Downregulation of the NudC gene BOB1 leads to heat-induced DNA damage in *Arabidopsis thaliana*

Mariyana Georgieva, Dimitar Todorov, Valya Vassileva

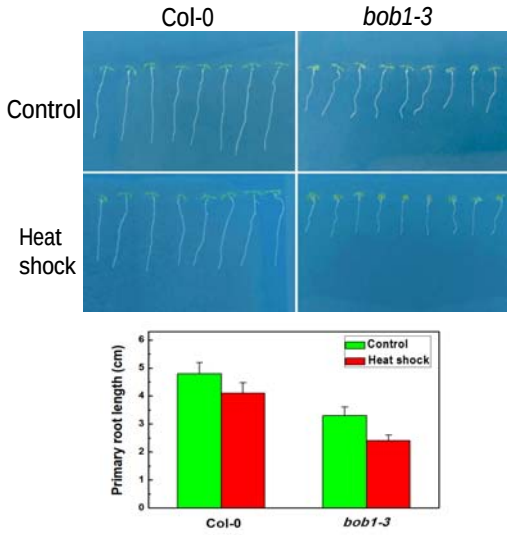
Institute of plant physiology and genetics, Bulgarian Academy of Sciences

Abstract

The global climate change resulting in an increased ambient temperature has been recognised as a major agricultural problem that affects many physiological, metabolic and genetic processes in plants. Responses of plants to extreme temperatures have been extensively studied, but how heat stress affects DNA integrity remain poorly understood. In this study, we focused on the BOB1 gene belonging to the Nuclear distribution C (NudC) gene family that contributes to abiotic stress tolerance and development of the plant model species *Arabidopsis thaliana*. Since BOB1 loss-of-function mutants are embryo lethal, we used the partial loss-of-function *bob1-3* mutant, which is vital and fertile. To examine heat sensitivity of *bob1-3*, a lesion-specific comet assay was performed. We compared heat-induced DNA damage (DNA strand breaks and oxidative base damage) in *bob1-3* and the wild-type Col-0 plants. Our results show that BOB1 may contribute to thermotolerance of *Arabidopsis* through maintenance of genome integrity, which can be used for screening of heat effects on plant genomes.

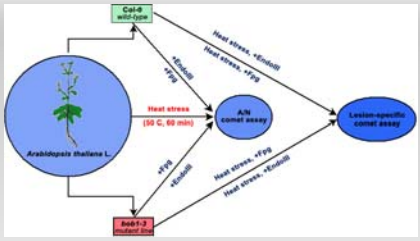
Results

Representative images of root phenotypes of bob1-3 mutant and wild-type Col-0 plants analysed for heat shock response.



Arabidopsis seedlings were grown on vertically oriented agar plates for 4 days at 22°C and heated to 45°C for 60 min, then grown for 3 more days at 22°C and analysed. The agar plates were scanned with an Epson Perfection V850 Pro scanner and ImageJ 1.52n software was used for primary root length measurements.
Stress by heat shock resulted in significantly shorter primary roots in Arabidopsis plants with downregulation of the *NudC* gene *BOB1*, compared to Col-0 control. The heat shock led to a decrease in root length of Col-0 plants by approximately 15%, whereas the mutant roots showed about 27% reduction. This observation suggests that the fully functional *BOB1* gene contributes to thermotolerance acquisition in Arabidopsis plants.

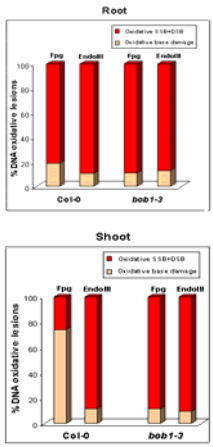
Experimental design



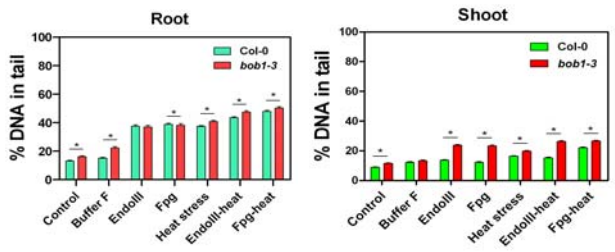
Lesion-specific A/N comet assay
***Fpg*-specific for oxidized purines**
***EndoIII*-recognize oxidized pyrimidines**
Categorization of comets included five classes according to the percentage of DNA in the 'comet' tail, class 1, no damage (<5%); class 2, low damage (5.1-17%); class 3, moderate damage (17–35%); class 4, high damage (35.1–60%); class 5, extremely high damage (over 60% of DNA material in the comet tail) (1)
Genetic damage index (GDI) was calculated using the formula GDI=[1 (*n1*)+2 (*n2*)+3 (*n3*)+4 (*n4*)/N (*n0-n4*)
Where, n0, number of cells with class 1; *n*1, number of cells with class 2; *n*2, number of cells with class 3; *n*3, number of cells with class 4; *n*4, number of cells with class 5. Afterward, oxidative DNA damage (OD) was obtained for each treatment using the formula OD=[(%GDI *buffer*+%GDI *enzyme* (Fpg or EndoIII)+%GDI *heat stress*)−(%GDI *buffer* +%GDI *heat stress*)] (2)

Results

Distribution of oxidative DNA damage type in Arabidopsis line with downregulation of NudC gene familv.

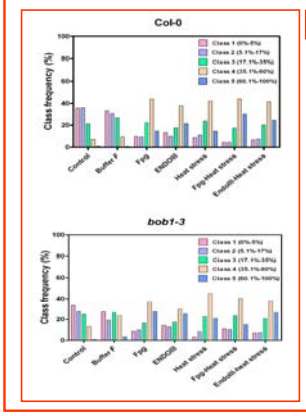


The effects of heating on genome integrity of Arabidopsis lines. Heat stress leads to accumulation of oxidative DNA damage in mutant line bob1-3 in comparison to wild type Col-0.

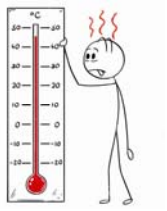


Oxidative DNA damage was assessed in *Arabidopsis* nuclei isolated from wild-type Col-0 and mutant line *bob1-3*, exposed to high temperature stress (50ºC, 60min) using the A/N protocol of comet assay and in the presence or absence of Fpg and EndoIII enzymes. The average Tail DNA % was assessed for root and shoot samples after heat stress and in the absence of Fpg and EndoIII, a modest increase in Tail DNA % was observed in shoot samples in both Arabidopsis lines.

Differences in DNA damage level in wild-type Col-0 and bob1-3 mutants. The* lesion-specific *A/N comet assay in Arabidopsis bob1-3 nuclei treated with enzymes Fpg and EndoIII revealed an increase in the frequency of nucleoids class 4 and 5.

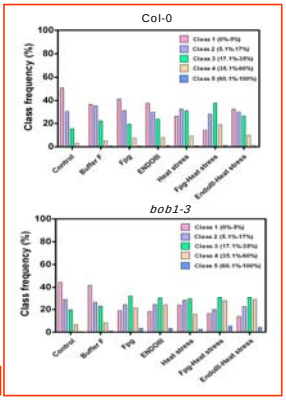


I. Roots

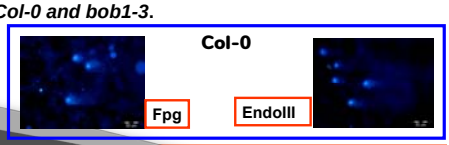


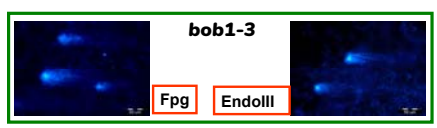
Frequency of nucleoid classes observed by A/N comet assay and lesion-specific A/N comet assay in *Arabidopsis* nuclei isolated from roots (I), shoots (II) and treated using heat stress (50°C, 60 min)

II. Shoots



Representative comet images from Fpg heat stress, EndoIII heat stress treated Arabidopsis isolated nuclei from Col-0 and bob1-3.





References

1. Mocali et al., 2005 *J Gerontol A Biol Sci Med Sci*.;60(6):695-701.
2. Pérez-Iglesias et al., 2017 *Ecotoxicol Environ Saf*.;142:503-508.

Acknowledgements

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