



Molecular cloning and stress-dependent regulation of potassium channel gene in Chinese cabbage (*Brassica rapa* ssp. *Pekinensis*)

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Summary

Potassium channels are important for many physiological functions in plants, one of which is to regulate plant adaption to stress conditions. In this study, *KCT2*, the gene encoding a membrane-bound protein potassium channel (GenBank accession number: *AY796219*), was isolated from Chinese cabbage (*Brassica rapa* ssp. *Pekinensis*) by RACE-PCR technique. Bioinformatics methods were performed for the gene structure and molecular similarity analysis. The *KCT2* expression patterns under various stress conditions were studied by semi-quantitative RT-PCR. DNA gel blot was used to analyze genomic organization. The putative *KCT2* was found to contain five membrane-spanning segments, a pore-forming domain (P-domain) between the last two transmembrane spans, a TxxTxGYGD motif in the P-domain and a putative cyclic nucleotide-binding-like domain within a long C-terminal region. *KCT2* is closest to *KAT2* in *Arabidopsis*. *KCT2* could be a one-copy gene with different isoforms or belong to a small gene family with four or five members. *KCT2* was expressed more strongly in leaves than in shoots and roots. *KCT2* transcription products were up-regulated by a 4-h-incubation in abscisic acid (ABA) and various stress treatment including cold stress (4 °C) for 24 h, drought stress for 1 h, and salt stress for 12 h. *KCT2* transcription was not affected by anoxia stress for 8 h and was

Abbreviations: P-domain, pore-forming domain; cNBD, cyclic nucleotide-binding domain; NUP, Nested Universal Primer; TAIR, The Arabidopsis Informational Resource; TMDs, transmembrane domains; UPM, Universal Primer A Mix

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down-regulated with cold stress for 48 h. *KCT2* was cloned for the first time from the genus *Brassica*. Expression analysis indicated that in the early stage of plant adaption to stress conditions *KCT2* is up-regulated, which results in a stimulation of potassium transport.

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Introduction

Potassium is an important cation in most biological systems. It is the most abundant cation in plant cells, constituting up to 10% of the total plant dry weight. It plays an important role in basic functions at the cellular and whole plant level, including maintenance of membrane potential, electrical neutralization of anionic groups, osmoregulation, control of cell membrane polarization, ion homeostasis, enzyme activation, signal transduction, and many other physiological functions (Shabala, 2003; Véry and Sentenac, 2003). The molecular mechanism of potassium uptake by plant roots, and transportation within plants has been intensively studied during the last decade. The first molecular analysis of potassium channels involved in membrane K^+ transport resulted in the identification of two *Arabidopsis* potassium channel genes, *AKT1* and *KAT1* in 1992 (Anderson et al., 1992; Sentenac et al., 1992). These channels are related to the animal K^+ channels of the Shaker family. Both *AKT1* and *KAT1* were characterized by functional complementation of yeast (*Saccharomyces cerevisiae*) mutant strains defective for K^+ uptake (Anderson et al., 1992; Sentenac et al., 1992). Modern molecular biological methods revealed several channel proteins were capable of potassium transportation in *Arabidopsis*, potato (*Solanum tuberosum*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), crystalline iceplant (*Mesembryanthemum crystallinum*), grapevine (*Vitis vinifera*), cenizaro (*Samanea saman*) and several other species (Müller-Röber et al., 1995; Buschmann et al., 2000; Su et al., 2001; Moshelion et al., 2002; Pratelli et al., 2002; Langer et al., 2004). These isolated plant potassium channel proteins can be divided into five groups: AKT1-, KAT1-, K^+ outward rectifier (SKOR)-like α -subunits, AKT2/3 and AtKC1 (Pilot et al., 2003b). Channels of the so-called Shaker family have been shown to play a role in many functions, such as K^+ uptake from the soil solution, K^+ -transport through the phloem and the stomatal movements (Maathuis et al., 1997; Zimmermann and Sentenac, 1999; Shabala, 2003; Véry and Sentenac, 2003). Similar to animal Shaker-type channels, KAT1 and AKT1 in *Arabidopsis* were

predicted to have six or seven membrane-spanning regions (Maathuis et al., 1997). A pore-forming domain (P-domain), between the fifth and sixth membrane span, determines cation selectivity and channel block (Anderson et al., 1995; Maathuis et al., 1997; Nakamura et al., 1997; Doyle et al., 1998). For the P-domain indications were obtained that a Thr-x-Thr-Thr-x-Gly-Tyr-Gly-Asp (T-x-T-T-x-G-Y-G-D) motif for determining K^+ selectivity (Anderson et al., 1995; Nakamura et al., 1997). Mutations in the G-Y-G-D region can alter the specificity of KAT1 (Uozumi et al., 1995). This motif is conserved in all of the potassium channels of the Shaker family in plants and animals.

The water status of plants can be regulated by the activity and number of ion channels, which, therefore, also affect the adaptation to drought stress of the plant (Zhu et al., 1998). In stressed conditions, the abscisic acid (ABA) content will influence the activity of potassium channels and the regulation of guard-cell ion transportation which promote stomatal closure resulting in a reduced transpiration (Qi and Spalding, 2004). *GORK*, one of the potassium channels, was found to be ABA insensitive and up-regulated by drought, salt and cold stresses in guard cells (Becker et al., 2003). *MKT1* and *KMT1*, the Shaker-like potassium channels from crystalline iceplant (*M. crystallinum*), were drastically regulated by salt stress condition (Su et al., 2001). The stress-dependent transcript patterns of the subfamily of potassium channels indicated that genes encoding potassium uptake systems display tissue specificity and a complex regulation in higher plants (Pilot et al., 2003a).

Chinese cabbage (*Brassica rapa* ssp. *Pekinensis*), originating from China, is an important leafy vegetable especially in the northern part of the country. Especially under stress conditions, potassium uptake is important for maintaining good yields and quality. The aim of the present study is therefore to clone and characterize potassium channel gene *KCT2* from Chinese cabbage and investigate its expression patterns under different stress conditions in order to learn more about the regulation of potassium uptake and its relation to the mechanism of stress tolerance.

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