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## Photosynthetic response to dehydration and high temperature in trehalose-producing transgenic tobacco

S-S Jun, HJ Choi, JY Yang, Y-N Hong

School of Biological Sciences, Seoul National University, Seoul 151-742, Korea,  
[ssjun@snu.ac.kr](mailto:ssjun@snu.ac.kr)

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### Introduction

Trehalose, a commonly found disaccharide in bacteria, fungi, and primitive plants is known to provide them a protective role against desiccation and high temperature (McDougall and Strøm 1994; Müller *et al.* 1999). Recently, endogenous production of trehalose even in higher plants was implicated in *Arabidopsis*, suggesting its functional role in higher plants (Blázquez *et al.* 1998; Goddijn and Smeekens 1998; Vogel *et al.* 1998). Engineered tobacco plants expressing yeast *TPS* showed enhanced tolerance against dehydration by air-drying in both keeping higher fresh weight and recovering after rehydration (Holmström *et al.* 1996). Tobacco plants expressing *E. coli otsA* and *otsB* were similarly drought-tolerant (Pilon-Smits *et al.* 1998). Some prominent physiological changes including morphological alterations were also observed in parallel with enhanced resistance to dehydration (Goddijn *et al.* 1997; Romero *et al.* 1997). The object of present work is to confirm earlier results in physiologically more relevant conditions and to assess the photosynthetic ability of trehalose-producing plants under drought and high temperature stress.

### Materials and methods

#### *Plant transformation.*

The coding region of *otsA* gene (Kassen *et al.* 1992; 1994) was obtained by PCR using Vent polymerase (New England Biolabs, Beverly, MA) and primers corresponding to its 5'- and 3'-ends with *Xba*I and *Eco*RI site, respectively, to clone into pWP90 vector containing a double 35S CaMV promoter and a CaMV polyA terminator. Transgenic tobacco was generated by the leaf disc transformation procedure and selected on MS medium containing kanamycin. Homozygous plants in F<sub>2</sub> or F<sub>3</sub> generations were used for the subsequent physiological experiments.

#### *Plant growth and treatment.*

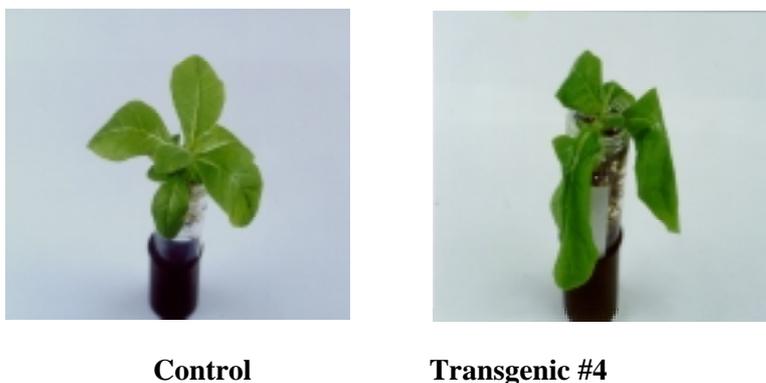
Tobacco (*Nicotiana tabacum* L. var SR1) plants were grown for 3 to 4 weeks in a growth chamber maintained at 25±1°C with a diurnal cycle of 16 h-light and 8 h-dark under the light intensity of 100 µmol m<sup>-2</sup> s<sup>-1</sup>. Dehydration treatment was done by immersing the roots in the Hoagland solution containing 10% (W/V) polyethylenglycol (PEG)-6000. Water potential of leaf was measured psychometrically using dewpoint microvoltmeter (HR-33T, Wescor, USA). High temperature treatment was done by incubating the whole plants in the incubator maintained at 40°C~45°C providing with full humidity.

### Measurement of photosynthesis.

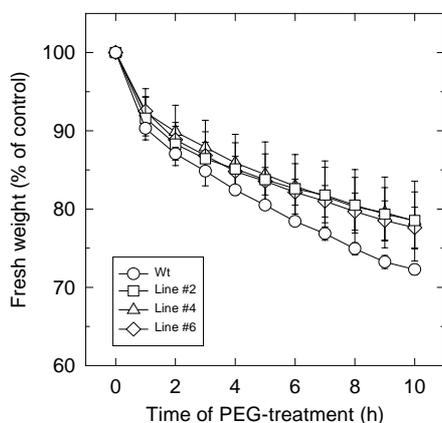
O<sub>2</sub> evolution and Chl fluorescence was measured simultaneously using a leaf disc (3.5 cm in diameter) in Hansatech (Kings Lynn, UK) LD2 leaf disc chamber with Clark type electrode and Walz PAM Chl fluorometer (Effeltrich, Germany). Each leaf disc was chosen to have approximately equal Chl content, when measured by Minolta Chl meter (SPAD-502, Minolta, Japan) and normalized to the control.

## Results and discussion

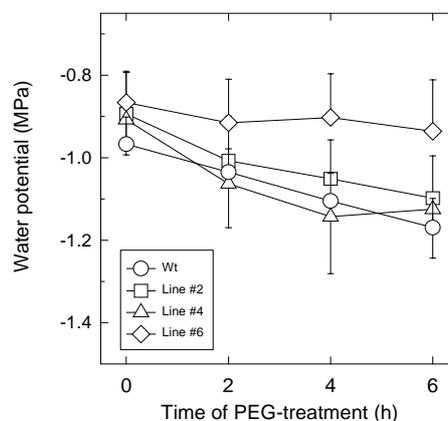
*Enhanced tolerance to dehydration in transgenic plants.* When dehydration of plants was induced by PEG-treatment, trehalose-producing tobacco plants maintained morphological intactness better than control plants. After 3 h of PEG-treatment, a representative transgenic plant (line #4) stayed turgid while a wild type plant withered visibly (Fig. 1). Decrease in fresh weight (FW) by PEG-treatment showed a comparable result. All lines of transgenic plants exhibited improved water retaining ability as shown by lesser reduction in FW after PEG-treatment (Fig. 2) and higher initial water potential (Fig. 3). However, water potential in the leaves was decreased similarly after PEG-treatment in both control and transformants (Fig. 3).



**Fig. 1.** Photographs of wild type and transgenic plants after 3 h of PEG-treatment. Note the difference in the withered state between wild type and transgenic plants.



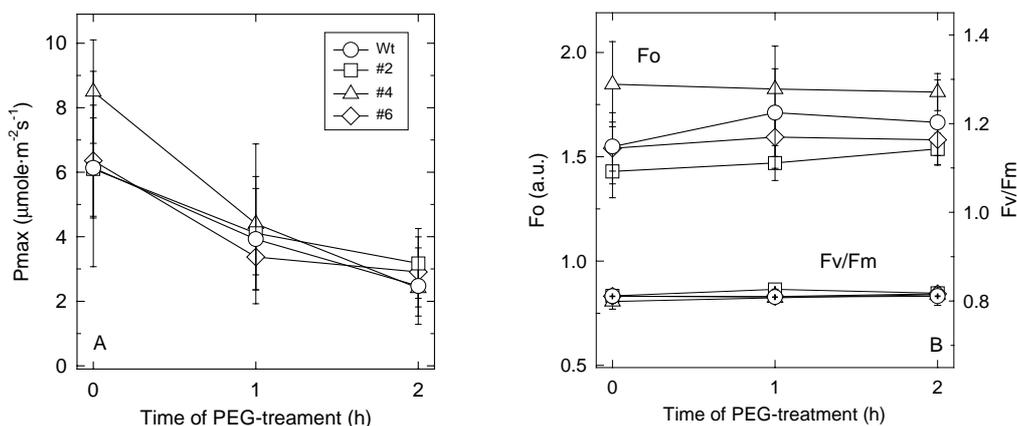
**Fig. 2.** Changes in relative fresh weight after PEG-treatment in wild type and transgenic plants.



**Fig. 3.** Changes in water potential after PEG-treatment in wild type and transgenic plants.

### Photosynthetic responses to dehydration

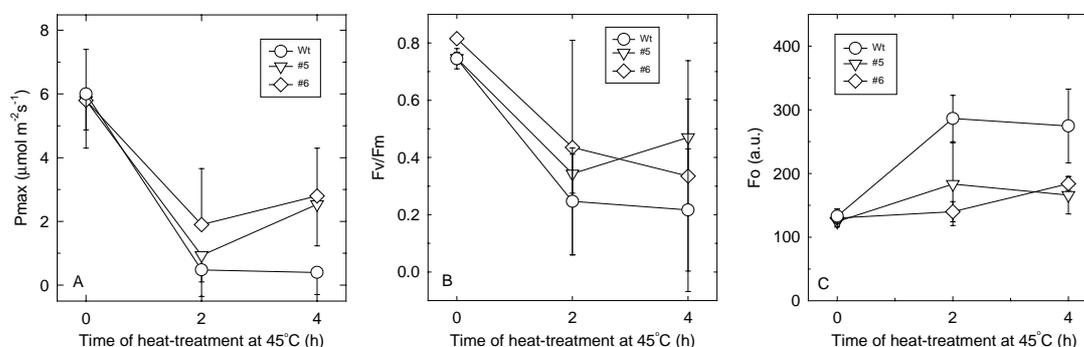
Upon dehydration, the maximal photosynthetic rate of O<sub>2</sub> evolution (P<sub>max</sub>) was decreased in a similar phase in both nontransformants and transgenic plants (Fig. 4A), but no significant changes in Chl fluorescence parameters (F<sub>o</sub> and F<sub>v</sub>/F<sub>m</sub>) were observed in both nontransformants and transgenic plants (Fig. 4B). It appears that trehalose confers transgenic plants improved water retaining ability against dehydration, but without keeping their photosynthetic capacity.



**Fig. 4.** Changes in P<sub>max</sub> (A), and Chl fluorescence parameters (B; F<sub>o</sub>, F<sub>v</sub>/F<sub>m</sub>) after PEG-treatment in wild type and transgenic plants.

### Photosynthetic responses to high temperature.

Since no apparent parameters were available to assess the tolerance against heating, photosynthetic performance after heat-treatment was directly used as a criterion for the resistance. In general, heating leads to the decline in P<sub>max</sub> and maximal photochemical efficiency (F<sub>v</sub>/F<sub>m</sub>), but increase in the initial Chl fluorescence (F<sub>o</sub>). After 2 h of heat-treatment at 45°C in the dark, P<sub>max</sub> was declined more than 80% in wild type plants while in transgenic plants P<sub>max</sub> was maintained close to 50% even after 4 h (Fig. 5A). Similarly, Chl fluorescence parameters (F<sub>o</sub> and F<sub>v</sub>/F<sub>m</sub>) remained more favourable in transgenic plants after heat treatment at 45°C than nontransformants (Fig. 5B and 5C). Heat-treatment at lower temperatures (40°C and 43°C) yielded identical results with lesser inhibition (not shown). It is concluded that trehalose confers improved tolerance against high temperature.



**Fig. 5.** Changes in P<sub>max</sub> (A), F<sub>v</sub>/F<sub>m</sub> (B), and F<sub>o</sub> (C) after PEG-treatment in wild type and transgenic plants.

## Conclusion

Induction of trehalose synthesis by genetic engineering seems to confer drought-resistance to transgenic plants as shown by improved water-retaining ability against dehydration and recovering ability. However, maintenance of high photosynthetic capacity under dehydration was not accompanied. On the other hand, trehalose-producing plants showed better ability in holding photosynthetic activity under heat treatment at 40°C~45°C. Transgenic plants exhibited higher P<sub>max</sub> and more favorable Chl fluorescence parameters (F<sub>o</sub> and F<sub>v</sub>/F<sub>m</sub>) after heat treatment. The results suggest that trehalose may provide wide range of tolerance against various stresses, but in terms of maintaining photosynthetic productivity it appears to be more effective in conferring enhanced tolerance against high temperature than dehydration. Test for the effectiveness of trehalose-producing plants against salt and chilling stress is in progress.

## Acknowledgments

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