Modulation of thermotolerance in *Arabidopsis thaliana* through variations in the expression of the *NudC* gene *BOB1*

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ABSTRACT

The rise of global temperatures and heat waves caused by climate change is a growing concern worldwide. As a result, it is increasingly challenging to maintain healthy plant species and protect them from environmental fluctuations. Heat stress can severely impact crucial biological processes in plants and hinder their growth and development. In order to identify regulators that can improve plant thermotolerance, we studied the *BOB1* gene, a member of the Nuclear distribution C (*NudC*) gene family, and evaluated the effects of both overexpression and downregulation of this gene in the plant model species *Arabidopsis thaliana*.

We exposed four Arabidopsis lines, namely, the wild type Col-0, the *BOB1* downregultion line (*bob1-3*) and two overexpressing lines (*35S:BOB1-1* and *35S:BOB1-2*) to heat stress and analyzed them using the alkaline/neutral comet assay. Our study revealed that the overexpression or suppression of the *BOB1* gene had varying effects on genome stability and plant growth. Notably, the amount of induced DNA strand breaks was comparable in control and overexpressing lines. These findings suggest that *NudC* genes plays a crucial role in activating plant thermotolerance and improving plant health.

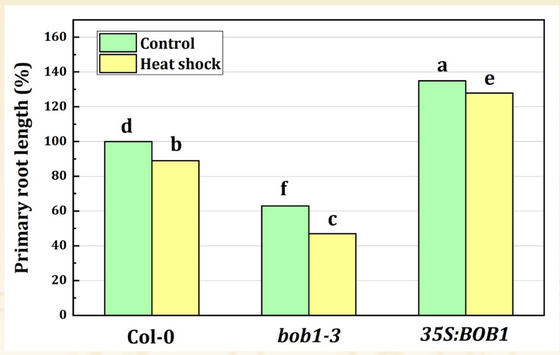
RESULTS

Root phenotype of lines with downregulation and overexpression of *BOB1* gene

Under favourable growth conditions, downregulating the *BOB1* gene decreased primary root length by 37%, while constitutive overexpression resulted in about 35% increase compared to the wild-type Col-0.

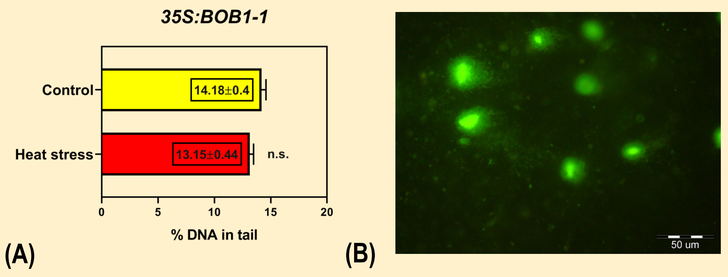
Interestingly, *BOB1*-overexpressing plants showed a relatively milder reduction in root length under heat stress compared to wild-type Col-0 and *bob1-3* mutant plants. The *bob1-3* mutant was most severely affected, exhibiting a decrease of approximately 16% compared to only 7% in the *35S:BOB1* plants.

Changes in root length in lines with downregulation or overexpression of *BOB1* gene after exposure to heat shock



35S:BOB1-1 OVEREXPRESSION LINE

(A) Levels of DNA strand breaks, expressed as percentage of DNA in comet tail, in nuclei isolated from the roots of 11-day-old plants of *35S:BOB1-1* overexpression line detected by the A/N comet assay.
(B) Representative microphotograph of the comets of the overexpression line *35S:BOB1-1* after heat stress.

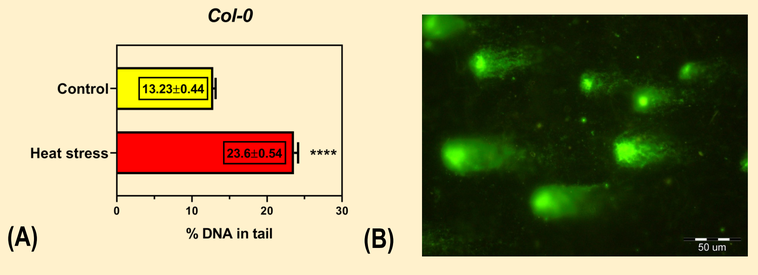


There is no significant differences between the control group and heat treated group indicating increased thermotolerance in *35S:BOB1-1* overexpression line.

n.s.- no significant differences (Mann-Whitney)

WILD-TYPE COL-0 LINE

(A) DNA damages were measured in *Arabidopsis* nuclei isolated from wild-type Col-0, exposed to heat stress (45ºC, 60min) using the A/N protocol of comet assay.
(B) Representative microphotograph of the comets of the wild type line Col-0 after heat stress.

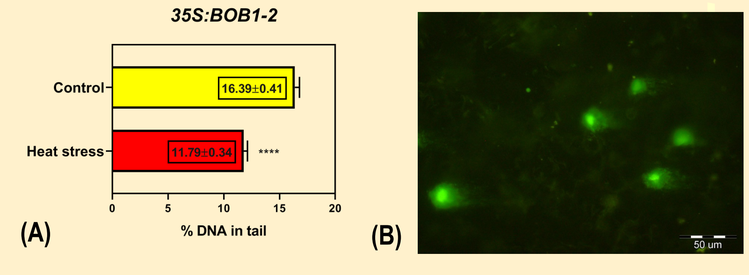


Data represent the mean values (±SE) of % DNA in tail parameter assessed for root samples after heat stress.

****P<0.0001, significantly different relative to the control - untreated nuclei. (Mann-Whitney)

35S:BOB1-2 OVEREXPRESSION LINE

(A) Levels of DNA strand breaks, expressed as percentage of DNA in comet tail, in nuclei isolated from the roots of 11-day-old plants of *35S:BOB1-2* overexpression line detected by the A/N comet assay.
(B) Representative microphotograph of the comets of the *35S:BOB1-2* overexpression line after heat stress.



The reduced number of heat- induced DNA damage in the nuclei isolated from *35S:BOB1-2* overexpression line indicate increased thermotolerance in this overexpression line.

****P<0.0001, significantly different relative to the control - untreated nuclei.(Mann-Whitney)

Overexpression of the *BOB1* gene increases thermotolerance and protects genome integrity

MATERIAL AND METHODS

Plant material

Eleven-day-old *Arabidopsis thaliana* seedlings were grown on ½ MS medium.
The following lines were used:
Col-0- wild type
bob1-3-downregultion line
35S:BOB1-1 ***35S:BOB1-2*** Overexpressing lines

Stress exposure

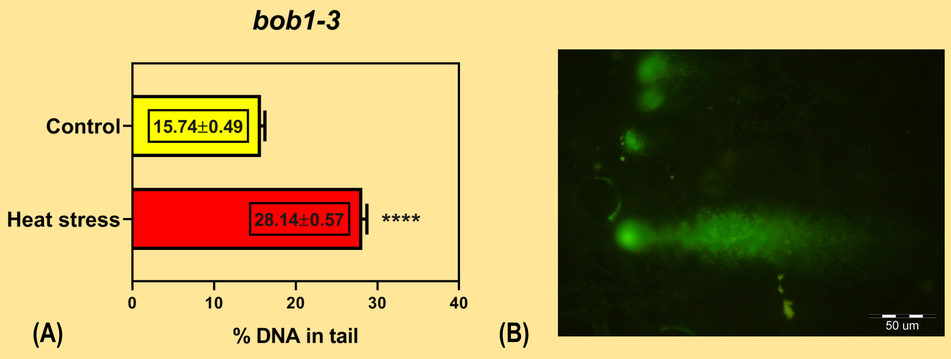
Heat stress was applied by exposure to 45°C for 60 minutes.

Methods

Plant root phenotyping
Isolation of nuclei
Plant alkaline/neutral (A/N) comet assay
Statistical analysis
Mann-Whitney test

bob1-3 mutant line

(A) Heat-induced DNA strand breaks in *Arabidopsis* mutant line with downregulation of *BOB1* gene (*bob1-3*) Compared to the wild-type line Col-0, this mutant line exhibits reduced thermotolerance and a heat-sensitive phenotype.
(B) Representative comet images of heat-treated Arabidopsis nuclei isolated of the downregulation mutant line *bob1-3* determined by alkaline/neutral comet assay.



Data represent the mean values (±SE).

****P<0.0001, significantly different relative to the control - untreated nuclei. (Mann-Whitney test)

CONCLUSIONS

Our findings reveal that both overexpression and suppression of the *BOB1* gene affect *Arabidopsis* root morphology and have differential genotoxic effects, manifested by changed root length and altered levels of DNA strand breaks. These findings indicates the crucial role of the *BOB1* gene in root growth and DNA damage response.

More detailed studies to understand the structural changes in genome organisation in plants with modified *NudC* gene expression, as well as the chaperone function of NudC proteins and their role in plant protection against abiotic stresses will be further conducted.

Acknowledgments

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