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Research article

Salt-responsive mechanisms in chromosome segment substitution lines of rice (*Oryza sativa* L. cv. KDML105)

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ABSTRACT

Two chromosome segment substitution lines of Khao Dawk Mali 105 (KDML105) rice that carry quantitative trait loci for drought tolerance located on chromosome 8 (DT-QTL8) designated CSSL8-94 and CSSL8-116 were investigated for co-expression network and physiological responses to salinity compared to their parents (KDML105; drought and salt sensitive recurrent parent, and DH103; drought tolerant QTL donor). These CSSL lines show different salt-response traits under salt stress (CSSL8-94 shows higher tolerance than CSSL8-116) and possess different segments of DT-QTL8. To identify specific biological process(es) associated with salt-stress response, co-expression network analysis was constructed from each DT-QTL segment. To evaluate differential physiological mechanisms responding to salt stress, all rice lines/cultivar were grown for 21 d in soils submerged in nutrient solutions, then subjected to 150 mM NaCl for 7 d. Physiological parameters related to co-expression network analysis (photosynthetic parameters) and salt responsive parameters (Na^+/K^+ ratio, proline content, malondialdehyde and ascorbate peroxidase activity; EC1.11.1.1) were investigated along with the expression analysis of related genes. Physiological responses under salt stress particularly photosynthesis-related parameters of CSSL8-94 were similar to DH103, whereas those of CSSL8-116 were similar to KDML105. Moreover, expression levels of photosynthesis-related genes selected from the co-expression networks (*Os08g41460*, *Os08g44680*, *Os06g01850*, *Os03g07300* and *Os02g42570*) were slightly decreased or stable in CSSL8-94 and DH103 but were dramatically down-regulated in CSSL8-116 and KDML105. These differential responses may contribute to the photosynthesis systems of CSSL8-94 being less damaged under salt stress in comparison to those of CSSL8-116. It can be concluded that the presence of the specific DT-QTL8 segment in CSSL8-94 not only confers drought tolerant traits but also enhances its salt tolerant ability.

1. Introduction

Thailand is one of the top rice producing and exporting countries of the world. Thai rice is very popular in the world market especially, Khao Dawk Mali 105 (KDML105) which is Thailand's most important economic crop and mostly cultivated in the northeastern part of Thailand. Drought is a major cause of relatively low productivity of rain-fed rice farming in this area. To solve this problem, chromosome segment substitution lines (CSSL) of KDML105 that carry quantitative trait loci for drought tolerance (DT-QTL) in various segments were developed (Kanjoo et al., 2012). The CSSLsused in this study were derived from a cross between KDML105 which is a drought and salt sensitive cultivar and a doubled haploid line 103 (DH103; drought tolerant). The QTL donor parent, DH103, was developed from a cross between CT9993 and IR62266, both of them are well-known for the drought tolerance ability and have been used as donors of drought-resistance genes in

Abbreviations: APX, ascorbate peroxidase; C, control; CEF, cyclic electron flow; CSSL KDML105 DT-QTL8, chromosome segment substitution lines of KDML105 that carry quantitative trait loci for drought tolerance on chromosome 8; CSSL8-94, CSSL KDML105 DT-QTL8 line 94; CSSL8-116, CSSL KDML105 DT-QTL8 line 116; DH103, doubled haploid line 103; *E*, transpiration rate; g_s, stomatal conductance; KDML105, Khao Dawk Mali 105; MDA, malondialdehyde; *NHX1*, Na^+/H^+antiporter 1; P_N, net photosynthesis rate; PIP, plasma membrane intrinsic proteins; Pro, proline; *SOS1*, salt overly sensitive 1; S, salt treatment; TIP, tonoplast intrinsic proteins; WUE, water use efficiency.

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many rice breeding programs (Lanceras et al., 2004). Because DT-QTL from the donor (DH103) is located on chromosome 8, this CSSL population of 31 lines is called CSSL KDML105 DT-QTL8.

Another limitation of rice production in northeast Thailand is not only drought but also saline soils. From the Land Resources in Thailand's data (Yuvaniyama, 2004), saline soils in the northeastern part of Thailand (ranging from severe, moderate, to low salinity) amount to 16.73% of the total land area. High concentration of salts is able to reduce the water in soil solutions and causes an osmotic effect by interfering with the balance of water uptake. Compatible solutes are believed to maintain water balance by the mechanism of osmotic adjustment and also enhance stress tolerance of plants by preserving membrane integrity (Zhu, 2001). Proline (Pro) is one of the most-studied compatible solutes. Demiral and Türkan (2005) presented that Pro accumulation contributed to osmotic adjustment during salinity in salt-sensitive rice because it accumulated higher Pro than in salt-tolerant rice under salt stress. Moreover, for controlling water movement in and out of plant cellular membranes, aquaporins (AQP) are involved in this process. AQP is a member of major intrinsic proteins (MIP) family which includes plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs) and nodulin−26−like intrinsic membrane proteins (NIPs). Transgenic *Arabidopsis* overexpressing *MaPIP1;1* (isolated from banana) showed elevated expression of *MaPIP1;1* together with higher primary root elongation, root hair numbers and survival rates compared to wild type under drought and salt stress conditions (Xu et al., 2014).

Salt stress also causes an ion toxicity effect from Na^+, Cl^-, SO_4^{2-} or Mg_2^+ and nutrient imbalance. A large accumulation of Na^+ and Cl^- ion leads to inhibition of K^+ uptake (Sairam and Tyagi, 2004). The response to salt in some plants are salt exclusion at the whole plant (or the cellular level) or reducing the concentration of Na^+ in the cytosol (by compartmentalizing salt in the vacuoles) or enhancing K^+ uptake activity, while keeping Na^+ out (Munns et al., 2006). Saltoverly sensitive (SOS) pathway initiated in the plasma membrane has been reported to play important roles on ion homeostasis by mediating cellular signal under salt stress (Ji et al., 2013). Furthermore, Na^+/H^+ exchanger (NHX) which is located in the tonoplast membrane also involved in Na^+ compartmentalization from cytosol into vacuoles. Transgenic *Arabidopsis* which overexpressed *SOS1 or SOS3* alone or in combination with *NHX1* showed higher salt tolerance ability compared to that of the wild type (Yang et al., 2009). Osmotic stress and ion toxicity also induced stomatal closure leading to limited CO_2 followed by electron leakage from the electron transport chain to oxygen thus generating over-accumulation of reactive oxygen species (ROS) (Miller et al., 2010). Stressed plants therefore enhanced the production of antioxidant enzymes such as catalase (CAT; EC1.11.1.6) and ascorbate peroxidase (APX; EC1.11.1.1) to protect plant cells from damaging ROS. Overproduction of ROS can cause oxidative damages to proteins, DNA and lipids. Products from lipid peroxidation such as malondialdehyde (MDA) have been widely used as a parameter for indication of cellular damage induced by ROS. An increase in antioxidant enzymes activity and lipid peroxidation in plants under salt stress has been observed in many plant species (Nounjan et al., 2012; Rasool et al., 2013; Medeiros et al., 2014).

Nowadays, bioinformatics is a global tool widely used in plant biology for prediction of unknown genes/proteins or studying gene ontology. Co-expression network is one of the bioinformatics applications which predict an interaction of expression correlation of genes under the same condition or study genes association in specific traits (Usadel et al., 2009; Lee et al., 2010). Numerous authors have used co-expression analysis to identify candidate genes or study gene functions in plants. For example, Kim et al. (2013) used this method to identify candidate radio marker genes responding under gamma irradiation treatment compared to other abiotic stresses in rice.

In general, the mechanisms of abiotic stress (e.g. drought and salt) tolerance in plants are related to physiological responses and share regulatory network of gene expression to defend plants from damages incurred by these stresses. This study aimed to identify specific biological process (es) associated with salt-stress responses in drought tolerant rice. Firstly, salt tolerance ability of the CSSL KDML105 DT-QTL8 population (31 lines; CSSL8-94 − CSSL8-124) was evaluated. Secondly, two CSSL8 lines which showed contrasting performance under salt stress and received different DT-QTL8 segments were selected as representative plant materials. Gene co-expression network of each line was constructed to identify abiotic stress-responsive gene sets. Finally, physiological parameters related to co-expression network analysis (photosynthetic parameters), salt responsive parameters (Na^+/K^+ ratio, Pro content, MDA and APX activity) and expression of related genes were investigated.

2. Material and methods

2.1. Plant materials and stress treatment

A set of 31 CSSL KDML105 DT-QTL8 lines developed by Kanjoo et al. (2012) was evaluated for salt tolerance according to Gregorio et al. (1997) (Supplemented data B). Two lines of CSSL (CSSL8-94 and CSSL8-116) which showed contrasting performance under salt treatment and receiving different DT-QTL segments (see Supplemented data A for the genotypes of CSSL8-94 and CSSL8-116) were selected as representative plant materials to analyze gene co-expression network and study salt responsive physiological parameters including expression of related genes compared to the QTL donor (DH103) and the recurrent parent (KDML105).

Seeds of the four rice lines/cultivar were germinated in distilled water for 2 d at room temperature, and then transferred to seedling trays containing loamy sand soils (73.64% sand; 23.57% silt; 2.79% clay; total N 0.023%; available P 33.36 mg kg^{-1}; exchangeable K 77.57 mg kg^{-1}; CEC 23.24 c mol kg^{-1}; organic matter 0.638%). The trays were placed in two cement tanks filled with nutrient solution (Yoshida et al., 1976) to cover the soil surface. The plants were allowed to grow without stress for 21 d under natural light condition in a greenhouse at the Faculty of Agriculture, Khon Kaen University. Average daily solar radiation during the experimental period was 417.07 W/m^2, the average temperature day/night was 31.2°C/27.8°C. The plants were then divided into two groups, 5 trays each: (1) control, the seedling trays were submerged under the nutrient solution, and (2) NaCl treatment, the seedling trays were submerged under nutrient solution containing 150 mM NaCl in another cement tank. Na^+ concentrations in the soil of the control and treatment groups were 0.2 g/kg and 1.6 g/kg, respectively. The soils were completely submerged under the nutrient solution (for control) or nutrient solution containing 150 mM NaCl. The EC of the solution in the salinized tank was daily adjusted to approximately 15 dS m^{-1}. At 7 d after exposure to 150 mM NaCl, leaf tissues were harvested. The collected samples were stored at −80°C and −20 °C for further analysis (for gene expression study and biochemical parameters determination, respectively).

2.2. Identification of putative genes/proteins, correlated genes and network construction

Based on genotype data, the DT-QTL segments were located between RM477 and RM4153 in CSSL8-94, and between RM3485 and RM1615 in CSSL8-116 (Supplemented data A). The putative genes situated within these markers were searched in Gramene