Self-DNA Early Exposure in Cultivated and Weedy *Setaria* Triggers ROS Degradation Signaling Pathways and Root Growth Inhibition

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Background

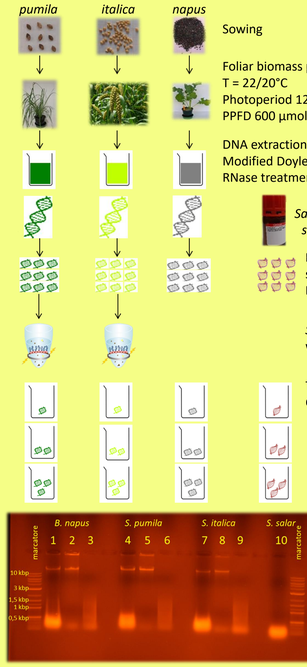
Recently, a species-specific concentration dependent **inhibitory effect** caused by **self-DNA** was discovered (Mazzoleni *et al.*, 2015). Non-self treatments showed a magnitude of the effect consistent with the **philogenetic distance** between DNA source and target species (Duran-Flores & Heil, 2017). The mechanisms underlying this phenomenon are still uncertain but recent papers have highlighted that self-DNA may act as a **damage-associated molecular pattern** (DAMP) triggering in the early response intercellular flux of Ca^{+2}, transmembrane depolarization, activation of mitogen-activated protein kinase, hydrogen peroxide and extrafloral nectar production (Barbero *et al.*, 2016; Duran-Flores & Heil, 2017). A recent transcriptomic profiling pointed out a significant differential expression of several pools of genes responsive to **abiotic stress** under self-DNA, mostly evident between 1 h and 8 h from exposure (Chiusano *et al.*, 2021). Potential applications include the production of DNA-based herbicide (Mazzoleni *et al.*, 2014).

Our questions

1. **Does the species-specificity of self-DNA inhibition still hold at infrageneric level, in particular in cultivated vs. weed *Setaria* congeneric species?**
2. **Which genes and molecular pathways are involved in the early response to self-DNA exposure in plants?**

ACTIVITY 1: CROSS-FACTORIAL EXPERIMENT TO INVESTIGATE SELF-DNA INHIBITION AT INFRAGENERIC LEVEL IN *SETARIA* SPECIES

I. DNA solution preparation



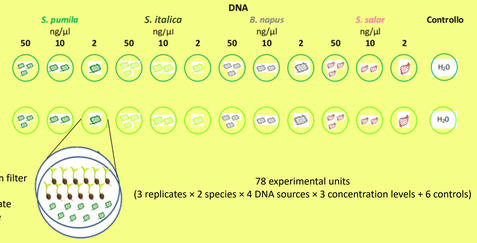
1, 4, 7: extracted DNA

2, 5, 8: post RNase

3, 6, 9: post sonication

10: commercially available low-weight DNA

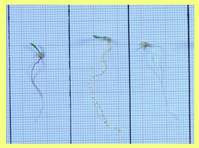
II. Exposure to DNA solutions



III. Root elongation measurement and statistical analysis

Photographs taken before and after exposure

ImageJ
Image Processing & Analysis in Java



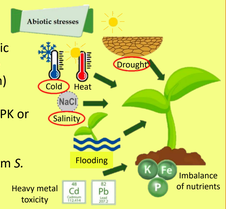
Root elongation expressed as percentage of control

- **3-ways ANOVA** model to test the significant effects of target species, DNA source and DNA concentration
- **Tuckey's post-hoc test**
- **One-sampe t-test** for multiple comparisons (Bonferroni's method) to assess the differences from the control

ACTIVITY 2: TARGETED GENE EXPRESSION ANALYSIS TO EXPLORE THE MOLECULAR MECHANISMS INVOLVED IN THE EARLY RESPONSE TO SELF-DNA IN *SETARIA* SPECIES

I. Target gene selection

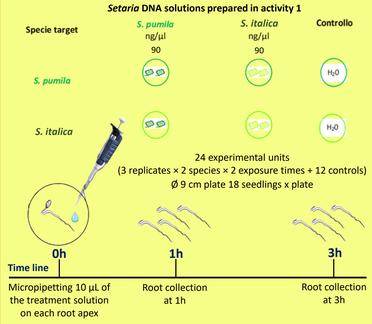
Gene	Gene Family	Function	Reference
Gene1_SiFSD2	SUPEROXIDE DISMUTASE (FeSOD)	Defence against ROS and apoptotic stimuli, convert superoxide(O_2^-) into hydrogen peroxide (H_2O_2) and dioxygen (O_2)	Wang T. *et al.* 2018. 3 Biotech. Dec;8(12):486.
Gene2_SiALDH22A1	ALDEHYDE DEHYDROGENASE (ALDH)	Oxidation of aldehydes to carboxylic acids, reducing the effect of lipid peroxidation under various environmental stresses	Chen Z. *et al.* 2014. Plos one. 9(7):e101136.
Gene3_SiALDH7B1	ALDEHYDE DEHYDROGENASE (ALDH)	Oxidation of aldehydes to carboxylic acids, reducing the effect of lipid peroxidation under various environmental stresses	Chen Z. *et al.* 2014. Plos one. 9(7):e101136.
Gene4_SiCSD3	SUPEROXIDE DISMUTASE (CuZnSOD)	Defence against ROS and apoptotic stimuli, convert superoxide(O_2^-) into hydrogen peroxide (H_2O_2) and dioxygen (O_2)	Wang T. *et al.* 2018. 3 Biotech. Dec;8(12):486.
Gene5_SiWD40-144	WD REPEATS include conserved tryptophan (W) and aspartic acid (D) residues and a repeat length of 40 amino acids	Scaffolding molecule, WD repeat-containing protein 26 isoform X2 (WDR26) may act as a negative regulator in MAPK signalling pathway	Mishra AK. *et al.* 2014. PLoS ONE 9(1): e86852.
Gene6_SiWD40-155	WD REPEATS include conserved tryptophan (W) and aspartic acid (D) residues and a repeat length of 40 amino acids	Scaffolding molecule, WD repeat-containing protein DWA2, known as a negative regulator of ABA signaling	Mishra AK. *et al.* 2014. PLoS ONE 9(1): e86852.
Gene7_SiMPK17-1	Mitogen-activated protein kinase (MAPK/MPK)	MPK signalling cascades transduce and amplify endogenous and exogenous stimuli. MPK17 can be firstly triggered by ROS production; its downregulation seems to significantly reduce growth	Lata C. *et al.* 2010. Biochemical and biophysical research communications. 393(4). 720–727.



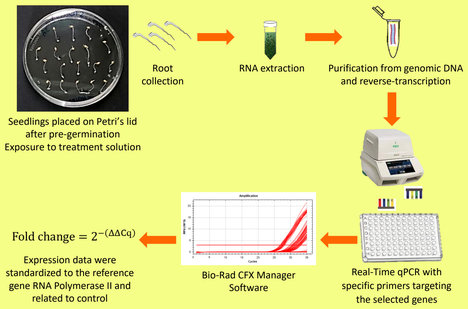
- Genes responsive to abiotic stress in *S. italica* over the relevant time-frame (1-3 h)
- Genes related to ROS, MAPK or signaling pathways
- Genes amplifiable also from *S. pumila* cDNA

dos Santos et al. 2022. Stresses: 2, 113-135. https://doi.org/10.3390/stresses2010009

II. Exposure to *Setaria* spp. DNA solutions



III. Experiment workflow



IV. Statistical analysis

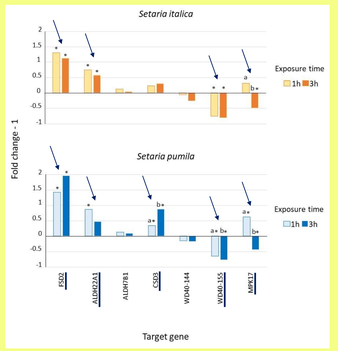
Two independent-sample t test for multiple comparisons (Bonferroni's method) to assess the differences in average ΔCq values between 1 h and 3 h treatment and between 1 h treatment and control and 3 h treatment and control within each target gene for both species

V. Results

Early and persistent activation of genes involved in ROS degradation and management (*FSD2*, *ALDH22A1*, *CSD3*)

Early activation followed by deactivation of MAPK genes (*MPK17*)

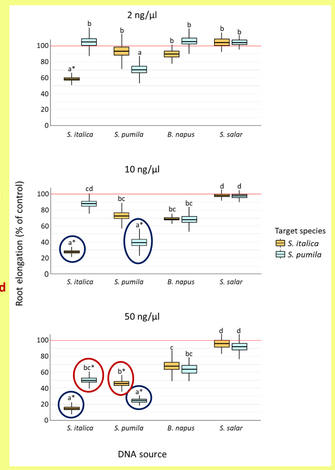
Deactivation of scaffolding molecules acting as negative regulators of stress signaling pathways (*WD40-155*)



IV. Results

Self-DNA inhibitory effect still holds at infra-generic level for congeneric species with different ecological traits, such as the weedy invasive *Setaria pumila* and the cultivated *Setaria italica*

Our research highlighted some critical issues, such as the extent of possible inhibition of crop species treated with DNA targeting closely related weeds



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CONCLUSIONS AND PERSPECTIVES

- **Self-DNA inhibitory effect still holds at infra-generic level. Possible side effect alert: partial inhibition of crop species treated with DNA targeting closely related weeds.**
- **Confirmed abiotic stress pathways' involvement and ROS production in the early response to self-DNA.**
- **Need for further investigation of the relationships between DNA exposure and stress signaling pathways with fully representative gene sets and a more comprehensive time frame.**

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