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TaSCL14, a Novel Wheat (*Triticum aestivum* L.) *GRAS* Gene, Regulates Plant Growth, Photosynthesis, Tolerance to Photooxidative Stress, and Senescence

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ABSTRACT

Rates of photosynthesis, tolerance to photooxidative stress, and senescence are all important physiological factors that affect plant development and thus agricultural productivity. GRAS proteins play essential roles in plant growth and development as well as in plant responses to biotic and abiotic stresses. So far few *GRAS* genes in wheat (*Triticum aestivum* L.) have been characterized. A previous transcriptome analysis indicated that the expression of a *GRAS* gene (*TaSCL14*) was induced by high-light stress in Xiaoyan 54 (XY54), a common wheat cultivar with strong tolerance to high-light stress. In this study, *TaSCL14* gene was isolated from XY54 and mapped on chromosome 4A. *TaSCL14* was expressed in various wheat organs, with high levels in stems and roots. Our results confirmed that *TaSCL14* expression was indeed responsive to high-light stress. Barley stripe mosaic virus (BSMV)-based virus-induced gene silencing (VIGS) of *TaSCL14* in wheat was performed to help characterize its potential functions. Silencing of *TaSCL14* resulted in inhibited plant growth, decreased photosynthetic capacity, and reduced tolerance to photooxidative stress. In addition, silencing of *TaSCL14* in wheat promoted leaf senescence induced by darkness. These results suggest that *TaSCL14* may act as a multifunctional regulator involved in plant growth, photosynthesis, tolerance to photooxidative stress, and senescence.

KEYWORDS: *Triticum aestivum* L.; *TaSCL14*; Photosynthesis; Photooxidative resistance; Senescence; BSMV−VIGS

INTRODUCTION

Photosynthesis is the primary source of dry matter production and grain yield in crop plants. Oxidative damage or early leaf senescence that is induced by harsh environmental conditions such as excessive amounts of light can cause dramatic yield losses in crops (Lim et al., 2007). Therefore, there is a potential to improve agricultural productivity through research and technological development focusing on improving rates of photosynthesis and tolerance to environmental stresses.

The GRAS proteins were named after the three initially identified gene products of *GIBBERELLIC ACID INSENSITIVE* (*GAI*), *REPRESSOR OF GA1* (*RGA*) and *SCARECROW* (*SCR*) (Bolle, 2004) which play diverse roles in plant growth

Abbreviations: A, CO_2 assimilation rate; bp, base pair; BSMV, barley stripe mosaic virus; *Ci*, intercellular CO_2 concentration; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; dpi, days post-inoculation; dNTPs, deoxyribonucleotide triphosphates; *E*, transpiration rate; ELISA, enzyme linked immunosorbent assay; *Fv/Fm*, maximal photochemical efficiency of photosystem II; GA, gibberellin; GAI, GIBBERELLIC ACID INSENSITIVE; GFP, green fluorescent protein; *Gs*, stomatal conductance; H_2O_2, hydrogen peroxide; *L*, light; MDA, malondialdehyde; MV, methyl viologen; *P.I.*, photosynthetic efficiency performance index; PPFD, photosynthetic photon flux density; PSII, photosystem II; qPCR, quantitative real-time PCR; RGA, REPRESSOR OF GA1; Rubisco (EC 4.1.1.39), ribulose-1,5-bisphosphate carboxylase/oxygenase; SCL, SCARECROW-LIKE; SCR, SCARECROW; SHR, SHORT-ROOT; TCA, trichloroacetic acid; VIGS, virus-induced gene silencing.

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and development. They belong to a plant specific transcription factor family sharing conserved domains of amino acid signature motifs in their respective C-termini and variable N-terminal regions (Bolle, 2004; Lee et al., 2008). At least 33 GRAS family members in *Arabidopsis thaliana* and 57 members in rice (*Oryza sativa* L.) have been identified to date (Tian et al., 2004; Lee et al., 2008). In *Arabidopsis*, the GRAS proteins are grouped into eight branches based on their amino acid sequences (Lee et al., 2008). Only one-third of the *Arabidopsis* GRAS proteins have been genetically analyzed. Phytochrome A signal transduction 1 (PAT1) and SCARECROW-LIKE13 (SCL13), which belong to branch I, are involved in phytochrome signaling (Torres-Galea et al., 2006). SHORT-ROOT (SHR), a member of branch III, is involved in root and shoot radial patterning; it functions by creating an SHR−SCR complex with SCR (Cruz-Ramírez et al., 2012). The branch IV members GAI, RGA, RGA-LIKE1 (RGL1), RGA-LIKE2 (RGL2), and RGA-LIKE3 (RGL3), are negative regulators of gibberellin (GA) signaling (Tyler et al., 2004). SCL23, a member of branch V, may play a role in yet unknown SHR-involved developmental pathways in the shoot system (Lee et al., 2008). SCR, another member of branch V, has an SHR-independent role in modulating sugar response; this role of SCR is important for root growth (Cui et al., 2012). In addition, SCR has been shown to regulate leaf growth by stimulating S-phase progression of the cell cycle (Dhondt et al., 2010). SCL3, a member of branch VI, regulates GA signaling *via* interaction with SHR−SCR and DELLAs (Heo et al., 2011). SCL6, SCL22 and SCL27, which belong to branch VIII, are known targets of *miR171* (Llave et al., 2002). The biological roles of the branch II members including SCL9, SCL11, SCL14, SCL30, SCL31, and SCL33 remain largely unknown. Fode et al. (2008) suggested that SCL14 may regulate the expression of genes involved in the detoxification of xenobiotics and possibly endogenous harmful metabolites. Additionally, SCL14 interacts with TGA and CYP81D11 (a cytochrome P450 protein) in defense against herbivorous insects (Matthes et al., 2010). GRAS proteins from other plant species have also been characterized. For example, Kamiya et al. (2003) proposed that OsSCR is involved not only in the asymmetric division of cortex/endodermis progenitor cells, but also involved in the formation of stomata and ligule by establishing polarization of cytoplasm in rice. Rice *Grain Size 6* (*GS6*), which clusters in the same clade as the branch VI members of the *Arabidopsis* GRAS gene family, is known to negatively regulate grain size (Sun et al., 2013). MONOCULM 1 (MOC1), a rice GRAS family nuclear protein, plays an important role in controlling rice tiller by initiating axillary buds and promoting their outgrowth (Li et al., 2003). Moreover, DWARF and LOW-TILLERING, two members of rice GRAS family, function in the positive regulation of brassinosteroid signaling (Tong et al., 2009). Overexpression of *SLENDER RICE-like 1* (*SLRL1*) in rice alters GA responses and causes a dwarf phenotype (Itoh et al., 2005). In poplar (*Poplar euphratica*), PeSCL7 plays an essential role in responses to salt and drought stresses (Ma et al., 2010). In white lupin (*Lupinus albus* L.), suppression of *LaSCR1* expression *via* RNA interference results in a decreased number of roots (Sbabou et al., 2010). In tomato (*Solanum lycopersicum*), the *Lateral suppressor* (*Ls*) gene encoding a GRAS protein is required for the initiation of axillary meristems (Schumacher et al., 1999). The expression of *GRAS* homologs in tobacco (*Nicotiana tabacum* L.) is known to be induced by hydrogen peroxide (H_2O_2) (Vandenabeele et al., 2003). In addition, in pine (*Pinus radiata* D. Don) and chestnut (*Castanea sativa* Mill.), PrSCL1 and CsSCL1 are induced by auxin and are involved in root formation (Sánchez et al., 2007). To date, there has been little research reported about the functions of GRAS proteins in wheat.

Barley stripe mosaic virus (BSMV)-mediated virus-induced gene silencing (VIGS) is a useful tool for gene functional analysis in cereals. VIGS exploits the RNA-mediated antiviral defense mechanism of plants to study the function of endogenous genes. BSMV, a single-stranded RNA virus consisting of a tripartite genome (α, β, and γ), has been shown to be a useful vector for VIGS in wheat (Scofield et al., 2005). A fragment of the target gene to be silenced can be inserted downstream of the stop codon of the BSMVγb gene to degrade the endogenous gene transcript (Holzberg et al., 2002); as a control for BSMV infection, the antisense strand of the green fluorescent protein gene (*GFP*) is inserted to represent a non-plant gene fragment (Hein et al., 2005). In recent years, the BSMV−VIGS system has become a popular method for studying the functions of genes in wheat. It has been used to study genes related to powdery mildew resistance (Scofield et al., 2005), stripe rust resistance, aphid resistance, and drought tolerance (Manmathan et al., 2013), as well as genes involved in the control of seedling growth (Wang et al., 2011).

Chinese winter wheat cv. Xiaoyan 54 (XY54), created by crossing bread wheat (*Triticum aestivum*, 2n = 42) with tall wheatgrass (*Thinopyrum ponticum*, 2n = 70), is known to have strong tolerance to high-light stress (Yang et al., 2006). In our transcriptome analysis of XY54 in response to high-light stress, a set of probes encoding *TaSCL14* were found to be significantly induced (unpublished data). Here, we cloned *TaSCL14* from XY54 and examined its expression patterns in different wheat organs and under high-light stress. Further, we silenced *TaSCL14* in XY54 using a BSMV−VIGS method and analyzed the morphological features, photosynthetic capacity, tolerance to photooxidative stress, and leaf senescence of the *TaSCL14*-silencing plants. Our results further the understanding of the function of *TaSCL14* in wheat and thus contribute to broader research efforts aimed at improving the responses of crop plants to high-light stress.

RESULTS

Isolation and phylogenetic analysis of *TaSCL14*

Using a Chinese Spring cDNA sequence (GenBank accession No. AK333956) as the reference sequence, *TaSCL14* was cloned from the XY54 genome. The genomic DNA sequence of *TaSCL14* consists of 2238 nucleotide base pairs (bp). The