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Rice RCN1/OsABCG5 mutation alters accumulation of essential and nonessential minerals and causes a high Na/K ratio, resulting in a salt-sensitive phenotype

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ABSTRACT

Mineral balance and salt stress are major factors affecting plant growth and yield. Here, we characterized the effects of rice (*Oryza sativa* L.) *reduced culm number1* (*rcn1*), encoding a G subfamily ABC transporter (OsABCG5) involved in accumulation of essential and nonessential minerals, the Na/K ratio, and salt tolerance. Reduced potassium and elevated sodium in field-grown plants were evident in *rcn1* compared to original line 'Shiokari' and four independent *rcn* mutants, *rcn2*, *rcn4*, *rcn5* and *rcn6*. A high Na/K ratio was evident in the shoots and roots of *rcn1* under K starvation and salt stress in hydroponically cultured plants. Downregulation of *SKC1/OsHKT1;5* in *rcn1* shoots under salt stress demonstrated that normal function of RCN1/OsABCG5 is essential for upregulation of *SKC1/OsHKT1;5* under salt stress. The accumulation of various minerals in shoots and roots was also altered in the *rcn1* mutant compared to 'Shiokari' under control conditions, potassium starvation, and salt and D-sorbitol treatments. The *rcn1* mutation resulted in a salt-sensitive phenotype. We concluded that RCN1/OsABCG5 is a salt tolerance factor that acts via Na/K homeostasis, at least partly by regulation of *SKC1/OsHKT1;5* in shoots.

1. Introduction

Rice is a major crop in many regions of the world, especially in Asian countries. It is a staple that provides 50–80% of daily calorie intake for more than 3 billion people [1]. Mineral balance is one of the main factors affecting plant yield. Potassium (K) is one of the three essential macronutrients required in the largest quantities for plant growth and yield and it plays a vital role in sustainable crop production systems [2,3]. As the most abundant cation in plant cells, K^+ is essential to physiological processes, including osmoregulation, cell growth, enzyme activity, membrane polarization, and photosynthesis [4]. The K content in glycophytes (plants that can tolerate only relatively low concentrations of salts) can reach 1.5–5% of plant dry weight, and the majority of K acquired from soil is transported to shoots [5]. A potassium content of less than 10 g/kg dry weight will lead to deficiency symptoms in most species [3,6]. K deficiency causes regional withering of old leaves and death of meristems, which eventually leads to reduced shoot growth and crop yield [4,5]. Due to intensive cropping practices and increased application of nitrogen (N) and phosphorus (P) fertilizers in recent years, K has become the most limiting nutrient for rice yields [7–9]. Genotypic differences in tolerance to low K have been reported by many researchers [9–13].

Salt stress is one of the most important soil stresses that limit plant growth and development. Soil salinity causes serious threats to agriculture worldwide, particularly in irrigated lands. In saline soil, rice plants experience osmotic stress, which results in reduced uptake of water and generates effects similar to those of water stress caused by drought. Exposure to salinity also causes accumulation of salts in plant tissues. These salts can eventually rise to toxic levels, especially in older leaves [20], and may cause sodium (Na) toxicity due to ionic stress, which can reduce nutrient acquisition or cause nutritional imbalances [21]. To adapt to the adverse environment, plants maintain ion and osmotic homeostasis with rapid

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ionic and osmotic signaling. Osmotic stress induced by high salinity increases the biosynthesis of abscisic acid (ABA). ABA then acts as a regulator initiating a second round of signaling in the salt stress response of the ABA-dependent pathway. There are also several salt stress inducible genes that are ABA independent [22].

In addition to the importance of K in plant developmental and reproductive processes, K acquisition acts as a defense mechanism against salt stress, which is mainly caused by excessive amounts of Na [14,15]. The Na/K ratio for plants growing in saline soils must be maintained by favoring accumulation of K over Na. High levels of Na or high Na/K ratios can disrupt enzymatic processes in the cytoplasm because of the ability of Na to compete with K for binding sites [16,17]. Physiological and genetic studies have indicated that K accumulation and Na exclusion in shoots are crucial for salt tolerance during salinity stress in glycophytes [18,19]. Though various channels, carriers and pumps act to maintain ion homeostasis in cells, salt stress leads to ionic imbalance. Genes encoding root K-uptake channels have been identified in many species, including *AKT1* and *AtKC1* in *Arabidopsis* [23,24], *SKT1* in potato [25], *LKT1* in tomato [26], *KDC1* in carrot [27], *ZMK1* in maize [28], and *OsAKT1* in rice [29]. OsAKT1 plays a role in K-uptake channels in roots and its expression is sensitive to salt stress [28]. The high-affinity K-transporter gene *OsHKT1;5* serves as a critical quantitative trait locus, *SKC1*, for salt tolerance in rice because the gene product functions as a Na-specific transporter, as does that of *AtHKT1;1* [30]. The recirculation of Na from shoots to roots by SKC1/OsHKT1;5 is important for detoxifying Na and contributes to salt stress tolerance in rice [30]. In addition, *OsHKT2;1* is expressed in roots and OsHKT2;1 takes up nutritional Na to maintain cell growth under low-K conditions [31].

ATP-binding cassette (ABC) transporters constitute one of the largest protein families and are present in organisms ranging from bacteria to humans [32]. In most cases, functional ABC transporters act as ATP-driven pumps and consist of two hydrophobic transmembrane domains (TMDs), which constitute the membrane-spanning pore, and two cytosolic domains, which are referred to as nucleotide-binding domains (NBDs) or nucleotide binding folds, as they contain ATP-binding Walker A and B motifs [33]. ABC proteins function in the transport of a wide variety of compounds including hormones, mineral ions, lipids, peptides, secondary metabolites and xenobiotics [34]. In plants, the ABC superfamily is divided into eight subfamilies, from ABCA to ABCH, and these proteins are highly abundant, with more than 120 isoforms in rice and *Arabidopsis thaliana* [35]. The ABCG subfamily exhibits a TMD–NBD–TMD–NBD structure and is divided into plant/fungal-specific pleiotropic drug resistance full-length transporters and eukaryotic white brown complex (WBC) half-size transporters that function as homo- or heterodimers [35]. This half-size G subfamily has 29 members in the Arabidopsis genome and 30 in the rice genome [36,37]. Arabidopsis AtABCG11 (COF1/DSO/AtWBC11) and AtABCG12 (CER5/AtWBC12) are required for the export of various cuticular lipids [38–43], AtABCG26 (AtWBC27) is involved in transport of sporopollenin precursors [44–47], and AtABCG13 (AtWBC13) is required for flower cuticle secretion and petal epidermis pattering [48]. In addition, AtABCG25/WBC25 and AtABCG22/AtWBC22 are involved in ABA transport and ABA responses [49,50]. More recently, it was shown that AtABCG9, AtABCG11 and AtABCG14 are involved in vascular pattering [51]. There has been rapid progress in understanding the functions of ABCG proteins in a dicot, Arabidopsis, whereas little is known about their functions in rice. Rice *reduced culm number 1* (*rcn1*) encodes a half-size G subfamily protein, OsABCG5 (OsWBC5) [52]. *RCN1/OsABCG5* is expressed in leaf primordia of main and axillary shoots, in vascular cells and leaf epidermis of older leaves, in crown root primordia, and in endodermis, pericycle and stele in roots [52]. In roots, *RCN1/OsABCG5* is upregulated following treatment with ABA, cytokinin or salicylic acid [53]. A possible role of RCN1/OsABCG5 in biosynthesis, transport, degradation, or signaling in these phytohormones remains to be clarified. Taking a different strategy, we characterized the phenotype of *rcn1* mutants and found that (1) plant growth shows no response to N, P, or K supply in a paddy field [54]; (2) N, P, and K shortage prolong flowering by more than 40 days and remarkably reduce shoot and root dry weight [55]; (3) *rcn1* lacks a response to nutrient shortage during root development involving the elongation of seminal, crown, and lateral roots and lateral root branching [56]. There remains a gap in connecting the molecular function of the RCN1/OsABCG5 transporter and this multiple aberrant phenotype. Furthermore, the independent *rcn* mutants *rcn2*, *rcn4*, *rcn5*, and *rcn6* show consistently reduced lateral root elongation, as does *rcn1* [56]. Therefore, assessing the differences in mineral accumulation between *rcn1* and other *rcn* mutants will help in understanding the role of RCN1/OsABCG5 in homeostasis of mineral balance.

In order to characterize RCN1/OsABCG5 in more detail, we performed further experiments and observed differences in growth between wild-type plants and the *rcn1* mutant under K starvation and salt stress. Here we present several lines of evidence that suggest the involvement of RCN1/OsABCG5 in accumulation of essential and nonessential minerals, Na/K homeostasis, and salt tolerance of rice.

2. Materials and methods

2.1. Element analysis of field-grown plants

Rice (*Oryza sativa* L. ssp. *japonica*) cultivar 'Shiokari' was used as the wild type. Five independent rice mutants originating from 'Shiokari,' S-97-61 (*rcn1-2*), N-174 (*rcn2*), N-187 (*rcn4-2*), N-185 (*rcn5*), and N-186 (*rcn6*) [31–33,37,38], were used in field experiments. Rice seeds were sterilized by immersion for 12 h in 0.2% benomyl hydrate solution (Sumitomo Chemical). Sterilized seeds were planted in soil compost. Plant materials were transplanted and grown at the Experiment Farm, Field Science Center for Northern Biosphere, Hokkaido University in 2006.

At 40 d after transplanting, field-grown plant samples were washed and dried for 3 d at 65 °C. The dried plant samples were then ground to a powder using a vibratory disk mill and analyzed for plant tissue elemental concentration as follows. N content was analyzed by dry combustion using a Vario EL III CHN elemental analyzer (Elementar Analysensysteme GmbH). Sulfur, chlorine, and silicon contents were measured using an XEPOS fluorescence X-ray analyzer (SPECTRO Analytical Instruments GmbH). Na, K, magnesium (Mg), and calcium (Ca) contents were analyzed using the Kjeldahl digestion method on a Z-5010 atomic absorption spectrophotometer (Hitachi High-Technologies Corporation). P, iron, and manganese concentrations in the Kjeldahl solution were analyzed by an ICPS-8100 inductively coupled plasma atomic emission spectrometer (Shimadzu Corporation). Each analysis involved at least 18 individual plant samples, and data were collected in triplicate.

2.2. Plant samples in controlled-growth cabinet

'Shiokari' and S-97-61 (*rcn1-2*) were used in controlled-growth experiments. Seeds were sterilized as described for field-grown plants. The sterilized seeds were sown and grown for 3 d on plastic mesh floating in a container filled with aerated (500 cc/min) reverse osmosis water in a growth chamber with a 16-h light (350 μmol/m^2/s, fluorescent tubes) (28 °C) and 8-h dark (24 °C) cycle. Five-day-old seedlings were divided into four treatments and were grown on plastic mesh in containers with various media: control treatment, 25% Murashige and Skoog (MS) medium