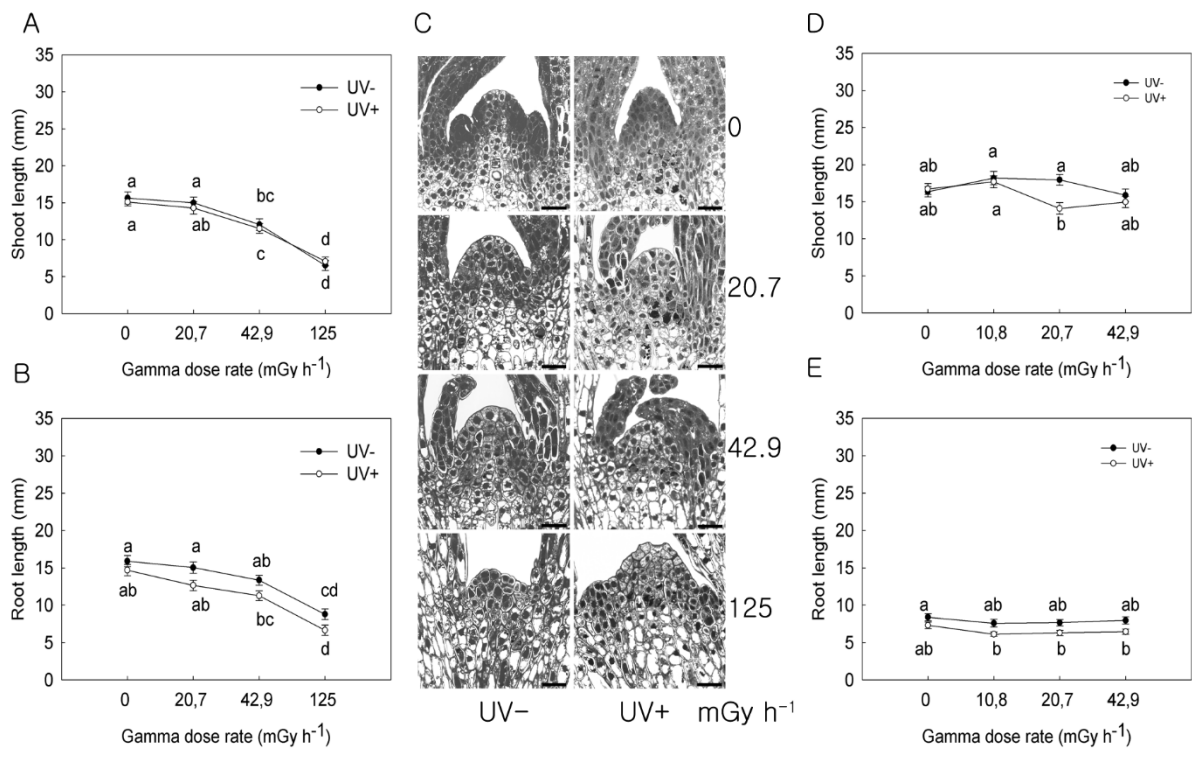


Fig. 1

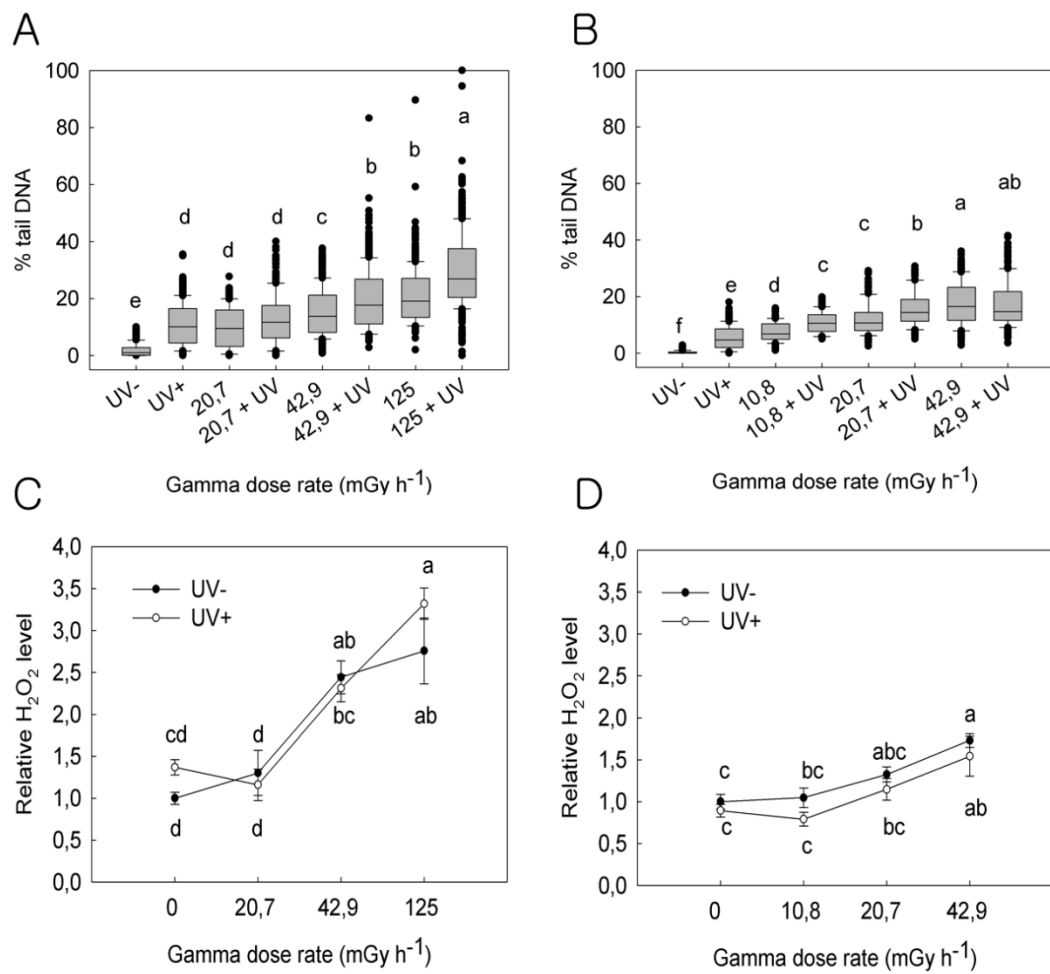


Fig. 2

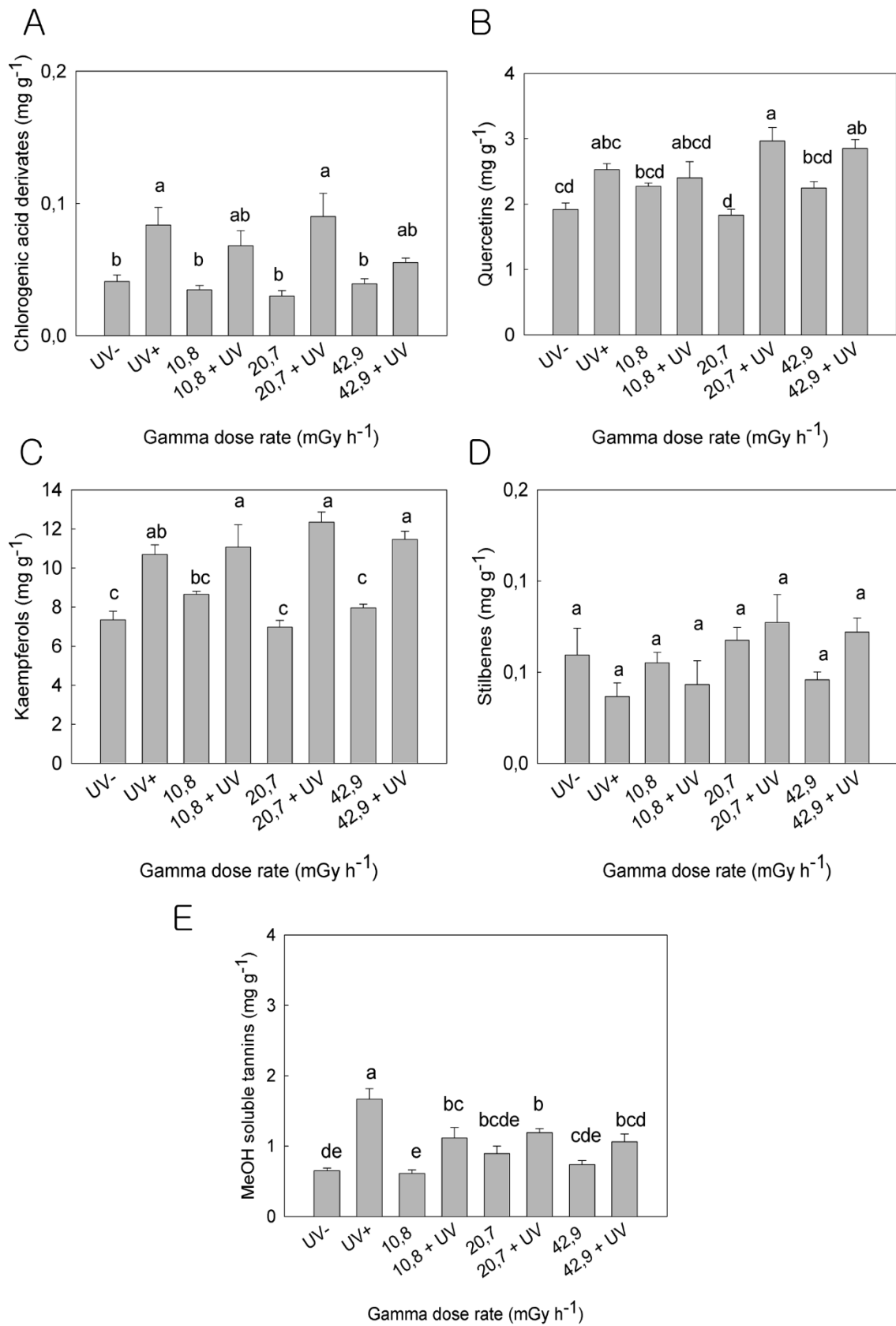


Fig. 3

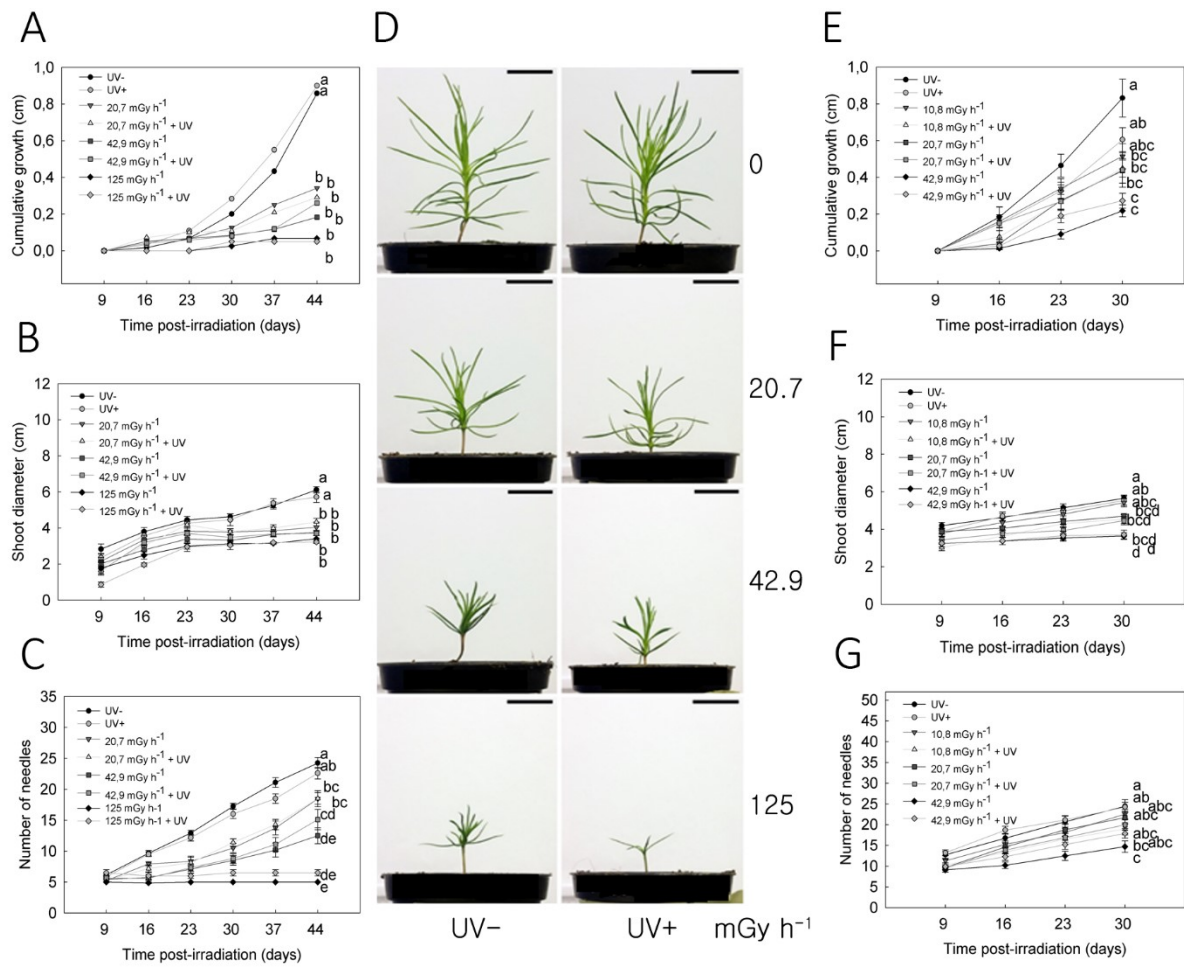


Fig. 4

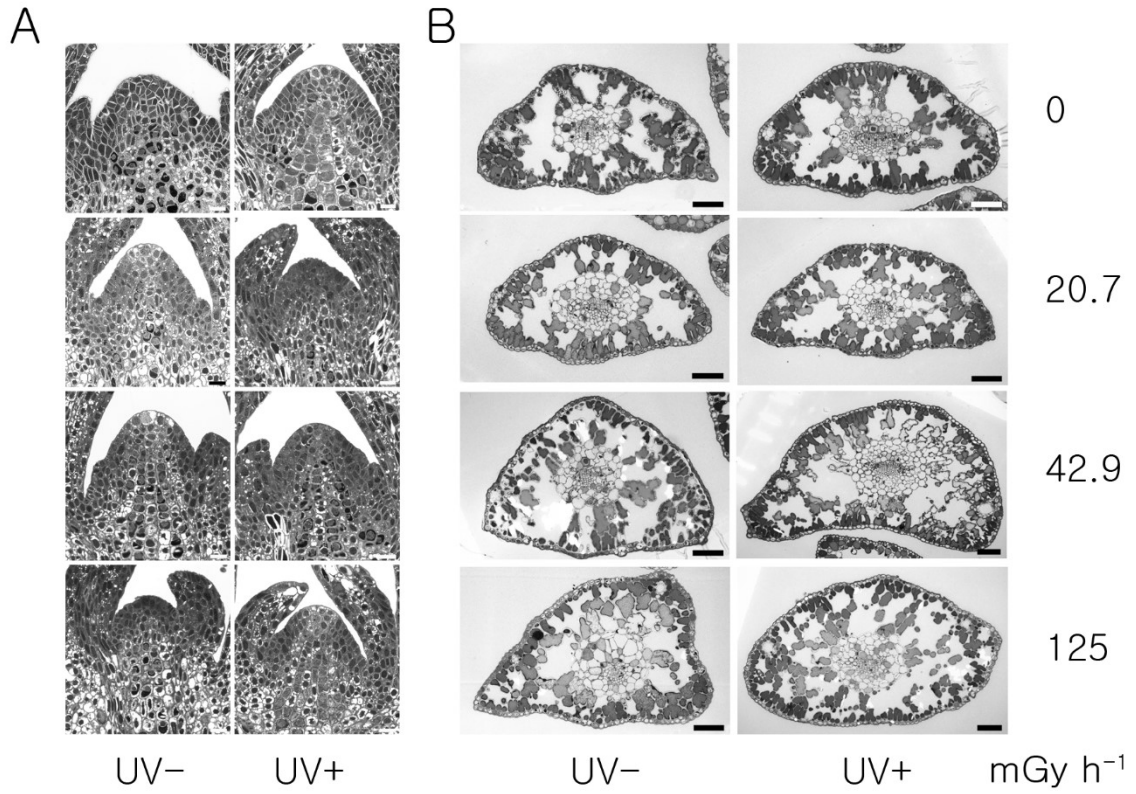


Fig. 5

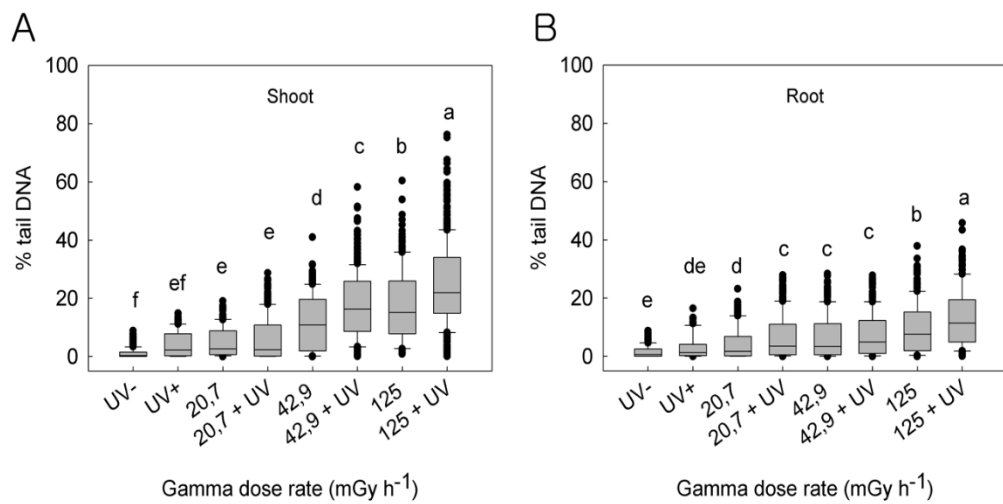


Fig. 6

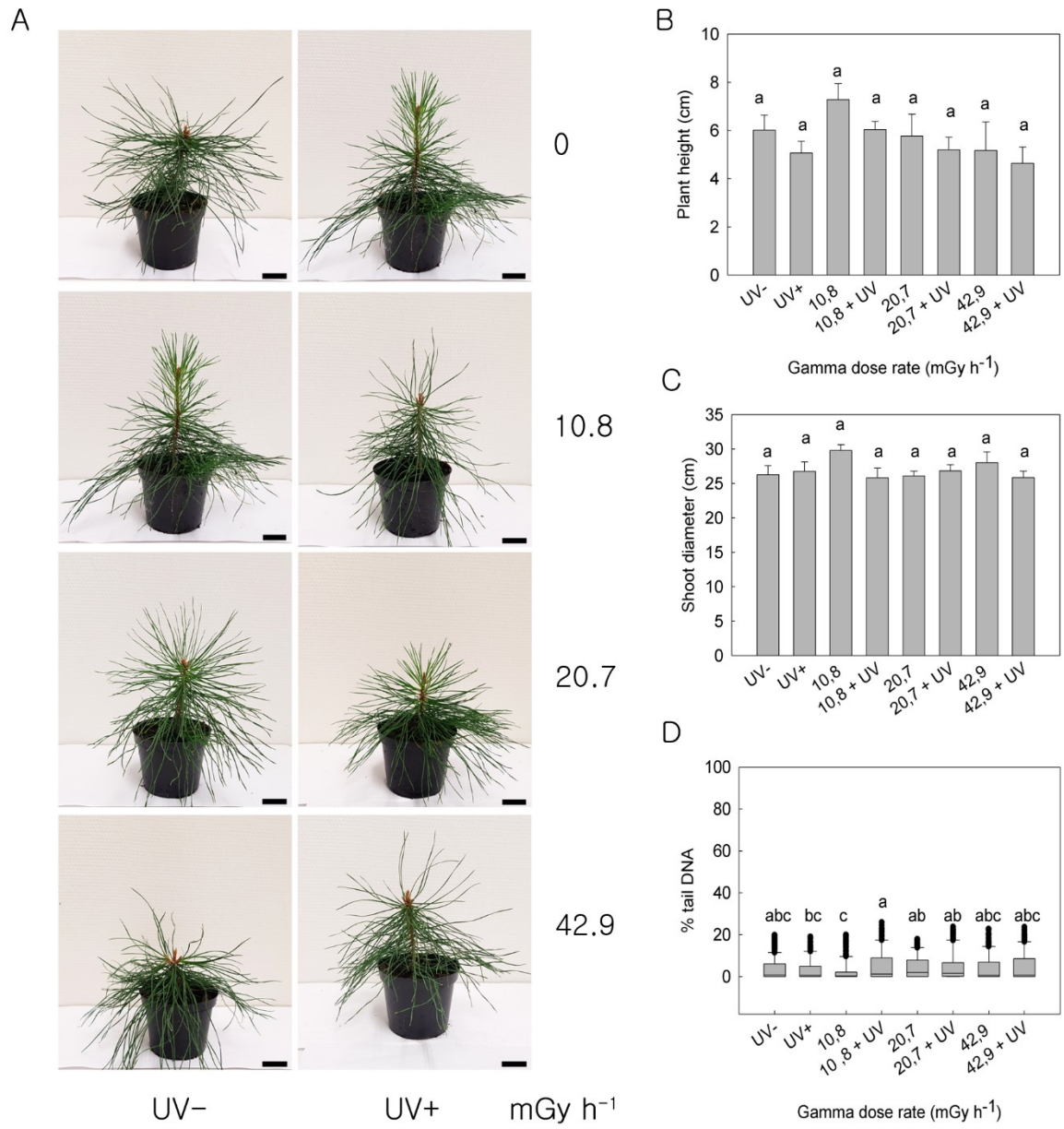


Fig. 7

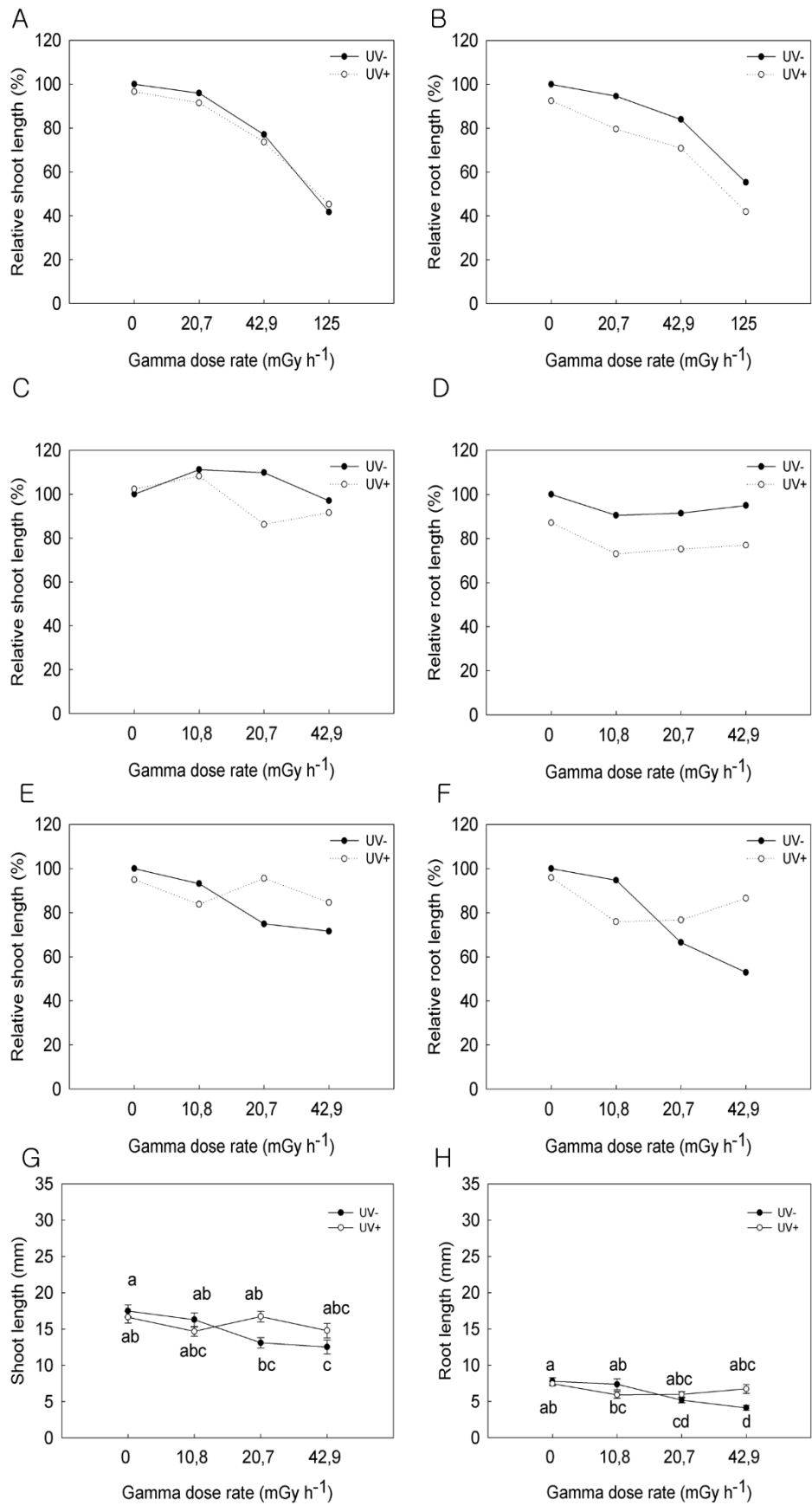


Fig. S1

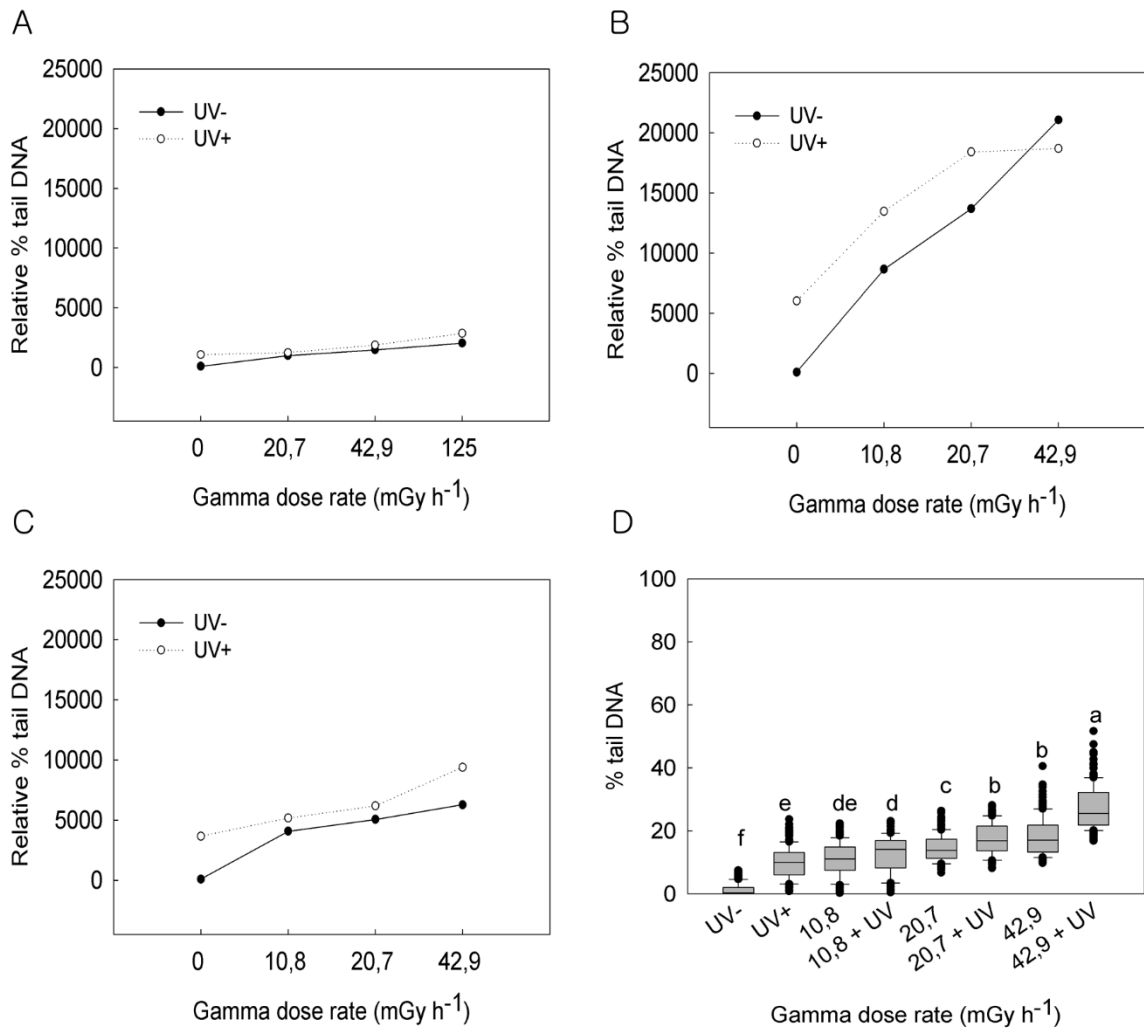
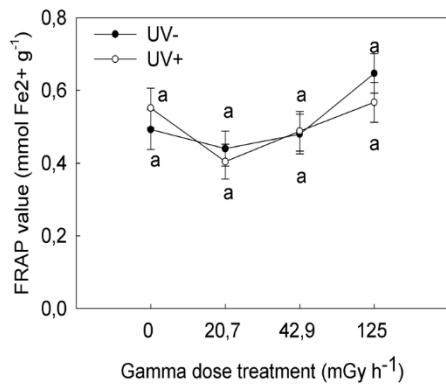


Fig. S2

A



B

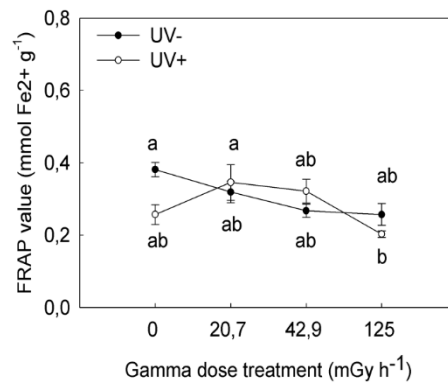


Fig. S3

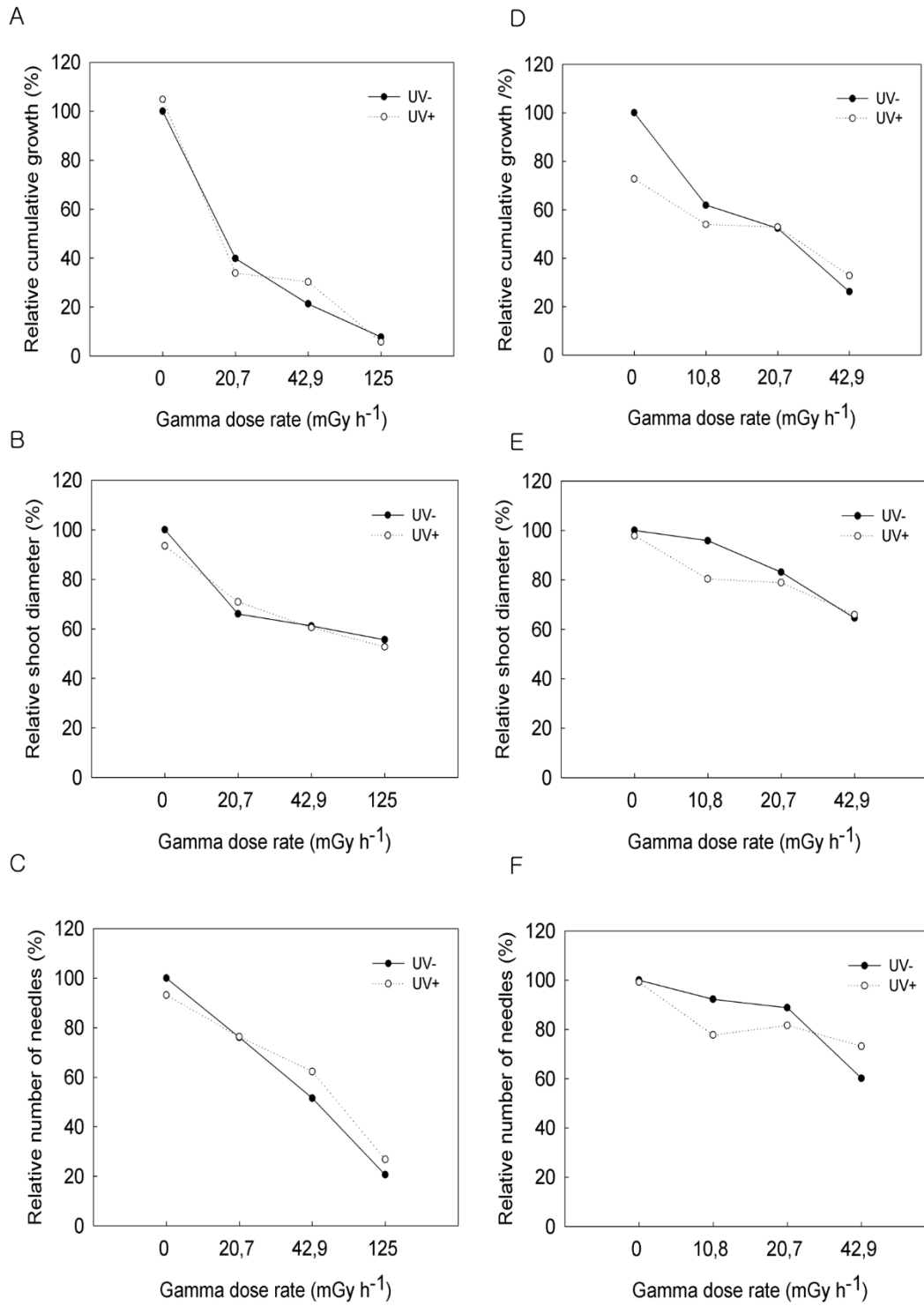


Fig. S4

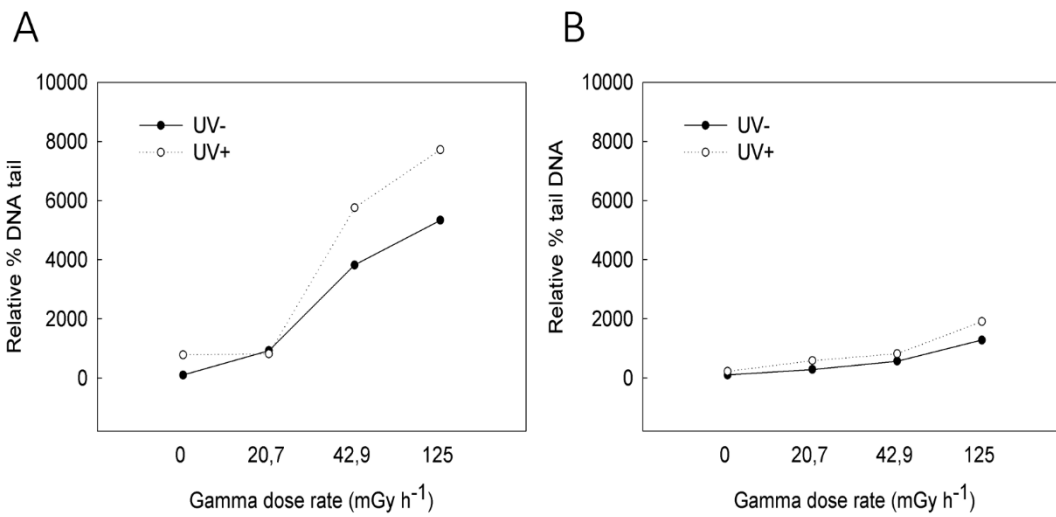


Fig. S5

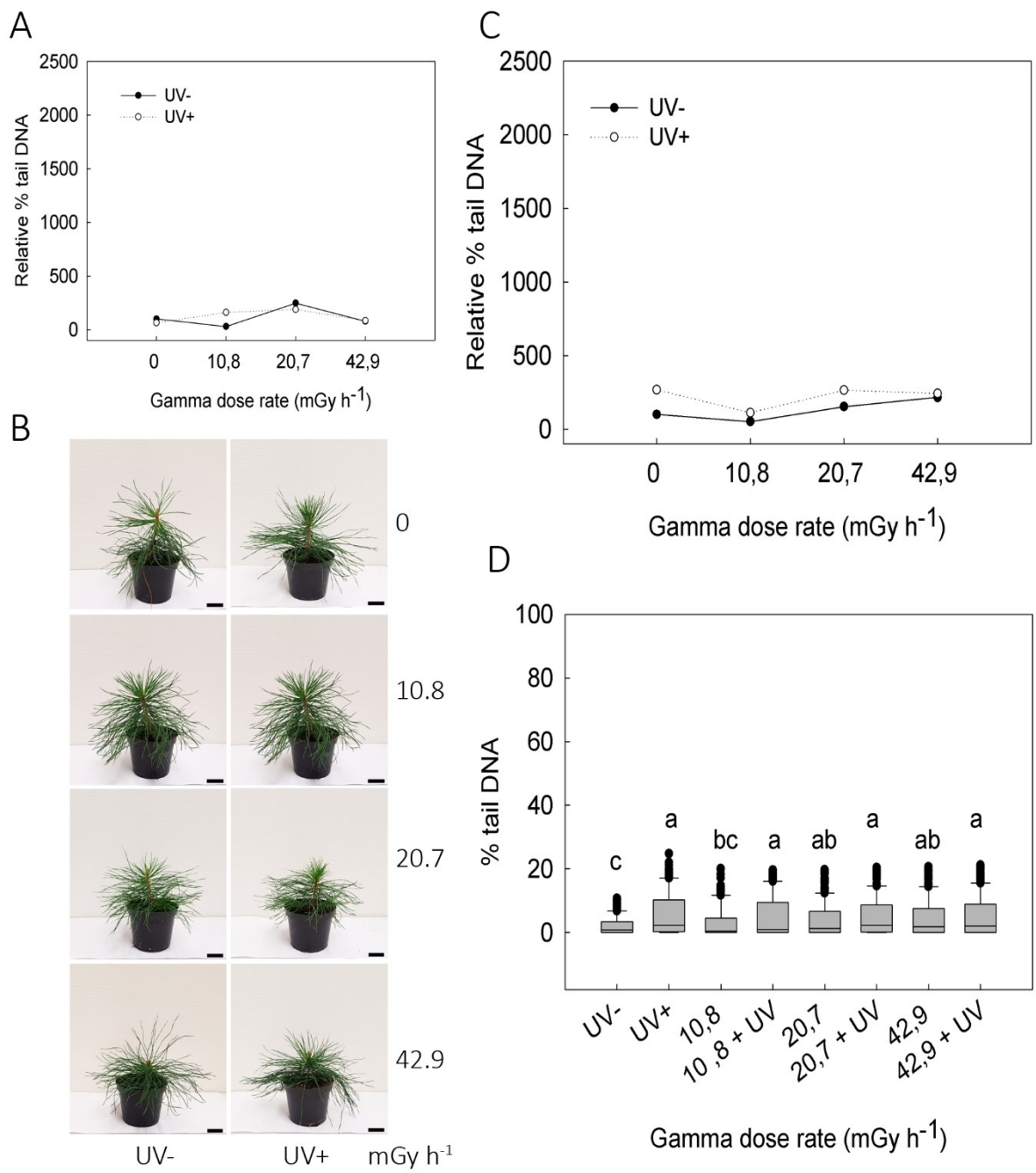


Fig. S6