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BnaA07.BBX22 enhances the salt tolerance of rapeseed by regulating ROS homeostasis

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Background:

Salt stress poses a grievous threat to global agricultural productivity and food security. Plant is typically accompanied by a growth deficiency and yield penalty under salt stress condition. Hence, we cloned a salt tolerance gene BnaA07.BBX22 by fine mapping using the RIL population in *Brassica napus*.

Objective:

In order to confirm the function of the salt tolerance gene BnaA07.BBX22 in rapeseed, and to analyse the molecular mechanism of the gene BnaA07.BBX22 under salt stress. Finally, to provide genetic resources and theoretical basis for the cultivation of novel varieties of salinity-tolerant rapeseed.

Methods:

Overexpress BnaA07.BBX22, BnabZIP, and BnaWRKY recombinant plasmids were transferred to Arabidopsis thaliana and rapeseed by Agrobacterium-mediated inflorescence immersion of Arabidopsis and hypocotyl infestation of rapeseed, respectively. DAB and NBT staining were used to measure the H2O2 and O2- contents in the overexpression lines and WT. Yeast two-hybrid, bimolecular fluorescence complementation (BiFC) and split luciferase (LCI) assays were used to demonstrate BnaA07.BBX22 could interact with BnabZIP. Yeast one-hybrid and dual-luciferase transient expression assay were used to show BnabZIP could bind the G-box or C-box motifs in the promoter of BnaWRKY. CRISPR/Cas9 system was used to generate the wrky mutants in BnaA07.BBX22-OE plants.

Results:

Overexpression of BnaA07.BBX22 conferred enhanced biomass and root length of rapeseed under salt and drought stress conditions by mediating reactive oxygen species (ROS) homeostasis. In addition, the salt-treated over-expressing *B. napus* plants exhibited significantly higher soluble protein and chlorophyll content (SPAD) contents, lower malondialdehyde (MDA) contents, and higher peroxidase (POD) activity compared to the control plants. Overexpression of BnaA07.BBX22 significantly increased the leaf numbers at seedling stage and the branch numbers at mature stage under normal growth conditions. Yeast two-hybrid, LCI, and BiFC experiment proved BnaA07.BBX22 interacts with BnabZIP, and overexpression of BnabZIP enhanced biomass and root length of Arabidopsis under salt stress condition. Yeast one-hybrid and dual luciferase experiment shown BnabZIP interacts the promoter of BnaWRKY, then activates the expression of BnaWRKY. Overexpression of BnaWRKY enhanced biomass and root length of Arabidopsis under salt stress condition by mediating ROS homeostasis. CRISPR wrky mutants in the overexpression-BnaA07.BBX22 lines exhibited salt-sensitive phenotype.

Conclusions:

Taken altogether, we propose the module BnaA07.BBX22-BnabZIP-BnaWRKY to improve plant salt tolerance by regulating ROS homeostasis in rapeseed. These findings provide insights into currently underexplored mechanisms of plant how to respond to stress tolerance that will lay a foundation for breeding higher stress-tolerance, higher yielding rapeseed for sustainable agriculture.