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Virus-induced Gene Silencing of *TaERECTA* Increases Stomatal Density in Bread Wheat

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Barley stripe mosaic virus (BSMV)-based virus induced gene silencing (VIGS) is an effective strategy for rapid determination of functional genes in wheat plants. *ERECTA* genes are reported to regulate stomatal pattern of plants, and manipulation of *TaERECTA* (a homologue of *ERECTA* in bread wheat) is a potential route for investigating stomatal development. Here, the leucine-rich repeat domains (LRRs) and transmembrane domains of *TaERECTA* were selected to gain BSMV:ER-*LR* and BSMV:ER-*TM* constructs, respectively, targeting *TaERECTA* for silencing in wheat cultivars 'Bobwhite' and 'Cadenza', to identify the function of *TaERECTA* on stomatal patterns. The results showed that reduced expression of *TaERECTA* caused an increased stomatal and epidermal cell density by average 13.5% and 3.3%, respectively, due to the significantly reduced size of leaf epidermal and stomatal cells, and this led to an increase in stomatal conductance. These suggest that modulation of *TaERECTA* offers further opportunities in stomatal engineering for the adaptation of photosynthesis in wheat.

Keywords: BSMV-VIGS, stomatal conductance, stomatal density, *TaERECTA*, *Triticum aestivum* L.

Introduction

Stomata are essential for plant growth and survival, and they coordinate gas exchange, water transpiration and contribute to crop production. Primary stomatal complexes undergo several asymmetric divisions to produce the epidermal cells and stomata, and stomata are almost invariably separated from each other by at least one epidermal cell (Nadeau and Sack 2002). Stomatal size has varied throughout the evolution of crops, and the mode of action of stomata is modulated by environmental factors (Berger and Altmann 2000); it is also subject to genetic regulation, such as in functional gene modulation and post-translational modification (Kim et al. 2010). In *Arabidopsis*, overexpression of the *WIN/SHN1* gene results in low stomatal density and strong drought tolerance, in which the expression of *SPCH*, *FAMA*, *YODA* and *MUTE* genes (related to stomatal development) are obviously reduced (Yang et al. 2011). The *GsGF14* transgenic lines of *Arabi-*

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dopsis showed a lower transpiration rate, and a slow net photosynthesis rate and growth suppression, in addition to reduced stomatal size (Sun et al. 2014). These results suggest the possibility of gene manipulation for the genetic modification of stomata in plant development.

The gene *ERECTA* was originally isolated from a natural *erecta* mutant of *Arabidopsis*, and encodes a Leucine-rich receptor-like-serine/threonine protein kinase (LRR-RLK) with an important role in plant morphogenesis (Torii et al. 1996). *ERECTA* are required to fine-tune the proliferation and growth of cells in the same tissues type during *Arabidopsis* organogenesis (Shpak et al. 2003). In addition, transpiration efficiency was regulated by *ERECTA* gene, as well as effects on stomatal and epidermal cell patterns, mesophyll cell proliferation and cell-cell contact in *Arabidopsis* (Masle et al. 2005). Compelling evidence suggests that three ER-family genes (*ER*, *ERL1* and *ERL2*) synergistically coordinate cell division in the cortex of *Arabidopsis*, complete functional loss of three ER-family genes lead to stomatal clustering (Shpak et al. 2005). Over-expression of the *PdERECTA* gene from *Populus nigra* × (*Populus deltoides* × *Populus nigra*) the early seedling establishment produces, larger leaf areas, and enhanced long-term water use efficiency in *Arabidopsis* (Xing et al. 2011). Transgenic tomato (*Solanum lycopersicum* cv. Micro-Tom) plants expressing a truncated ER protein from *Arabidopsis* (AtΔKinase) develop the diminished growth and surface area (Villagarcia et al. 2012). Thus *ER* appears to be a specificity switch for the manipulation of the growth of crop species.

To investigate functions of the *ERECTA* family in bread wheat, we previously isolated *TaERECTA* (Huang et al. 2013), which is up-regulated under various abiotic stresses (Zheng et al. 2016). Correlation analysis across a diverse panel of 48 bread wheat genotypes reveals that *TaERECTA* is positively correlated with flag leaf area (FLA), photosynthesis rate and biomass yield per plant (Zheng et al. 2015). Here, functions of *TaERECTA* on stomatal development was characterized by using wheat plants, in which Barley Stripe Mosaic Virus (BSMV)-mediated virus-induced gene silencing (VIGS) was used to silence *TaERECTA* in two wheat genotypes (Yuan et al. 2011). The aim of the present study is to verify the *TaERECTA* effect on the development of stomatal and epidermal cells, and provide a crucial foundation for using *TaERECTA* as the candidate gene in the improvement of wheat gas exchange and production.

Materials and Methods

Plant materials and growth conditions

Nicotiana benthamiana was used for preparation of BSMV sap inoculum, and the bread wheat cultivars 'Bobwhite' and 'Cadenza' were used for BSMV infection. All plants were grown in pots (20 cm diameter × 15 cm height), in a greenhouse at 20 °C/23°C night/day with an 8 h/16 h dark/light photoperiod (photosynthetic photon flux density of 525 $\mu mol \cdot s^{-1}\ m^{-2}$).

BSMV-VIGS of TaERECTA

The BSMV-VIGS system described by Yuan et al. (2011), consisting of three T-DNA binary plasmids, pCaBS-α, pCaBS-β and pCa-γbLIC, was used in this study. Two fragments of the leucine-rich repeat domain (LRR) (BSMV:*ER-LR*) and transmembrane domain (BSMV:*ER-TM*), predicted to generate small RNAs of 21–24 nucleotides triggering *TaERECTA* gene silencing, were selected and cloned into pCa-γbLIC in antisense orientation via ligation-independent cloning (LIC), to gain VIGS constructs to generate BSMV-VIGS constructs. The siRNA-Finder (Si-Fi) software (http://labtools.ipk-gatersleben.de/), database with the LRR-RLK family, was used to aid the selection of effective target gene-specific fragments of *TaERECTA*.

The selected target gene fragments were generated using RT-PCR with total RNA extracted from peduncle tissue of the wheat cultivar Cadenza as a template, using the LIC primers listed in Table 1. KOD FX DNA polymerase (KFX-101, TOYOBO) was used to amplify the gene fragments.

BSMV:*asGFP* (Lee et al. 2014) was used as a negative control as it does not trigger silencing of wheat genes. The magnesium chelatase subunit H (*MgChlH*) was introduced into BSMV to gain a positive control that predictably triggers the chimeric phenotype of chlorophyll deficiency.

Transformation of the BSMV binary plasmids into *Agrobacterium tumefaciens* strain GV3101 and agroinfiltration into 3–4 week-old *N. benthamiana* plants was carried out as described previously (Lee et al. 2012; 2014). Sap from the infiltrated *N. benthamiana* leaves was used to mechanically inoculate the leaves of wheat plants at the seven-leaf stage with four tillers (Z17) (Zadoks et al. 1974). Six plants of each cultivar of Bobwhite and Cadenza were inoculated for each BSMV treatment.

Table 1. Primers used in the BSMV construction and qRT-PCR analysis

Target primer	Forward (5'-3')	Reverse (5'-3')	Usage
ER:LR	**AAGGAAGTTTAA** ACTGCCCGGACGG TGATTCTT	**AACCACCACCACCGT** CGACGAGCAATCCCCA ATCTC	BSMV-VIGS construct using LRR coding region
ER:TM	**AAGGAAGTTTAA** GGCTGGCGTTGTC CCTACCGA	**AACCACCACCACCGT** GGAGGACTGTGCGG CCTG	BSMV-VIGS construct using transmembrane region
eIF4E	TGGCAAGCAGTGG AAGGAGT	TCACGGGATCAAAC GGTGTAG	Reference gene for qRT-PCR
TIP41	TGCAGCAAAATGG AAATTCA	TGCGTAGCATCTTG GTTCAG	Reference gene for qRT-PCR
TaERECTA-Q	AACTGAGCTTGAG ACTGTCGGC	CCAGAGGCTGCCAT TTTCCATG	Identification of *TaERECTA* expression by qRT-PCR

Quantification of TaERECTA expression

Tissue from systemically infected leaves (i.e. newly developed, not directly-inoculated leaves) was harvested at 20 days post-virus inoculation (dpi) and flash-frozen in liquid nitrogen for RNA preparation and cDNA synthesis. *TaERECTA* gene expression was quantified using the real time PCR system ABI 7300 (Applied Biosystems, USA) using the primers of *TaERECTA-Q*; two housekeeping genes *eIF4E* and *TIP41* were used to normalize the relative gene expression (Zheng et al. 2015) (Table 1).

Assays for leaf anatomy

Leaf epidermal samples were collected from both the adaxial (top) and abaxial (bottom) surface of newly and fully expanded leaves from six wheat plants of each BSMV treatment and used to determine stomatal density (SD). The leaf surface was brushed with 1 cm^2 of transparent nail polish for approximately 20 s, and covered with sellotape, avoiding veins if possible. The sellotape was then removed and placed on a microscope slide. Leaf epidermal samples were observed with a Zeiss Axiophot upright light microscope (Zeiss, Germany). Images were recorded using a QImaging Retiga Exi CCD digital camera (QImaging, Canada) and the MetaMorph Microscopy Automation & Image Analysis software (Molecular Devices, USA). An epidermal area free of debris was selected and oriented to place as many stomata as possible inside the area of the image acquired (viewing area). Four images were collected from each slide. The total number of stomata and epidermal cells in each image were counted, respectively; the mean SD and epidermal cell density (ED) were estimated, respectively, by using the following formula:

$$SD\,/\,ED(No.mm^{-2}) = \frac{Number\ of\ stomata/\text{epidermal cells}\ (No.)}{View\ area\ (mm^2)}$$

Stomatal size for the same four images, from each of six wheat plants of each BSMV construct treatment, was measured using the scale tool in Adobe Photoshop CS6 software. The mean stomatal index was estimated using the following formula:

$$Stomatal\ index(\%) = \frac{Number\ of\ stomata\ (No.)}{Number\ of\ stomata\ (No.) + Number\ of\ epidermal\ cells\ (No.)} \times 100$$

Measurement of stomatal conductance

Stomatal conductance of the newly expanded leaves of silenced and BSMV:*asGFP* infected control plants were investigated at 20 dpi using a leaf porometer (SC-1, America) under greenhouse conditions. Measurements were taken at approximately noon (11:00–13:00) (Itoh et al. 2005). Six wheat plants per BSMV treatment for each cultivar of Bobwhite and Cadenza were measured to calculate the mean stomatal conductance.

Data analysis

Analysis of variance (ANOVA) was used to assess variation of *TaERECTA* expression, stomatal density, stomatal size and stomatal conductance in the BSMV plants and corresponding control. All analyses were conducted using SPSS Statistics Software version 19.0 (IBM SPSS Statistics, USA).

Results

Systemic symptoms of BSMV infected plants

Plants of the wheat cultivars Cadenza and Bobwhite were infected by each BSMV. Inoculated leaves gradually withered and died after mechanical inoculation of sap from the infiltrated *N. benthamiana*, transformed with the BSMV binary plasmids (Fig. S1*). Neighbouring or interval-infected leaves developed a viral yellow symptom after 7 dpi, whereas the newly developed leaves showed the natural phenotype, and were used to assay leaf anatomy and stomatal conductance. The BSMV:*MgChlH* infected plants displayed the chimeric phenotype of chlorophyll deficiency as opposed to the non-inoculated plants (mock). Plants infected with BSMV:*ER-LR* showed more seriously viral symptoms than that of BSMV:*ER-TM* infection. The infected leaves of Bobwhite developed serious systemic mosaic viral symptoms, including stunting, necrosis and curling of leaves, and Cadenza infected by BSMV displayed less severe symptoms. When compared to the mock (the non-inoculated plants), the inoculated plants reduced height and delayed development, probably due to the viral suppression of BSMV construct after agroinfiltration transformation.

TaERECTA expression in inoculated plants

After plants infection with BSMV:ER, the relative expression of *TaERECTA* was significantly reduced in both cultivars, Bobwhite and Cadenza ($P < 0.01$), compared to the BSMV:asGFP infected plants (Fig. S2). However, reduced expression of *TaERECTA* was variable in each plant infected by BSMV:ER-LR or BSMV:ER-TM, as detected by individual quantification of quantitative Real-Time PCR (qRT-PCR). Under BSMV:ER-*LR* and BSMV:ER-*TM* infection, in Bobwhite, expression of *TaERECTA* was significantly reduced by 48% and 31%, respectively; in Cadenza, expression of *TaERECTA* was significantly reduced by 53% and 50%, respectively. These results suggested that *TaERECTA* transcript levels were efficiently reduced in plants infected with both BSMV:ER constructs. BSMV symptoms of wheat plants were more visible in Cadenza than Bobwhite, possibly due to the rapid growth of Bobwhite as a spring wheat cultivar, and it developed relatively mature leaves at inoculation.

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

TaERECTA contributes to leaf anatomy

The anatomy of newly and fully expanded leaves of BSMV-VIGS plants showed a tendency for smaller stomatal cells and epidermal cells (Fig. S3). Compared to BSMV:*asGFP* infected plants, stomatal density (SD) of fully expanded leaves was significantly higher by an average of 17% and 20% on the adaxial and abaxial surface in Bobwhite infected with BSMV: *ER-LR* ($P < 0.05$), respectively; whereas by average of 8% increase on the adaxial and abaxial surfaces after BSMV:*ER-TM* infection. In Cadenza, SDs of plants infected with BSMV:*ER-LR*, increased by an average of 26% on the adaxial surface ($P < 0.05$) and 11% on the abaxial surface, as well as an average increase of 14% and 4% on adaxial and abaxial surfaces, respectively, after infection with BSMV: *ER-TM*. Epidermal cell density (EDs) of fully expanded leaves of BSMV plants was also obviously higher than that of BSMV:*asGFP* infected plants in both Bobwhite and Cadenza, while there was no significant difference between *TaERECTA* silenced plants and BSMV:*asGFP* infected plants (Table 2).

Table 2. Stomatal and epidermal cells density on the young fully-expanded leaves of wheat plants infected with BSMV constructs targeting *TaERECTA* for silencing

Items ($No.mm^{-2}$)	BSMV constructs	Bobwhite		Cadenza	
		adaxial	abaxial	adaxial	abaxial
Stomatal density	BSMV:*asGFP*	60±2.0	43±2.2	50±2.1	45±2.0
	BSMV:*ER-LR*	70±4.5(17%)*	52±3.1(20%)*	63±2.0(26%)*	50±2.1(11%)
	BSMV:*ER-TM*	65±2.9(8%)	47±3.3(8%)	57±2.4(14%)	47±1.5(4%)
Epidermal cells density	BSMV:*asGFP*	289.15±26.9		216.22±7.5	
	BSMV:*ER-LR*	297.65±27.1(2.9%)		225.68±35.0(4.4%)	
	BSMV:*ER-TM*	298.56±11.9(3.3%)		221.34±24.0(2.4%)	

Values were represented as means ±s.d. (n = 24 images), data in parentheses are the percentage of increased value relative to BSMV:*asGFP*-infected plants. Asterisks represent difference significant between plants infected with BSMV:*ER* constructs and BSMV:*asGFP* ($P < 0.05$).

BSMV:*asGFP*: control; BSMV:*ER-LR* and BSMV:*ER-TM*: BSMV constructs containing LRR and transmembrane domains of *TaERECTA*, respectively.

Table 3. Stomatal size on the adaxial surface of young fully-expanded leaves of wheat plants infected with BSMV constructs targeting *TaERECTA* for silencing

BSMV-VIGS constructs	Bobwhite		Cadenza	
	length (μm)	width (μm)	length (μm)	width (μm)
BSMV:*asGFP*	76.21±1.2	37.33±0.6	89.28±1.9	44.12±2.5
BSMV:*ER-LR*	73.58±1.3*	38.92±1.7	83.94±5.1**	38.01±3.1**
BSMV:*ER-TM*	72.92±1.1*	35.85±0.5*	79.62±1.4**	40.21±1.8*

Values were represented as means ±s.d. (n = 24 images). Asterisks represent difference significant between plants infected with BSMV:ER constructs and BSMV:*asGFP* (*$P < 0.05$, **$P < 0.01$).

BSMV:*asGFP*: control; BSMV:*ER-LR* and BSMV:*ER-TM*: BSMV constructs containing LRR and transmembrane domains of *TaERECTA*, respectively.

Complementary measurement of stomatal size showed the significantly reduced length and width of stomatal cells in plants infected with both BSMV:*ER-LR* and BSMV:*ER-TM* in Bobwhite ($P < 0.05$), except for stomatal width with BSMV:*ER-LR* infection. Meanwhile, stomatal length and width in plants infected with both BSMV constructs in Cadenza was greatly and significantly reduced ($P < 0.01$), with the exception of stomatal width for BSMV:*ER-TM* infection ($P < 0.05$) (Table 3). Nevertheless, stomatal index showed no difference between plants infected with both BSMV:ER and BSMV:*asGFP* in both wheat cultivars (Fig. S4). Therefore, the number of stomatal and epidermal cells of wheat leaves greatly increased in plants after BSMV infection, as size of stomatal cells significantly reduced, whereas stomatal index displayed stable variation, these predicted that the reduced expression of *TaERECTA* might synergistically affect the size of epidermal cells, largely lead to a clustering pattern of leaf cell types.

Reduced TaERECTA expression increased stomatal conductance

ERECTA is essential for patterns of stomata in response to photosynthesis and transpiration in *Arabidopsis* (Masle et al. 2005). Therefore, the response of stomatal conductance (gs) was investigated to detect the variable stomatal closure of plants infected with BSMV:ER. Infected plants induced an increase in stomatal conductance (gs) in cultivars Bobwhite and Cadenza, with relatively higher gs in the adaxial than in the abaxial surface of wheat leaves (Fig. S5). In Bobwhite, stomatal conductance of plants infected with BSMV:*ER-LR* and BSMV:*ER-LR* was greatly and significantly higher by 27% and 40% in the adaxial surface ($P < 0.01$), whereas the increase was only 16% ($P < 0.05$) and 22% ($P < 0.01$) in the abaxial surface, respectively. However, in Cadenza infected with both BSMV:*ER-LR* and BSMV:*ER-LR*, stomatal conductance was significantly higher by 14% and 13% in the adaxial surface ($P < 0.05$), respectively, as well as a 21% increase for BSMV:*ER-LR* infection ($P < 0.05$), and only an increase of 10% for BSMV:*ER-TM* infection ($P < 0.01$). These suggest that reduced expression of *TaERECTA* increased gs of BSMV silenced plants due to the mutative anatomy in leaf size as described above.

Discussion

In this study, the data showed that *TaERECTA*, a homologue of *ERECTA*, was involved in stomatal regulation in bread wheat. Wheat plants with reduced expression of *TaERECTA* were observed, and it was concluded that, in wheat, *TaERECTA* was also involved in the regulation on the stomatal cells and epidermal cells size, and therefore affected stomatal conductance.

Virus-induced gene silencing through BSMV system has been widely used for functional gene analysis in monocots. Ten-day-old wheat at the two-leaf stage is highly susceptible and useful for assessment of the effects of mildew pathogenesis (Yuan et al. 2011; Panwar et al. 2013). However, in a preliminary experiment, the winter wheat cultivar 'Riband' with a narrow blade trait was used to inoculate with BSMV constructs at the two-leaf stage (Z12) (Zadoks et al. 1974). No distinct virus symptoms were found on leaf

surfaces, and leaf was severely destroyed after sap inoculation, probably due to the relatively much younger plants (data not shown). Therefore, spring wheat Bobwhite and winter wheat Cadenza with relatively wide blades were selected again and inoculated with BSMV-ER construct at the late seven-leaf stage (Z15) (Zadoks et al. 1974). This led to systematic viral symptoms of infected leaves, and the newly-development of fully-expanded leaves showed no viral symptoms, were used to investigated stomatal patterns, in addition to the more distinct symptoms of Cadenza over Bobwhite, due to the relatively rapid development of the latter. These results suggest that effect of *TaERECTA* BSMV constructs partially rests with different wheat cultivars, either spring or winter wheat cultivars with right growth traits, and the optimal inoculation stage.

TaERECTA is a typical transmembrane LRR-RLK protein, consisting of three elements of LRRs, transmembrane (TM) and serine/threonine kinase (SK) domains (Huang et al. 2013). Here, *ER-LR* and *ER-TM* were selected to gain BSMV constructs, respectively, and plants infected with BSMV:*ER*-LR and BSMV:*ER*-TM showed a reduced expression of *TaERECTA* when compared to control (BSMV:*asGFP* infected plants), as well as the more distinct trend in plants infected with BSMV:*ER*-LR than BSMV:*ER*-TM, this implies LRRs may contribute greatly to the function of *TaERECTA,* and are the crucial elements in the identification of *TaERECTA* BSMV. LRRs domain has a receptor signal participating in the protein-protein interaction (Afzal et al. 2013), TM transduces extracellular signals into the cells to control a wide range of physiological responses (Lease et al. 1998), this provide a new insight of *TaERECA* interaction with other signal ligand upstream.

Loss-of-function *ERECTA* mutants exhibit lower WUE as a result of reduced biochemical capacity for photosynthesis (Masle et al. 2005). Compelling evidence shows that stomata division is negatively controlled by the putatively secreted peptides EPF1 and EPF2 (Hara et al. 2007), with synergistic regulation requiring TOO MANY MOUTHS (TMM) and ERECTA in *Arabidopsis* (Hara et al. 2009; Hunt and Gray 2009). Here, down expression of *TaERECTA* in bread wheat caused an increase in stomatal and epidermal cell density, i.e. significantly increased variation on the adaxial surface of plants infected with BSMV:*ER-LR*. Further investigation showed plants infected with BSMV constructs had a significantly lower stomatal length and width in Bobwhite and Cadenza, whereas there was no significant difference in the stomatal index. These results allow assessment of the hypothesis that the size of epidermal cells was also synergistically reduced, and that the reduced size of leaf anatomy induced the increased numbers of leaf mesophyll cells, which probably affected the gas exchange on leaf surface in bread wheat, i.e. significantly increased stomatal conductance of plants infected with BSMV constructs, when complete loss of *TaERECTA* gene.

In rice, stomatal conductance is known to be highly correlated with leaf photosynthesis, from experiments involving the high-yielding indica rice cultivar 'Takanari' (Xu et al. 1997). Wheat plants infected with *TaERECTA* BSMV constructs showed significantly increased stomatal conductance on adaxial and abaxial surfaces, probably in response to biochemical stimuli introduced from *TaERECTA* inoculation. In principle, an increase in stomatal conductance can allow plants to increase their CO_2 uptake and subsequently

enhance photosynthesis (Kusumi et al. 2012). However, the relationship of stomatal conductance with CO_2 uptake and photosynthesis is not so simple under multiple environmental factors. Under controlled environmental conditions, stomatal conductance can become the primary determinant of photosynthetic capacity, so modulation of *TaERECTA* provides a new tool for the further examination of stomatal engineering for photosynthetic adaptations in wheat.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at https://akademiai.com/loi/0806

Electronic Supplementary *Figure S1*. Symptom of wheat plants infected with BSMV constructs in cultivars Bobwhite and Cadenza. Mock: no-inoculated plants; BSMV:*asGFP*: control; BSMV:*ER-LR* and BSMV:*ER-TM*: plants infected with BSMV constructs containing regions of LRR and transmembrane domains of *TaERECTA*, respectively; BSMV:*MgChlH*: positive control

Electronic Supplementary *Figure S2. TaERECTA* expression level in young fully-expanded leaves of wheat plants infected with BSMV constructs targeting *TaERECTA* for silencing. Values were represented as means ± s.d. (n = 6), values above histograms are the decreased expression of *TaERECTA* relative to control. Asterisks represent difference significant between plants infected with different BSMV constructs and BSMV:*asGFP* ($P < 0.01$). BSMV:*asGFP*: control; BSMV:*ER-LR* and BSMV:*ER-TM*: BSMV constructs containing regions of LRR and transmembrane domains of *TaERECTA*, respectively

Electronic Supplementary *Figure S3*. Stomatal patterns on the adaxial surface of young fully-expanded leaves of wheat plants infected with BSMV constructs targeting *TaERECTA* for silencing. Four images were collected from each of six wheat plants per BSMV construct treatment. BSMV:*asGFP*: control; BSMV:*ER-LR* and BSMV:*ER-TM*: BSMV constructs containing regions of LRR and transmembrane domains of *TaERECTA*, respectively

Electronic Supplementary *Figure S4*. Stomatal index of young fully-expanded leaves of wheat plants infected with BSMV constructs targeting *TaERECTA* for silencing. The mean stomatal index, for the four images, from each of six wheat plants per BSMV construct treatment, was assessed on the adaxial and abaxial surface. BSMV:*asGFP*: control; BSMV:*ER-LR* and BSMV:*ER-TM*: BSMV constructs containing regions of LRR and transmembrane domains of *TaERECTA*, respectively

Electronic Supplementary *Figure S5*. Stomatal conductance of young fully-expanded leaves of wheat plants infected with BSMV constructs targeting *TaERECTA* for silencing. Values were represented as means ± s.d. (n = 6). Asterisks represent difference significant between plants infected with different BSMV constructs and BSMV:*asGFP* (**$P < 0.01$, *$P < 0.05$). BSMV:*asGFP*: control; BSMV:*ER-LR* and BSMV:*ER-TM*: BSMV constructs containing regions of LRR and transmembrane domains of *TaERECTA*, respectively