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Overaccumulation of glycine betaine makes the function of the thylakoid membrane better in wheat under salt stress

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ABSTRACT

Wheat (*Triticum aestivum* L.) lines T1, T4, and T6 were genetically modified to increase glycine betaine (GB) synthesis by introduction of the *BADH* (betaine aldehyde dehydrogenase, BADH) gene from mountain spinach (*Atriplex hortensis* L.). These transgenic lines and WT of wheat (*T. aestivum* L.) were used to study the effect of increased GB synthesis on wheat tolerance to salt stress. Salt stress due to 200 mmol L^{-1} NaCl impaired the photosynthesis of the four wheat lines, as indicated by declines in net photosynthetic rate (P_n), stomatal conductance (G_s), maximum photochemical efficiency of PSII (F_v/F_m), and actual photochemical efficiency of PSII (Φ_{PSII}) and an increase in intercellular CO_2 concentration (C_i). In comparison with WT, the effect of salinity on the three transgenic lines was mild. Salt stress caused disadvantageous changes in lipids and their fatty acid compositions in the thylakoid membrane of the transgenic lines and WT. Under salt stress, the three transgenic lines showed slightly higher chlorophyll and carotenoid contents and higher Hill reaction activities and Ca^{2+}-ATPase activity than WT. All the results suggest that overaccumulation of GB resulting from introduction of the *BADH* gene can enhance the salt tolerance of transgenic plants, especially in the protection of the components and function of thylakoid membranes, thereby making photosynthesis better. Changes in lipids and fatty acid compositions in the thylakoid membrane may be involved in the increased salt stress tolerance of the transgenic lines.

Abbreviations: BA, betaine aldehyde; BADH, betaine aldehyde dehydrogenase; C_i, intercellular CO_2 concentration; DGDG, digalactosyl diacylglycerol; F_m, maximum fluorescence in the dark; F_o, initial fluorescence in the dark; F_v/F_m, maximum photochemical efficiency of PSII; GB, glycinebetaine; G_s, stomatal conductance; MGDG, monogalactosyl diacylglycerol; OA, osmotic adjustment; PC, phosphatidylcholine; PG, phosphatidylglycerol; Pn, net photosynthetic rate; ROS, reactive oxygen species; SQDG, sulfoquinovosyl diacylglycerol; SS, salt stress; WT, wild type; Φ_{PSII}, actual photochemical efficiency of PSII.

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1. Introduction

Crops in their natural habitats are frequently exposed to various environmental stresses, such as salinity, drought, and high temperature, and have developed mechanisms that allow them to withstand stresses. One such mechanism involves the accumulation of compatible solutes, which are defined as water-soluble organic compounds of low molecular mass that are nontoxic at high concentration [1].

Glycine betaine (GB) is regarded as one of the most effective compatible solutes. It has been reported that GB protects cells from stresses by maintaining an osmotic balance with the surrounding environment [2] and by stabilizing the quaternary structures of complex proteins, such as antioxidant enzymes and the oxygen-evolving PSII complex [3]. Some studies indicate that GB plays an important role in enhancing plant tolerance to drought and heat stress [4,5]. However, to date, there have been few reports confirming that GB can protect wheat plants against salt stress by overaccumulation of GB.

To cope with the major ecological problem of excess salt in soil, efforts have been undertaken to enhance the salt tolerance of economically important crops by genetic engineering approaches. Wheat can accumulate GB naturally, like spinach and beet, but the GB level in vivo is too low to bring about adequate osmotic adjustment under stress conditions. Transgenic wheat with *BADH* (betaine aldehyde dehydrogenase, BADH) cDNA can overcome this deficiency. The gene encoding BADH catalyzes the transformation of betaine aldehyde (BA) into GB and has an essential role in the synthesis of GB. However, it has been reported that GB-synthesizing transgenic plants can accumulate GB at levels lower than natural accumulators, but still confer tolerance to various abiotic stresses [6].

As the foundation of crop growth and yield, photosynthesis is one of the most important metabolic processes. The maintenance of high photosynthetic activity is thus a key factor in maintaining crop yield under stress. The photosynthetic activity of the chloroplast is one of the most stress-sensitive physiological processes. Stress damages the thylakoid membrane, disturbs its functions, and ultimately decreases photosynthesis and crop yield [7,8]. The function of the thylakoid membrane is based on fluidity and integrality, properties determined by its composition of lipids, membrane proteins, pigments, ions, and other biological substances. Stress can cause changes in the fluidity and composition of membranes [9].

Our previous experiments suggested that the expression of an exogenous *BADH* gene in the transgenic wheat lines T1, T4, and T6 resulted in GB levels significantly higher than that of the WT. GB at these levels enhanced the salt tolerance of transgenic plants by regulating ion homeostasis, enhancing osmotic adjustment (OA), and scavenging reactive oxygen species (ROS) [10]. In the present study, we investigated the effect of increased GB content on photosynthetic parameters, pigment contents and lipid composition of transgenic wheat lines under salt stress.

2. Materials and methods

2.1. Plant materials

Seeds of three transgenic lines (T1, T4, and T6) and WT of wheat (*Triticum aestivum* L.) were kindly provided by the Institute of Genetics and Developmental Biology of the Chinese Academy of Sciences, China. Transgenic wheat lines overexpressing *BADH*, encoding betaine aldehyde dehydrogenase (BADH), were designated as T1, T4, and T6 and generated by introduction, by microprojectile bombardment, of a pABH9 plasmid encoding the *BADH* gene cloned from *Atriplex hortensis* L. [11] under the control of a maize ubiquitin promoter and a *bar* gene.

2.2. Wheat seedling incubation and salt stress (SS) treatments

Wheat seeds sterilized with 0.2% sodium hypochlorite for 5 min were germinated for 24 h on filter papers moistened with water. The germinating seeds were grown on nylon gauze of an appropriate density and cultured in trays (25 cm × 18 cm × 5 cm) with water. The germinating seeds were then cultured in an incubator with a photosynthetic photon flux density of 150 μmol m^{-2} s^{-1}, constant temperature (25 °C), 75%–80% relative humidity (RH), and a photoperiod of 12/12 h light/dark cycle. After 10 days, the seedlings with one expanded and one expanding leaf were subjected to NaCl treatments. To avoid salt shock, the plants were initially subjected to low NaCl concentration (50 mmol L^{-1}) for two days through the roots. After the second day, treatment concentrations were increased by 50 mmol L^{-1} per day until a final concentration of 200 mmol L^{-1} was reached. After two and four days of treatment with 200 mmol L^{-1} NaCl (corresponding to seven and nine days since first exposure to salt stress), the first expanded leaves of the seedlings were harvested.

2.3. Determination of gas exchange parameters

Net photosynthetic rate (P_n), stomatal conductance (G_s), and intercellular CO_2 concentration (C_i) were measured with an open-system infrared gas analyzer (IRGA) (CIRAS-1, Hertfordshire, UK) between 10:00 and 12:00 a.m., at 360 μg g^{-1} CO_2 concentration and 21% O_2 concentration, irradiance of approximately 1200 μmol m^{-2} s^{-1}, 80% RH, and 24–30 °C temperature. The measurements of these photosynthetic parameters lasted approximately 10 min, during which no significant recovery was observed. Readings were taken directly from the instrument.

2.4. Determination of chlorophyll fluorescence parameters

Determinations of initial fluorescence in the dark (F_o), maximum fluorescence in the dark (F_m), and variable fluorescence in the dark (F_v) were performed with a pulse modulated fluorescence monitoring system (FMS-2, Hansatech, UK) using a modified procedure originally from Ma et al. [12] Maximum photochemical efficiency of PSII (F_v/F_m) was calculated as $F_v/F_m = (F_m - F_o)/F_m$.