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Original Paper

Overexpression of the tomato *glutamate receptor-like* genes *SlGLR1.1* and *SlGLR3.5* hinders Ca^{2+} utilization and promotes hypersensitivity to Na^{+} and K^{+} stresses

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Abstract Thirteen *glutamate receptor-like* genes have recently been identified in tomato plants; however, their functions have not been fully elucidated. We overexpressed *SlGLR1.1* and *SlGLR3.5* in *Arabidopsis* and found that transgenic plants showed symptoms such as curled and deformed leaves, dwarf stature and retarded growth resembling those of Ca^{2+} deficiency. The results revealed that the levels of Ca^{2+} in aerial tissues did not differ between wild-type and transgenic plants, suggesting that overexpression of *SlGLR1.1* and *SlGLR3.5* did not affect Ca^{2+} uptake. Transgenic lines were hypersensitive to K^{+} and Na^{+} ionic stresses, which was rescued by addition of Ca^{2+} to the growth medium. Ectopic expression of *SlGLR1.1* and *SlGLR3.5* resulted in reduced efficiency of Ca^{2+} utilization, suggesting that these genes may play a role in calcium assimilation in tomato plants by controlling ionic transport across the membrane.

Key words: Glutamate receptor-like, tomato, overexpression, calcium, ion hypersensitivity.

Plant membranes contain a variety of receptors involved in the transport of nutrients, ions and metabolites (Davenport 2002). Glutamate is a ubiquitous plant cell ligand recognized by glutamate receptors located in the plasma membrane (Forde and Lea 2007). Glutamate receptors have been studied in both animals and humans as its ligand, glutamate, is an important excitatory neurotransmitter sensed by glutamate receptors at brain synapses (Baluska 2010). As a major excitatory neurotransmitter, glutamate activates two receptor families: metabotropic glutamate receptor proteins (mGluR), which activate biochemical cascades, and ionotropic glutamate receptors (iGluR), which form cation-selective ion channels belonging to the pore-loop subfamily (Mayer 2006). The iGluRs are glutamate-gated ion channels involved in the movement of Na^{+} and Ca^{2+} across the post-synaptic plasma membrane (Watkins 2000).

The first indication that glutamate receptors were present in organisms lacking a nervous system came from the discovery of a family of *glutamate receptor-like* (*GLR*) genes in *Arabidopsis thaliana* (Lam et al. 1998). Based on the *Arabidopsis* genome sequence data, 20 *AtGLR* genes were identified (Chiu et al. 1999; 2002). Recently, a new family of 13 *SlGLR* genes was identified in the tomato, *Solanum lycopersicum* (Aouini et al. 2012). The predicted structure of these *Arabidopsis* and tomato GLRs contains four transmembrane domains, the second of which does not span the membrane (similar to the structure of animal iGluRs).

In animals, most iGluRs are ligand-gated non-selective cation channels that are permeable to Ca^{2+} in the post-synaptic membrane (Swanson and Sakai 2009). Glutamate receptors can belong to different classes of ion channels that provide a calcium-permeable pathway across the plasma membrane. In *Arabidopsis* roots, glutamate specifically triggers the transient elevation of $[Ca^{2+}]$ and membrane depolarization, which are both sensitive to the Ca^{2+} antagonist La^{3+} (Dennison and Spalding 2000). Two T-DNA insertion lines of *AtGLR3.3* were identified and shown to be defective in the fast electrical response to both glutamate and Ca^{2+} (Qi et al. 2006). Possible alternative roles for the *AtGLR* genes were proposed independently by a transgenic

Abbreviations: GLR, glutamate receptor-like; SlGLR, *Solanum lycopersicum* glutamate receptor-like; AtGLR, *Arabidopsis thaliana* glutamate receptor-like; iGluR, ionotropic glutamate receptor; B5 medium, Gamborg B5 medium.
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plant study and a physiological study. Overexpression of *AtGluR2* in *Arabidopsis* caused symptoms of Ca^{2+} deficiency and sensitivity to other ions that could be rescued by providing external Ca^{2+}, suggesting that *AtGluR2* may function in calcium translocation in plants (Kim et al. 2001). Ectopic expression of *AtGLR3.1* causes impairment of Ca^{2+} oscillation-induced stomatal closure, suggesting a role for *AtGLR3.1* in Ca^{2+} signaling in guard cells (Cho et al. 2009). In addition, Kang et al. (2006) suggested that a putative small radish glutamate receptor (RsGluR) acts as a Ca^{2+} channel in the plasma membrane based on the fact that *Arabidopsis* cells transformed with *RsGluR* were hypersensitive to glutamate. Using a pharmacological approach, Michard et al. (2011) showed that D-Ser plays an important role as the most active agonist of GLR activity in the pollen tubes of tobacco and *Arabidopsis*. D-Ser activates 2 GLRs (AtGLR1.2 and AtGLR3.7) in the apical region of pollen tubes, allowing Ca^{2+} permeation into the cytoplasm, thereby shaping the Ca^{2+} signature by modulating both Ca^{2+} influx intensity and oscillation amplitude. Physiological studies show that GLRs are important for pollen function and are not mere by-products of signaling cascades controlling pollen tube growth and navigation in female tissues (Michard et al. 2011).

The structural similarity of GLRs to iGluRs, coupled with the results from previous genetic and physiological studies, indicates that GLRs play a role in Ca^{2+} homeostasis and signaling. GLR proteins have been proposed to function as ligand-gated Ca^{2+}-permeable channels (Lacombe et al. 2001; Meyerhoff et al. 2005), although direct proof of the function of these proteins is lacking. In the present study, we report the functional characterization of two tomato *GLR* genes, *SlGLR1.1* and *SlGLR3.5*, and examine their role in calcium utilization in transgenic *Arabidopsis* plants overexpressing these genes. These genes were selected based on the distinctive expression pattern; *SlGLR1.1* showed higher transcript level in root while *SlGLR3.5* was detectable in flower.

Materials and methods

Plant material

Arabidopsis thaliana ecotype Columbia (Col-0) was used in the study. Seeds were surface-sterilized with 75% (v/v) ethanol and 1 ml washing solution (Sodium hypochlorite) (10%). After five washes with sterile distilled water, seeds were stratified at 4°C in the dark for 3 days before germination.

Plant growth and measurements

Plants were either grown in composite soil in a growth chamber, or in controlled culture on sterile 1/4 B5 agar plates in Petri dishes (22°C/18°C, 16 h light/8 h dark cycle). For ionic sensitivity analysis, surface-sterilized seeds were grown for 2 weeks in Ca^{2+}-depleted 1/4 B5 medium (Gamborg et al. 1968) supplemented with the appropriate ions (Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}).

Plant transformation

The coding regions of tomato *glutamate receptor-like* genes *SlGLR1.1* and *SlGLR3.5* (GenBank accession numbers: AB623193 and AB623205) were amplified by polymerase chain reaction (PCR) using full-length cDNA clones (LEFL1011BE01 and LEFL1007BF10) as templates and the following gene-specific primers: *SlGLR1.1*, sense 5′-ATGCTTTATTAGTGG GGAAGA-3′, antisense 5′-GAACGGTCAGTTCTTGAA CCA-3′ and *SlGLR3.5*, sense 5′-ATGAAATATGGCTCA TTTCAAAC-3′, and antisense 5′-ATCCCCAAATACCGT GCCATAG-3′. PCR products were cloned into the pCR8/GW/TOPO vector (Invitrogen). The inserts were transferred to the pBI-OX-GW1 binary vector (Inplanta Innovations Inc.) downstream of a cauliflower mosaic virus (CaMV) 35S promoter. The resulting constructs were transformed into *Agrobacterium tumefaciens* (strain GV2260) by electroporation and introduced into *A. thaliana* Col-0 ecotype using the floral dip method (Clough and Bent 1998). Briefly, unopened *Arabidopsis* flower bud clusters were submerged in a suspension of 1/2 Murashige and Skoog (MS) basal medium (Murashige and Skoog 1962), 5% sucrose, 0.005% Silwet, 44 nM benzylaminopurine, and *Agrobacterium* cells. Plants were inoculated for 5 s with gentle agitation, then covered with a plastic film and kept in the dark for 24 h. Plants were grown for a further 3–5 weeks to allow self-fertilization and the resulting T_1 seeds were harvested from brown and dried siliques.

To select the transgenic plants, T_1 seeds were surface-sterilized and spread on MS medium agar plates containing 100 mg l^{-1} kanamycin as a selective marker. Plates were allowed to dry for 1 h, cold-treated at 4°C for 3 days and then transferred to a growth chamber. After 14 days, viable plants with green leaves and long, strong roots were transplanted to soil and grown to maturity. Genomic DNA was extracted from the leaves of the transformants to test for the presence of the desired gene by PCR. The selected lines were homozygous, as indicated by the 100% kanamycin resistance in the T_3 generation, and for each gene we examined two independent transgenic lines.

RNA extraction and quantitative real-time RT-PCR analysis

Total RNA was extracted from *Arabidopsis* wild-type and transgenic seedlings using the RNeasy Plant Mini Kit (Qiagen) and treated with DNase (Qiagen). RNA concentration and quality were analyzed using a spectrophotometer and agarose gel electrophoresis, respectively. First-strand cDNA was synthesized from 2 μg of total RNA using the SuperScript VILO cDNA Synthesis Kit (Invitrogen). The first-strand cDNA was diluted to 100 ng μl^{-1} and 1 μl of the dilution was used for quantitative RT-PCR with SYBR Premix Ex Taq (Takara Bio). The PCR conditions were based upon the manufacturer's instructions. The *Arabidopsis* ubiquitin gene, *AtUBQ10* (TAIR accession number: AT4G05320.2) was included as an

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internal standard. Specific single transcript amplifications were confirmed by agarose gel electrophoresis, single dissociation peaks, and calibration curves. All analyses were repeated a minimum of two times using independent RNA samples. The primer sequences used for the *AtUBQ10*, *SlGLR1.1* and *SlGLR3.5* genes are described in Supplemental Table1.

Calcium analysis

Calcium content analysis was carried out with 2 week-old *in vitro*-grown plants by placing them in sterile 1/4 B5 agar plates supplemented with $CaCl_2$ at different concentrations (0.1, 1 and 10 mM). The aerial portions of the plants were harvested and fresh weight was measured individually. Dry weight was measured after heating the material at 70°C for 72 h. The dried samples were then used for calcium measurements. To determine the calcium content, samples were ground to a powder and treated with 1 ml of 6 N HCl at 100°C until the samples were perfectly ashed. The resulting digestate was re-suspended to 10 ml with deionized water. The total Ca^{2+} content per gram dry weight was determined by an inductively coupled plasma-optical emission spectrometer (ICP-OES; Optima 7300DV, Perkin Elmer).

In situ hybridization and histological analysis of SlGLR3.5 in floral organs

The probe was amplified using PCR from a full length cDNA. The corresponding primers used were: sense, 5′ GAGGTCTCG ATCC-3′ and antisense, 5′ GGTTGTTGTAAGG-3′. The sense and anti-sense digoxygenin-labelled riboprobes were generated by run-off transcription using T7 RNA polymerase according to the manufacturer's protocol (Roche). The corresponding T7 RNA polymerase primers used were: sense, 5′ T7-TGTAAT ACGACTCACTATAGGGCTGAGGTCTCGATCC-3′ and antisense, 5′ T7-TGTAATACGACTCACTATAGGGCTGGT TGTTGTAAGG-3′. For histological analysis, flower buds (0.5 to 15 mm in length) were fixed in FAA solution (2% formalin, 50% ethanol, and 5% acetic acid) for 4 h at room temperature. After fixation, the tissues were dehydrated in ethanol and then, embedded in paraffin. At least 10 buds were sampled and checked. Sections 8 μm thick were fixed in 3-aminopropyl-triathoxylan-coated slides, deparaffinished in Histosol Plus (Life Sciences International). Synthesis of digoxygenin-11-rUTP-labeled riboprobes, hybridization, washes, and immunological detection of the hybridized probes were carried out as previously described (Joubes et al. 2001). Slides were observed under a microscope (Zeiss-Axioplan).

Results

Morphological phenotype of transgenic Arabidopsis plants overexpressing SlGLR1.1 and SlGLR3.5

To elucidate the roles of tomato *glutamate receptor-like* genes, *SlGLR1.1* and *SlGLR3.5* were overexpressed in *Arabidopsis thaliana* by expressing the corresponding cDNA under the control of the CaMV 35S promoter. Transgenic lines were tested for expression of the transgene by real-time RT-PCR. In wild-type *Arabidopsis* plants, the *SlGLR1.1* and *SlGLR3.5* mRNAs could not be detected in total RNA extracted from the whole seedling. Homozygous transgenic lines showed increased expression of both genes, while the level of expression varied across the different transgenic lines. Two independent transgenic lines highly overexpressing *SlGLR1.1* (G1-1 and G1-11) and *SlGLR3.5* (G3-1 and G3-6) were used for further analysis (Supplemental Fig. 1).

Figure 1 shows the growth characteristics of representative transgenic lines for each gene. Seed germination and plant growth were severely retarded, and necrosis of the tips and margins of young leaves was observed. Transgenic plants showed a dwarf stature, and produced undeveloped lateral shoots. The flowers and siliques of the transgenic plants were similar to those of the wild-type in terms of size and structure. These morphological characteristics of the transgenic plants resembled symptoms of Ca^{2+} deficiency observed in transgenic *Arabidopsis* overexpressing *Arabidopsis glutamate receptor* (*AtGluR2*) (Kim et al. 2001) and the *RsGluR* (Kang et al. 2006).

Calcium content of wild type and transgenic plants

To examine whether overexpression of *SlGLRs* resulted in Ca^{2+}-related phenotypes, the Ca^{2+} content of the transgenic lines was investigated. Plants were grown for two weeks in Ca^{2+}-depleted 1/4 B5 medium supplemented with 0.1, 1 and 10 mM of $CaCl_2$. Ca^{2+} levels in the aerial parts did not differ between wild-type and transgenic plants (Fig. 2). Thus, the responses to Ca^{2+} nutrition between the overexpressors and wild-type plants could not be explained by differences in Ca^{2+} within the plant shoots. This indicates that the observed phenotype is not due to reduced transport or uptake of Ca^{2+}.

The sensitivity of transgenic plants to ionic stresses

Physiologically, symptoms of Ca^{2+} alteration are often diagnosed by an increased sensitivity to other ions in crop plants (Scaife and Turner 1984). Furthermore, Kim et al. (2001) reported that the overexpression of *AtGluR2* gene caused reduced efficiency of Ca^{2+} utilization with hypersensitivity to K^+ and Na^+ in transgenic plants. Therefore, we tested the sensitivity of the overexpressors to different cations and mannitol by growing wild-type and transgenic plants in 1/4 B5 medium with added K^+, Na^+ or Mg^{2+} for 2 weeks. Compared with that in wild-type plants, the growth of the transgenic lines was inhibited by different concentrations of Na^+ and K^+, suggesting hypersensitivity to these ions. In contrast,

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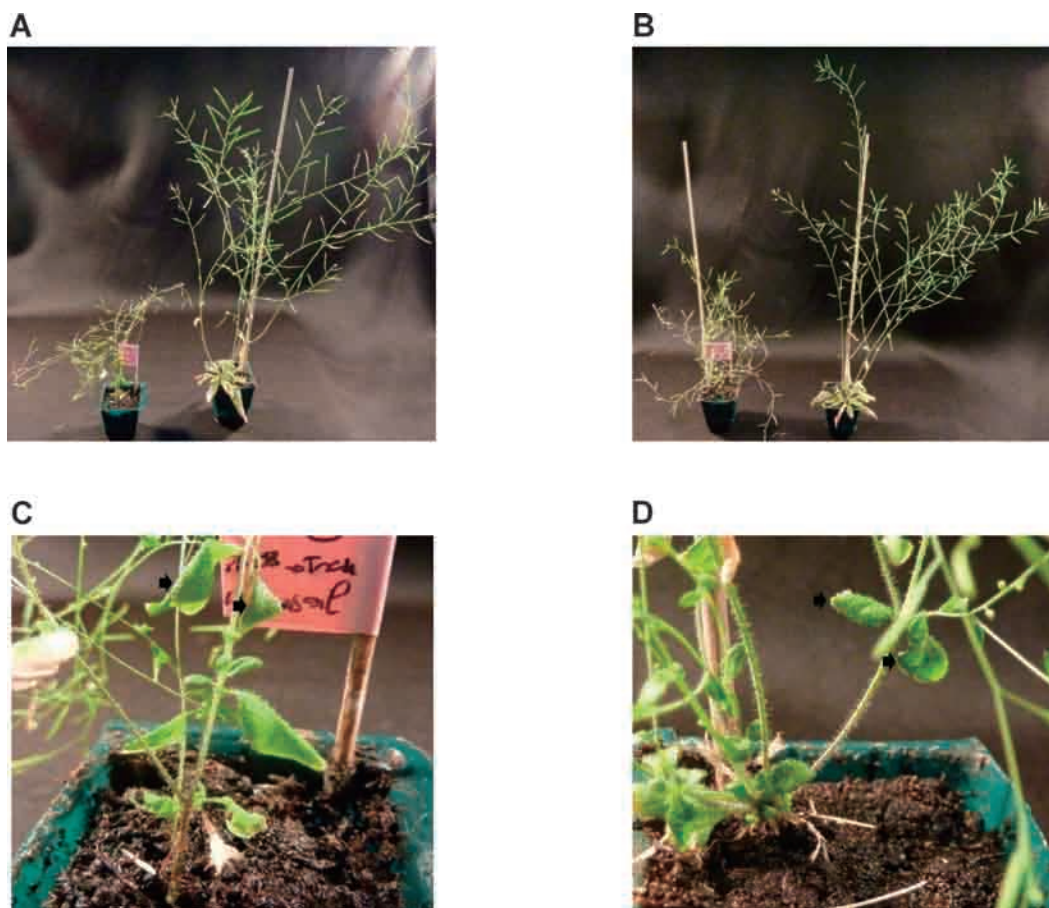

Figure 1. Morphological phenotype of transgenic *Arabidopsis* overexpressing *SlGLR1.1* and *SlGLR3.5*. (A and B). Transgenic *Arabidopsis* plants showing dwarfism. (A). Wild-type plant (right), *SlGLR1.1* overexpressing plant (G1-1, left). (B). Wild-type (right) and *SlGLR3.5* overexpressing plants (G3-6, left). (C and D). Curled and deformed leaves indicated by arrows in transgenic plants overexpressing *SlGLR1.1* (C) and *SlGLR3.5* (D).

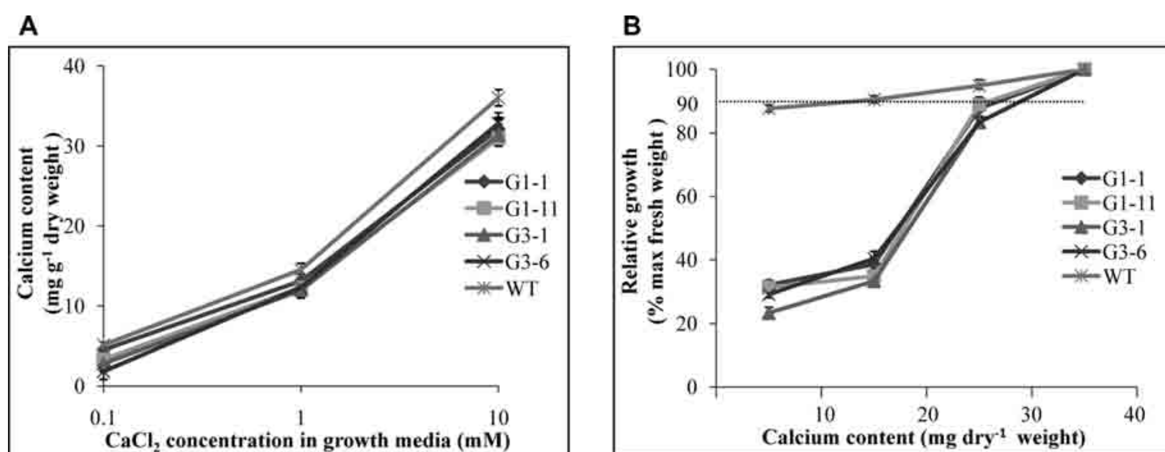

Figure 2. Effect of Ca^{2+} supplementation on calcium content in the aerial parts of the plant. (A). Plants were grown in 1/4 B5 medium supplemented with different concentrations of $CaCl_2$ (0.1, 1, 10 mM). (B). The fresh weight of 2-week-old seedlings was measured independently and then plants were dried for Ca^{2+} analysis. The Ca^{2+} content was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES).

no difference in growth was observed in the presence of Mg^{2+}. Transgenic plants did not display hypersensitivity to mannitol, suggesting that the phenotypes observed for K^+ and Na^+ are not caused by general osmotic stress (Fig. 3).

Effect of calcium supplementation on ion hypersensitivity

To assess the effect of Ca^{2+} supplementation on the ionic stress response in the transgenic lines, plants were grown in K^+ (20 mM KCl) and Na^+ (40 mM NaCl) stress media

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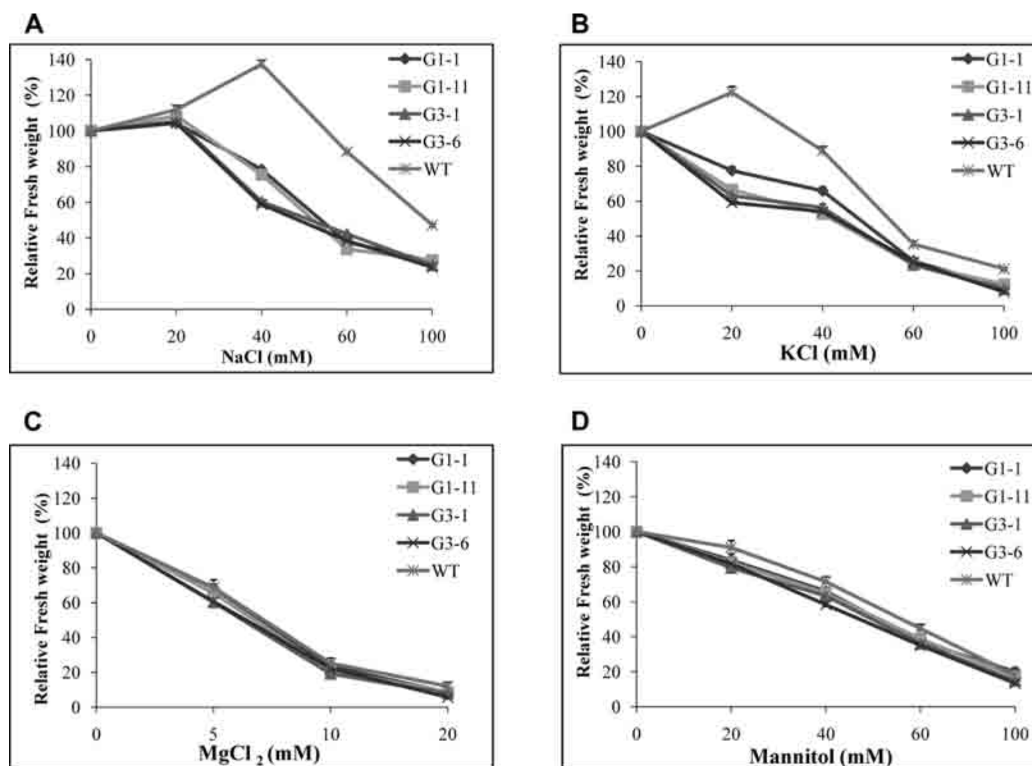

Figure 3. Sensitivity of transgenic plants to ionic (Na^+, K^+, Mg^{2+}) and osmotic (mannitol) stresses. Fresh weight is presented as a percentage of the fresh weight in 1/4 B5 medium. Plants were grown in 1/4 B5 media supplemented with different concentrations of NaCl, KCl, $MgCl_2$, and mannitol. Data represent the average ±SE of three independent assays (20 seedlings were examined for each assay). Sensitivity to NaCl (A), KCl (B), $MgCl_2$ (C) and mannitol (D).

with added Ca^{2+} (5 mM $CaCl_2$) for 2 weeks. The $CaCl_2$ dose was selected based on preliminary experiments in which supplementation with 5 mM $CaCl_2$ resulted in a significant reduction in the sensitivity of transgenic plants to ionic stress. In *Arabidopsis* overexpressing *SlGLR1.1* and *SlGLR3.5*, Ca^{2+} supplementation in the presence of other ions (K^+ and Na^+) rescued and attenuated the sensitivity of plants to ionic stress (Fig. 4).

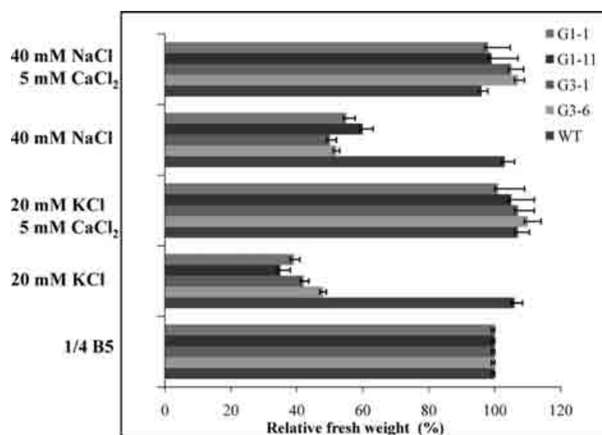

Figure 4. Effect of calcium supplementation on ion hypersensitivity. Relative fresh weight as a function of the concentration of ions added to the medium. Twenty seedlings were used for each measurement. Values shown are relative to the fresh weight of seedlings grown in 1/4 B5 medium. Error bars indicate standard error (SE).

Discussion

Since the first reports of *glutamate receptor* genes in plants (Lam et al. 1998; Chiu et al. 1999; 2002) the function of plant glutamate receptors has become a topic of increasing interest. The role of plant *glutamate receptor-like* has been studied extensively. Plant *GLR*s are associated with light signal transduction (Lam et al. 1998), calcium homeostasis (Kim et al. 2001; Kang et al. 2006), cell division and survival (Li et al. 2006), Ca^{2+}-induced stomatal closure (Cho et al. 2009), and pollen tube growth and morphogenesis (Michard et al. 2011). However, their role in the physiology and development of plants remains unclear. Recently, a family of 13 tomato *GLR* genes was identified (Aouini et al. 2012). To investigate the physiological roles of the *SlGLR* genes, we generated transgenic lines of *Arabidopsis* overexpressing *SlGLR1.1* and *SlGLR3.5*. Transgenic plants exhibited necrosis on the leaf tips, dwarf stature with multiple secondary inflorescences, curled leaves, and delayed growth (Fig. 1). These symptoms, which are similar to those of transgenic *Arabidopsis* overexpressing *AtGluR2* (Kim et al. 2001) and *RsGluR* (Kang et al. 2006), are indicators of Ca^{2+} deficiency in sink tissues due to altered Ca^{2+} utilization, reduced transport of Ca^{2+} from root

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to shoot, or reduced uptake of Ca^{2+} in the root (Saure 2001). In the present study, *SlGLR1.1*- and *SlGLR3.5*-overexpressing and wild-type plants exhibited similar calcium contents in aerial parts (Fig. 2). This indicates that Ca^{2+} deficiency-like symptoms were probably due to altered Ca^{2+} utilization, because uptake of the ion was not altered. In addition, transgenic plants displayed a hypersensitivity to ionic stress that was rescued by supplementation of the medium with exogenous Ca^{2+} (Fig. 4). The ability to suppress the ion sensitivity of the transgenic plants with exogenous Ca^{2+} strongly suggests that Ca^{2+} homeostasis of these plants is altered. It is reasonable that the symptoms of Ca^{2+} deficiency in the transgenic plants might be caused by an impairment of Ca^{2+} utilization coupled with Na^+ and K^+ sensitivity. Thus, the *SlGLR1.1*- and *SlGLR3.5*-overexpressing phenotypes suggest that these genes might be implicated in the maintenance of Ca^{2+} homeostasis. These results suggest that, similar to *AtGluR2* (Kim et al. 2001) and *RsGluR* (Kang et al. 2006), *SlGLR1.1* and *SlGLR3.5* might play a role in plant growth and development. Kim et al. (2001) demonstrated that *AtGluR2* overexpression produced symptoms of Ca^{2+} deficiency in *Arabidopsis* plants, indicating that AtGluR2 is involved in balancing Ca^{2+} during plant growth. Kang et al. (2006) reported that RsGluR functions as a plasma membrane Ca^{2+} channel and that increased GluR levels result in hypersensitivity to glutamate. More recently, glutamate receptor-like channels were shown to facilitate Ca^{2+} influx across the plasma membrane, modulating the apical $[Ca^{2+}]_{cyt}$ gradient and consequently affecting pollen tube growth and morphogenesis (Michard et al. 2011).

In animals, iGluRs are ligand-gated non-selective cation channels that allow the flow of Na^+ and K^+ and sometimes Ca^{2+} in response to glutamate binding (Tabassum and Feroz 2011). It is possible that overexpression of the *SlGLR1.1* and *SlGLR3.5* genes cause excess Na^+ or K^+ uptake if SlGLR1.1 and SlGLR3.5 are permeable to Ca^{2+} as well as monovalent cations as in the case of the animal iGluRs. Increased uptake of Na^+ or K^+ could compete with Ca^{2+}, thus reducing the efficiency of Ca^{2+} utilization. Overexpression of the *SlGLR1.1* and *SlGLR3.5* genes led to Ca^{2+} deficiency-like symptoms and ionic hypersensitivity (Na^+ and K^+) that was alleviated by increasing the external Ca^{2+} concentration. In fact, previous studies have suggested that *AtGLR3.2*, *AtGLR3.3* and *RsGluR* are all implicated in Ca^{2+} signaling (Kim et al. 2001; Meyerhoff et al. 2005; Kang et al. 2006). The results of the present paper also suggest a role for *SlGLR1.1* and *SlGLR3.5* in Ca^{2+}-mediated signaling. When activated these receptors allow the flow of Ca^{2+} as well as Na^+ and K^+ in response to glutamate binding and this would explain the hypersensitivity of transgenic plants to Na^+ and K^+.

Phylogenetic analyses in tomato enabled the classification of GLR family genes into three clades (Aouini et al. 2012). SlGLR3.5 was included in clade III and SlGLR1.1 was included in clade I. The classification of SlGLRs revealed the divergence of a distinct Clade I represented by two genes (*SlGLR1.1* and *SlGLR1.2*) and indicated that this clade is possibly specific to the Solanaceae family. These genes displayed different expression patterns, showing higher *SlGLR1.1* expression in roots, whereas *SlGLR3.5* expression was mostly detected in flowers (Supplemental Fig. 2). Although *SlGLR1.1* and *SlGLR3.5* are expressed in different tissues and classified into distinct clades, they appear to play similar roles in Ca^{2+} homeostasis and signaling. Kang et al. (2006) reported that *RsGluR* has high sequence similarity with *AtGluR2* (2001), which belongs to *Arabidopsis* clade III. *SlGLR3.5* is classified into tomato clade III, which is homologous to *Arabidopsis* clade III. These relationships suggest that *SlGLR3.5* may have similar roles to *AtGluR2* and *RsGluR* in plant growth and development, and SlGLRs likely function in Ca^{2+} and monovalent cation transport and form constitutively active ion channels as reflected by their protein sequence (Aouini et al. 2012). Furthermore, using a domain swapping approach and heterologous expression in *Xenopus* oocytes, an analysis of chimeric proteins that contain the ion pore domains of each of 17 *Arabidopsis* GLR proteins in either rat GluR1 or GluR6 receptors suggested that *AtGLR1.1* and *AtGLR1.4* have functional Na^+, K^+ and Ca^{2+} permeable pore domains, and that currents through the *AtGLR1.1* pore resemble the glutamate-activated currents observed in plants (Tapken and Hollmann 2008).

Moreover, *SlGLR3.5* mRNA was detectable in reproductive organs, namely the carpel and anther, at stage 18 (Brukhin et al. 2003) (Supplemental Fig. 2). SlGLR3.5 would be an interesting study target to support an active role for GLRs in plant development, especially reproduction. Michard et al. (2011) described the importance of *Arabidopsis* GLRs in pollen tube growth by using two T-DNA insertion lines, *Atglr1.2* and *Atglr3.7*, in which pollen tubes grew more slowly than in wild-type plants and showed different abnormalities. Both lines also displayed a decreased number of seeds per silique, suggesting partial male sterility. *SlGLR1.1*, which is expressed in tomato roots (Aouini et al. 2012), may be a good candidate for studying the role of GLRs in the root tip. Li et al. (2006) revealed that the short-root phenotype of a rice mutant defective in the expression of the *OsGLR3.1* gene is due to the disruption of meristematic activity in the root apex, suggesting that this *GLR* gene plays an essential role in the maintenance of the root meristem.

In this study, we provide preliminary evidence suggesting a role for tomato GLRs in calcium

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homeostasis. Further investigation into the localization, biochemistry and electrophysiological function of SlGLRs would of great interest. As an important Solanaceous crop, the tomato is a promising material for further study on the function of *GLR* genes.

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