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Article

Genome-Wide Comparative *in silico* Analysis of Calcium Transporters of Rice and Sorghum

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Abstract

The mechanism of calcium uptake, translocation and accumulation in Poaceae has not yet been fully understood. To address this issue, we conducted genome-wide comparative *in silico* analysis of the calcium (Ca^{2+}) transporter gene family of two crop species, rice and sorghum. Gene annotation, identification of upstream *cis*-acting elements, phylogenetic tree construction and syntenic mapping of the gene family were performed using several bioinformatics tools. A total of 31 Ca^{2+} transporters, distributed on 9 out of 12 chromosomes, were predicted from rice genome, while 28 Ca^{2+} transporters predicted from sorghum are distributed on all the chromosomes except chromosome 10 (Chr 10). Interestingly, most of the genes on Chr 1 and Chr 3 show an inverse syntenic relationship between rice and sorghum. Multiple sequence alignment and motif analysis of these transporter proteins revealed high conservation between the two species. Phylogenetic tree could very well identify the subclasses of channels, ATPases and exchangers among the gene family. The *in silico cis*-regulatory element analysis suggested diverse functions associated with light, stress and hormone responsiveness as well as endosperm- and meristem-specific gene expression. Further experiments are warranted to validate the *in silico* analysis of the predicted transporter gene family and elucidate the functions of Ca^{2+} transporters in various biological processes.

Key words: *in silico* comparison, calcium transporter, rice, sorghum, genomic annotation, synteny

Introduction

Calcium plays a central role in plants in the regulation of growth and development by acting as a second messenger in the signal transduction pathways. The membrane-associated calcium (Ca^{2+}) transporter proteins are essential to maintain calcium homeostasis under normal physiological conditions and provide tolerance against various stresses. There are three major classes of Ca^{2+} transporter proteins: channels, ATPases (pumps) and exchangers (*1*-*6*). Members of these transporter proteins may differ in their cellular and tissue distribution and the regulation by other signaling pathways (*7*, *8*). The spatial and temporal regulation of calcium concentration in plant cells depends on the coordinated activities of channels and active transporters located on different organelles and membranes (*9*). Calcium channels, pumps and ex-

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changers, which differ in their cellular distribution and mechanism of transport, operate the complex and tight regulation of calcium homeostasis. Specific isoforms of these proteins are responsible for increasing or reducing free calcium in the cytosol (*10*).

Whereas diffusion of molecules across membranes (either intracellular or across the plasma membrane) is mediated by calcium channels, the calcium pump (ATPase) is a membrane-bound Ca^{2+} transporter that uses energy derived from ATP hydrolysis to transport Ca^{2+} across membranes against their concentration gradient (*11*). There are two major Ca^{2+} ATPase families (*12, 13*): P-type IIA and P-type IIB. The P-type IIA family lacks N-terminal auto-regulatory domain while the IIB family of plant is characterized by the presence of an auto-inhibitory N-terminal domain containing a Ca^{2+}/CaM-binding site and a serine phosphorylation site (*14*). Calcium exchanger is a secondary active transporter using energy from the flow of one ion (for example, Na^{+}) down its concentration gradient to transport Ca^{2+} against its concentration gradient (*10*). Ca^{2+} transporters on various membranes play an important role in orchestrating diverse biological processes. The results of electrophysiological studies and molecular analyses indicate the existence of many species of Ca^{2+} transporter proteins (*15, 16*). Although a burgeoning number of Ca^{2+} transporters have been identified, it is often difficult to associate functions with particular transporters. Thus, there is a dire need of in-depth understanding of structural and functional roles of such transporter family, which can be elucidated by comparing the Ca^{2+} transporter gene family in the grass species of rice and sorghum.

Grasses, covering 20% of the earth's land surface, are ecologically well adapted (*17*). This group of plants is especially important to agriculture, contributing a large portion of the calories consumed in the human diet (*18*). Both rice and sorghum belong to the Poaceae family and represent two types of carbon metabolism: C3 and C4, respectively. The evolution of C4 photosynthesis in the Sorghum lineage involved redirection of C3 progenitor genes as well as recruitment and functional divergence of both ancient and recent gene duplicates (*19*). Analysis of the sorghum sequence provides new insights into the recruitment of C3 genes to the C4 pathway, allowing us to identify more orthologs in cereals.

Though there exists great diversity among cereals in terms of genome size, ploidy level and chromosome numbers, attempts have been made to reveal the existing synteny and colinearity on the basis of comparative genomics (*19*). A total of 19,929 (57.8%) sorghum gene models were collinear with rice. About 60% of sorghum genes are located in syntenic regions to rice and orthologous relationships are well established by genetic markers as well as whole-genome comparisons (*19, 20*). The identification of specific genes and their function would help to understand the development, evolution and differences of crop plants. Additionally, the comparative analysis of sorghum and rice will also offer new insights into the role of different Ca^{2+} transporters like calcium channel, pump and exchanger. Furthermore, examination of comparative synteny mapping between two monocot model species will allow understanding how genes evolve within monocot species and thus allow better identification of target genes for Ca^{2+} signaling. In view of the un-availability of sufficient information on the molecular mechanism(s) associated with calcium nutrition, transport and accumulation in cereal grains, we focused on comparing the Ca^{2+} transporter gene family in the grass species rice and sorghum.

In the present study, we performed genome-wide *in silico* identification of Ca^{2+} transporter gene family of sorghum from its recently sequenced genome (*19*), with annotation for chromosomal location(s), gene structure and phylogenetic tree construction. The putative functions of the predicted Ca^{2+} transporter genes in sorghum were investigated by analyzing the *cis*-regulatory elements present in the promoter region of these genes. Moreover, the comparative phylogeny and syntenic mapping with rice transporter gene family were also analyzed.

Results and Discussion

Genome-wide annotation of Ca^{2+} transporter gene family

The availability of sequenced genome of sorghum (*19*) provided ample opportunity to annotate and predict the complete set of information of Ca^{2+}