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Physiological Evaluation of Transgenic Rice Developed for Drought Tolerance

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Abstract

Evaluation of physiological performance of trehalose-producing transgenic rice line was conducted to investigate drought tolerance at early growth stage. Under artificially induced drought condition of 8% polyethylene glycol 6000, this transgenic rice line had leaf photosynthetic rate of 11.08 μmol CO_2 $m^{-2}s^{-1}$, leaf transpiration rate of 8.38 mmol H_2O $m^{-2}s^{-1}$ and leaf water potential of -1.12 MPa after 96 hours of treatment. Nakdongbyeo, the parent of this transgenic rice line, had photosynthetic rate of 15.42 μmol CO_2 $m^{-2}s^{-1}$, leaf transpiration rate of 8.04 m mol H_2O $m^{-2}s^{-1}$ and leaf water potential of -0.88 MPa. The other variety used in this experiment for comparison, IR 72, showed higher values than both transgenic rice line and variety Nakdonbyeo on all three parameters; leaf photosynthetic rate of 20.61 μmol CO_2 $m^{-2}s^{-1}$, leaf transpiration rate of 12.88 mmol H_2O $m^{-2}s^{-1}$, and leaf water potential of -0.82 MPa. So this transgenic rice line did not show superior performance in leaf transpiration rate, leaf photosynthetic rate and leaf water potential compared to variety Nakdongbyeo. This result along with visual observation on leaf rolling and drying during the experimental period indicated poor physiological performance of this transgenic rice line. Further studies on metabolic status of stress-induced trehalose, along with study on physiological response of this transgenic rice line during drought stress would shed more light on overall physiological performance of this transgenic rice line.

Key words: Drought tolerance, leaf water potential, trehalose-producing transgenic rice, polyethylene glycol.

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Introduction

Drought is an unfavorable condition mainly characterized by inadequate water for a plant to grow normally and complete its life cycle (Cabuslay et al. 2002). It is one of the most important constraints of rice production in many rice-producing countries of the world (Herdt 1991). Rice is grown widely under rain fed conditions. In Asia about 45% of total rice area is estimated to have no irrigation input (Crosson 1995). The erratic and unpredictable monsoon pattern in this region has seriously challenged rice production in the rain fed ecosystem. In this context, development of well adapted drought tolerant rice varieties for rain fed lowland conditions would improve productivity and stability in the lowland rice growing environment (Jongdee et al. 2002). Despite focused efforts to improve crop for resistance to abiotic stresses such as drought by traditional breeding, success has been limited (Garg et al. 2002). Owing to the slow progress of drought tolerant rice cultivars by conventional breeding, there is increasing research efforts in engineering stress-tolerant rice varieties over the last decade.

A non-reducing disaccharide, trehalose (α-D-gluco-pyranosyl-[1-1]-α-D-glucopyranoside), is commonly found in a wide variety of organisms including vascular plants. It not only serves as carbohydrate reserve, but also protects biological molecules in response to different stress conditions in organisms (Penna 2003). In *E.coli*, biosynthesis of trehalose is a two-step process consisting of the conversion of UDP-glucose and glucose-6-phosphate to trehalose-6-phosphate by trehalose-6-phosphate synthase (TPS) and subsequent dephosphorylation of trehalose-6-phosphate to trehalose by trehalose-6-phosphate phosphatase (TPP). The *E. coli* bacterial genes involved in trehalose biosynthesis were subsequently introduced in plants. The constitutive overexpression of TPS and or TPP genes from yeast or *E. coli* in tobacco or potato plants resulted in undesirable pleiotropic effects, including stunted growth and altered metabolism under normal growth conditions (Goddijn et al. 1997). By overcoming undesirable effects of these two enzymes at individual states, Seo et al. (2000) constructed the bifunctional fusion enzyme TPSP from TSP and TPP. Garg et al. (2002) reported that trehalose accumulation in rice plants would confer high tolerance levels to different abiotic stresses including drought, salinity and cold. Subsequently, Jang et al. (2003) generated trehalose-producing transgenic rice by the transformation of a Korean rice variety, Nakdongbeyo. The gene encoding a bifunctional fusion (TPSP) of the trehalose-6-phosphate synthase (TSP) and trehalose-6-phosphate phosphatase (TPP) was introduced in the rice variety Nakdongbyeo under the control of maize ubiquitin promoter. The resultant transgenic rice was reported to produce and accumulate higher amount of trehalose with high catalytic efficiency of fusion enzyme in TPSP rice plants with increased tolerance to drought, salt and cold stresses.

Although metabolic engineering for enhanced accumulation of trehalose in plants has been the recent focus of attention, very few reports have been done on the physiological evaluation of transgenic rice so far. The objective of this study was to evaluate the physiological performance of trehalose-producing transgenic rice line developed for drought tolerance under artificial drought conditions at early growth stage.

Materials and Methods

Plant Materials

Three different rice varieties used in the study were; trehalose-producing transgenic line developed by Jang et al. (2003) and acquired from Korea Reserch Institute for Bioscience and Biotechnology, Taejon, Korea, and varieties Nakdongbyeo and IR 72, both obtained from the germplasm collection of Kyungpook National University, Daegu, Korea. Rice variety Nakdongbyeo, which is also parent of the transgenic line, is one of the popular varieties in Korea. It was the first rice variety of Korea demonstrated to give fertile transgenic plants (Lee et al. 1995). So Nakdongbyeo has been included for comparative analysis of the physiological traits in transgenic line during drought stress. Another variety IR 72 was chosen as a check, since it has been extensively used in drought studies and characterized as mild drought tolerant variety (Cabuslay et al. 2002). Moreover, IR 72 is reported as one of the most advanced and successful rice variety released by International Rice Research Institute providing very high yield (Datta et al. 1992).

Growth Conditions

Germinated seeds were grown on a seed nursery filled with sand and Yoshida nutrient solution (374.8 μM NH_4NO_3, 128.1 μM $NaH_2PO_4 \cdot 2H_2O$, 172.1 μM K_2SO_4, 340 μM $CaCl_2 \cdot 2H_2O$, 81.1 μM $MgSO_4 \cdot 7H_2O$, 7.1 μM $FeSO_4 \cdot 7H_2O$, 2 μM $MnSO_4 \cdot 5H_2O$, 8 μM H_3BO_3, 0.6 μM $ZnSO_4 \cdot 7H_2O$, 0.04 μM $CuSO_4 \cdot 5H_2O$, 0.004 μM $(NH_4)_6 MO_7O_{24} \cdot 4H_2O$) was supplied for two weeks (Yoshida et al. 1976). Following the method described by Kang et al. (2005), two-week old rice seedlings were transplanted at 4 cm interval on a piece of styrofoam plate placed on nutrient solution in a plastic container with size (25 cm × 20 cm × 15 cm) and rice seedlings were acclimated to hydroponics for 24 hours in the controlled environment chamber. On the next day, five liters of Yoshida nutrient solution was maintained in each plastic container. Nutrient solution was treated with PEG 6000 to make it 8 and 16% PEG 6000 solution.

For artificial induction of water stress, the addition of non-ionic osmotic agents such as PEG to liquid media is often preferred since it is mimic to specific levels of soil water stress (Lu et al. 1998). Because PEG is not readily taken up by cell, it cannot cross membranes such that the water potential gradient in the tissue is not altered. PEG 6000 solution has been considered better for work with plant than PEG solutions of lower molecular weight (Burlyn et al. 1973).

Untreated control pots were kept with only the nutrient solution and no PEG treatment. The culture solution was not changed during the period of 96 hours. All the treated and untreated control rice plants were grown in controlled condition in the growth chamber maintained in 16 hours, 25℃ during day and 8 hours, 20℃ during night. A mixture of cool-white fluorescent and halogen lamps provided light intensity of 1000 μmol $m^{-2}s^{-1}$ at the level of shoot (Kang et al. 2005). Water potential of the different PEG solution were; 8% PEG: -0.24 MPa and 16% PEG: -0.32 MPa, respectively.

Measurement of transpiration and photosynthetic rate

A portable leaf carbon dioxide analyzer (LCA-4) and a Parkinson chamber (PLC-4, ADC system, UK) were used to determine the photosynthetic and transpiration rate per unit leaf area on the fully expanded leaves. The measurements were taken at 96 hours after drought treatment. Leaf photosynthetic rate was determined at carbon dioxide concentration of 350 parts per million under 1600 μmol $m^{-2}s^{-1}$ light intensity in the growth chamber in 8% PEG treatment and in untreated control.

Measurement of leaf water potential

Leaf water potential (LWP) was measured on the uppermost fully expanded leaves of four individual plants of both 8 and 16% PEG treatment at 96 hours after drought exposure, using a dew point micro voltmeter (HR-33T, Wescor, USA). Visual observation was taken for four consecutive days after drought treatment. Plants were specifically observed for leaf rolling and leaf drying. During visual observation, photographs (not given in this paper) were also taken and assessed along with other measured physiological parameters.

Statistics

Completely randomized design (CRD) was used in this experiment. Measurement in four replicates was taken for leaf water potential and measurement in five replicates was taken both for leaf photosynthetic rates and leaf transpiration rates, respectively. All results of transpiration and photosynthetic rates were subject to one-way analysis of variance with Duncan multiple range test (DMRT) between mean values.

Results and Discussion

Leaf transpiration

Leaf transpiration rate at 8% PEG treatment condition for transgenic rice was 8.38 mmol H_2O $m^{-2}s^{-1}$, for Nakdongbyeo was 8.04 mmol H_2O $m^{-2}s^{-1}$ and for IR 72 was 12.88 mmol H_2O $m^{-2}s^{-1}$. The transpiration rate of transgenic line at 8% PEG was not significantly different from Nakdongbyeo but was significantly lower than IR 72. At untreated control condition, the means of transpiration rates among three lines were not significantly different (Table 1). When compared with respect to relative transpiration rate, transgenic rice had relative transpiration rate of 77%, Nakdongbyeo of 77% and IR 72 of 111%. Relative transpiration in transgenic rice was the same to that of its parent, Nakdongbyeo, but lower than check variety IR 72. It was evident that transgenic line and Nakdongbyeo showed transpiration rates lower at stressed condition than at non-stressed control conditions. Conversely, variety IR72, having higher transpiration rate at drought condition of 8% PEG could have overcome the adverse effects of drought by opening up more stomata and maintaining gas exchange, thereby balancing transpiration and photosynthetic rates in that variety. Cabuslay et al. (2002) reported that having a high transpiration in IR 72 could be a desirable trait only under the conditions of mild water stress. Rice variety with relative transpiration close to that of untreated control might be relatively more tolerant to drought stress and could possess genes to withstand water deficit.

Leaf photosynthesis

Leaf photosynthetic rate of transgenic rice line at 8% PEG was 11.08 μmol CO_2 $m^{-2}s^{-1}$ and its untransformed parent Nakdongbyeo was 15.42 μmol CO_2 $m^{-2}s^{-1}$. IR 72 showed the highest value 20.61 μmol CO_2 $m^{-2}s^{-1}$ among the three lines. Mean of photosynthetic rates of transgenic line was significantly lower than that of Nakdonbyeo and IR 72 at 8% PEG. Similarly at untreated control condition, the mean photosynthetic rate of transgenic rice line was significantly lower than both Nakdongbyeo and IR 72. Simultaneously, the mean photosynthetic rate of Nakdongbyeo was not significantly different from IR 72 (Table 2). When compared among three rice lines with respect to relative photosynthesis, IR 72 had the highest value of 97% followed by transgenic rice of 70% and Nakdongbyeo of 69%. It could be a clear indication that the transgenic rice line is not significantly superior to its untransformed parent Nakdongbyeo and at the same time might not be as good as IR 72 in photosynthetic performance. Comparable study conducted by Siddique et al. (1999) on wheat varieties at early vegetative stage also reported similar finding that photosynthetic rate significantly decreases under water stress condition compared with non-stressed plants. Although there was no significant difference in transpiration rates between transgenic line and its untransformed parent, Nakdongbyeo at 8% PEG, the photosynthetic rate of transgenic rice line was lower than Nakdongbyeo. Variety IR72 grown at drought level of 8% PEG maintained photosynthetic rate closer to that of non-stressed control.

Table 1. Effect of drought on Leaf transpiration rates of rice varieties after 96 hours of drought treatment.

Rice varieties	Leaf transpiration rate		[1] Relative transpiration
	Untreated control	8% PEG	
	---------- mmol H_2O $m^{-2}s^{-1}$ ----------		
Transgenic rice	10.95±2.60^a	8.38±0.35^b	77%
Nakdongbyeo	10.40±0.65^a	8.04±0.20^b	77%
IR 72	11.63±2.54^a	12.88±0.29^a	111%

Each value is mean ± standard error (n=5).
Means followed by same letter within the same column are not significantly different at 5% by DMRT.
[1] Relative transpiration rate was obtained by dividing the mean value obtained from the measurement of transpiration rate in the drought treated plants with the corresponding mean value obtained from the measurement of the untreated control plants and multiplied by 100.

Table 2. Effect of drought on Leaf photosynthetic rates of rice varieties after 96 hours of drought treatment.

Rice varieties	Leaf photosynthetic rate		[1] Relative photosynthetic rate
	Untreated control	8% PEG	
	----------- μmol CO_2 $m^{-2}s^{-1}$ -----------		
Transgenic rice	15.85±0.96[b]	11.08±0.39[c]	70%
Nakdongbyeo	21.78±0.52[a]	15.04±0.88[b]	69%
IR 72	21.19±0.98[a]	20.61±0.64[a]	97%

Each value is mean ± standard error (n=5).
Means followed by same letter within the same column are not significantly different at 5% by DMRT.
[1] Relative photosynthetic rate was obtained by dividing the mean value obtained from the measurement of photosynthetic rate in the drought treated plants with the corresponding mean value obtained from the measurement of the untreated control plants and multiplied by 100.

Table 3. Effect of drought on leaf water potential of rice varieties after 96 hours of drought treatment.

Rice varieties	Leaf water potential		
	Untreated control	8% PEG	16% PEG
		----------- (MPa) -----------	
Transgenic rice	-0.90±00 [1]	-1.12±00	-1.17±00
Nakdongbyeo	-0.76±0.01	-0.88±0.04	-0.93±0.03
IR 72	-0.69±00	-0.82±0.02	-1.36±0.02

[1] Each value is mean ± standard error (n=4)

Leaf water potential (LWP)

LWP decreased continuously when PEG concentration increased regardless of rice varieties used. Transgenic rice line was found with the lowest LWP values of -0.90 MPa and -1.12 MPa at untreated control and 8% PEG, respectively. Nakdongbyeo, the parent of transgenic rice line showed higher LWP values of -0.76 MPa and -0.88 MPa at control and 8% PEG conditions, respectively than those of transgenic rice line. IR 72 on the other hand showed LWP values of -0.69 MPa and -0.82 MPa at control and 8% PEG, respectively. The capacity of rice varieties to maintain higher LWP value during drought has been considered as a promising trait for selection to improve tolerance against drought. As a result, maintenance of LWP by plant is often used as a selection criterion for a breeding program with the objective of improving drought tolerance in rice (Jongdee et al. 2002). In this observation, lower water potential values in higher negative magnitudes integrated with poor visual score (data are not shown) of transgenic line than that of Nakdongbyeo confirmed the poor physiological performance of this transgenic line. Higher transpiration rate in IR 72 (Table 1) might be the reason behind the lowest LWP values of this line at 16% PEG.

This study on physiological evaluation of transgenic rice developed for drought tolerance has elucidated response of a transgenic rice line in water deficit at seedling stage. Based on similar findings reported by various authors (Cabuslay et al. 2002; Fukai et al. 1999; Jongdee et al. 2002) and physiological traits of the transgenic line evaluated in this study, this transgenic line was not better than Nakdongbyeo and IR 72 up to the drought level equivalent to 8% PEG in Yoshida solution under the growth chamber conditions. In one study, Cabuslay et al. (2002) reported that under mild stress conditions, high transpiration rate of IR 72 was associated with maintenance of leaf moisture and leaf area, which allowed photosynthetic activity and provided assimilates for continued growth. However, under severe stress condition, high transpiration might cause excessive moisture loss, which, during prolonged exposure, could lead to death of rice plants. So, desirable rice varieties should have balanced photosynthetic and transpiration rates to cope up drought condition at the minimum cost of energy. Transgenic rice line, though found to have lower initial leaf water potential, was more consistent than two other varieties such as Nakdongbyeo and IR 72, in maintaining leaf water potential from 8 to 16% PEG treatment. That trait of transgenic line might be attributed to the osmotic adjustment by higher amount of trehalose accumulation than Nakdongbyeo as reported by Jang et al. (2003).

Verslues et al. (2006) has reported that evaluating the stress responses of transgenic plants is often the most challenging type of experiment because the objective would be to evaluate whether the plant's overall performance under stress has been altered; it has been suggested that a broader approach than just measuring parameters such as changes in gene expression or metabolite levels of the developed transgenic lines be used. In another recent experiment, Tarek et al. (2005) has reported that accumulation of trehalose in wheat under drought stress condition was tissue and species specific. Based on that finding, it could be that shoot tissues might have different response to drought from root tissues. Since our study was limited to physiological parameters of rice shoots in the growth chamber conditions, further study on root parameter of this transgenic line could be worth considering.

Penna (2003) has reported that low or undetectable levels of trehalose in transgenic plants could account for a specific trehalase activity where trehalase contributes to trehalose degradation. For further understanding of poor physiological performance of this transgenic line, more experiments on synthesis and degradation of stress-induced trehalose during drought stress need to be conducted along with study on physiological responses of this line to drought. Finally, the result of this study supports a generalized thesis that rigorous analyses of key physiological traits along with evaluation on changes in gene expression or metabolite levels should be conducted before disseminating transgenic rice developed for drought tolerance from the laboratory to general cultivation.

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