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COR15B expression is affected by chloroplast functionality and its role in response to salt stress in *Arabidopsis thaliana*

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Abstract

Cold-responsive (*COR*) genes participate in the response of plants to low-temperature stress. In this study, we isolated and characterized a cold-responsive and light-inducible gene *COR15B* from *Arabidopsis thaliana*. Chloroplast damage caused by mutations (albino mutants *seca1*, *secy1*, and *tic20*) or by a norflurazon (NF) treatment resulted in a reduction of *COR15B* transcription. A semi-quantitative RT-PCR analysis shows that *COR15B* was induced by the salt stress in an abscisic acid-dependent manner. An over-expression of *COR15B* in *Arabidopsis* resulted in transgenic lines more sensitive to the NaCl treatment than the wild type. However, *COR15B* knockdown did not significantly affect the sensitivity of the *cor15b* mutant to the salt stress. Furthermore, we found that the expression of *COR15A,* a homologous gene of *COR15B,* was significantly elevated in *cor15b* mutant plants. All these results suggest that plants acquire the ability to fully express *COR15B* only after development of functional chloroplasts. The expressional reprogramming and functional backup may exist between *COR15* homologues in *Arabidopsis*.

Additional key words: abscisic acid, expressional reprogramming, low temperature, mutants, NaCl, norflurazon.

Introduction

Plants respond to stresses, such as drought, low temperature, or high salinity, with a number of physiological and molecular changes (Hasegawa *et al.* 2000, Seki *et al.* 2003, Yamaguchi-Shinozaki and Shinozaki 2006, Silveira *et al.* 2012, Meng *et al.* 2014). In *Arabidopsis thaliana*, a large number of cold-regulated genes have been identified (Lee *et al.* 2005, Yamaguchi-Shinozaki and Shinozaki 2006, Li *et al.* 2013). Interestingly, *KIN1* and *KIN2*, *COR15A* and *COR15B*, and *RD29A* and *RD29B* appear to be pairs of homologues, the number of each being tightly linked on the chromosome in tandem array (Kurkela and Borg-Franck 1992, Wilhelm and Thomashow 1993, Yamaguchi-Shinozaki and Shinozaki 1993, Wang *et al.* 1994).

COR15A, a cold-responsive (*COR*) gene, firstly isolated from the model plant *Arabidopsis*, encodes a 15 kDa protein with significant similarities in its amino acid sequence to those encoded by late embryogenesis abundant (*LEA*) genes (Lin and Thomashow 1992). A homologue of *COR15A, COR15B*, has also been identified in *Arabidopsis* (Wilhelm and Thomashow 1993). Both COR15A and COR15B are encoded by nuclear genes and are located in the chloroplast (Wilhelm and Thomashow 1993, Nakayama *et al.* 2007, Candat *et al.* 2013). Although these two nuclear genes are aligned in tandem on the 2nd chromosome, their expression profiles are not absolutely consistent. For example, an increased amount of mRNAs of *COR15A* occurs in plants in response to cold, drought, and abscisic acid (ABA), however, *COR15B* is strongly responsive to cold and ABA, but not drought (Wilhelm and Thomashow 1993). In addition to *Arabidopsis*, two *COR15* copies have also been discovered in a variety of other species, such as *Brassica*, *Rorippa*, and *Draba* (Weretilnyk *et al.* 1993, Zhou *et al.* 2009). Previous studies have demonstrated that the constitutive expression of *COR15A* enhances cold tolerance in *Arabidopsis* (Artus *et al.* 1996, Steponkus

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Abbreviations: ABA - abscisic acid; COR - cold-responsive; DMSO - dimethyl sulfoxide; LEA - late embryogenesis abundant; NF - norflurazon; RT-PCR - reverse transcription-polymerase chain reaction; UTR - untranslated regions.

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et al. 1998). Transgenic tobacco plants over-expressing *CbCOR15A*/*B* from *Capsella bursa* also exhibite enhanced cold tolerance (Wu *et al.* 2012, Zhou *et al.* 2012).

During photomorphogenesis, chloroplasts develop from undifferentiated proplastids in meristematic cells (Vothknecht and Westhoff 2001, López-Juez 2007, Waters and Langdale 2009). Since more than 95 % of chloroplast proteins are encoded by nuclear genes (Inaba and Schnell 2008), it is not surprising that there is an inter-organelle communication between the chloroplast and nucleus, called retrograde signalling. Over the last decades, chloroplast-to-nucleus retrograde signalling have been studied by analyzing the photosynthesis-related nuclear gene expression in response to chloroplast damage (Leister 2005, Nott *et al.* 2006, Koussevitzky *et al.* 2007, Kakizaki *et al.* 2012). Recently, a few studies have investigated the role of retrograde signalling in regulating the expression of nuclear gene during several stresses including high irradiance, drought, heat, and excess of ammonium (Fernandez and Strand 2008, Estavillo *et al.* 2011, Li *et al.* 2012, Yu *et al.* 2012).

Although the expression profiles and functional characterizations of *Arabidopsis COR15B* in plant response to low temperature are well studied, the functional investigations of this gene involved in salt stress are scarcely reported (Wilhelm and Thomashow 1993, Wu *et al.* 2012). We have been interested in the relationship between chloroplast functionality and *COR15B* expression, and in the functional character-rization of *COR15B* in response to salt stress. In the present study, a cold-responsive gene *COR15B* was isolated, its expression and its potential role involved in salt stress were studied.

Materials and methods

Plants and growth conditions: *Arabidopsis thaliana* (L.) Heynh. ecotypes Columbia-0 (Col-0) and Wassilewskija (Ws), were used for experiments. All T-DNA insertions mutants, *seca1* (SALK_063371), *secy1* (CS16483), *tic20* (SALK_039676), *aba1* (SALK_059469), and *cor15b* (SALK_085398) were obtained from the Arabidopsis Biological Resource Center (Alonso *et al.* 2003). Seeds were surface sterilized in a 25 % (m/v) solution of natrium hypochlorite plus 0.01 % (v/v) *Triton X-100* for 10 min and washed with sterilized water for 15 times. Following 3 d of stratification in the dark at 4 ºC, the seeds were germinated and grown on plates containing a half strength Murashige and Skoog (MS) medium with 0.75 % (m/v) agar, 1 % (m/v) sucrose (pH 5.7) at a temperature of 22 ± 1 ºC, a 16-h photoperiod, an irradiance of 90 µmol m^{-2} s^{-1}, and a relative humidity of 60 %. For norflurazon (NF) treatment, NF was diluted from 100 mM stock solutions prepared in dimethyl sulfoxide (DMSO) to 5 µM, and equivalent volume of DMSO was included in NF-free-treated controls. Plants were grown on an agar medium containing 5 µM NF under continuous irradiance for 5 d. For salt-stress and ABA treatments, the sterilized seeds were germinated on a 1/2 MS solid medium for 7 d, and then the seedlings were transferred to a 1/2 MS liquid medium containing 200 mM NaCl for 2 and 4 h, or 100 µM ABA for 4 h.

Identification of T-DNA insertion mutants: As suggested by the Salk Institute Genomic Analysis Laboratory (http://signal.salk.edu/tdnaprimers.2.html), a PCR-based method was used to identify T-DNA insertion mutants. A T-DNA left border primer *LBb1.3* and gene-specific primers *COR15B-DF* and *COR15B-DR* for *cor15b* (SALK_085398) were used in PCR. Other mutants, *seca1* (SALK_063371), *secy1* (CS16483), *tic20* (SALK_039676), and *aba1* (SALK_059469) have been described in more details previously (Morris *et al.* 2006, Liu *et al.* 2010, Kasmati *et al.* 2011, Skalitzky *et al.* 2011). Primers presented in this paper are listed in Table 1 Suppl.

Construct, plant transformation, and crossing: For the construction of *35S::COR15B* unit, the full-length coding sequence (CDS) corresponding to the *COR15B* gene (At2g42530) locus was cloned by using RT-PCR from *Arabidopsis*. The pair of primers used in the PCR was *OEcor15b-F* and *OEcor15b-R* (*Nco*I and *Bst*EII sites were introduced). The specific PCR fragment was then inserted into a binary vector pCAMBIA1301 between *Nco*I and *Bst*EII sites, replacing the *GUS* gene, to create the recombinant transcription unit *35S::COR15B*. The recombinant plasmids were then introduced into an *Agrobacterium tumefaciens* strain GV3101 and transformed into *A. thaliana* (Col-0) plants using the floral dip method (Clough and Bent 1998). Transformants were screened on a half-strength MS medium containing 50 µg cm^{-3} hygromycin. Within 2 to 3 weeks, the seedlings with green true leaves were identified as transformants and transferred to soil. All primers used are listed in Table 1 Suppl.

To obtain *seca1/cor15b* double mutants, *cor15b* (the female parent) was crossed to *seca1/+* (the male parent). Homozygous plants were identified by a segregation analysis, by comparison with the parental phenotypes, and by PCR-based genotyping in the F$_2$ progeny.

RNA extraction, cDNA synthesis, and gene expression analysis: Total RNA was extracted from plant tissues with *TRI* reagents followed by treatment with RNase-free DNase I (*TaKaRa*, Dalian, China) at 37 ºC for 1 h to degrade genomic DNA. First strand cDNA was synthesized from 2.0 μg of total RNA using *ImProm-II*™ reverse transcriptase (*Promega*, Madison, WI, USA) in 0.01 cm^3 of a reaction mixture, following the manufacturer's instructions with minor modifications. For semi-quantitative RT-PCR analysis, the PCR reaction contained 0.001 cm^3 of a template, 1.5 U *ExTaq* (*TaKaRa*), dNTPs (0.2 mM each), and primers in a final volume of 0.03 cm^3. Three RT-PCR reactions were repeated independently using *TIP41-like* as internal control. The real-time RT-PCR analysis was performed as described previously (Liu *et al.* 2010). The gene-specific primers are available in Table 1 Suppl.

Salt stress analysis: In plate germination assays, the seeds were sown on a 1/2 MS agar medium supplemented with 150 mM NaCl following 3 d of stratification in the dark at 4 ºC. Germination (defined by the emergence of the radicle) rates were recorded for 3 d after sowing. On the 5th day, the cotyledon expansion and greening rates of the seedlings were measured.

Results and discussion

In *Arabidopsis*, COR15B is encoded by a nuclear gene and is located in chloroplasts (Wilhelm and Thomashow 1993, Candat *et al.* 2013). A previous study has shown a *COR15B* mRNA accumulation in leaves but not in roots (Hundertmark and Hincha 2008). We analyzed putative *cis*-acting elements in the *COR15B* promoter by using the *Plant CARE* software (Lescot *et al*. 2002). Some *cis*-elements involved in light and ABA responsivenesses were found in the 1 158-bp promoter fragment of the *COR15B* gene (Table 1). These results prompted us to study whether the *COR15B* expression is dependent on irradiance. We detected *COR15B* mRNA in light-grown seedlings but not in dark-grown seedlings and its amount was dramatically elevated following exposures to the irradiance of 90 μmol m^{-2} s^{-1} for 4, 8, and 12 h (Fig. 1). This distribution profile implies that the presence of undifferentiated chloroplasts affected the transcript accumulation of *COR15B*. To corroborate this line of evidence, we examined the expression of *COR15B* in three mutants affected in the *AtcpSecA*, *AtcpSecY*, and *AtTic20* genes, all of which exhibiting severe defects in both the structure and function of chloroplasts (Roy and Barkan 1998, Liu *et al.* 2010, Kasmati *et al.* 2011, Skalitzky *et al.* 2011). The results show that the expression of *COR15B* was significantly impaired (Fig. 2*A-F*). The treatment of seedlings with a phytoene desaturase-inhibitor, norflurazon (NF), leads to photo-bleaching chloroplasts and the loss of light-induced expression of photosynthesis-related genes. The experiment was next carried out to examine whether the expression of *COR15B* can be affected by the NF treatment. The results show that the accumulation of *COR15B* mRNA was severely inhibited by the NF treatment (Fig. 2*G*,*H*). We also monitored the expression of the marker genes *LHCB1* and *RBCS* which were used as positive controls for retrograde signalling. These two

Table 1. Putative *cis*-elements involved in radiation and ABA responsivenesses in the *COR15B* promoter.

Site name	Plant species	Position (from ATG)	Strand	Matrix score	Sequence	Function
3-AF1 binding site	*Solanum tuberosum*	1056	+	10	TAAGAGAGGAA	light responsive element
AT1-motif	*Arabidopsis thaliana*	88	-	9	AATCTAATCT	part of a conserved DNA module involved in light responsiveness
Box 4	*Petroselinum crispum*	366	+	6	ATTAAT	part of a conserved DNA module involved in light responsiveness
Box I	*Pisum sativum*	653	-	7	TTTCAAA	light responsive element
G-box	*Antirrhinum majus*	226	+	6	CACGTA	*cis*-acting regulatory element involved in light responsiveness
	Pisum sativum	143	+		CACGTG	
	Daucus carota	226	-	6	TACGTG	
	Arabidopsis thaliana	143	+	6	CACGTG	
	Brassica napus	144	+	8	ACACGTGT	
GAG-motif	*Hordeum vulgare*	855	-	7	GGAGATG	part of a light responsive element
GT1-motif	*Arabidopsis thaliana*	481	-	6	GGTTAA	light responsive element
I-box	*Solanum tuberosum*	837	+	10	TATTATCTAGA	part of a light responsive element
MRE	*Petroselinum crispum*	563	-	10	AACCTAA	MYB binding site involved in light responsiveness
Sp1	*Zea mays*	29	+	5	CC(G/A)CCC	light responsive element
ABRE	*Arabidopsis thaliana*	226	-	6	TACGTG	*cis*-acting element involved in abscisic acid responsiveness
		143	+	6	CACGTG	

genes were downregulated after the NF treatment, which is an expected result due to retrograde signalling triggered by impaired chloroplast function.

In order to investigate the effect of NaCl stress on the *COR15B* expression, a semi-quantitative RT-PCR analysis was carried out using RNA extracted from the 7-d-old *Arabidopsis* seedlings treated with 200 mM NaCl. An induced expression of *COR15B* was observed

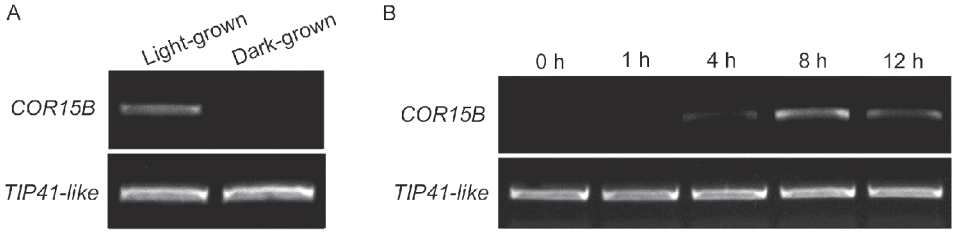

Fig. 1. *A* - The expression of *COR15B* in 4-d-old wild-type (Col) light-grown and dark-grown seedlings. *B* - The transcription of *COR15B* during light-induced greening of etiolated wild-type (Col) seedlings. After growth in darkness for 4 d, the etiolated seedlings were irradiated for 1, 4, 8, and 12 h. The *TIP41-like* gene was used as internal control. The typical results from three independent experiments.

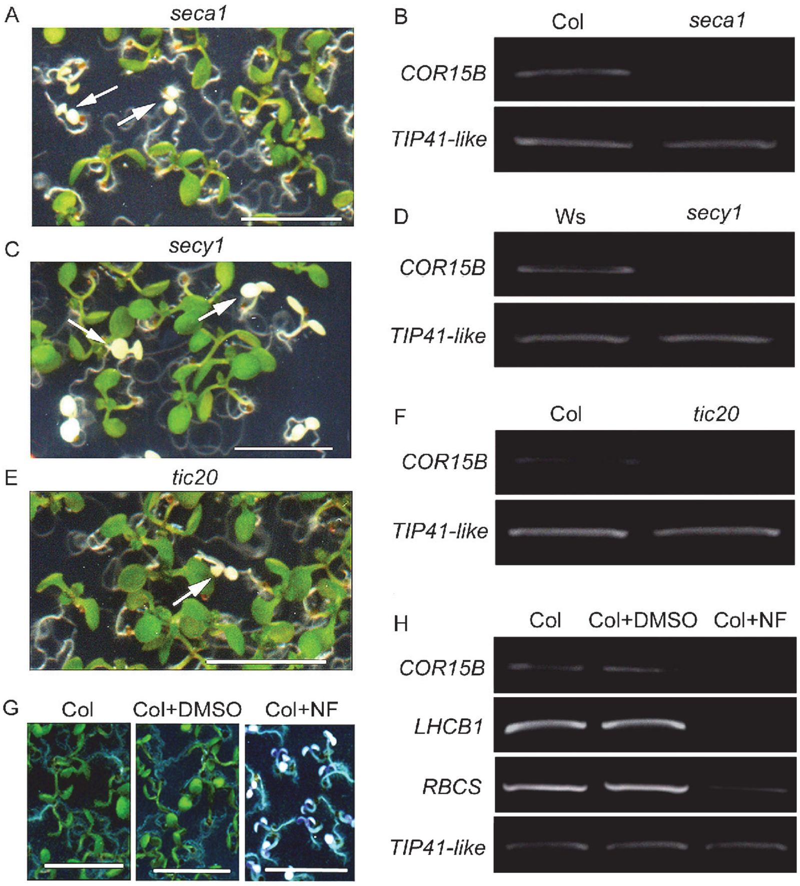

Fig. 2. *A, C, E* - Progenies of self-pollinated *seca1, secy1*, and *tic20* heterozygous plants grown on a 1/2 MS medium with 1 % (m/v) sucrose for 7 d. The *seca1, secy1*, and *tic20* homozygous plants are albino (some indicated by the *arrowheads*). The *scale bar* = 5 mm. *B, D, F* - Steady-state mRNA amounts of *COR15B* are significantly decreased in *seca1, secy1*, and *tic20* homozygous mutants. Total RNA extracted from the rosette leaves from 4-leaf-stage seedlings of wild-type (Col or Ws), *seca1, secy1*, and *tic20* homozygous plants was used for semi-quantitative RT-PCR analyses to detect the accumulation of *COR15B* mRNA. *G* - the changes in phenotype of wild-type (Col) seedlings grown on DMSO (Col+DMSO) or norflurazon (Col+NF) for 5 d. The *scale bar* = 5 mm. *H* - The RT-PCR analyses of *COR15B*, *LBCB1*, and *RBCS* transcriptions in wild-type (Col) seedlings treated with DMSO or NF. Total RNA prepared from 5-d-old wild-type seedlings. The *TIP41-like* gene was used as internal control. The typical results from three independent experiments.

at 2 h of the NaCl treatment, and the salt stress-induced expression of *COR15B* was further enhanced at 4 h (Fig. 3*A*). Next, we examined whether the salt stress induction of *COR15B* depended on the synthesis of endogenous ABA. The results showed that *COR15B* expression was induced considerably by exogenous ABA, the inductive effects of NaCl on *COR15B* expression, however, were reduced significantly in the ABA-deficient *aba1* mutant (Fig. 3*B,C*).

To further investigate the potential role of *Arabidopsis* COR15B in plant responses to salt stress, a total of nine hygromycin-resistant *35S::COR15B* transgenic lines were generated and verified by PCR tests (data not shown). Of these, three homozygous T_3 transgenic lines (B14, B19, and B20) were obtained. The semi-quantitative RT-PCR analysis confirmed a higher *COR15B* transcription in the transgenic plants than in the wild-type (Col-0) plants (Fig. 4*A*). We also detected the expression of *COR15A*, a homologous gene to *COR15B*, in the wild-type and transgenic plants. Interestingly, the expression of *COR15A* was significantly impaired in the *COR15B*-overexpressing plants as compared with the corresponding wild-type plants (Fig. 4*A*). Further, we determined whether COR15B is involved in the plant response to the salt stress. Two homozygous transgenic lines (B14 and B20) were randomly selected for analyzing their phenotypes. Under 200 mM NaCl, the germination rate of seeds from the two *COR15B*-overexpressing lines was much more reduced than that of wild-type seeds (Table 2). When the wild-type and transgenic seeds were germinated on a 1/2 MS medium containing 150 mM NaCl and allowed to growth for 5 d, the cotyledon greening rate of the transgenic seedlings decreased more severely than that of the wild type (Table 2).

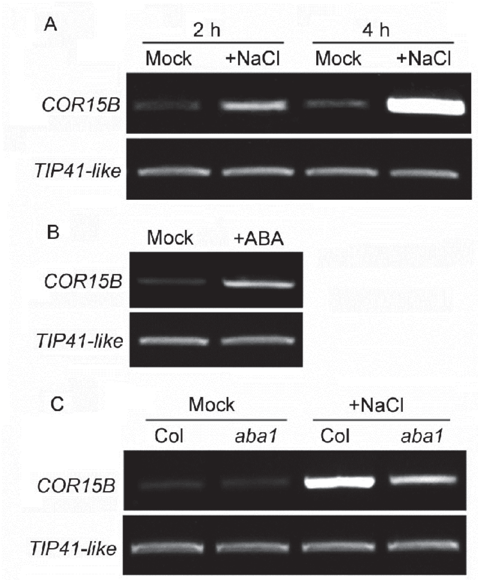

Fig. 3. *A* - The accumulation of *COR15B* transcripts under NaCl treatment. Total RNA samples were extracted from 7-d-old seedlings treated with 200 mM NaCl for 2 and 4 h. Untreated plants were used as mock. *B* - The accumulation of the *COR15B* transcript under ABA treatment. Total RNA samples were extracted from 7-d-old seedlings treated with 100 µM ABA for 4 h. Untreated plants were used as mock. *C* – The expression analysis of the *COR15B* transcript in 7-d-old wild type (Col) and *aba1* mutant seedlings treated with 200 mM NaCl for 4 h. Untreated seedlings were used as mock. The *TIP41-like* gene was used as internal control. The typical results from three independent experiments.

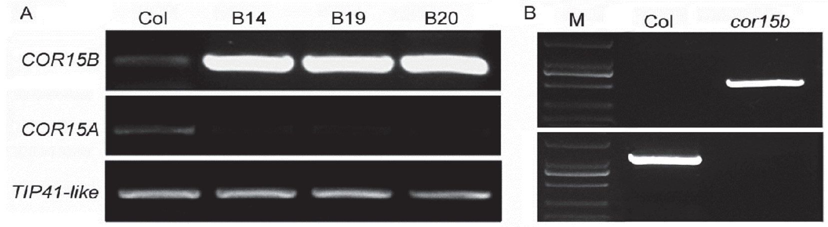

Fig. 4. *A* - The expressions of *COR15A/B* in wild type (Col) and homozygous transgenic *Arabidopsis* lines (B14, B19, and B20). Total RNA extracted from 14-d-old seedlings was used for semi-quantitative RT-PCR analyses to detect the accumulation of *COR15A/B* mRNA with the *TIP41-like* as internal control. The typical results from three independent experiments. *B* - The PCR analysis of the T-DNA insertion in *cor15b* mutant plants. The upper panel: 14-d-old wild-type and *cor15b* mutant plants were identified with the primers *COR15B-DF* and *LBb1.3*, respectively. The lower panel: 14-d-old wild-type and *cor15b* mutant plants were identified with the primers *COR15B-DF* and *COR15B-DR*, respectively. M - DL2000.

The data presented above show that the over-expression of *COR15B* in *Arabidopsis* enhanced plant sensitivity to the salt stress. This observation prompted us to test whether a knocked down expression of *COR15B* affected plant sensitivity to the salt stress. We isolated a T-DNA insertion mutant (SALK_085398) of the *COR15B* gene from *Arabidopsis Biological Resource Center* and designated it as *cor15b*. T-DNA was found to be located at the 3′-untranslated region (UTR) of the *COR15B* gene by PCR amplification and a sequencing analysis (Fig. 4*B* and Fig. 1 Suppl.). Further, we used real-time quantitative PCR to amplify *COR15B* mRNA in wild-type and *cor15b* mutant plants. As compared with the wild type, the expression of *COR15B* was largely reduced but not completely suppressed in the *cor15b* mutant plants (Table 3) suggesting that the T-DNA

Table 2. Seed germination and cotyledon greening rate of wild-type and *COR15B* transgenic lines B14 and B20 treated with 0 and 150 mM NaCl. Means ± SD, $n = 3$. Values followed by different letters are significantly different according to the Student's *t*-test at at $P < 0.05$.

Parameter	NaCl [mM]	Time [d]	Wild type	B14	B20
Germination [%]	0	2	95.25 ± 2.55a	92.92 ± 5.58a	91.25 ± 4.85a
		3	98.35 ± 0.57a	95.25 ± 3.57a	94.75 ± 3.86a
	150	2	15.32 ± 1.49a	4.85 ± 1.56b	7.21 ± 1.23b
		3	71.80 ± 2.76a	41.28 ± 4.95b	48.55 ± 7.64b
Cotyledon greening [%]	0	5	98.55 ± 2.05a	96.85 ± 2.42a	98.40 ± 1.95a
	150	5	41.15 ± 6.72a	24.60 ± 3.67b	27.75 ± 4.35b

Table 3. The real-time quantitative PCR analysis of *COR15A/B* transcripts in 14-d-old wild-type and *cor15b* mutant plants. The *TIP41-like* gene was used as internal control. Means ± SD, $n = 3$. Values followed by different letters are significantly different according to the Student's *t*-test at $P < 0.05$. The expression level in the wild-type was set to 1.

Gene	Wild-type	*cor15b*
COR15B	1.00 ± 0.09a	0.23 ± 0.04b
COR15A	1.00 ± 0.12a	4.37 ± 0.06b

insertion in *cor15b* knocked down the expression of *COR15B*. To investigate the role of *Arabidopsis* COR15B in plant responses to the salt stress, germination rates were then determined for both the wild-type and *cor15b* mutant seeds with or without NaCl (Table 4). There were no obvious differences in germination rates between the *cor15b* mutant and wild-type seeds with or without NaCl. Similarly, when the *cor15b* mutant and wild type seeds were germinated and grown on media containing 150 mM NaCl, the cotyledon greening rate of *cor15b* mutant seedlings showed no obvious difference compared with that of the wild-type seedlings (Table 4).

We also detected the expression of *COR15A* in the *cor15b* mutant and wild-type plants. Interestingly, the expression of *COR15A* was significantly elevated in the *cor15b* mutant as compared with the wild-type plants (Table 3). It indicates that the increased *COR15A* mRNA content in the *cor15b* mutant might compensate for the reduced expression of *COR15B* by expressional reprogramming between the two homologous genes. Since *COR15B* expression is associated with chloroplast functionality, we also investigated whether chloroplast functionality affected expressional reprogramming between the two *COR15* homologous genes. The RT-PCR analyses show that the induction of *COR15A* was partially reduced in the *seca1/cor15b* double mutant when compared with the *cor15b* single mutant, despite the enhanced expression of *COR15A* was not affected in the *seca1/cor15b* double mutant as compared with the wild type (Fig. 2 Suppl.).

The genomic sequence analysis indicates that *CORl5A* and *COR15B* are organized in tandem on the second chromosome (Fig. 3*A* Suppl.). The high similarity existing between these two nuclear genes together with their close linkage and the fact that they have the same transcriptional orientation, clearly suggests a common origin, probably by two consecutive duplications of an ancestral gene and subsequent divergence through mutations (Zhou *et al.* 2009). Kafri *et al.* (2005) proposed that transcriptional reprogramming is the major factor of functional compensation in *Saccharomyces cerevisiae*: when one gene is mutated, the expression of another is reprogrammed to recover the original function. This expressional reprogramming between the *COR15* homologous genes was also found in our work. The expression of *COR15A* was significantly up-regulated in

Table 4 Seed germination and cotyledon greening rate of wild-type and *cor15b* mutant plants treated with 0 and 150 mM NaCl. Means ± SD, $n = 3$. Values followed by different letters are significantly different according to the Student's *t*-test at $P < 0.05$.

Parameter	NaCl [mM]	Time [d]	Wild type	*cor15b*
Germination [%]	0	2	96.05 ± 2.08a	97.75 ± 3.76a
		3	98.02 ± 1.56a	99.40 ± 2.94a
	150	2	16.36 ± 3.74a	19.83 ± 4.16a
		3	74.05 ± 4.62a	79.46 ± 5.83a
Cotyledon greening [%]	0	5	96.08 ± 2.82a	98.74 ± 3.67a
	150	5	41.95 ± 3.62a	45.32 ± 5.07a

a knockdown mutant of *COR15B*. Furthermore, under the normal conditions or salt stress, no obvious phenotypic differences were observed between the wild-type and *cor15b* mutant plants. Since we have still not isolated an available mutant for the *COR15A*, we cannot rule out the possibility that mutations in both *COR15A* and *COR15B* genes do not confer any noticeable phenotype on plants.

Previous studies have shown that COR15A is encoded by a nuclear gene and is located in the stromal compartments of chloroplasts in *Arabidopsis* (Lin and Thomashow 1992, Nakayama *et al.* 2007). A recent study reported that COR15B is also targeted to the chloroplast as COR15A (Candat *et al.* 2013). The program *ChloroP 1.1* (http://www.cbs.dtu.dk/services/ ChloroP/) predicted that the first 40 and 41 amino acids were the chloroplast transit peptides of COR15A and COR15B, respectively (Fig. 3*B* Suppl.). Radiation, which triggers the differentiation of proplastids into fully functional chloroplasts, is one of the most important signals influencing chloroplast development (López-Juez 2007, Waters and Langdale 2009). In this study, the *COR15B* expression was found to be light-inducible. To further investigate whether light-regulated chloroplast development might also affect the expression of *COR15B*, several *Arabidopsis* albino mutants, *seca1*, *secy1*, and *tic20* (which exhibited severe defects in both structure and function of chloroplast) were used to detect the accumulation of *COR15B* mRNA. It was shown that the accumulation of *COR15B* mRNA in these albino plants was significantly impaired. These results suggest that the accumulation of *COR15B* mRNA depended on the chloroplast functionality.

It has been reported that the expression of nuclear-encoded photosynthetic genes could be inhibited through chloroplast-to-nucleus retrograde signalling (Leister 2005, Nott *et al.* 2006, Koussevitzky *et al.* 2007, Kakizaki *et al.* 2009, 2012). In the present study, we found that the expression of *COR15B* was reduced when chloroplasts were damaged by the NF treatment. Furthermore, the accumulation of *COR15B* mRNA was severely inhibited in the albino mutants. All these results suggest that the expression of *COR15B* was regulated by chloroplast-to-nucleus retrograde signalling. Early analyses of the retrograde chloroplast signalling pathways primarily monitored a few particular photosynthesis-related genes like *LHCB* and *RBCS* (Oelmuller and Mohr 1986, Susek *et al.* 1993, Mochizuki *et al.* 2001, Koussevitzky *et al.* 2007). This work identified an additional target gene, *COR15B,* which could be used to define distinctive retrograde pathways.

Despite the known expression profiles and functional characterizations of *COR15B* in response to low temperature (Wilhelm and Thomashow 1993, Wu *et al.* 2012), the function of this gene in salt stress has been scarcely reported. Data presented in this paper reveal a high expression of *COR15B* in the *Arabidopsis* seedlings treated with NaCl, which is similar to the elevated expression of *COR15B* during cold acclimation (Wilhelm and Thomashow 1993, Zuther *et al.* 2012). It is well-known that plant responses to environmental stresses are regulated by ABA (Quesada *et al.* 2000, Xiong *et al.* 2001). Previously it was proposed that *COR* genes expressed through an ABA-independent pathway (Gilmour and Thomashow 1991, Shinozaki and Yamaguchi-Shinozaki 2000). Nevertheless, the exogenous application of ABA highly increased the expression of members in the *COR* gene family (Knight *et al.* 2004). Our experimental data also demonstrate that the *COR15B* expression was up-regulated by the salt stress in an ABA-dependent manner. These results suggest a potential ABA involved signalling regulatory network of *COR15B* in response to the salt stress.

Several studies have shown that both COR15A and COR15B are positive regulators of plant tolerance to low temperature. For example, a constitutive expression of *Arabidopsis COR15A* enhances freezing tolerance of chloroplasts and protoplasts (Artus *et al.* 1996). Mature *Arabidopsis* COR15A functions as cryoprotective protein which forms oligomers in the chloroplast stroma to prevent the formation of interbilayer hexagonal II phases in membrane dispersions containing monogalactosyl-diacylglycerol (MGDG) and protect the chloroplast from freezing injury (Wilhelm and Thomashow 1993, Steponkus *et al.* 1998, Nakayama *et al.* 2007, Thalhammer *et al.* 2010,). Two recent papers also published that transgenic tobacco plants over-expressing *CbCOR15A* and *CbCOR15B* from *Capsella bursa* exhibit enhanced cold tolerance at the whole plant level (Wu *et al.* 2012, Zhou *et al.* 2012). In this study, the over-expression of *COR15B* in *Arabidopsis* resulted in displaying hypersensitivity to salinity. However, the *COR15B* knockdown did not significantly affect the sensitivity of the *cor15b* mutant to the salt stress at seed germination and post-germination stages. Furthermore, expressional reprogramming existed between the *COR15* homologous genes. All these results suggest that the two COR15 isoforms may be redundant in response to salt stress in *Arabidopsis*.

In summary, a tight correlation between the chloroplast functionality and expression of *COR15B* was revealed in *Arabidopsis*. It would be of great interest to investigate the molecular mechanism by which chloroplasts influence the expression of *COR15B*. The present study also contributes to shed light on the role of *COR15B* in response to salt stress, and deepens knowledge of the functional relationship between *COR15* homologous genes.

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