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Isolation, expression analysis and chromosomal location of *P5CR* gene in common wheat (*Triticum aestivum* L.)

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Abstract

Many organisms, including higher plants, accumulate free proline under osmotic stress. P5CR is the last enzyme in the cascade for proline synthesis. We isolated *TaP5CR* in wheat with a homologous sequence method. It is 1025 bp in length and contains a complete open reading frame of 864 bp that encodes a polypeptide of 288 amino acids. A Southern blot showed that *TaP5CR* had more than two copies in wheat. The *TaP5CR* gene which we isolated was located on chromosome 3D and expressed at much higher levels in radicles, flowers and leaves than other organs. Its expression was upregulated by salt, PEG, ABA and heat stress. *TaP5CR* overexpression in *Arabidopsis* can enhance root growth under salt stress and increase proline content and decrease MDA content under NaCl, PEG and ABA stress. The results showed the possibility to use *TaP5CR* to enhance transgenic *Arabidopsis* stress tolerance.

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Keywords: P5CR; Stress tolerance; Wheat

1. Introduction

Osmotic adjustment is one of the most important mechanisms for plants to adapt to their environment (Csonka, 1981). Proline, one of the most important osmolytes, accumulates in plants subjected to drought and salt stress (Van Rensburg et al., 1993). It has been suggested that proline not only plays a role as an osmoregulator (Venekamp et al., 1989; Delauney and Verma, 1993), inhibitor of programmed cell death (PCD)(Chen and Dickman, 2005), but is also involved in redox regulation (Bellinger and Larher, 1989), as well as radical scavenging (Smirnoff and Cumbes, 1989; Bohnert and Shen, 1999; Hong et al., 2000). Salt stress increases lipid peroxidation or induces oxidative stress in plant tissues (Mittal and Dubey, 1991; Hernandez et al., 1994). Lipid peroxidation is the symptom readily ascribed to oxidative damage, and malondialdehyde (MDA) as the cytotoxic production of lipid peroxidation is often used as an indicator of free radical production (Kunert and Ederer, 1985).

Proline accumulation mainly results from enhancing synthesis of glutamate via two successive reductions that are catalyzed by pyrroline-5-carboxylate synthesis (P5CS) and pyrroline-5-carboxylate reductase (P5CR). Several studies have indicated that P5CS is the limiting rate enzyme in proline biosynthesis (Szoke et al., 1992; Kavi Kishor et al., 1995). P5CR is the last and critical enzyme in proline biosynthesis, for it lies at the converging point of the glutamate and ornithine pathways. Although P5CR does not catalyze a limiting step (Szoke et al., 1992), its expression (Delauney and Verma, 1990; Williamson and Slocum, 1992; Verbruggen et al., 1993) and activity (Treichel, 1986; Laliberte and Hellebust, 1989; Rayapati et al., 1989; Argandona and Pahlich, 1991; Ashraf, 1994; Mattioni et al., 1997; Giridara Kumar et al., 2003) is upregulated under salt stress in several plant species. Proline accumulation, in the presence of salinity, may be due to increased production of pyrroline-5-carboxylate (P5C) which is then converted to proline by normal or enhanced levels of P5CR activity. A correlation between increased levels of P5CR activity and proline accumulation has been reported in *Chorella* (Nocek et al., 2005), NaCl-adapted cells of *Mesembryanthemum*

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(Treichel, 1986), *B. juncea* (Madan et al., 1995), and in the S1 genotype of *Morus alba* (Giridara Kumar et al., 2003).

Overexpression of *P5CR* in soybean can increase its tolerance to abiotic stress (Madan et al., 1995). In addition to osmotic stress, the transcription of *P5CR* is increased in response to cold and biotic stress (Madan et al., 1995; Misener et al., 2001; De Ronde et al., 2004; Nocek et al., 2005; Yang et al., 2006).

In higher plants, *P5CR* cDNAs have been isolated from soybean (Delauney and Verma, 1990), pea (Williamson and Slocum, 1992), *Arabidopsis thaliana* (Verbruggen et al., 1993), barley (GenBank accession no., AY177684), rice (GenBank accession no., AK067368), *Zea mays* (GenBank accession no., DQ026301), kiwifruit (Walton et al., 1998), *Lycopersicon esculentum* (GenBank accession no., BT013373) and *Vigna unguiculata* (GenBank accession no., AB056453). To date, a *P5CR* gene in wheat has not yet been reported. The aim of the present investigation was to isolate *P5CR* from wheat using a salt-tolerant variety Chadianhong. *P5CR*'s role in salt tolerance was investigated, using the salt-sensitive variety Chinese spring, salt-tolerant variety Chadianhong and *Arabidopsis*, in which *TaP5CR* was over-expressed. The cloning of the *TaP5CR* gene will contribute to the study of mechanisms of abiotic tolerance in wheat and also provide an additional gene for the genetic improvement of stress tolerance in wheat.

2. Materials and methods

2.1. Plant materials

Seeds of bread wheat (*Triticum aestivum* L., the salt-tolerant variety Chadianhong and the salt-sensitive variety Chinese Spring (Weng, 1999)) were germinated at 22 °C for 4 days and then planted in holes of thin foam board, which floated on half strength Hoagland's solution. As the first leaf emerged, NaCl was added to the solution to a concentration of 250 mM. The growth condition photoperiod was 14 h light/10 h dark, 25 °C in light and 18 °C in dark (300–500 $\mu mol \cdot m^{-2} \cdot s^{-1}$). *A. thaliana* Columbia plants grew in 1/2 Murashige and Skoog (MS) medium or soil supplied with 1/2 MS at 22 °C with the photoperiod of 16 h light/8 h night (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 75% relative humidity.

2.2. DNA and RNA isolation and reverse transcription

Total RNA was isolated using TRIZOL reagent following the instructions of the manufacturer (Tiangen Co. Beijing, China). Reverse transcription using total RNA as template was performed according to the SuperScript™||| Reverse Transcriptase provided by Invitrigen Company. Genomic DNA was isolated using the SDS procedure for wheat leaves (Devos and Gale, 1992) and the CTAB method for *Arabidopsis* rosette leaves (Stewart and Via, 1993).

2.3. Isolation of the full-length cDNA of P5CR from bread wheat

Using the sequence of a full-length *P5CR* (GenBank accession no, AY177684) from barley, a blast search against the wheat EST database (http://www.tigr.org/tigr-scripts/tgi/T-index.cgi?species=wheat) was conducted. We identified a tentative consensus (TC) sequence of 1140 bp in length whose score value was 354.5, e-value 2.1e-155, and identity 86%. It contains an open reading frame of 864 bp. Using regions of high sequence conservation, one pair of primers (primer1) was designed to cover the entire sequence and was synthesized by Boya Biotech Co (Shanghai, China). The sequences for primer1 are: 5′-AGCCGCCGCGCCCCTCTCCTAC-3′ and 5′-ACA-CAGCTAGACCAGGAAAAT-3′. The procedure for PCR was 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 49 °C for 45 s, 72 °C for 1 min, 72 °C for 15 min for extension.

2.4. DNA sequencing and sequence analysis

The PCR product was purified and cloned into the pGEM-T vector (Promega, USA). The sequencing reactions were performed using the BigDye terminator method using a XL3730 DNA analyzer. Nucleotide and amino acid sequences were compared with those released in GenBank databases. The full-length sequence of *TaP5CR* has been deposited in GenBank databases with the accession number AY880317.

2.5. Southern blotting

The genomic DNA from Chinese Spring, *Triticum urartu* (A genome), *Aegilops speltoides ssp speltoide* (S genome), *Aegilops. tauschii ssp strangulate* (D genome) was digested with *Eco*R|, *Eco*RV and *Hin*d|||, separated on a 1% (w/v) agarose gel and then transferred to a nylon membrane (Amersham-Pharmacia, UK) for Southern blot. The *TaP5CR* coding region was used as the probe and labeled by the ^{32}P-dCTP. Blots were hybridized and washed according to the Sambrook procedure (2001). Autoradiographic exposures were captured using a Personal FX phosphoimager (Bio-Rad).

2.6. Chromosome location of the TaP5CR gene

The chromosomal location of the *TaP5CR* genes was done by PCR analysis using the nullisomics–tetrasomics (NT) lines of Chinese Spring. The primers (primer2) were designed according to the sequence of *TaP5CR*. The sense primer for primer2 was 5′ TTGGACAGCTGAAGAAAAATA3′ and the antisense primer for primer2R was 5′ ACACAGCTAGACCAGGAAAAT3′. The PCR reaction mixture was subjected to 35 cycles of 94 °C denaturation for 1 min, annealing 55 °C for 30 s and 72 °C extension for 1 min, plus a final extension at 72 °C for 10 min. The PCR products were separated on 1% (w/v) agarose gel.

2.7. RT-PCR analysis

Total RNA was treated with DNAaseI (Promega) to remove the genomic DNA contamination. The first strand of *TaP5CR* was synthesized by using 5 μg of total RNA as the template with a cDNA synthesis Kit (Promega), and subjected to RT-PCR analysis. The specific primers and PCR conditions were the same as those used for the chromosomal location of *TaP5CR* but only with 30 cycles. The PCR products were

separated on 1% (w/v) agarose gel. A wheat tubulin gene was used as a control, which was amplified with primers 5′ AGAACAGTGTTGTAAGGCTCAAC3′ and 5′GAGCTT-TACTGCCTCGAACATGG3′and the PCR procedure of at 94 °C for 5 min, 28 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min, and final extension of 15 min at 72 °C.

2.8. Plant transformation and tolerance analysis

A *Bgl*||/*Nhe*|fragment encoding *TaP5CR* cDNA in the sense orientation was ligatured into the *Bgl*||/*Nhe*|site of vector Cambia p3301 under the control of 35S promoter from the cauliflower mosaic virus. The Cambia p3301-*TaP5CR* construct was transformed into *Agrobacterium tumefaciens* GV3101 by electroporation and the Cambia p3301 empty vector was also transformed into *A. tumefaciens* GV3101 by electroporation as control. *Arabidopsis* plants were transformed by the floral dip method. Seeds were harvested and fifty eight independent transgenic plants were screened by spraying 0.5% phosphino-thricin (PPT). In order to confirm the expression of the *TaP5CR* gene in the transgenic plants, total RNA for different transgenic plants was fractioned on a 1.2% (w/v) agarose/0.4 M formaldehyde RNA gel and transferred to a nylon membrane (Amersham-Pharmacia, UK) for Northern blot. The *TaP5CR* coding region was used as the probe and labeled by the ^{32}P-dCTP. Blots were hybridized and washed according to the Sambrook (2001) procedure. The T3 seeds (homozygous progenies for PPT resistance) were used for further studies.

2.8.1. Root length assay

To measure salt tolerance, seeds were sown in 1/2 MS medium at 4 °C for 3 days, incubated in a growth chamber under continuous light at 22 °C for 4 days (1/2 MS medium with 0.07% PPT for the transgenic plant, 0% PPT for the control), then the PPT resistant plants were transferred to the new medium with different concentrations of NaCl (0 mM, 50 mM, 100 mM, 150 mM) for 7 days. After 7 days, the root length was measured according to Shi et al. (2003). Each value included statistical data from 25 seedlings with at least three replicates. Variance analysis was performed by Fisher test and the means were statistically tested using a 2-sided *t*-test. Statistical significance is assumed at $p<0.01$, the significance was tested using least significant difference (LSD) at 1%. The SPSS 10.0 (for Windows) statistical package was used for the statistical analysis.

2.8.2. The growth recovery for the salt stress transgenic plant

Having grown in the different concentrations of NaCl (0 mM, 50 mM, 100 mM, 150 mM) for 7 days, the transgenic plants were transferred to 8 cm pots, filled with soil, and kept growing under normal growth conditions (without stress) for 10 days.

2.8.3. Determination of proline and MDA content

Seeds were sown in 1/2 MS medium at 4 °C for 3 days, and then incubated in a growth chamber under cycles of 16 h day/8 h night 22 °C for 10 days (1/2 MS agar medium with 0.07% PPT for transgenic plant, 0% PPT for the control). Then 10 day old plants with PPT resistance were transferred onto Whatman filter paper soaked with 0.125×MS salts, 0.125×MS salts +150 mMNaCl, 0.125×MS salts +50 μM ABA, 0.125×MS salts +8% PEG, and left for 18 h, respectively. After 18 h, the content of free proline was determined according to the method of Bates (1973), and MDA content was measured according to Tang (1999). Values of proline and MDA contents were taken from measurements of three replicates (18 independent seedlings per replicate) and SE of the means were calculated by Excel software.

3. Results

3.1. cDNA sequencing and sequence analysis

A 1025 bp amplicon was made by RT-PCR. The sequencing result suggested that the sequence contains an open reading frame (ORF) of 864 bp and would encode a peptide of 288 amino acids with a molecular mass of 29.3 KDa and a calculated isoelectric point (PI) of 8.43. This protein has 96%, 87%, 67%, 37% and 24% identity to that in barley, rice, *Arabidopsis*, *E. coli* and yeast respectively. The deduced amino acid sequence contains a complete P5CR (ProC) domain. Thus, this gene may encode a P5CR enzyme, and was designated as *TaP5CR*. The sequence of *TaP5CR* gene was submitted to the GenBank as AY880317.

3.2. Southern blot analysis of TaP5CR

The number of *TaP5CR* copies in the A, B, D genomes was estimated by Southern blot analysis using its full-length cDNA as a probe. When the genomic DNA was digested by *Eco*R| (there are no restriction site for *Eco*R| in the PCR genome

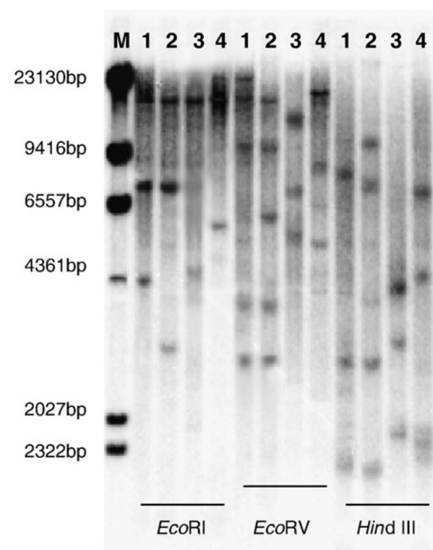

Fig. 1. Genomic DNA gel blot of the *TaP5CR* gene. Lane 1: Chinese spring; Lane 2: *T. urartu* A genome; Lane 3: *Ae. speltoides ssp speltoide* S genome; Lane 4: *Ae. stauchii ssp strangulate* D genome. Restriction enzymes for digestion are shown on the bottom of the panel. DNA size markers (bp) are indicated.

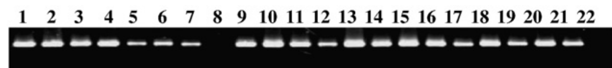

Fig. 2. Chromosome location *TaP5CR* genes by PCR using nullisomics–tetrasomica (NT) line of Chinese Spring. Chromosome location can be determined by missing a specific band in certain NT lines (1: N1AT1D; 2: N1BT1D; 3: N1DT1B; 4: N2A2B; 5N2BT2D; 6: N2DT2B; 7: N3AT3D; 8: N3DT3A; 9: N3BT3D; 10: N4AT4B; 11: N4DT4B; 12: N4BT4A; 13 N5AT5D; 14: N5DT5A; 15:N5BT5D; 16: N6AT6B; 17: N6BT6A; 18: N6DT6A; 19: N7AT7B; 20: N7BT7D; 21: N7DT7B; 22: water).

sequence), the Southern blot analysis showed that the hexaploid Chinese Spring had three bands, and A, B, D genome had more than two bands respectively. When the genomic DNA was digested by *Eco*R V and *Hin*d III (there was no restriction site for *Eco*R V and two restriction sites for *Hin*d III in the P5CR genome sequence), all of the hexaploid Chinese Spring and A, B, D genome had more than two bands. These results indicated that wheat genome might contain more than two copies of *TaP5CR* (Fig. 1).

3.3. Chromosome location of the TaP5CR gene

Chromosome location of the *TaP5CR* gene was determined by PCR using the nullisomics–tetrasomics (NT) lines of Chinese Spring (Fig. 2). The result showed that all of the NT lines gave one band except the N3DT3A line (a line with a deletion of the whole chromosome 3D by replacing the 3A chromosome). It suggests that the *TaP5CR* genes might be located on the 3D chromosome.

Fig. 4. Northern blot analysis of *TaP5CR* in *Arabidopsis* plants. Twenty micrograms of total RNA extracted from fully expanded leaves of 4-week-old T2 plants was analyzed by RNA gel blotting using a *TaP5CR* cDNA probe. Lane 1 was for the wild type; lane 2 was for the p3301; lane 3, 4, 5 were for the 35S: *TaP5CR*-18, 35S: *TaP5CR*-16 and 35S: *TaP5CR*-13 lines, respectively. Ethidium bromide-stained rRNA, before transfer, is shown for an indication of RNA loading.

3.4. Expression level of TaP5CR in wheat

TaP5CR transcripts were detected in radicles, flowers, leaves, seedlings, root and young spike but not in the stem. We also studied the expression of *TaP5CR* in response to different stresses (Fig. 3). The abundance of *TaP5CR* transcripts in the salt-tolerant variety Chadianhong increased in response to 250 mM NaCl, 15% PEG, 38 °C heat stress and 50 μM ABA stress, but had no response to 4 °C cold stress. The expression of *TaP5CR* was induced by salt, dehydration, heat and exogenous ABA treatments but not by cold treatment. The *TaP5CR* mRNA was induced within 2 h of salt stress in the salt-tolerant variety Chadianhong, but did not increase until after 8 h in the salt-sensitive Chinese Spring and then decreased after 12 h. The *TaP5CR* mRNA also appeared within 4 h after the start of PEG treatment and its level continued to increase up to 48 h. Strong accumulation of *TaP5CR* mRNA in response to hot stress was observed 4 h after starting hot treatment. Rapid accumulation of *TaP5CR* mRNA in response to exogenous ABA was also observed within 2 h and continued for 48 h, and the level of accumulation was higher than that in response to other stress conditions.

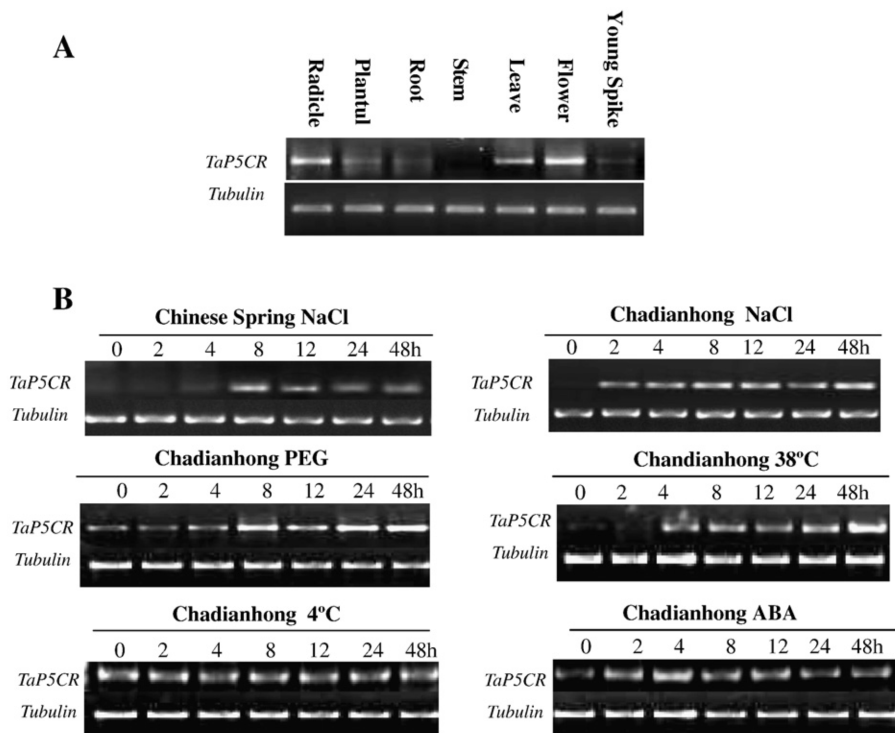

Fig. 3. Expression of *TaP5CR* in wheat. (A) *TaP5CR* expression in different organs as detected by RT-PCR. (B) *TaP5CR* expression in response to various treatments. The seedlings were treated with the following solutions: 250 mM NaCl, 15% (w/v) polyethylene glycol 6000 solution, 50 μM ABA, placed at 38 °C or 4 °C for the indicated time respectively, the *TaP5CR* expression in leaves as detected by RT-PCR. The *TaP5CR* specific fragment was amplified by RT-PCR with 30 cycles. A *Tubulin* fragment was also amplified by RT-PCR as an internal control with 28 cycles.

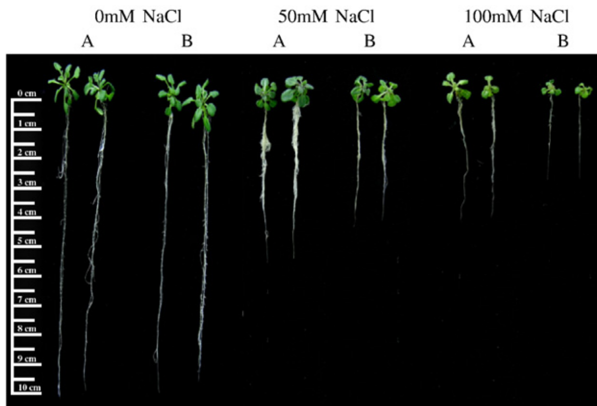

Fig. 5. The root length assay of the transgenic plants over-expressing *TaP5CR* under different NaCl treatments for 16 days growth. A: 35S:*P5CR*, B: p3301. The left ruler had the least unit scale 1 cm.

3.5. Overexpression of *TaP5CR* confers salt tolerance in *Arabidopsis*

To test the function of *TaP5CR*, it was transformed and expressed in *Arabidopsis* driven by the cauliflower mosaic virus (CaMV) 35S promoter. We obtained 58 lines of T1 transgenic plants, and three of them were selected to examine the expression of *TaP5CR* by Northern blot and grown to get a T3 generation for further analysis. As shown in Fig. 4, *TaP5CR* transcripts accumulated in three of the 35S::*TaP5CR* transgenic lines.

3.5.1. Root length was increased in the transgenic plant under stress condition

To determine whether overexpression of *TaP5CR* regulates root growth, we measured the root length of T3 transgenic plants on MS medium containing different concentrations of NaCl (Fig. 5, Table 1). The results showed that the root growth of transgenic plants was less inhibited by NaCl treatment. Under 50 mM NaCl treatment, the root length was 4.88 cm for the control plants (plants transformed with empty vector) and 6.78 cm for the transgenic plants, the relative growth in length (65.4%) of the transgenic plants was higher than that of the control plants (only 52.1%). Under 100 mM NaCl treatment, the root growth was severely inhibited, but the root length of the transgenic plants was significantly higher than that of the control. The relative growth length of the transgenic plants was 40.9% and only 31.3% for the control plants. Higher concentrations of NaCl (150 mM) severely inhibited *Arabidopsis* growth and led to bleached rosette leaves in the control plants, but most of the transgenic lines still had green rosette leaves (data not shown).

3.5.2. The greater recovery growth for the transgenic plants

After different salt treatments, seedlings in the 1/2 MS medium were transplanted into soil and grown under normal growth conditions for 10 days. The results showed that the transgenic line can quickly recover normal growth from 50 and 100 mM NaCl treatment. This result can be attributed to the overexpression of *TaP5CR* not present in the control lines. However, there are no significant differences between the transgenic line and the control after 150 mM NaCl treatment (Fig. 6). This shows that the transgenic plants cannot tolerate 150 mM NaCl stress.

3.5.3. The free proline content and MDA content

To determine if the overexpression of *TaP5CR* in *Arabidopsis* was related to proline levels, we measured proline contents. Under normal growth conditions, transgenic seedlings were found to produce 3 fold more proline than the control seedlings (Fig. 7A). Under salt stress, the proline in transgenic lines was about 2.5–4 times higher than that in the control seedlings.

MDA is a product of lipid peroxidation and has been extensively used as an indicator of oxidative stress (Gomez et al., 1999). In order to identify whether the overexpression of *TaP5CR* can protect the plant cell membrane from salt injury, we measured the MDA content (Fig. 7B). The result showed that under normal growth conditions, there was no difference for the MDA content between control and the transgenic plants, while under 150 mM NaCl treatment, the MDA content in the transgenic plants was lower than that in the control plant. Under PEG stress, the MDA content in the control plants was about two times more than that in the transgenic line. Under ABA stress, there were significant differences in the MDA content between the control and the transgenic plants. This finding suggests that the product of the exogenous *TaP5CR* gene might play a role in a protective anti-oxidation system, reducing oxidative damage to the transgenic Arabidopsis plants, because MDA content was decreased.

4. Discussion

P5CR is an enzyme functioning at the converging point of the proline and ornithine biosynthetic pathways and have a very important role. The mRNA level of *P5CR* in *Arabidopsis* was significantly higher in roots and ripening seeds than levels in green tissue (Williamson and Slocum, 1992). Higher levels were also found in rapidly dividing cells and cells experiencing developmentally programmed osmotic adjustments, such as root tips, shoot meristem, guard cells, hydathodes, veins, pollen grains, ovules and developing seeds (Hare and Cress, 1996; Hua

Table 1
The root length and root relative growth rate of the transgenic plants

	0 mM	50 mM		100 mM	
	Root growth length (cm)	Root growth length (cm)	Relative root growth length (%)	Root growth length (cm)	Relative root growth length (%)
P3301	9.37(0.22) ^{A}	4.88 (0.31) ^{B}	52.08	2.93 (0.11) ^{B}	31.27
35S:: *TaP5CR*	10.37(0.23) ^{A}	6.78 (0.33) ^{A}	65.38	4.25 (0.26) ^{A}	40.98

The PPT resistant seedlings were transferred to the 1/2 MS salt with 0, 50, 100, 150 mM NaCl and grown for 7 d and the roots were measured. The experiment was repeated three times. Values with different superscript letters indicate significant difference at $p<0.01$ (Fisher's protected LSD test). Numbers in parentheses are standard deviations ($n=25$).
Means with different superscript letters A and B within each attribute are significantly different ($p=0.01$). LSD, least significant difference.

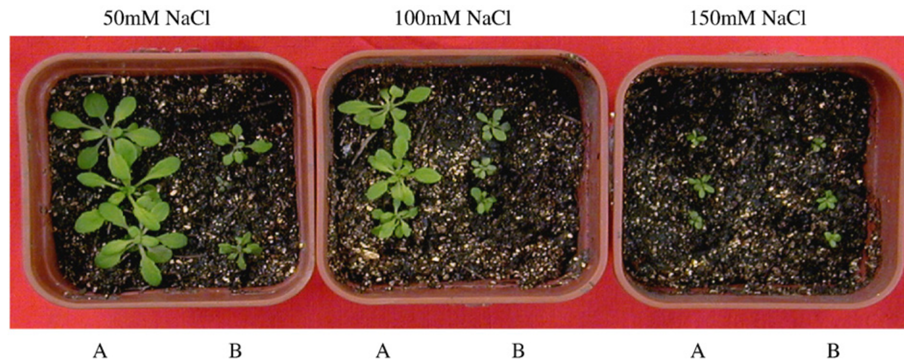

Fig. 6. The growth recovery of the transgenic plants. The salt treated seedlings were transferred to soil and the images were captured after the seedlings were grown under normal growth conditions for 10 days. A: 35S:*P5CR*, B: p3301.

et al., 1997). Our results showed that the *TaP5CR* mRNA level was significantly higher in radicles, flowers, leaves and seedlings when compared to expression in roots and young spikes. The transcript level of *TaP5CR* in stem was not identified, under normal growth conditions, suggesting radicle growth and flower development might need higher levels of *TaP5CR* expression to synthesize proline as an energy source.

Salt treatment increased the level of *P5CR* mRNA in soybean seedlings (Delauney and Verma, 1990), as well as in *Arabidopsis* (Verbruggen et al., 1993; Hong et al., 2000; Hua et al., 2001) and pea (Savoure et al., 1995). Mattioni et al. (1997) concluded that the increase in P5CR activity in *T. durum* seedlings during dehydration and salt stresses was unrelated to levels of the corresponding transcript. Our result showed that *TaP5CR* transcript levels increased under salt, osmotic, ABA and heat stress, The results may be due to the fact that the *TaP5CR* we isolated was not the same as that of Mattioni and may be a homolog of *P5CR* (Mattioni et al., 1997) or that the transcript accumulation may arise from differences in the carbohydrate status of the stressed plants (Hare et al., 1999). Moreover, the salt-tolerant variety accumulated higher levels of *P5CR* transcripts when compared to the salt-sensitive variety, which was in line with those of Verbruggen et al. (1993). In our study, *TaP5CR* transcript levels did not increase under cold stress, which may be the reason why Chadianhong is a winter wheat variety and has other mechanisms of tolerance to cold stress.

Overexpression of *TaP5CR* in *Arabidopsis* showed a much higher accumulation of proline than the control plants, resulting in the plants increased growth recovery after the salt stress. These results were similar to those of De Ronde et al. (2004). However, they slightly differed from the results of Szoke et al. (1992). Szoke et al. (1992) showed that transgenic plants had significant increases in P5CR activity. They also reported slightly higher levels of *P5CR* transcription than control plants after salt treatment, but no significant increases in proline accumulation. It showed that the amount of P5CR normally present in the plant appears to be sufficient for contributing the high level of proline. *P5CR* has multiple copies in plants and perhaps different copies have either different functions or show some functional redundancy. There are two copies of *P5CR* isolated in *Arabidopsis* (Verbruggen et al., 1993), more than one copy in soybean (Delauney and Verma, 1990), two and three copies in pea (Williamson and Slocum, 1992) and two copies in spinach (Murahama et al., 2001). Southern blot in this report showed that wheat *P5CR* had more than two copies.

MDA had been used as an indictor for membrane injury in many organisms (Esterbauer et al., 1991). In this study, the *P5CR* transgenic *Arabidopsis* plants had a lower level of MDA

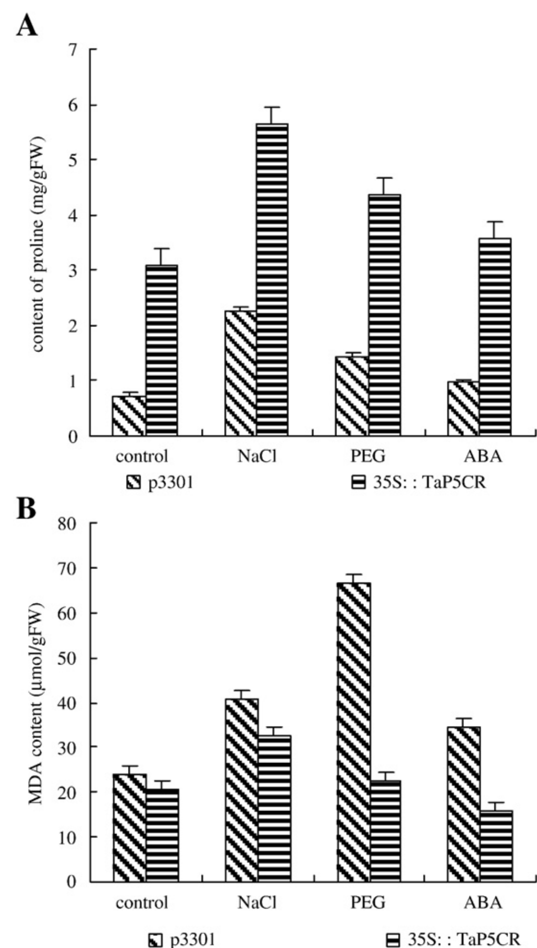

Fig. 7. Effects of different stresses on the proline and MDA content. After varying treatments for 18 h, seedlings were measured for their proline and MDA content. Mean values represent three replicates, 18 independent seedlings per replicate for control and transgenic plants. Error bars represent standard error and FW represent Fresh weight.

content than the wild type (Fig. 7). Decreased MDA content suggested that the product of the exogenous *TaP5CR* gene had a role in a protective anti-oxidation system and functions to reduce oxidative damage to the transgenic *Arabidopsis* plants. The data also showed that over-expressing *TaP5CR* can enhance the reduction of P5C to proline. The conversion of P5C to proline provided a metabolic shuttle of redox equivalent between the cytosol and mitochondrion (Hare and Cress, 1997), reduced cellular acidification induced by stress and decreased the stress injury to the membrane, at the same time, primed oxidative respiration to provide energy needed for recovery. Therefore, the transgenic plants had a higher degree of salt tolerance and recovered more rapidly from the salt stress.

In addition, we located the wheat *P5CR* gene on chromosome 3D which also has other genes related to tolerance, such as salt tolerance genes (Annette et al., 2004), genes controlling water stress-induced apical sterility (Shahram, 2006), and genes for Slow-Rusting Resistance (Liu et al., 2006). Whether *P5CR* and other genes related to tolerance are located on the same loci of chromosome 3D can only be answered with the fine-mapping of *TaP5CR*. However, the location of gene *P5CR* on chromosome 3D is very important for map-based cloning genes related to salt tolerance and wheat genome sequence in the future. The cloning, functional analysis and locating of *TaP5CR* in wheat will contribute to the study of mechanisms of abiotic tolerance and also provide an additional gene for the genetic improvement of stress tolerance in wheat.

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