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RESEARCH ARTICLE

Molecular cloning and characterization of RNA binding protein genes from the wild radish

Eunsook Chung · Selvam Ayarpadikannan · Chang-Woo Cho · Hyun-Ah So · Kyoungmee Kim · Soonok Kim · Myounghai Kwak · Kee-Young Kim · Doh Hoon Kim · Seon-Woo Lee · Jai-Heon Lee

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Abstract
Two cDNA clones encoding RNA binding proteins (RBPs) were isolated from a cDNA library constructed from salt-treated leaf tissues of wild radish (*Raphanus sativus* var. *hortensis* for *raphanistroide*). The deduced amino acid sequence of either *RsRBP1* or *RsGRP1* contains an RNA-recognition motif (RRM) at the carboxy or amino terminal. Comparative sequence analysis of RsRBP1 reveals extensive homology (63-84%) to known RBPs from other plants. RsGRP1 was shown to be most homologous to AtGRP7 (93%) out of eight members of Arabidopsis glycine-rich RBPs. Transcript levels of *RsRBP1* was up-regulated slowly and reached its maximum at 9 h during salt stress. On the other hand, RNA expression of *RsGRP1* was up-regulated rapidly but significantly was reduced at 9 h after salt stress. The RsRBP1 and RsGRP1 proteins were detected in the nucleus and cytoplasm. Characterization of the transgenic Arabidopsis plants overexpressing *RsRBP1* and *RsGRP1* revealed that both transgenic lines displayed enhanced growth under the osmotic stress conditions. Overexpression of *RsGRP1* resulted in delayed germination rates under the osmotic stress conditions, whereas *RsRBP1* overexpression Arabidopsis did not display any difference in germination rates during osmotic stress. These results suggest that *RsRBP1* and *RsGRP1* may be involved in the responses to osmotic stress in plant.

Keywords EST; Gene expression; Osmotic stress; *Raphanus sativus*; RNA binding protein; Tolerance

Introduction

Regulation of gene expression is achieved through both transcriptional regulation and post-transcriptional control of RNAs in eukaryotes. RNA binding proteins (RBPs) regulate gene expression at the translation and the post-transcriptional level such as pre-mRNA splicing, nucleocytoplasmic mRNA transport, mRNA stability and decay, or through chromatin remodeling to post-translational modifications (Dreyfuss et al., 1993; Lorković, 2009). RBPs are characterized by the presence of one or more RNA-binding domains such as RNA recognition motif (RRM), the K-homology (KH) domain, RGG-box, and zinc-finger motif (Lorković, 2009). The RRM contains two short consensus sequences, RNP1 (octamer) and RNP2 (hexamer) located in a structurally conserved region of about 80 amino acids (Lorković and Barta, 2002). The RRM domains appear to be ancient protein structures as they have been found in organisms ranging from bacteria to humans (Burd and Dreyfuss, 1994).

Plant RBPs function as regulatory factors in floral transition, floral patterning, ABA signaling, stress response, circadian rhythms, and chromatin modification (Lorković, 2009; Ambrosone et al., 2012). More than 200 putative *RBP* genes have been identified in the *Arabidopsis thaliana* genome (Lorković, 2009) and about 250 *RBP* genes in *Oryza sativa* (Cook et al., 2011). The large variety of possible RNA targets implies the existence of a large number of RBPs with different binding specificities. The function of some RRM containing RBP proteins can be predicted based on the similarity with

E. Chung and S. Ayarpadikannan contributed equally to this work.

E. Chung · S. Ayarpadikannan · C.-W. Cho · H.-A. So · K. Kim · D. H. Kim · S.-W. Lee · J.-H. Lee (✉)
BK21 Center for Silver-Bio Industrialization, College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea
e-mail: jhnlee@dau.ac.kr;

S. Kim · M. Kwak
National Institute of Biological Resources, The Integrated Environmental Research Park, Kyoungsedong, Seogu, Incheon 404-708, Korea

K.-Y. Kim
National Academy of Agricultural Sciences, RDA, Suwon 441-707, Korea

their counterparts of other organisms such as poly(A)-binding proteins (PABPs), snRNPs and spliceosome-associated RRM proteins (Lorković and Barta, 2002). FCA and FPA are the plant-specific RRM containing proteins, which control flowering time in Arabidopsis (Lorković, 2009; Lorković and Barta, 2002). However, the functions of most Arabidopsis RRM containing RBP proteins have been elusive. Furthermore, detailed analyses of RRM containing proteins have previously been restricted to only a few major model species, e.g., Arabidopsis, rice, and tobacco (Kim et al., 2010; Lee et al., 2009; Lorković and Barta, 2002).

A number of GRPs have been demonstrated to be involved in a variety of stress conditions (Lorković, 2009). It has been shown that an Arabidopsis GRP, designated *atRZ-1a* characteristic of the CCHC-type zinc fingers, is related with seed germination and seedling growth at low temperatures, and plays a role in the enhancement of freezing tolerance in Arabidopsis (Kim et al., 2005). Recently, three Arabidopsis AtGRP family genes including *AtGRP2*, *AtGRP4*, and *AtGRP7* have been shown to be associated with osmotic stress tolerance (Kim et al., 2007, 2008, 2010; Kwak et al., 2005). Most of GRPs have been shown to be found in nucleus or cytoplasm, while AtGRP2 is localized into mitochondria of Arabidopsis, implying that *AtGRP2* may play a role in mitochondrial gene expression at the post-transcriptional regulation (Vermel et al., 2002). The different structural features and subcellular localization of each GRP can attribute to its diverse roles in plants.

Raphanus sativus var. *hortensis* for *raphanistroides* (wild radish: Brassicaceae) grows mainly on beaches in East Asia. Wild radish grows primarily on sand dunes or on sandy cliffs near the sea in East Asia. It is herbaceous, diploid biennial, with 2n = 18 (Kitamura and Murata, 1987). The plant height is 30-60 cm with purple or whitish pink flowers. However, the genetic information of wild radish is limited for salt tolerance. In this study, we have characterized two wild radish genes encoding RBP containing RRM. Their expression was significantly upregulated under the salinity stress conditions. Nuclear and cytoplasmic localization of wild radish RBPs implies their role in post-transcriptional process in plant cell. Using the over-expression transgenic Arabidopsis plants, each wild radish *RBP* gene may exert differential roles in stress tolerance and germination process under the osmotic stress conditions.

Materials and Methods

Complementary DNA library construction and EST sequencing
Raphanus sativus var. *hortensis* for *raphanistroide* seeds (The Wild Plant Seed Bank of Korea) were germinated and grown in a soil culture in greenhouse conditions. Two-month-old plants were treated with 200 mM NaCl (0, 3, 6, 12 or 24 h). Total RNA was isolated from leaf tissues with RNA extraction kit (Ambion, USA). Complementary DNA (cDNA) from both control and NaCl treated plants were prepared according to manufacturer's instructions (Stratagene, USA). Poly (A^+) messenger RNAs (mRNAs) were isolated from the total RNA using an oligo dT cellulose column. For the library construction, cDNA was synthesized with 4.8 μg of mRNA using a Zap - cDNA Gigapack III Gold cloning kit (Stratagene, USA). Size-fractionated cDNA fragments were pooled and directionally cloned into *Eco*RI and *Xho*I site of pBluescript SK^- vector (Stratagene, USA). The ligated plasmids were transformed into *Escherichia coli* DH5α XL1-Blue MRF`, which yielded the cDNA library of titer 0.8×10^8. Subsequent EST analyses carried out as previously described (Ayarpadikannan et al., 2012).

Northern blot analyses
Total RNAs were isolated from leaf tissues of *R. sativus* treated with 200 mM NaCl (0, 3, 6, 9, 12, 24 h) by RNA extraction kit (Ambion, USA). Fifteen microgram of total RNA was fractionated on a 1% agarose-formaldehyde gel, and subsequently transferred to the nylon membrane (GE Healthcare, USA). The PCR products amplified with gene-specific primers were used for probe labeling as follows: RsRBP1, F (5'-ATGGCGAACTTAGAAGCTGAAGC-3') and R (5'-CTTTGTCAGAATCTCAGCACCAT-3'); RsGRP1, F (5'-ATGGCGTCCGCTGATGTTGAGT-3') and R (5'-TTACCAACCACCACCACCGCTT-3'). Probes were labeled with ^{32}P-dCTP using Ladderman™ labeling kit (Takara, Japan). The membrane was hybridized with ^{32}P-dCTP labeled probe and washed as described by Church and Gilbert (1984). The membrane was exposed to X-ray film (Fujifilm, Japan) at -70ºC.

Generation of GFP fusion constructs and subcellular localization
Each full-length *RsRBP1*or *RsGRP1* cDNA was PCR-amplified with CACC-forward primer and Reverse-No Stop primer, respectively. Gene-specific primers are as follows: RsRBP1, F (5'-CACCATGGCGAACTTAGAAGC-3') and R (5'-AAAACGTCTTCTCGGGCTTCG-3'); RsGRP1, F (5'-CACCATGGCGTCCGCTGATG-3') and R (5'-CCAACCACCACCACCGCTT-3'). Each PCR amplified DNA fragment was cloned between *attB1* and *attB2* sites of the pENTR/D TOPO vector (Invitrogen, USA; http://www.invitrogen.com/), respectively. *RsRBP1* or *RsGRP1*, was then recombined as an N-terminal fusion of GFP into the Gateway destination binary vector, pK7FWG2 (Plant Systems Biology, Belgium; http://www.psb.ugent.be/), yielding 35S:RsRBP1-GFP, 35S:RsGRP1-GFP by a LR recombination reaction.

Each 35S:GFP, 35S:RsRBP1-GFP, or 35S:RsGRP1-GFP construct was transformed into *Agrobacterium* sp. strain C58c1, respectively. For the transient expression of GFP proteins *in planta*, the transformed *Agrobacterium* cells containing 35S:GFP, 35S:RsRBP1-GFP, or 35S:RsGRP1-GFP were in-

oculated into the leaves of *Nicotiana benthamiana* plants as previously described (Chung et al., 2004). The protoplasts were isolated from the *Agrobacterium*-infiltrated *N. benthamiana* leaves as described by Abel and Theologis (1994). A Zeiss LSM700 (Germany) confocal microscope was used to observe fluorescence as described previously (Chung et al., 2009).

For the stable expression of GFP protein, *Arabidopsis* was transformed with *Agrobacterium* containing either 35S:GFP, or 35S:RsGRP1-GFP binary construct using a floral dipping method (Clough and Bent, 1998). Transgenic 35S:GFP, and 35S:RsGRP1-GFP plants (T_1 generation) were selected on MS media containing kanamycin (50 μg/mℓ). Leaf and root tissues from the transgenic 35S:GFP, and 35S:RsGRP1-GFP *Arabidopsis* seedlings (2 week-old) were used to localize fluorescent signals. To stain nucleus, seedlings were incubated in DAPI dye solution (4', 6-diamidino-2-phenylindole; Invitrogen, USA) in deionized water. Stained tissues were observed with a Zeiss Axiophot fluorescence microscope fitted with fluorescein isothiocyanate filters; excitation filter BP 543 nm and emission filter LP 590 nm to visualize the green fluorescence, excitation filter G365 nm and emission filter LP 420 nm to visualize 4'-6-Diamidino-2-phenylindole (DAPI) staining.

Vector construction of binary vectors and generation of overexpression transgenic plants

To produce the 35S-RsRBP1 and 35S-RsGRP1 transgenic plants, the coding region of 35S-RsRBP1 or 35S-RsGRP1 was amplified by PCR with CACC-forward primer and the reverse primer, respectively. Primers used for vector construction are as follows: RsRBP1, F (5'-CACCATGGCGAACTTAGAAGC-3') and R (5'-TCAAAAACGTCTTCTCGGGCT-3'); RsGRP1, F (5'-CACCATGGCGTCCGCTGATG-3') and R (5'-TTACCAACCACCACCACCGCT-3'). PCR products were cloned into the pENTR vector (Invitrogen) and then recombined into the Gateway destination binary vector, pB7WG2D (Plant Systems Biology), in which transgene expression is under the control for the CaMV 35S promoter. Transformation of Arabidopsis was performed by the vacuum infiltration method (Clough and Bent, 1998) using *A. tumefaciens* strain C58c1. For the phenotypic analysis, T3 homozygous lines were used after basta selection.

Osmotic stress tolerance and germination tests

Each plant was grown in the same conditions, and the seeds were collected at the same time. The wild-type, 35S-RsRBP1, and 35S-RsGRP1 transgenic seedlings (10-d old) were transferred to MS media with or without NaCl (150 mM) or mannitol (300 mM) and were subsequently grown for 10 or 12 d. Root length of the seedlings grown under normal and osmotic stress conditions was measured with three replications. Germination (full emergence of radicles) was scored on MS medium (2% Sucrose and 0.8% agar) without or with NaCl (0, 150 mM) or mannitol (0, 300 mM) as indicated. Plates were chilled at 4°C in the dark for 3 d (stratified) and moved to 22°C with a 16-h-light/8-h-dark cycle. The percentage of seed germination was scored after 4 d with 3 repetitions.

Results and Discussion

Cloning and sequence analyses of *RsRBP1* and *RsGRP1*

Wild radish (*R. sativus* var. *hortensis* for *raphanistroide*) cDNA library was constructed from the leaf tissues treated with salt stress. About 288 ESTs were randomly sequenced and 240 unigenes were obtained. Northern blot analyses revealed that two stress-responsive candidate genes were differentially expressed under the high salinity stress conditions (Fig. 1, Table 1). Among the salt stress related genes found in our EST, the molecular function of *RsRBP1* and *RsGRP1* was further studied. The cDNA of *RsRBP1* is composed of 603 base pairs (bp) encoding a polypeptide (200 aa) of 22.4 kDa (Genbank accession number, JQ780056), which shares very high homology (63-84%) characteristic of the C-terminal RRM containing proteins from other plants (data not shown). RsRBP1 is most homologous to RBPs of *A. lyrata* (84%) and *A. thaliana* (81%) (Table 1). It seems that RsRBP1 is the orthologue of AtY14, which interacts with AtMago and AtPYM (Park and Muench, 2007). *Mago* was first identified required for axis formation during *Drosophila* oogenesis, and implicated in normal localization of *oskar* mRNA to the posterior pole of the oocyte (Boswell et al., 1991; Newmark and Boswell, 1994). Y14, known as *Tsunagi* in *Drosophila*, was identified as a binding partner of Mago in yeast two hybrid screens (Zhao et al., 2000). It has been proposed that Arabidopsis AtMago and AtY14 may act as the components of the exon junction complex (EJC) associated with post-transcriptional processes (Park and Muench, 2007). However, their precise function has not been elucidated in plants.

The coding region of *RsGRP1* gene is composed of 462 bp encoding a polypeptide (153 aa) of 15 kDa (Genbank accession number, JQ780055) (Table 1). Deduced amino acid sequence of *RsGRP1* belongs to the glycine-rich RBPs (GRPs) (Table 1). A class of plant GRPs consists of small polypeptides with an N-terminal RNA recognition motif (RRM) and a C-terminal glycine-rich region, so called RGG box (Burd and Dreyfuss, 1994). Numerous evidences suggest the involvement of GRPs in the plant response to various stress conditions (Lorković, 2009). RsGRP1 contains one RRM in the N-terminus, which is commonly found in other classes of GRPs (data not shown) (Lorković and Barta, 2002; Lorković, 2009). The RRM contains the highly conserved RNP2 ('CFVGGL'; 10-15 aa) and RNP1 ('RGFGFVTF'; 39-46 aa) (data not shown). Among the Arabidopsis eight GRPs, RsGRP1 shows the extremely high homology to the AtGRP7 (93%) implying that *RsGRP1* is an orthologue of *AtGRP7*. It has been recently

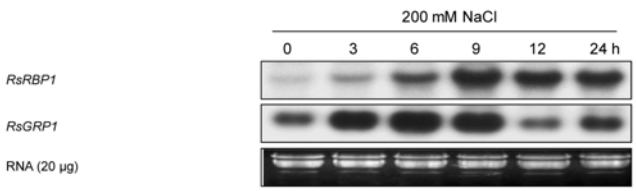

Figure 1. Northern blot analyses of *R. sativus* salt stress responsive genes. *R. sativus* leaves were treated with 200 mM NaCl (0, 3, 6, 9, 12 or 24 h). Equal loading of the total RNA (20 μg) was confirmed by EtBr staining, shown as total RNA below the signal panel. Following electrophoresis, RNA was transferred to a nylon membrane and hybridized with the gene-specific probe.

demonstrated that *AtGRP7* has RNA chaperone activity during the cold adaptation process in *Escherichia coli* (Kim et al., 2007). On the line with this report, overexpression of *AtGRP7* enhanced freezing tolerance in Arabidopsis (Kim et al., 2008). It has been proposed that *AtGRP7* may be involved in RNA export from nucleus to cytoplasm during cold stress in plants (Kim et al., 2008).

Subcellular localization of RsRBP1 and RsGRP1

In order to determine the subcellular localization of the RsRBP1 and RsGRP1 protein, a transient expression assay was performed using GFP fusion protein (Fig. 2). Localization studies can provide some clues to possible roles for RsRBP1 and RsGRP1 proteins in RNA post-transcription metabolism. It was shown that RsRBP1-GFP protein was mostly localized in the nucleus (Fig. 2a). This coincides with the previous report that AtY14, an orthologue of RsRBP1, was found in the nucleoplasm predominantly (Park and Muench, 2007). It was proposed that the AtY14-AtMago heterodimer in the nucleolus could be important in a role as EJC components (Park and Muench, 2007). Compared to the obvious nuclear localization of RsRBP1, RsGRP1-GFP protein was observed in the nucleus and also in the cytoplasm as aggregates (Fig. 2a). In order to ensure its nuclear localization, transgenic Arabidopsis overexpressing RsGRP1-GFP was generated and examined (Fig. 2b). Nucleus stained with DAPI was shown to be colocalized with green fluorescence signals in the root tissues of the transgenic RsGRP1-GFP Arabidopsis (Fig. 2b). This confirms that RsGRP1 is targeted to the nucleus. This observation coincides with the previous reports about the nuclear localization of the several GRPs including white mustard GRP, maize MA16 and tobacco RZ-1 proteins (Albà et al., 1994; Heintzen et al., 1994; Hanano et al., 1996; Sachetto-Martins et al., 2000). In addition, it was proposed that AtGRP7 (AtGRP7) can be detected in

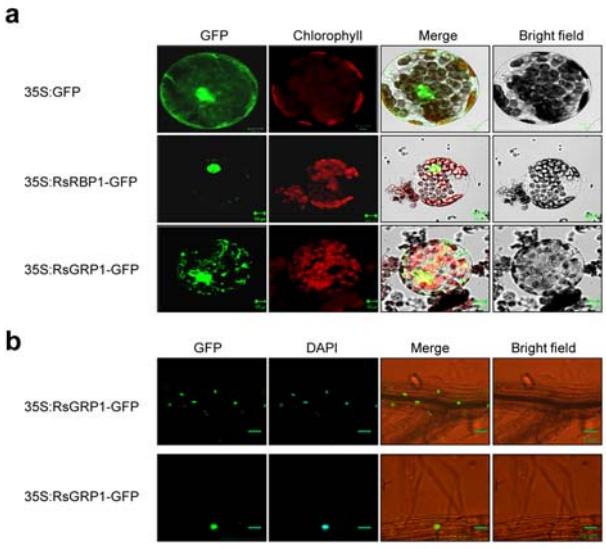

Figure 2. Subcellular localization of RsRBP1-GFP, and RsGRP1-GFP fusion proteins. (a) Each 35S:GFP, 35S:RsRBP1-GFP, and 35S:RsGRP1-GFP construct was transformed into tobacco leaves via *Agrobacterium*-infiltration respectively. Protoplasts were isolated from the infiltrated leaves after 36 h. Localization of fusion proteins was visualized by a confocal microscopy. (b) The 35S:RsGRP1-GFP construct was transformed into Arabidopsis. Root tissues from 35S:RsGRP1-GFP transgenic Arabidopsis plants were germinated and stained with DAPI. The samples were examined using a fluorescence microscopy at x 400 (upper panel) or x 800 (lower panel) magnifications.

both cytoplasm and nucleus (Ziemienowicz et al., 2003). Based on their nuclear localization, GRPs were shown to bind to RNA or DNA *in vitro* (Sachetto-Martins et al., 2000). It was proposed that *AtGRP7* is involved in the export of mRNAs from the nucleus to the cytoplasm under cold stress conditions (Kim et al., 2008). This led us to the hypothesis that *RsRBP1* and *RsGRP1* may be involved in RNA processing, maturation or control of gene expression on the modulation of the pathways activated by osmotic stress.

Test of Arabidopsis transgenic plants overexpressing *RsRBP1* and *RsGRP1* during osmotic stress

RBPs in plants have essential roles in diverse developmental processes and adaptation to various environmental conditions by post-transcriptional gene regulation (Lorkovíc, 2009). Numerous evidences are emerging that *GRPs* are involved in a variety of stress conditions. *AtGRP2*, *AtGRP4*, and *AtGRP7* out of eight AtGRP family members in Arabidopsis, have been shown to display changes in osmotic stress-associated pheno-

Table 1. Representative salt stress related genes of *R. sativus* analyzed by Northern blot.

Functional annotation	GenBank accession no.	Organism	E value	GenBank accession no.
RNA binding protein	JQ780056	*A. thaliana*	2.00E-70	AAG52616.1
Glycine-rich RNA binding protein	JQ780055	*Sinapis alba*	2.00E-37	P49311.1

types such as seed germination, seedling growth, and stress tolerance of Arabidopsis plants under diverse stress conditions (Kwak et al., 2005; Kim et al., 2007, 2008, 2010). Salt stress-inducible expression of wild radish *RBP* genes inspired us to further investigate the function of *RsRBP1* and *RsGRP1* in relation to the osmotic stress response using the overexpressing transgenic Arabidopsis. Independent transgenic lines showed their constitutive expression based on RT-PCR (data not shown). To determine whether overexpression of *RsRBP1* and *RsGRP1* alters tolerance response to the osmotic stresses in Arabidopsis, root growth of wild-type, 35S-RsRBP1 and 35S-RsGRP1 transgenic seedlings were compared under osmotic stress conditions (Fig. 3). Ten-d old seedlings of the wild-type and 35S-RsRBP1 overexpression transgenic plants were transferred to the media containing 150 mM NaCl or 300 mM mannitol and incubated for 12 d (Fig. 3a). Transgenic plants overexpressing *RsRBP1* showed the same phenotype as wild-type plants under nonstress conditions (Fig. 3a). By contrast, under the stress conditions, the 35S-RsRBP1 overexpression transgenic plants displayed better shoot and root growth in media containing mannitol or NaCl compared to the wild-type (Fig. 3a). Ten-d old seedlings of the wild-type and 35S-RsGRP1 overexpression transgenic plants were transferred to media containing 150 mM NaCl or 300 mM mannitol and incubated for 10 d (Fig. 3b). *RsGRP1* overexpressing plants showed the similar growth as wild-type plants grown under nonstress conditions (Fig. 3b). Transgenic plants overexpressing 35S-RsGRP1 displayed better shoot and root growth with mannitol stress, but not much difference under NaCl stress conditions compared to the wild-type (Fig. 3b). This result implies that the function of *RsRBP1* and *RsGRP1* is different in stress tolerance mechanism depending on the diverse stress treatments in plant.

To further examine the role of *RsRBP1* and *RsGRP1* in relation to osmotic stress responses in germination process, we carried out germination tests of wild-type and the overexpression transgenic plants under the NaCl, and mannitol stress conditions (Fig. 4). There was no substantial difference in the germination rates of the wild-type and 35S-RsRBP1 overexpression transgenic plants (Fig. 4a). On the other hand, we observed a small reduction in the germination rate of 35S-GRP1 overexpression transgenic plants compared to the wild-type under NaCl (150 mM), and mannitol (300 mM) conditions (Fig. 4b). Collectively, these results indicate that *RsRBP1* and *RsGRP1* may acts different roles in response to osmotic stress and developmental signals in plants.

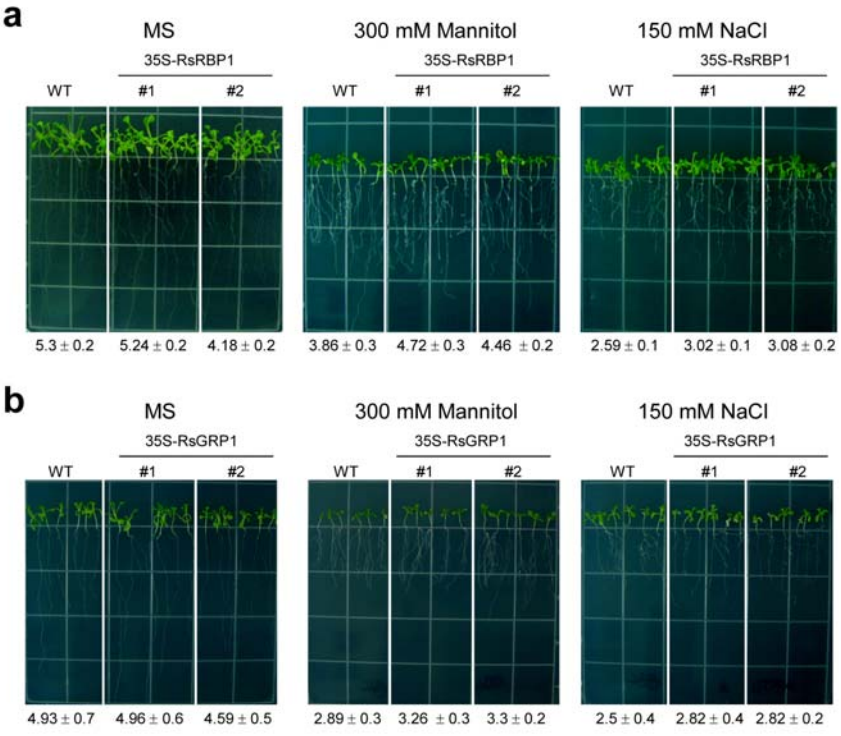

Figure 3. Tolerance tests of wild-type, 35S-RsRBP1 and 35S-RsGRP1 Arabidopsis transgenic plants under osmotic stress conditions. (a) Ten-d-old seedlings of the wild-type and *35S:RsRBP1* Arabidopsis transgenic lines (#1 and #2) were transferred to MS medium containing 2% (w/v) sucrose and 0.8% (w/v) phytoagar supplemented either mannitol (300 mM) or with NaCl (150 mM). Root length was monitored after 12 days. The values are the means ± SD (n = 3). This experiment was carried out three times with consistent results. (b) Ten-d-old seedlings of the wild-type and *35S:RsGRP1* Arabidopsis transgenic lines (#1 and #2) were tested for osmotic stress tolerance as described above. Root length was monitored after 10 days.

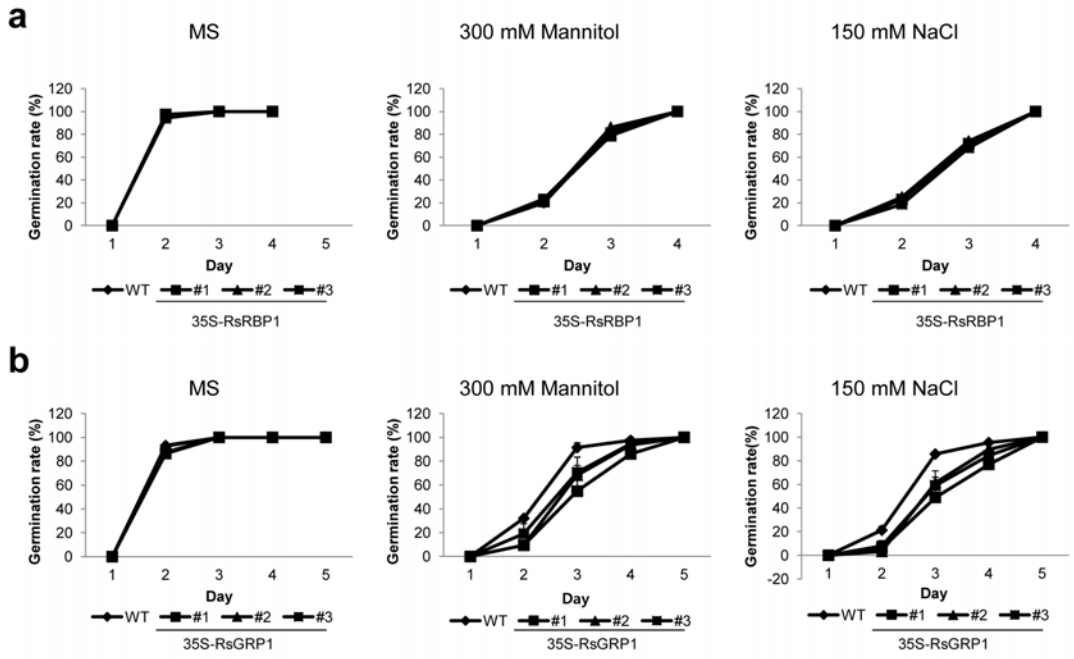

Figure 4. Germination rates of the wild-type, 35S-RsRBP1, and 35S-RsGRP1 Arabidopsis transgenic lines (#1, #2 and #3) under osmotic stress or ABA conditions. (a) Seeds of wild-type (Col-0) and 35S:RsRBP1 transgenic plants were germinated in media supplemented with NaCl (150 mM), or mannitol (300 mM). Seed germination rate was recorded on the indicated days. (b) Seed germination rate of the wild-type and 35S:RsRBP1 on MS media without or with NaCl (150 mM), or mannitol (300 mM) was scored on the indicated days. Mean values and standard errors were obtained from three independent experiments. At least 100 seeds per line were measured in each replicate.

Drosophila Y14 was shown to be required for transport of *oskar* mRNA during germ cell development (Boswell et al., 1991; Newmark and Boswell, 1994). However, any function of *AtY14*, a homolog of Y14, has not been identified in Arabidopsis. In this study, we have shown that wild radish *RsRBP1* homologous to *AtY14* is involved in response to osmotic stress. Further knock-out mutation or complementation test of *AtY14* may help us better understand its role in stress response and development in Arabidopsis. A number of literatures regarding diverse roles of *GRP* genes outnumber the functional studies related to *RsRBP1* in plants. It has been suggested that *AtGRP7*, an orthologue of *RsGRP1*, functions as a RNA chaperone in facilitating mRNA folding in the nucleus during cold stress (Kim et al., 2007; Kim et al., 2008). Above all, *AtGRP7* was shown to have positive effect on freezing stress tolerance but a negative effect on seed germination and seedling growth under salt or dehydration stress conditions (Cao et al., 2006; Kim et al., 2008). Delayed seed germination by overexpression of *RsGRP1* was observed as same in *AtGRP7* overexpression transgenic Arabidopsis (Kim et al., 2008) (Fig. 4b). Overall seedling growth of *RsGRP1* overexpression transgenic Arabidopsis was better than that of the wild-type plants under the osmotic stress conditions (Fig. 3b). This implies that *RsGRP1* may be involved in defense mechanism in response to various osmotic stresses in plants.

GRPs are believed to play their roles through binding to target RNAs and the regulation of RNA processing, including pre-mRNA splicing, capping, polyadenylation, mRNA transport, stability, and translation (Kim et al., 2008). It has been proposed that *AtGRP7* is likely to interact with the 3' UTR of its transcript rich in G/U sequences (Kim et al., 2007; Kim et al., 2008; Staiger et al., 2003). It will be essential to identify the specific RNA targets of particular RBPs responding to the developmental or environmental signals in plants. In conclusion, two wild radish genes differentially expressed during salinity stress encode RBP protein targeted to the nucleus and cytoplasm implying their involvement in post-transcriptional regulation under the abiotic stress conditions. The results of overexpression stress tests also provide evidence that each wild radish *RBP* gene may differ in function in tolerance and germination process during the osmotic stress conditions.

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