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Seed Gamma Irradiation of Arabidopsis thaliana ABA-Mutant Lines Alters Germination and Does Not Inhibit the Photosynthetic Efficiency of Juvenile Plants

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Abstract

Plant growth response to γ-irradiation includes stimulating or inhibitory effects depending on plant species, dose applied, stage of ontogeny and other factors. Previous studies showed that responses to irradiation could depend on ABA accumulation and signaling. To elucidate the role of ABA in growth and photosynthetic responses to irradiation, lines Col-8, *abi3-8* and *aba3 -1* of *Arabidopsis thaliana* were used. Seeds were γ-irradiated using ^{60}Co in the dose range 50-150 Gy. It was revealed that the dose of 150 Gy affected germination parameters of *aba3 -1* and Col-8 lines, while *abi3-8* line was the most resistant to the studied doses and even showed faster germination at early hours after γ-irradiation at 50 Gy. These results suggest that susceptibility to ABA is probably more important for growth response to γ-irradiation than ABA synthesis. The photosynthetic functioning of 16-day-old plants mainly was not disturbed by γ-irradiation of seeds, and no indication of photosystem II photoinhibition was noticed, revealing the robustness of the photosynthetic system of *A. thaliana*. Glutathione peroxidase activity and ABA concentrations in plant tissues were not affected in the studied dose range. These results contribute to the understanding of germination and photosynthesis fine-tuning and of mechanisms of plant tolerance to ionizing radiation.

Keywords

gamma irradiation, ABA signaling, *aba3 -1*; *abi3-8*, glutathione peroxidase, photosynthetic parameters, germination indices

Introduction

Seed germination is one of the most important stages of the plant life cycle, and even subtle changes at this stage eventually affect the whole plant growth and productivity.[1] Germination process occurs as a crosstalk of many signaling molecules, including plant phytohormones such as abscisic acid, gibberellins, and ethylene, and reactive molecules such as hydrogen peroxide, nitric oxide, and hydrogen sulphide.[1,2] Abiotic stress factors are capable of disrupting this complex network and can lead to delay or, in specific cases, acceleration of germination and early growth processes. Therefore, the intensity of abiotic stress factors and changes in growth and development of plants do not usually have a linear relationship.[3,4] Responses of plants to low-dose stress exposure include a broad range of stimulating (hormetic) effects and have possible agricultural implementation.[5] Seed treatment can induce the same or even more pronounced stimulating effect as a treatment on vegetative stages of development,[6] and this makes seeds an appropriate choice for studying responses to low-dose stress exposure.

Plants are constantly exposed to ionizing radiation from natural and anthropogenic sources, while elevated levels of radioactivity can be considered as an abiotic stress factor.

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γ-Radiation is capable to induce overproduction of reactive oxygen species (ROS), highly reactive and potentially harmful molecules.[7-9] ROS are involved in the regulation of many metabolic processes,[10] including stress response,[11] stomatal movement,[12] photosynthesis and photorespiration.[13] Therefore, plants have evolved very efficient ROS-scavenging mechanisms employing enzymatic (for example, various peroxidases, catalase, superoxide dismutase, glutathione reductase, etc.) and non-enzymatic components (phenolic compounds, carotenoids, flavonoids, ascorbate, glutathione, thioredoxins, alkaloids, α-tocopherol, and some amino acids).[14] In the middle of the complex network of stress responses many researchers place crosstalk between abscisic acid (ABA) and ROS-related signalling pathways.[15,16] ABA is a phytohormone, the level of which rises in response to different stresses.[17] It is essential for reserves' acquisition and desiccation tolerance during seed development and it induces dormancy by inhibiting germination.[18] ABA plays an important role in hormetic responses in plants, which was proven by the wide range of growth-related hormetic dose-responses, which were observed after application of exogenous ABA to different *Arabidopsis thaliana* L. lines.[19] There is also evidence of important role of ABA and ROS interactions during growth responses of plants to ionizing radiation. Qi et al[7] showed that 50 Gy irradiation led to acceleration of growth processes in wild-type plants of *A. thaliana*, but not for the ABA-deficient mutant line *aba2 -1*. This line also did not accumulate H_2O_2 after irradiation, while wild-type plants had higher concentration of this ROS.[7]

To prove the role of ABA and ROS interconnection in response to γ-irradiation, a transcriptomic analysis of barley embryos after acute low-dose and high-dose γ-irradiation of seeds has recently been performed. It revealed expression programs that might be responsible for growth stimulation and inhibition, respectively, at the later stages of ontogeny.[9] The authors concluded that the positive effect on growth and development of low-dose irradiated plants might be related to the modulation of ROS-related responses that, in turn, might be connected to changes in phytohormone signaling pathways, namely to balance ABA signaling suppression and ROS accumulation, while the high-dose irradiation mostly activated DNA damage-related responses and various peroxidases.[9]

Following the important role of ABA in plant responses to seed irradiation, in this study we sought to evaluate the responses to acute γ-radiation in the model radioresistant plant species *A. thaliana* ABA-mutant lines in order to reveal any dose-dependent or genotype-dependent response in terms of different aspects of germination dynamics, growth, photosynthetic efficiency, ABA content, and also glutathione peroxidise (GPX) activity. Plant glutathione peroxidases can function as redox transducers in ABA signaling, and their increased activity may confer tolerance to various abiotic stresses.[20] The chosen mutant lines are deficient in ABI3 protein (*abi3-8* line), which is essential for seed maturation and regulates the transition between embryo maturation and early seedling development, and ABA3 protein (*aba3 -1* line), which is involved in the last step of ABA biosynthesis and takes part in various abiotic stress responses.

Results

Germination Assay

We aimed to assess the dose-dependent and genotype-dependent responses of *A. thaliana* germination processes to γ-irradiation of seeds in doses 50, 100, and 150 Gy. Twenty-two single-value germination indices were estimated (Supplementary Data 1). Important aspects that characterize the germination dynamics, such as time, rate, homogeneity, and synchrony[21] were measured. Several indices showing the most evident germination changes after γ-irradiation of seeds are visualized on Figure 1.

Statistically significant differences were observed for *aba3 -1* genotype in 150 Gy γ-irradiated group, compared with non-irradiated seeds in terms of the mean germination time, also called germination resistance (the average length of time required for maximum germination of a seed lot) that was higher for the irradiated *aba3 -1* seeds ($p = 0.007$, Figure 1A).

The weighted germination percentage, calculated by giving maximum weight to the seeds that germinate first and decreasing weight to the seeds that germinate afterwards, was significantly decreased in Col-8 plants irradiated with 150 Gy, compared to non-irradiated Col-8 ($p = 0.047$). The weighted germination percentage was also significantly lower for *aba3 -1* seeds γ-irradiated with 150 Gy dose, compared to non-irradiated *aba3 -1* seeds ($p = 0.035$, Figure 1B).

The Kotowski's coefficient of velocity of germination (CVG, which is the inverse of mean germination time multiplied by 100%), was calculated for the assessment of the germination rate. This index was significantly lower for the seeds of *aba3 -1* irradiated with a dose of 150 Gy, as compared to non-irradiated seeds ($p = 0.009$, Supplementary Data 2, Figure 14 in Supplementary Data 3).

Timson's index or germination energy index (GEI), which is the progressive total of cumulative germination percentage recorded at specific intervals for a set period of time, is also used to measure the germination rate. Unlike CVG that measures the germination frequency, GEI measures the time in decreasing order, attributing to the faster seeds a weight corresponding to the greater time.[19] GEI values were decreasing with dose for *aba3 -1* seeds, reaching statistically significant decline in seeds γ-irradiated at the dose of 150 Gy (with $p < 0.05$). GEI value for Col-8 seeds was also significantly decreased after 150 Gy irradiation (Figure 1C).

Synchronization index or uncertainty of the germination process (U index) is a measurement of germination synchrony, low values of which indicate more synchronized germination.[21] Thus, *aba3 -1* seeds irradiated with γ-rays at maximal studied dose of 150 Gy showed significantly higher value of uncertainty of the germination process ($p = 0.008$) compared to non-irradiated control *aba3 -1* seeds (Supplementary Data 2, Figure 20 in Supplementary Data 3).

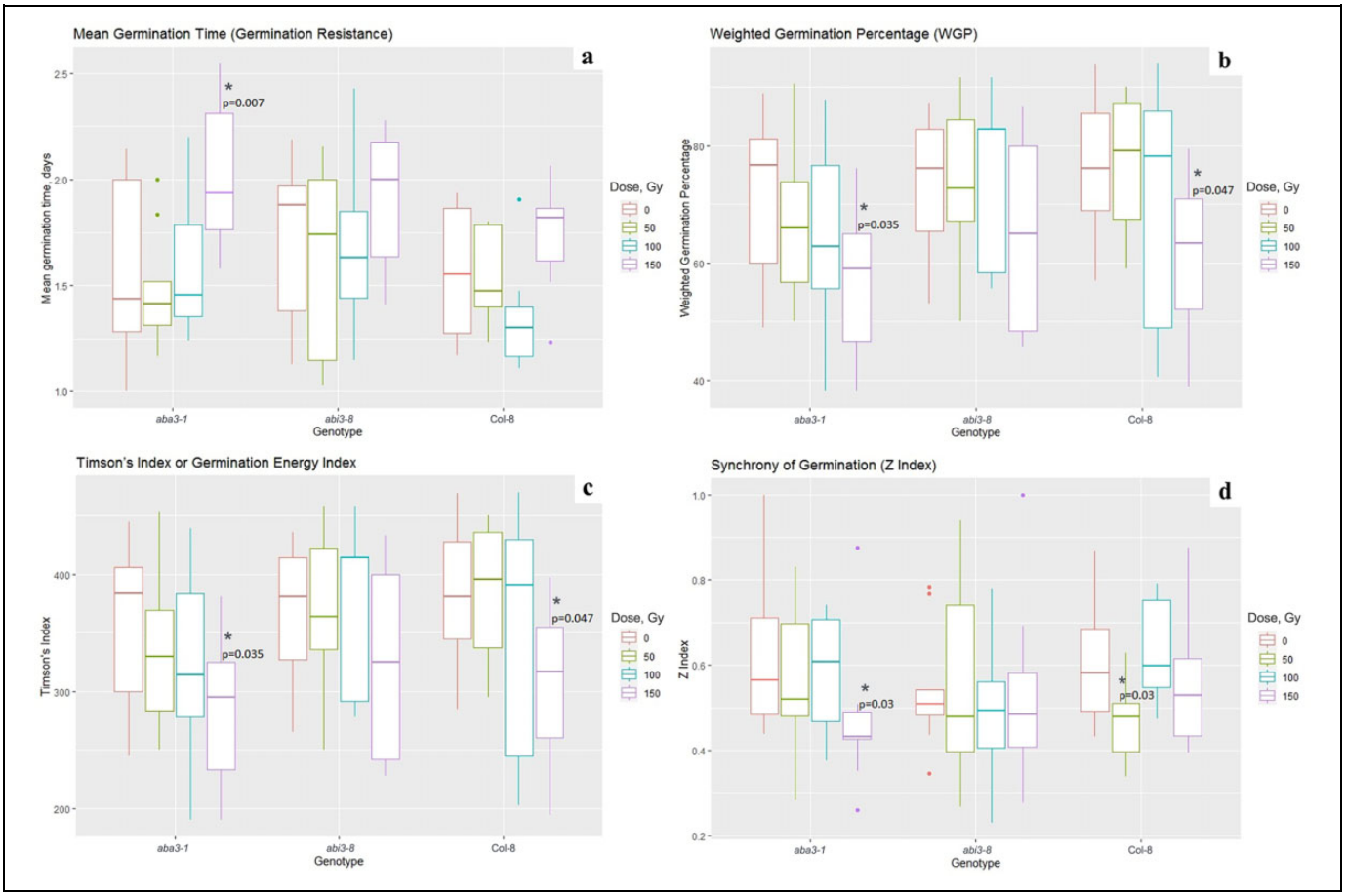

Figure 1. Main germination parameters assessed (R version 3.6.3). **a.** Mean germination time, days. **b.** Weighted germination percentage, %. **c.** Germination energy index. **d.** Germination synchrony Z index. * – Statistically significant difference ($p \leq 0.05$) compared with the non-irradiated control of the same genotype (Mann-Whitney U-test). The exact p-values are denoted under asterisk. Germination parameters were assessed based on the 3 independent experiments, 60-90 plants per genotype per dose in each.

Z index is another parameter of the germination synchrony. Z produces a number if and only if there are 2 seeds finishing the germination process at the same time, showing thus the degree of germination overlapping.[21] Z index is equal to 1 when the germination of all seeds occurs at the same time, and $Z = 0$ when at least 2 seeds could germinate, one at each time.[21] Z index showed statistically significant differences between higher values for non-irradiated *aba3 -1* seeds and lower values for *aba3 -1* γ-irradiated at 150 Gy ($p = 0.03$), as well as between higher Z for non-irradiated Col-8 and lower Z values for Col-8 seeds γ-irradiated at the dose of 50 Gy ($p = 0.03$) (Figure 1D).

Non-irradiated seeds of the 2 ABA-mutant lines showed differences in terms of Coefficient of variation of the germination time and standard error of germination rate, that were higher for *aba3 -1* ($p = 0.06$ and 0.04, respectively) (Supplementary Data 2, Figures 22, 23 in Supplementary Data 3).

There were no dose-dependent significant changes of germination indices in *abi3-8* seeds. This line however showed a faster germination at earlier hours (up to 31 hour) after lower dose irradiation (50-100 Gy, Figure 2). This stimulating effect was statistically significant in 50 Gy irradiated group (Figure 2). Genotype-dependent features of irradiated plants over time are also shown on Figure S1.

Leaf Area and Biomass

Figure 3 demonstrates the average leaf area and the average weight of one plant. In plants grown from non-irradiated *aba3 -1* seeds the mean value of the leaf area was significantly lower than in non-irradiated Col-8 ($p = 0.011$). In *aba3 -1* irradiated with the dose of 150 Gy the mean leaf area value was significantly lower than in the non-irradiated control ($p = 0.004$). The leaf area decreased significantly in Col-8 and *abi3-8* irradiated with the doses of 100 and 150 Gy as compared to the control of the same genotype (Figure 3A).

The fresh weight significantly decreased in plants grown from seeds irradiated with the doses of 100 and 150 Gy for all studied genotypes (Figure 3B).

As expected, the leaf area and the biomass accumulation values were correlated. Pearson's correlation coefficient is equal to 0.93 ($p = 2.2 \cdot 10^{-16}$).

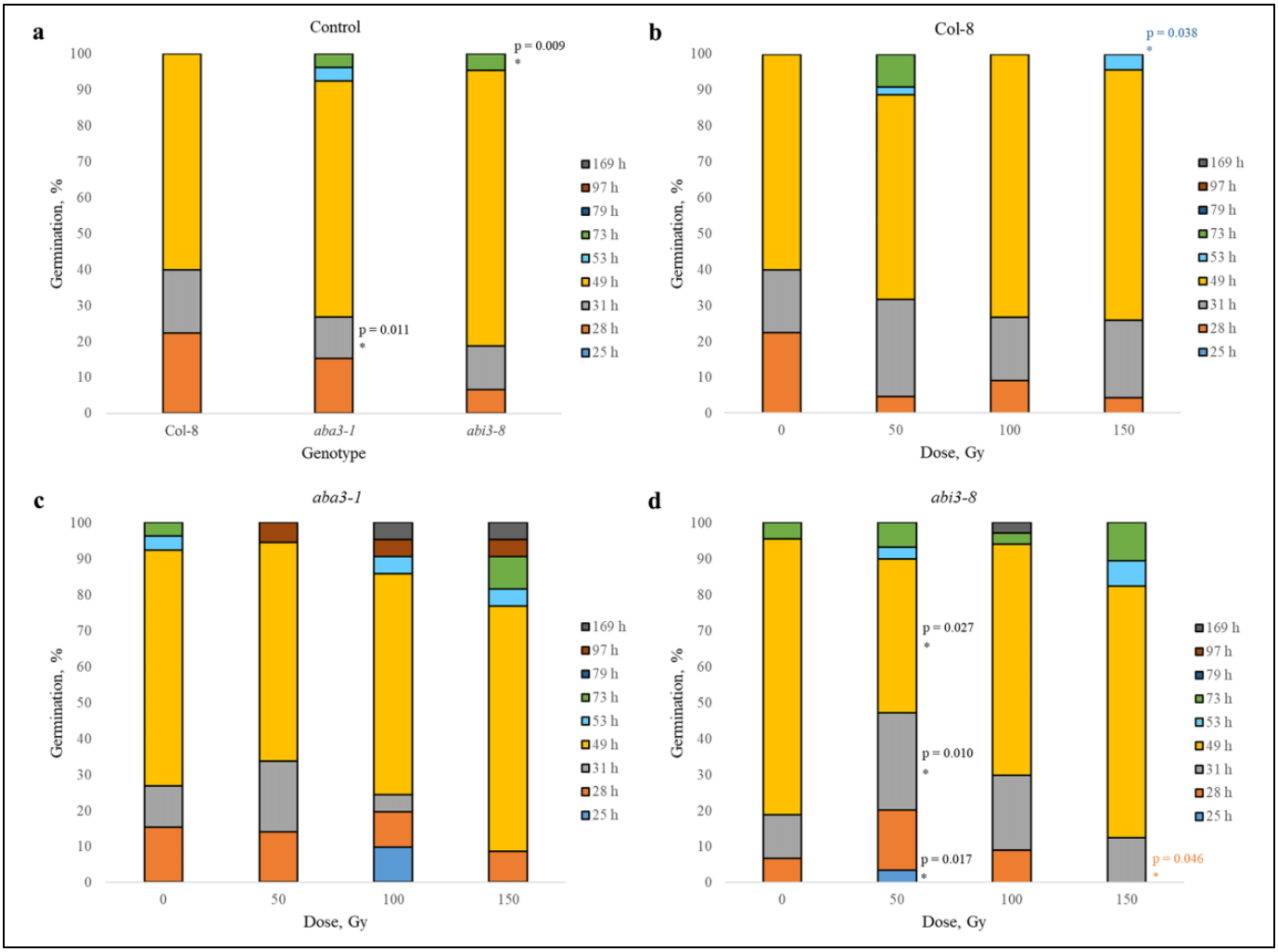

Figure 2. Time dynamics of seed germination. **a.** Comparison of germination of wild-type and mutant lines without γ-irradiation of seeds. **b.** Dynamics of germination of Col-8 line after γ-irradiation of seeds (0-150 Gy). **c.** Dynamics of germination of *aba3 -1* line after γ-irradiation of seeds (0-150 Gy). **d.** Dynamics of germination of *abi3-8* line after γ-irradiation of seeds (0-150 Gy). Y axis—percentage of seeds that germinated at a specific hour, taking the number of germinated seeds as 100%; X axis—the applied dose of γ-radiation; *—statistically significant differences (Mann-Whitney U-test) as compared to (**a.**) wild type Col-8 or (**b., c., d.**) non-irradiated seeds of the same genotype. The exact p-values are denoted above asterisk. Germination parameters were assessed based on the 3 independent experiments, 60-90 plants per genotype per dose in each.

GPX Activity and ABA Content

Abscisic acid content is presented in Table 1. Figure 4 shows the glutathione peroxidase activity assessed for the analysed genotypes. There were no statistically significant differences between the groups.

Photosynthetic Parameters

We sought to investigate the remote effects of gamma-irradiation of *A. thaliana* seeds on the juvenile plants' photosynthetic performance in the 2 aba-impaired mutants and the wild type. Table 2 shows the studied chlorophyll fluorescence parameters.

Fv/Fm ratio provides an estimate of the maximum quantum efficiency of PSII photochemistry (PSII maximum efficiency). In plants grown from non-irradiated *aba3 -1* seeds the mean value of this parameter was significantly higher than Fv/Fm ratio in non-irradiated Col-8 and *abi3-8* plants (as assessed by Kruskal-Wallis test, $p = 0.0001$, Figure 5B). The values of PSII maximum efficiency were approximately the same in plants grown from γ-irradiated Col-8 and *aba3 -1* seeds as compared to the non-irradiated controls, while in *abi3-8* plants Fv/Fm increased slightly reaching a significantly higher mean value in plants grown from irradiated with the dose of 150 Gy *abi3-8* seeds as compared to non-irradiated *abi3-8* ($p = 0.004$, Table 2).

Coefficients of photochemical fluorescence quenching qP and qL estimate the fraction of open PS II reaction centres. The qP is based on concept of separated PS II antennae, while the qL assumes interconnected PS II antennae, which appears the more realistic situation in leaves.[22] For an accurate assessment of the redox state of the QA (primary quinone electron

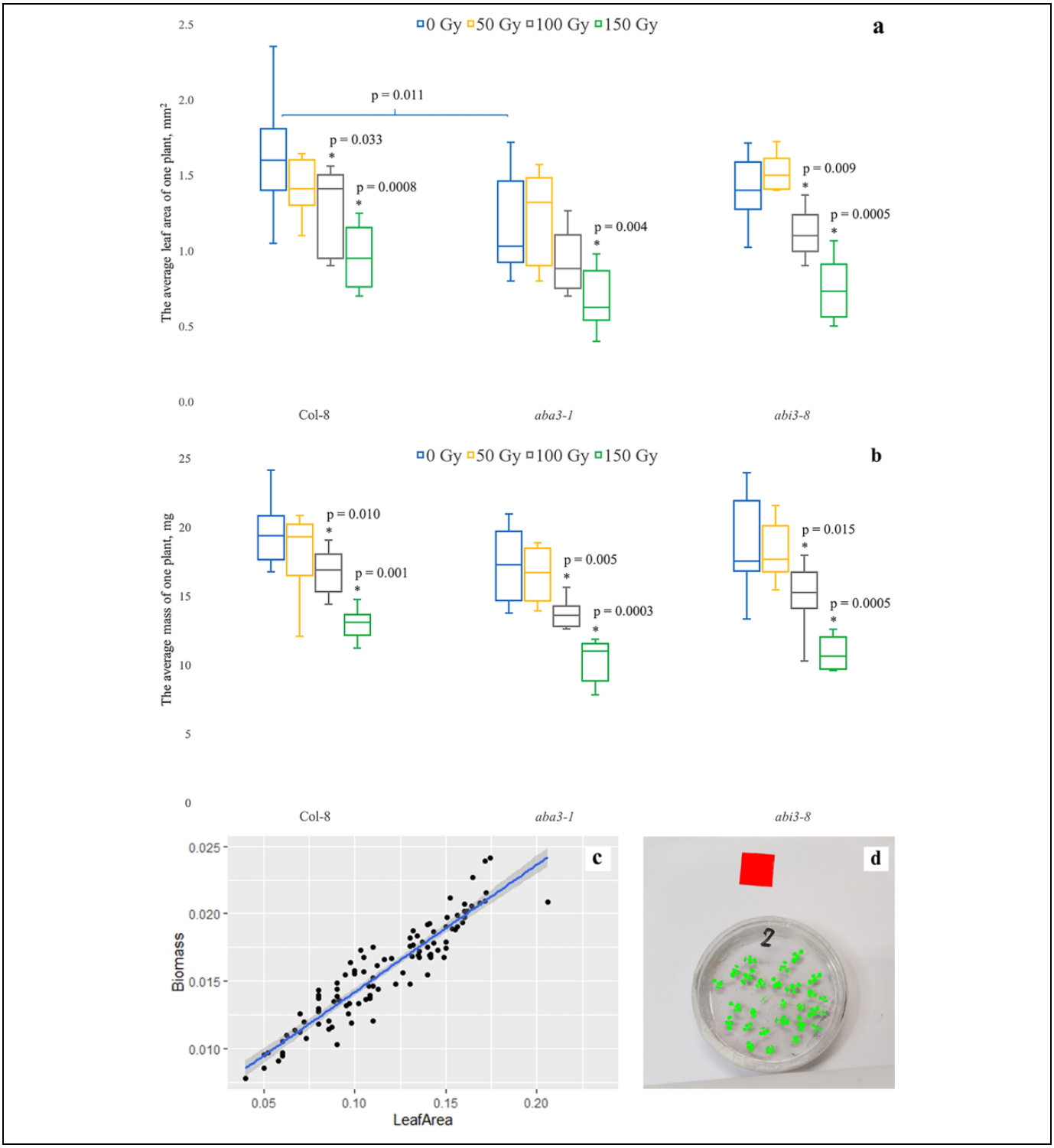

Figure 3. Morphological parameters of irradiated plants. **a.** The average leaf area of one plant, mm^2. **b.** The average weight of one plant, mg. **c.** QQ plot of linear relationship between biomass and leaf area. **d.** The example of Easy Leaf Area software usage for assessing average leaf area. * – Statistically significant difference ($p \leq 0.05$) compared with the non-irradiated control of the same genotype (Mann-Whitney U-test). The exact p-values are denoted under asterisk. Morphological parameters were assessed based on the 3 independent experiments, 60-90 plants per genotype per dose in each.

Table 1. ABA Concentrations in Leaves of 16-Day-Old *A. thaliana* Plants After Gamma Irradiation of Seeds.

	Col-8			*aba3-1*			*abi3-8*		
	Min	Med	Max	Min	Med	Max	Min	Med	Max
Dose					μM				
0 Gy	0.194	0.260	0.406	0.179	0.190	0.195	0.111	0.209	0.225
50 Gy	0.117	0.170	0.178	0.146	0.276	0.287	0.142	0.165	0.186
100 Gy	0.219	0.230	0.241	0.148	0.154	0.160	0.119	0.157	0.190
150 Gy	0.104	0.125	0.146	0.080	0.094	0.107	0.075	0.102	0.130

Note: ABA analysis was performed for the third independent experiment. 2-3 pooled replicates (0.3 g) was used for each condition, 2 technical replicates were performed. Med—median value; Min—minimal value; Max—maximal value. No statistically significant differences were found.

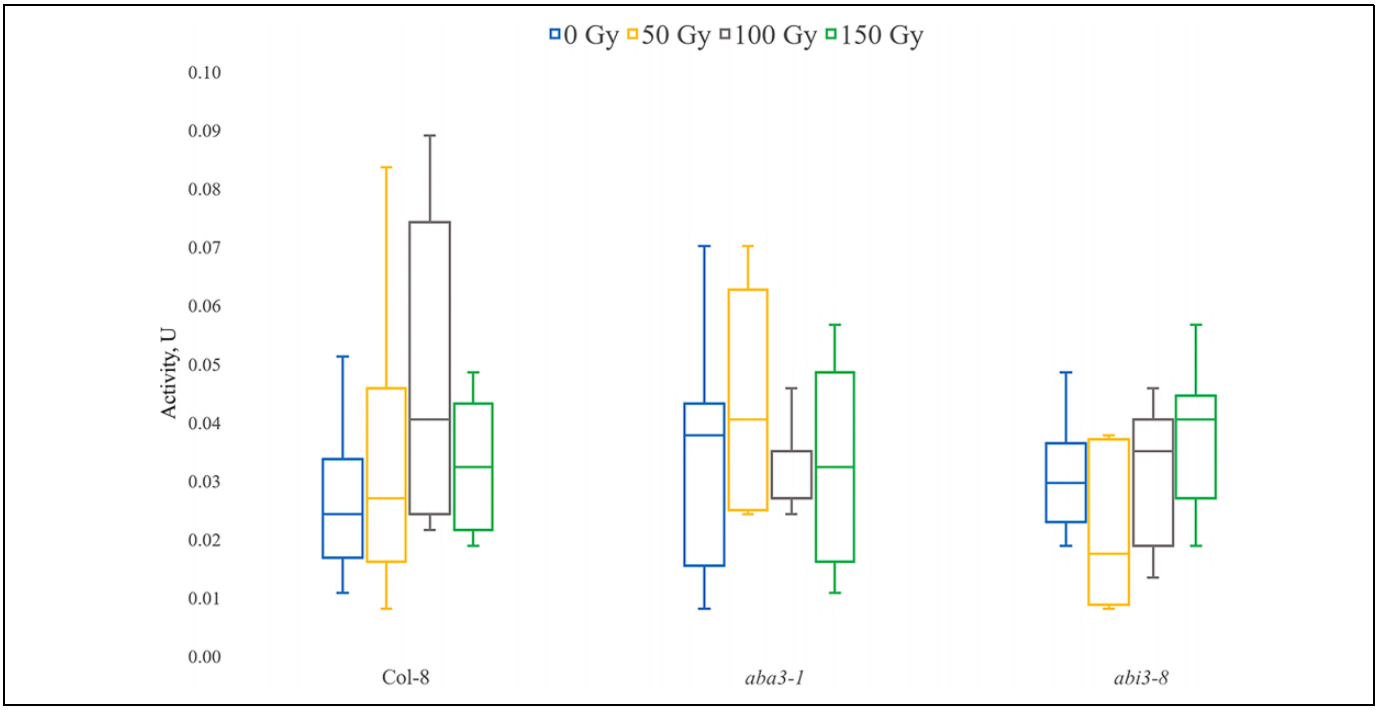

Figure 4. Glutathione peroxidise activity, International Units (U). GPX activity was assessed based on the 3 independent experiments. Three pooled samples (0.13 g) were analysed for each dose for each genotype in each experiment.

acceptor of PSII) pool qL and not qP is recommended to be used.[23] Both coefficients of photochemical fluorescence quenching were higher in non-irradiated *abi3-8* plants as compared to non-irradiated wild type ($p = 0.029$ for qL and $p = 0.04$ for qP) (Figure 5C, D). Both qL and qP were significantly higher in *abi3-8* irradiated with 50 Gy and in Col-8 irradiated with doses of 100 and 150 Gy as compared to non-irradiated plants of the same genotype (Table 2).

Both qN and NPQ are associated with non-photochemical quenching of excitation energy by thylakoid lumen pH- and zeaxanthin-dependent processes. NPQ values in plants grown from non-irradiated seeds of wild type and ABA-impaired genotypes showed a statistically significant difference between *aba3 -1* and *abi3-8* ($p = 0.039$) and between *aba3 -1* and Col-8 ($p = 0.007$) with lower values in *aba3 -1* (Figure 6, Table 2).

Y(II) is referred to as operating efficiency of PSII photochemistry.[24] This parameter estimates the efficiency at which light absorbed by PSII is used for QA reduction and has previously been termed $\Delta F/Fm'$ and $\phi PSII$.[23] Y(II) values of non-irradiated plants showed a significant difference between *aba3 -1* and *abi3-8* ($p = 0.1 \cdot 10^{-4}$) and between *aba3 -1* and Col-8 ($p = 0.2 \cdot 10^{-4}$) with higher mean values in *aba3 -1* (Figure 5A, Table 2). *abi3-8* plants irradiated with the dose of 150 Gy had higher operating efficiency of PSII photochemistry compared with the non-irradiated control of the same genotype ($p \leq 0.01$, Table 2).

Y(II) is used to estimate the relative rate of linear electron transport ETR. ETR was significantly lower in Col-8 irradiated with the dose of 50 Gy compared to the control ($p \leq 0.05$). For *abi3-8* genotype the 100 Gy irradiated plants showed the highest values of Y(II) ($p \leq 0.01$, Table 2).

Table 2. Photosynthetic Parameters Estimated for Leaves of 16-Day-Old *A. thaliana* Plants After Gamma Irradiation of Seeds.

	Dose, Gy	Fv/Fm	qP	qL	qN	NPQ	Y(II)	ETR
Col-8	0	0.755 ± 0.004	0.978 ± 0.003	0.919 ± 0.009	0.083 ± 0.006	0.087 ± 0.005	0.731 ± 0.003	1.711 ± 0.249
	50	0.756 ± 0.004	0.981 ± 0.003	0.930 ± 0.009	0.089 ± 0.005	0.087 ± 0.003	0.737 ± 0.003	**0.793 ± 0.051****
	100	0.756 ± 0.003	**0.987 ± 0.002****	**0.950 ± 0.008****	0.091 ± 0.005	0.089 ± 0.003	0.733 ± 0.003	1.280 ± 0.134
	150	0.754 ± 0.004	**0.986 ± 0.002***	**0.948 ± 0.007***	0.092 ± 0.004	0.086 ± 0.003	0.729 ± 0.003	1.160 ± 0.085
aba3-1	0	**0.772 ± 0.003**$^{\#\&}$	0.982 ± 0.003	0.931 ± 0.009	0.086 ± 0.005	**0.081 ± 0.003**$^{\#\&}$	**0.749 ± 0.002**$^{\#\&}$	1.108 ± 0.103
	50	0.778 ± 0.002	0.985 ± 0.002	0.938 ± 0.008	0.090 ± 0.004	0.078 ± 0.004	0.750 ± 0.002	1.047 ± 0.082
	100	0.776 ± 0.004	0.986 ± 0.002	0.926 ± 0.011	0.092 ± 0.004	0.076 ± 0.004	0.753 ± 0.002	1.166 ± 0.149
	150	0.763 ± 0.006	0.985 ± 0.003	0.949 ± 0.010	0.091 ± 0.006	0.081 ± 0.006	0.748 ± 0.003	0.922 ± 0.069
abi3-8	0	**0.755 ± 0.003**$^{\&}$	**0.986 ± 0.002**$^{\#}$	**0.949 ± 0.007**$^{\#}$	0.097 ± 0.004	**0.087 ± 0.003**$^{\&}$	**0.731 ± 0.003**$^{\&}$	1.226 ± 0.180
	50	0.761 ± 0.002	**0.992 ± 0.002***	**0.969 ± 0.006***	0.096 ± 0.004	0.081 ± 0.004	0.738 ± 0.002	1.037 ± 0.148
	100	0.760 ± 0.003	0.987 ± 0.002	0.953 ± 0.006	0.101 ± 0.003	0.089 ± 0.003	0.733 ± 0.003	**1.378 ± 0.176***
	150	**0.766 ± 0.002****	0.990 ± 0.002	0.959 ± 0.007	0.115 ± 0.006	0.090 ± 0.004	**0.741 ± 0.002****	0.807 ± 0.064

Data represents the mean ± SEM of 5 plants from the 3 biological replicates of the 3 individual experiments.
* – Statistically significant difference (U-test, $p \leq 0.05$) compared with the non-irradiated control of the same genotype.
** – Highly significant difference (U-test, $p \leq 0.01$) compared with the non-irradiated control of the same genotype.
# – Statistically significant difference (U-test, $p \leq 0.05$) compared with the non-irradiated wild type.
& – Statistically significant difference (U-test, $p \leq 0.05$) between the non-irradiated mutant lines.

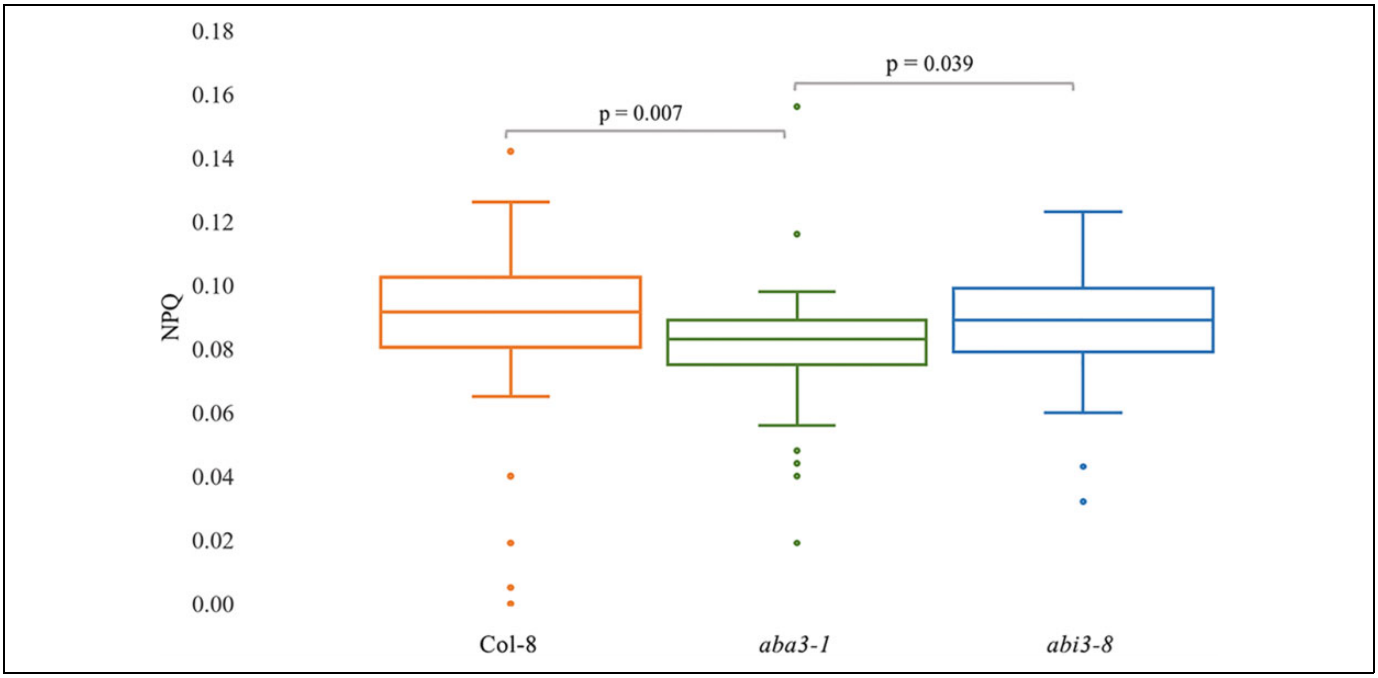

Figure 5. Photosynthetic parameters in plants grown from non-irradiated seeds of wild type and the 2 ABA-impaired mutants: **a.** Y(II). **b.** Fv/Fm ratio. **c.** qP. **d.** qL. Statistically significant differences (Kruskal-Wallis test followed-up by Dunn's test with Bonferroni adjustment) are shown as exact p-values.

Discussion

Abscisic acid is the main phytohormone responding to abiotic stresses. ABA regulates stomata closure as well as production of protective compounds.[25] During seed development, ABA controls mid to late stages of embryo maturation and desiccation tolerance through the B3 domain transcription factor family, including ABSCISIC ACID INSENSITIVE 3 (ABI3).[26] It also participates in plant vegetative development in a concentration-dependent manner, stimulating growth at low concentrations and inhibiting growth at high concentrations.[27] ABA important role in biphasic responses of plants to moderate stress exposure was confirmed in experiments with application of exogenous ABA.[19] Its involvement in germination dynamics and growth changes induced by γ-irradiation of *A. thaliana* seeds was studied in this work.

ABI3 and abi3-8

ABI3 (AT3G24650) is essential for seed maturation. It is a regulator of the transition between embryo maturation and

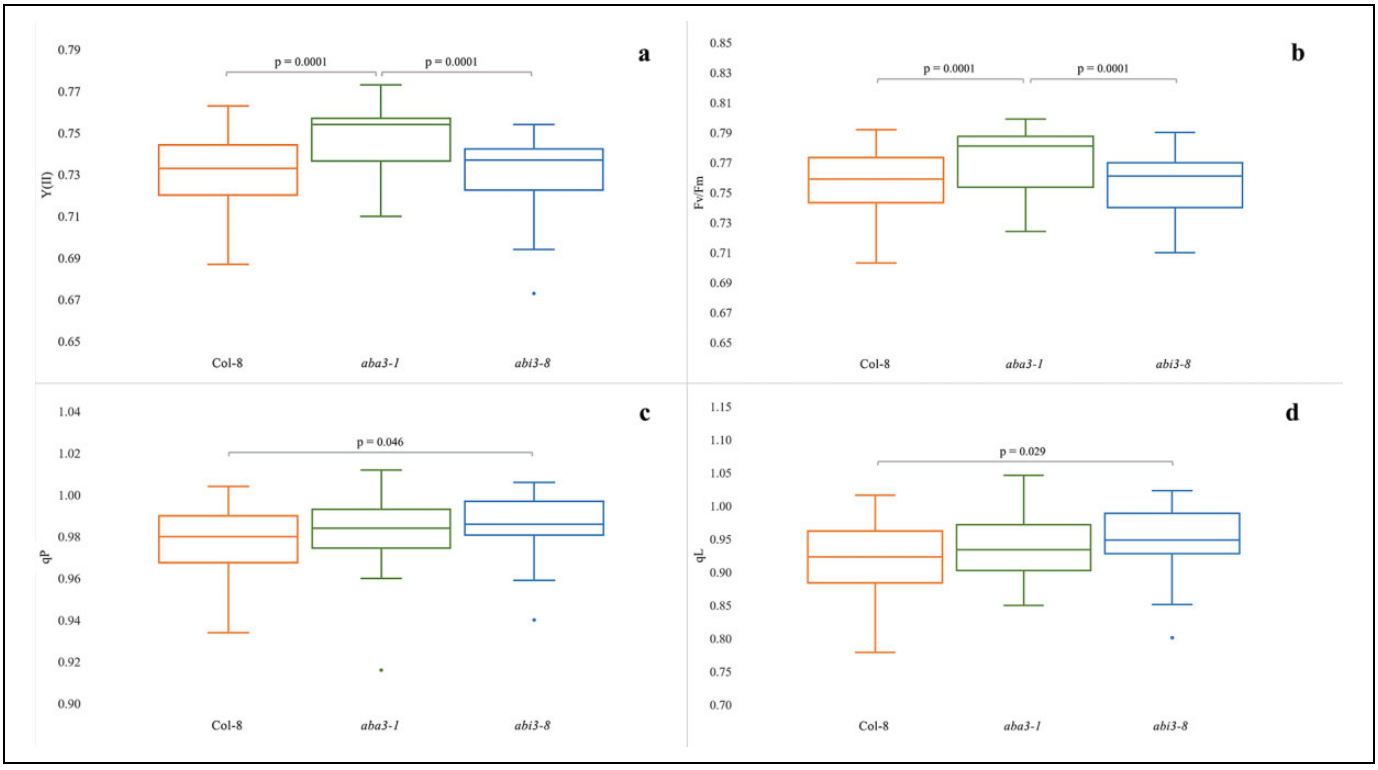

Figure 6. NPQ in plants grown from non-irradiated seeds of wild type and 2 ABA-impaired genotypes. Statistically significant differences (Mann-Whitney U-test) are shown as exact p-values. NPQ is based on analysis of 45 plants from 3 individual experiments.

early seedling development and is a central regulator in ABA signaling (TAIR, www.arabidopsis.org).

The abi3-8 mutation causes an amino acid substitution with conversion of leucine 298 to a phenylalanine within the B1 domain (C-to-T transition at position 1297).[28] This leucine is invariant in ABI3 orthologs identified so far. Perhaps this mutation disrupts the ABI3-ABI5 interaction, thus resulting in a preferential insensitivity to (+)-ABA.[28] Moreover, abi3-8 is also glucose-insensitive, showing green cotyledons and root growth on glucose plus ABA[28] and demonstrates a moderate level of thermoinhibition resistance at 34°C.[29] The abi3-8 is a "weak" allele with normal seed color, unlike embryos of strong *abi3* alleles (such as *abi3-3*, *abi3-4*, *abi3-5*, and *abi3-6*) that remain green throughout development.[30]

A recent assessment of transcriptomes in response to ABI3 by comparing developing *abi3-5* and wild type Ler seeds in order to ascertain direct and indirect ABI3-responsive target genes showed 884 up-regulated genes in *abi3-5* seed 15-16 days after flowering and 627 genes that were down-regulated compared to Ler.[31] Among up-regulated were the genes which products are involved in plastid translation, chlorophyll biosynthetic processes and photosynthesis.[31] It was therefore suggested that ABI3 regulates part of the light program in seeds and vegetative tissues.[32] The red-light signaling pathway and response to blue light were repressed in response to ABI3 (induced in *abi3-5* mutant[31]). These findings might be a possible explanation of the significantly higher values for coefficients of photochemical fluorescence quenching, estimating the fraction of open PSII reaction centers, in non-irradiated *abi3-8* plants as compared to non-irradiated wild type (Figure 5C, D).

ABA3 and aba3 -1

ABA3 (*AT1G16540*) encodes molybdenum cofactor sulfurase that is involved in the last step of ABA biosynthesis, the conversion of ABA-aldehyde to ABA. ABA3 participates in a plethora of biological processes such as auxin-activated signaling pathway, stomatal movement, defence response to bacteria, sugar mediated signalling pathway, response to abiotic stress, such as cold, heat, osmotic stress (TAIR, www.arabidopsis.org). ABA3 is also involved in protein import into chloroplast stroma and the *aba3* mutants are impaired in chloroplast protein import.[33] Watanabe et al[34] showed that anthocyanins in *aba3* plants after oxidative stress treatment accumulated at much lower levels, compared with wild type and *aba2* plants. In addition to the reduced anthocyanin pigmentation, *aba3* plants exhibited a typical symptom of oxidative injuries and reduced chlorophyll content.[34] This disrupted ability to cope with oxidative stress may be reflected in sufficiently more pronounced deterioration of morphological parameters of *aba3 -1* line after γ-irradiation in comparison with Col-8 (Figure 3).

The *aba3 -1* plants carry a G-to-A mutation in position 3707.[35] The *aba3 -1* mutant has reduced levels of ABA in vegetative tissues under normal and stress conditions, nevertheless endogenous ABA levels are higher than in most other

ABA-deficient mutants.[36] Because of excessive water loss, the ABA-deficient mutant *aba3 -1* is susceptible to water stress. Leaf temperature, measured by thermal imaging, was used as an indication of stomata conductance under water deficit, and *aba3 -1* had colder leaves than wild type.[35]

Although the mean leaf area value was significantly lower in plants grown from non-irradiated *aba3 -1* seeds than in non-irradiated Col-8, the effective photochemical quantum yield of photosystem II, Y(II), that estimates the photochemical use of excitation energy in the light was significantly higher for *aba3 -1* compared to the wild type. In plants grown from non-irradiated *aba3 -1* seeds the mean value of PSII maximum efficiency was also significantly higher than Fv/Fm in non-irradiated Col-8 and *abi3-8* plants (Figure 5). Fv/Fm measures the intrinsic quantum yield of PSII, and as such it should correlate with the maximum quantum yield of photosynthetic gas exchange[37] that is related to impaired stomatal closure in *aba3 -1*. Y(II) and the quantum yield of CO_2 assimilation with increasing light in leaves are characterized by linear relationship from a wide range of species, in which photorespiration was absent or suppressed.[38,39] The rate of consumption of NADPH and ATP are major factors that determine PSII operating efficiency in many situations. The supply of CO_2 from the atmosphere to the sites of carboxylation via the stomata, photorespiration, and the rate of transport of carbohydrates out of the cell can all influence the rate of NADPH and ATP utilization, and consequently the PSII operating efficiency.[23]

Excess light energy absorbed by the antenna light-harvesting proteins of PSII can be dissipated as heat through the non-photochemical quenching process. The extent of NPQ has been suggested to be associated with the number of quenching centers in the light-harvesting antenna.[40] Important to notice that NPQ trait itself shows significant biphasic response to increasing doses of stress.[41] A statistically significant decrease of NPQ observed in *aba3 -1* non-irradiated plants as compared to non-irradiated wild type and *abi3-8* plants might be explained by impaired function of stomata in ABA-deficient mutant, as heat dissipation depends on stomatal closure.[42] The dissipation of excess absorbed light energy is believed to play a key role in regulating light harvesting and electron transport and appears to be critical for the prevention of photooxidative damage to the photosynthetic apparatus.[23] The extent of NPQ in plants is strongly correlated with the levels of zeaxanthin and antheraxanthin that are formed from violaxanthin via the xanthophyll cycle, the process that might be weakened in *aba3* mutants impaired in chloroplast protein import.

Dose-Dependent Changes

Qi et al[7] provided strong evidence for the role of ABA in low dose gamma radiation-induced eustress. They showed that the responses of the ABA-deficient mutant line *aba2 -1* to low-dose gamma irradiation revealed that the germination index, primary root length, and fresh weight of 50 Gy gamma-irradiated mutant seedlings were not significantly different from those of the mutant control samples, whereas wild type plants showed growth stimulation under the same irradiation conditions compared with the wild type controls.[7] They showed that H_2O_2 concentration was not increased by low dose gamma irradiation in the ABA-deficient mutant *aba2 -1* compared with the mutant control, while in wild-type plants irradiation induced the accumulation of H_2O_2.[7]

In our study we have not observed growth stimulation after γ-irradiation of the studied genotypes within the dose range of 50–150 Gy, except for the *abi3-8* seeds that showed a faster germination at earlier hours (up to 31 hour) in 50 Gy irradiated group (Figure 2, Figure S1). Gamma-irradiation at a dose of 150 Gy significantly affected germinability, germination time, energy and synchrony of germination of the ABA-deficient *aba3 -1* mutant and to a lesser extent negatively affected the germination of Col-8 seeds. The line with ABA-impaired receptibility did not show any dose-dependent differences of germination parameters. The leaf area and biomass were mainly decreased after higher dose irradiation in all genotypes.

In irradiated *aba3 -1* photosynthetic parameters resembled those of control conditions. Coefficients of photochemical fluorescence quenching were significantly higher in *abi3-8* irradiated with 50 Gy and in Col-8 irradiated with doses of 100 and 150 Gy as compared to non-irradiated plants of the same genotype (Table 2). The values of PSII maximum efficiency and operating efficiency of PSII photochemistry increased slightly in *abi3-8* plants reaching a significantly higher mean value in irradiated with the dose of 150 Gy *abi3-8* as compared to non-irradiated *abi3-8* (Table 2), showing thus an increased ability to oxidize Q_A.

The relatively high ability of plants to maintain steady photosynthetic parameters under irradiation was recently shown by Van Hoeck et al[43] Sub-chronic (7 days) low-dose exposure of *Lemna minor* L. plants to ^{137}Cs even led to a slight increase of photosynthetic parameter, and authors considered this as a eustress beneficial response to low level of exposure.[43] Even the highest doses of irradiation used in that study (450 mGy/h) did not lead to the decrease of pigment content. However, in our previous work, seedlings of irradiated *Hordeum vulgare* L. seeds (100 Gy) showed significant transcriptional down-regulation on photosynthesis-related processes.[9] Considering higher radiosensitivity of *H. vulgare* to acute irradiation in comparison to *A. thaliana* and *L. minor*, the relative stability of photosynthesis after different modes of ionizing radiation exposure is species-specific and probably can be predicted from known values of LD_{50}.

Materials and Methods

Plant Material

Arabidopsis thaliana (L.) Heynh. seeds of 3 genotypes were studied: wild type Columbia-8 (Col-8) and 2 mutant lines: *abi3-8* with increased insensitivity to (+)-(S)-ABA vs. (−)-(R)-ABA[26] and ABA-deficient *aba3* -1.[36] Col-8 and mutant lines seeds were collected in 2018 and stored at room temperature in the dark dry place until analysis in 2019.

The line abi3-8 was developed as a result of a screen for mutants that took advantage of the ability of wild type *Arabidopsis* seeds to respond to (−)-(R)-ABA, an enantiomer of the natural (+)-(S)-ABA. The premise of the screen was to identify mutations that preferentially alter their germination response in the presence of one stereoisomer vs. the other.[28]

The line *aba3 -1* contains a recessively inherited mutation of the locus *AT1G16540* that encodes molybdenum cofactor sulfurase, which is involved in the conversion of ABA-aldehyde to ABA, the last step of abscisic acid biosynthesis. It was selected in screening of germination in the presence of the gibberellin biosynthesis inhibitor paclobutrazol.[27] The *aba3 -1* mutant shows reduced seed dormancy and excessive water loss, i.e. a phenotype characteristic for ABA-deficiency.[27]

Experimental Setup and Irradiation Conditions

Dry *A. thaliana* seeds of each genotype were randomly and homogeneously distributed in 4 groups: 1 control and 3 groups irradiated at different doses. Seeds were irradiated in paper envelopes at room temperature using a ^{60}Co gamma source (GUR-120, the Russian Institute of Radiology and Agroecology) with doses of 50, 100, and 150 Gy at a dose rate of 463 Gy/h. Non-irradiated seeds of each genotype served as control groups. Three experiments (with independent irradiation) were performed and 3 biological replicates (3 Petri dishes (60-90 plants) per genotype per experimental condition) were analyzed in each experiment.

The seeds were surface sterilized in 70% ethanol containing 1% bleach for 10 min on a mini-shaker (3D, Sunflower mini-shaker, Biosan), then rinsed with 70% ethanol 3 times and washed with distilled water at least 3 times. The seeds were planted on Petri dishes containing half-strength Murashige and Skoog medium (MS/2) supplemented with 3% (w/v) sucrose and 1% (w/v) agar, and placed at 4°C in the dark for 48 h. After stratification the seeds were transferred to the growth chamber (Sanyo MLR-351 H, Japan) at 21°C under long-day conditions (16 h light / 8 h dark, a photosynthetically active photon flux density of 80 μmol photons m^{-2} s^{-1}, 55% relative humidity) and grown for 16 days. Hereinafter the transfer of the seeds to the growth chamber is the start point for all time estimations.

In order to achieve homogeneity of growth conditions, in each independent experiment Petri dishes with control and irradiated plants were simultaneously placed in the growth chamber. Petri dishes were mixed often: each 2-8 h in the first 5 days of germinations, and every day after 5th day of germination.

Morphological Assessments

Seed germination was assessed during 1st-5th and 8th day after the transfer to the growth chamber on a macroscopic scale as endosperm rupture and visible radicle emergence. Calculation of germination indices was performed through the Germinationmetrics Package[44] using the partial germination count data.

The leaf area was estimated on the 11th day of cultivation through a fast and non-destructive method using Easy Leaf Area software.[45] For each Petri dish, we calculated the average total leaf area for a plant and the median value of this trait for each condition.

The biomass (fresh weight) of the plants was measured on the 16th day using analytical balance PA213C (Ohaus), and the average weight of one plant was calculated. All plants were afterwards frozen and stored in liquid nitrogen for further tests.

Assessment of Photosynthetic Parameters

The photosynthetic activity was assessed for 16-day-old plants on Petri dishes, before sampling for biomass measurements. The fluorescence parameters were measured for 5 plants per Petri dish, using 3 Petri dishes in one independent experiment (9 in total) after adaptation to darkness for at least 30 minutes, using JUNIOR-PAM chlorophyll fluorometer (Heinz Walz GmbH, Germany). For each plant, we used the biggest leaf for measurements. The intensity of actinic light was 125 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The duration of SAT-pulse was 0.8 s under intensity of 5 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

The photosynthetic parameters were calculated using WinControl-3 software (Heinz Walz GmbH, Germany). The following parameters were measured *in vivo*: chlorophyll (Chl) fluorescence parameters F_o (initial), F_m (maximum), photosynthetic active radiation (PAR, μmol photons m^{-2} s^{-1}), maximum photochemical quantum yield of photosystem II (PSII) from a dark-acclimated sample (Fv/Fm), coefficients of photochemical fluorescence (qP and qL), effective photochemical quantum yield of photosystem II (Y(II)), electron transport rate (ETR, μmol electrons m^{-2} s^{-1}), coefficient of non-photochemical fluorescence quenching (qN), non-photochemical fluorescence quenching (NPQ).

Glutathione Peroxidase (GPX) Activity Analysis

GPXs belong to an important class of enzymes involved in the reduction of H_2O_2 and some other peroxides using thioredoxin. Their enhanced activity has been reported in many plants under different abiotic stresses,[46] and recent evidence suggests that these enzymes can be implicated in plant growth and development.[20] There is also an indication that GPXs participate in ABA signalling.[47]

The activity of glutathione peroxidase was quantified in 16-day-old plants on a NanoDrop-2000 spectrophotometer (Thermo Fisher Scientific, USA). Three samples of 0.13 g per each experimental point were measured. GPX activity was evaluated by the rate of NADPH oxidation by glutathione reductase.[48] It should be noted that the use of glutathione as a reducing agent for assaying of the total GPX activity is expected to be less efficient than usage of thioredoxin as a reducing agent.[49]

ABA Content Measurement

The abscisic acid concentration was measured in samples of 16-day-old plants using a LC-30 Nexera high-performance liquid chromatograph system (Shimadzu, Japan) according to the protocol.[50] The results were processed using LabSolutions software (Shimadzu). 2 to 3 replicates were used for each experimental point (sample weight of 0.3 g). Each sample was analysed twice to eliminate instrumental errors. The ABA measurements were performed in the 3 rd independent experiment.

Data Analysis

Three experiments with independent irradiation of samples were performed, and in each experiment for each condition, we used 3 Petri dishes (9 in total per condition). The results presented in the paper are based on analysis of combined data among all experiments performed. Statistically significant differences were determined by the Mann-Whitney U-test, regression analysis, or Kruskal-Wallis test. Analyses were performed with Statistica 8.0, MS Office Excel 2019, and R version 3.6.3. Outliers were determined through the Dixon's Q test in MS Office Excel 2019 and removed (except for germination assay).

Germination indices were computed using R version 3.6.3. The multiple regression analysis or unpaired Wilcoxon rank sum test (Mann-Whitney U test) were applied. U test was used when the assumptions of the regression model (global statistics, skewness, kurtosis, link function, and heteroscedasticity) were not satisfactory. Results with p-value less or equal to 0.05 were considered statistically significant.

Conclusions

To study the effect of γ-radiation on the dynamics of seed germination of *A. thaliana* with impaired ABA synthesis and reception, the wild type Col-8 and 2 ABA-mutants *abi3-8* and *aba3 -1* were subjected to acute irradiation at doses 50, 100, and 150 Gy. The assessment of germination parameters revealed that γ-irradiation at a dose of 150 Gy significantly affects germinability, germination time, energy and synchrony of the ABA-deficient *aba3 -1* mutant. This dose also negatively affected germination of Col-8 seeds, although to a lesser extent than *aba3 -1* seeds. The *abi3-8* line with S-(+)-ABA reception defect turned out to be the most resistant to the impact of the studied doses of γ-radiation. Moreover, *abi3-8* seeds of 50 Gy irradiated group showed a faster germination at earlier hours (up to 31 hour).

Our results demonstrated that photosynthetic functioning of young plants (16-day-old) was not disturbed by acute γ-radiation of seeds with doses of 50 to 150 Gy and that no indication of PSII photoinhibition was noticed, revealing the robustness of the photosynthetic system of radioresistant species *A. thaliana*. The differences of photosynthetic parameters in plants grown from non-irradiated seeds of ABA-impaired genotypes suggest that ABA-related modifications of plant physiology might influence the performance of the photosynthetic apparatus.

Altogether our results contribute to the understanding of the fine-tuning of germination and photosynthesis and eventually to the resistance of plants to ionizing radiation.

Authors' Note

M.P. and D.B. planned the experimental layout and participated in all experimental procedures and data analysis; E.B. participated in experimental procedures, analyzed germination indices and photosynthetic parameters, wrote the first draft of the article; E.K., A.P., and E.S. performed glutathione peroxidase activity analysis; S.B. performed ABA analysis; M.P. partly analyzed the data and participated in photosynthetic parameters assays; A.M., I.G., and A.P. contributed in photosynthetic parameters assays; P.V. supervised the experiment; all co-authors contributed to the final version of the manuscript.

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Declaration of Conflicting Interests

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Supplemental Material

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