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Chloroplast targeting of *Brassica rapa* metallothionein gene type 1 (BrMT1) in Arabidopsis enhances environmental stress tolerance

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Objectives

1. Construction of plant expression vectors for chloroplast target of MT genes used transit sequence of rubisco small subunit (RbcS)
2. Development of transgenic plant targeted MT in chloroplast
3. To test whether chloroplast targeting of MT effects to environment stress resistance

Materials and Methods

1. Plant: *Arabidopsis thaliana* (Col-0), *Agrobacterium tumefaciens* stain: GV 3101
2. Paraquat bleaching experiment: Leaves of *RbcS-BrMT1* and wild type plants were incubated on 1/10 MS medium containing 0.025% Tween 20 with 0.5, 1.0 and 1.5 M and or without paraquat for 40hrs under continuous light.
3. Detection of H_2O_2 in arabidopsis leaves: After 24 hrs stabilization in water containing 0.5 and 1.0 M paraquat, the leaves were supplied with 1mg/ml DAB-HCl solution for 5~8 hr at 25 ? under continuous light.

Results and Discussion

Metallothioneins (MTs) are known to low molecular weight cysteine-rich proteins that can bind heavy metals. Chloroplast is the primary target of environmental stresses, in the present study, chloroplast targeting engineering of MT was used to enhance the capacity of plants for environmental stress resistance.

We have isolated the metallothionein gene type 1 from *Brassica rapa* cDNA library. We made plant expression vectors for chloroplast targeting of BrMT1 using transit sequence of rubisco small subunit (RbcS). This construct was introduced in the *Arabidopsis thaliana* (*col-0*) by *Agrobacterium* mediated transformation. Western blot using the GFP antibody showed RbcS-BrMT1-GFP proteins existed in chloroplast fraction. Also green fluorescence of *RbcS-BrMT1-GFP* was observed in chloroplast. Furthermore *RbcS-BrMT1* and *BrMT1* transgenic plants grew better than wild-type plants on half strength (1/2) MS agar medium containing 50 M, 60 M and 70 M Cd(II). We using leaves of *RbcS-BrMT1*, *BrMT1* and WT plants for paraquat (ROS-generating non-selective herbicide) bleaching experiment. The results showed that, *RbcS-BrMT1* plants were more tolerance to oxidative stress than *BrMT1* and WT. In the DAB staining experiment for H_2O_2 detection in paraquat treatment leaves, *RbcS-BrMT1* plant showed low accumulation of H_2O_2 caused by paraquat.

Our results suggest that the chloroplast targeting of *BrMT1* was effective to detoxification of ROS stress. Also it will be applicable for the development of plant with enhanced tolerance to environmental stresses.

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