Ionising radiation-induced DNA methylation changes in *Arabidopsis thaliana* plants exposed to gamma radiation over multiple generations

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Introduction

As sessile organisms, **plants** have to adapt to environmental changes, this includes adapting to **ionising radiation** (IR) in contaminated locations. **Epigenetic** modifications such as **DNA methylation** changes are one mechanism in overcoming shifts in the environment. Both potential **adaptation**[1,2,3] to IR and IR-induced DNA methylation[4] in plants has already been hinted at. Here, the effect of gamma radiation on DNA methylation, the effect of this methylation, and its function across **multiple generations** of ***Arabidopsis thaliana*** plants are studied.

Objectives

- Study the influence of IR on the DNA methylation profile of multigenerationally exposed *Arabidopsis* plants
- Identify whether differentially methylated regions (**DMR**s) are linked to transposable elements or genes and the methylation context
- Indicate potential methylation regulated pathways involved with the IR-response through functional analysis of DMR associated genes

Experimental Set-up

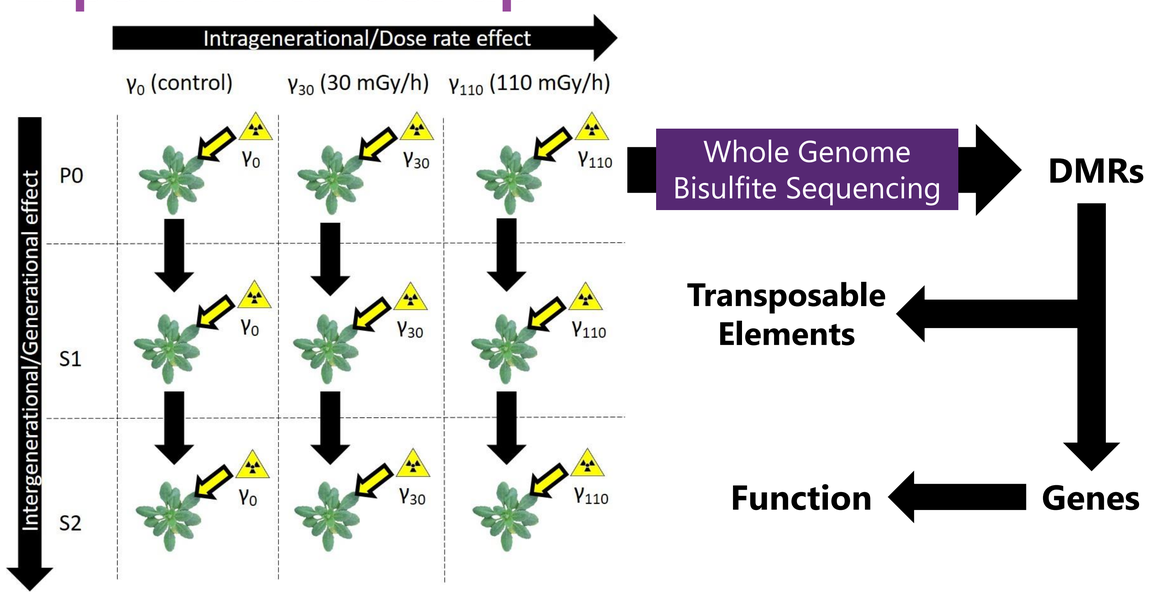

Fig. 1 Experimental set-up. Three generations of A. thaliana (P0 (Parent generation), S1 (generation 1), S2 (generation 2)) were exposed to three IR conditions. P0: parental generation with no previous history of IR exposure. S1 came from a previously exposed generation, S2 from a line with two previously exposed generations.

Results

Table 1 DMRs (CG) found in the promoter associated regions (<2 kbp upstream), gene bodies, and TEs divided in hypo- and hyper methylation.
(Meth. diff. >20%) (p≤0.05) (γ30 (30 mGy/h), γ110 (110 mGy/h), γ0 (<0.1µGy/h)

	Hypo			Hyper		
	Promoter Associated Region	Gene body	TEs	Promoter Associated Region	Gene body	TEs
Intergenerational (Generational effect)						
P0γ_{30} vs S1γ_{30}	33	34	15	31	38	12
P0γ_{30} vs S2γ_{30}	34	42	5	69	60	35
S1γ_{30} vs S2γ_{30}	255	345	95	327	464	134
S1γ_{110} vs S2γ_{110}	0	3	0	5	1	3
Intragenerational (Dose rate effects)						
S1γ_0 vs S1γ_{30}	31	20	10	27	26	14
S1γ_0 vs S1γ_{110}	0	0	0	1	0	0
S1γ_{30} vs S1γ_{110}	7	6	4	7	12	4
S2γ_0 vs S2γ_{30}	90	140	23	165	189	75
S2γ_0 vs S2γ_{110}	3	2	2	1	0	0
S2γ_{30} vs S2γ_{110}	2	2	1	1	3	0

Changes occurred predominantly in CG context, not in CHG or CHH. Hypermethylation was higher than hypomethylation. Most changes were observed at the lower dose rate (γ30). No changes were seen in the parent generation but only in the 1st and 2nd generation. DMRs were found to be associated with TEs and genes. Genes affected by DMRs showed an enrichment for stress response and DNA repair processes.

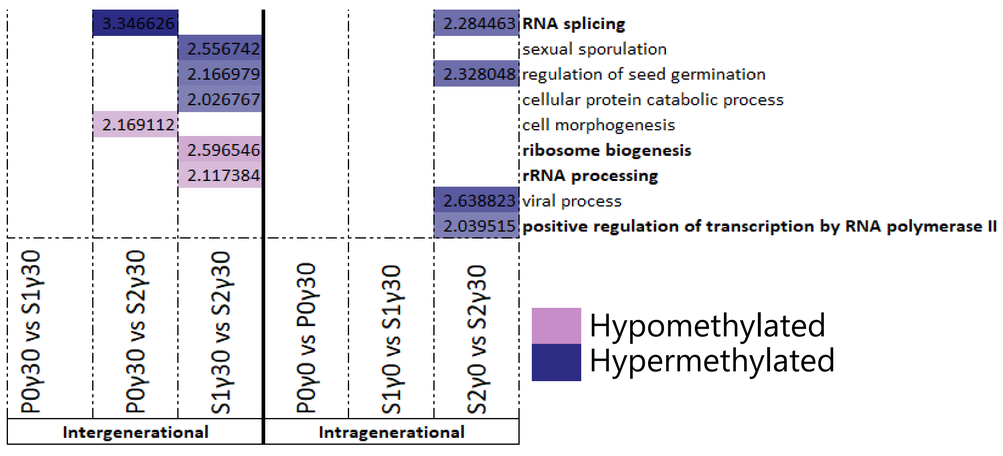

Fig. 2 GO term enrichment of genes associated with DMRs in their promoter associated regions.

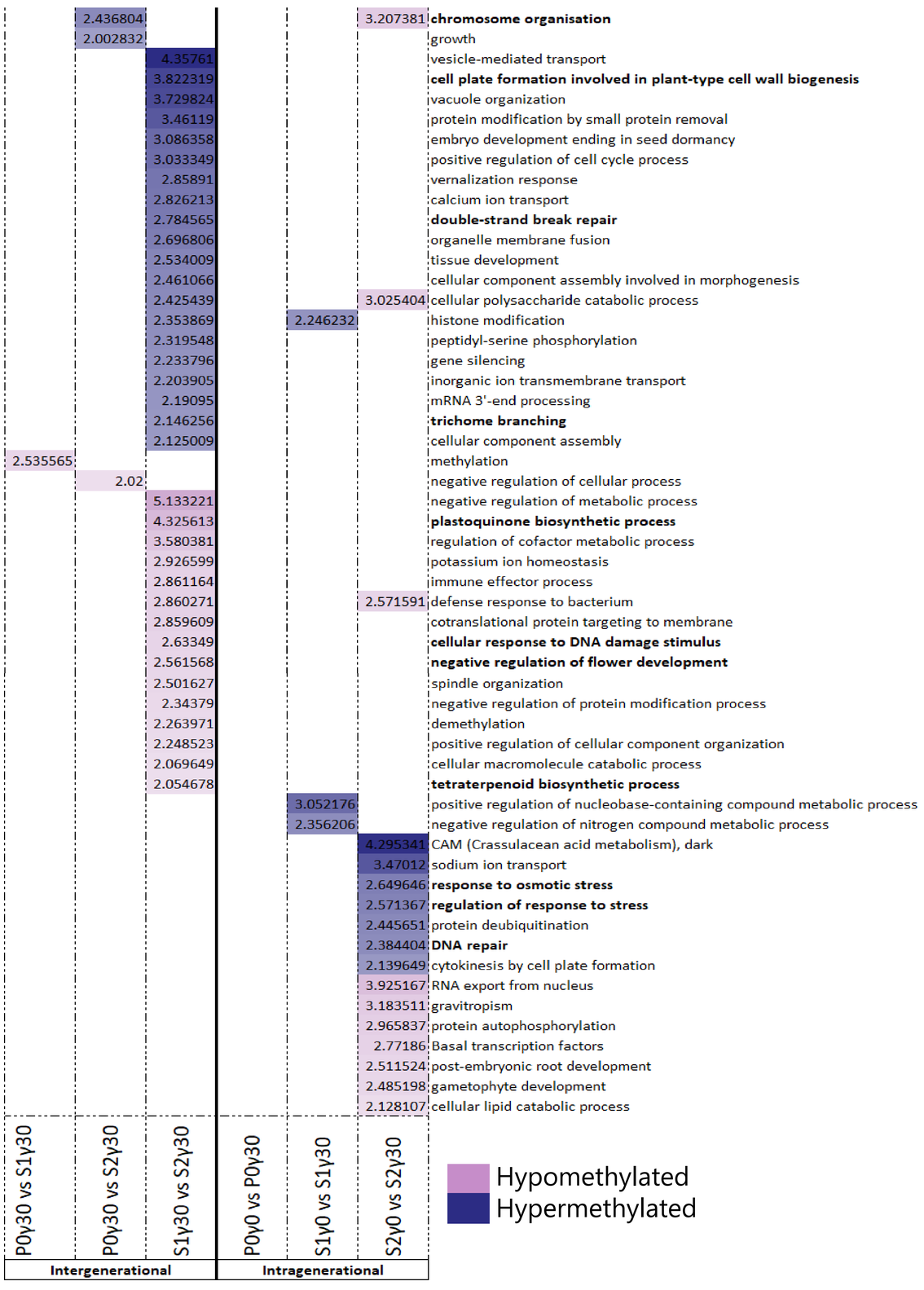

Fig. 3 GO term enrichment of genes associated with DMRs in their gene bodies.

Discussion and Conclusion

Findings indicate a regulatory role of DNA methylation in IR-stress response and potential stress adaptation.

DNA methylation changes occurred **most in CG context** which could indicate a potential IR specific methylation response. Additionally, a **preference for hypermethylation** was observed which has been hypothesised to **increase genome stability**, for one part by **reducing TE mobilisation**. This is also backed up by the significant number of (hypermethylated) DMRs associated with TEs. No DMRs were found between the parent generations over the different dose rates, but were observed in the first and second generation, indicating a potential **stress priming**. Counterintuitively, most changes occurred at the lower dose rate and not at the higher, which could hint at a **non-linear dose response**. A significant number of DMRs were associated with **stress response genes**, this in combination with their association with TEs indicates that IR-induced DNA methylation is not random.

References
1. Geras'kin, S., (2016). (J Environ Radioact, 162-163: p.347-357.) 2. Kovalchuk, I., et al., (2004). (Plant Physiol, 135: p.357-363.) 3. Kovalchuk, O., et al, (2003). (Mutat Res, 529: p.13-20.) 4. Horemans, N., et al., (2019). (Environ Pollut, 251: p.469-483.)

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