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Enhanced tolerance of the transgenic potato plants overexpressing Cu/Zn superoxide dismutase to low temperature

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ABSTRACT

Low temperature stress is one of the major factors for damage in plants, especially in food crops. Cu/Zn superoxide dismutase plays an important role in the processes of scavenging reactive oxygen species (ROS) caused by environmental stresses. In order to study transgenic potato plants whether improved tolerance to low temperature, in this research, *StSOD1* gene was overexpressed under the control of CaMV 35S promoter through *Agrobacterium tumefaciens*-mediated transformation in potato. The results showed that after 4 °C treatment for 48 h, superoxide dismutase (SOD) activity of the transgenic potato lines (OE lines) overexpressing *StSOD1* was 1.38-fold enhanced compared to the non-transgenic plants (NT lines). On the other hand, the activity of the transgenic potato lines (RNAi lines) that inhibiting expression of *StSOD1* decreased compared with NT lines. In addition, the effect of increased SOD activity on lipid peroxidation was determined by measuring malondialdehyde (MDA) contents in plants during cold treatment. The result showed that MDA content increased by 2.02-fold and 1.78-fold in NT lines and RNAi lines after 4 °C treatment for 48 h, respectively. In contrast, the OE lines showed only small changes and were lower than the NT lines. Meanwhile, the activity of peroxidase (POD) and catalase (CAT) were also enhanced in OE lines compared with NT lines after cold treatment. Through observing the phenotype, importantly, this research found that the leaves of NT lines and RNAi lines have wilted severely while the OE lines were slightly affected. In all, these results indicated that genetic engineering technology could be used to regulate the antioxidant enzymes activity in plants to improve tolerance to cold stress.

1. Introduction

ROS (Reactive oxygen species), which include superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (·HO) (Ron, 2002), are produced by the cellular metabolism in response to environment stresses (Saed-Moucheshi et al., 2014). At high concentrations, these active molecules can damage proteins, nucleic acids and lipids inside cells as well as cell membrane (Ajay et al., 2002; Apel and Hirt, 2004; Gill and Tuteja, 2010; Maksymiec, 2007), causing metabolic disorders. Therefore, plants have been evolved a complete set of antioxidant system and defense mechanism to reduce or even eliminate those damage caused by ROS under stress condition like drought, salinity, heavy mental and extreme temperature during evolution (Yousuf et al., 2012). Antioxidant system includes some antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), and some non-enzymatic molecules such as vitamin A, vitamin C, ascorbate and glutathione. SOD plays a key role in the process of eliminating ROS among those antioxidant enzymes (Masaru et al., 2002). It can convert the superoxide anion radical into hydrogen peroxide(H_2O_2), then the H_2O_2 is decomposed to water and oxygen by CAT、APX and AsA-Glu circulatory system (Azra et al., 2013), thereby removing the toxic effect of ROS. In all, SOD plays very important roles in protecting the plant cells structure, proteins, nucleic acids and membrane system from damaging effect of ROS. Three different types of SOD has been identified in plants, including iron SOD, manganese SOD and copper-zinc SOD (Cu/Zn SOD), which are classified by the differences in covalently linked catalytic metal ions and cellular location (Mahanty et al., 2012). Mn-SOD, Fe-SOD mainly located in the mitochondria and chloroplast respectively, while Cu-Zn SOD is found in the cytosol and in chloroplasts (Igor et al., 2002; Zambounis et al., 2002).

In recent years, with the increasingly aggravated global warming, the extreme weather was arose more frequently. The environmental stress has increasingly become one of the important factors to influence the growth of plants, such as salinity, drought, high temperature, chilling and heavy metal (Jahnke et al., 1999; Janas et al., 2010; Yu and

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Rengel, 1999; Zhang et al., 2010). Some studies confirmed that salt stress can induce increase of total SOD activity while other research found no changes or opposite tendency of total SOD activity. For example, SOD activity was increased in the leaves of *Cicer arietinum*, *Beta vulgaris*, and *Brassica juncea*, while it was reduced in *Vigna unguiculata* (Bor et al., 2003; Hernandez et al., 2006; Kumar et al., 2013; Rasool et al., 2012; Sunita et al., 2008). These results indicated that SOD activity was influenced by several conditions, depending on plant species, growing stage and plant organs used for measurement. When plants suffered drought, a decline of CO_2 fixation would arises because of abscisic acid controlled stomatal closure, which will cause the decrease in photosynthetic activity and morphological disorders (Alexieva et al., 2001). The SOD activity and its isoforms was increased in the leaves of *Olea europaea* (Doupis et al., 2013). But in *Gossypium* and two grasses (*Festuca arundinacea* and *Poa pratensis*), the SOD activity of leaves was first elevated then diminished (Deeba et al., 2012).

Potato (*Solanum tuberosum* L.) is a vital crop with high nutrition value and high yield. It adopts well in the cool environment, but can't resist the frost. Potato seedlings will suffer the chilling injury at the temperature ranges of -0.5 to -0.8 °C and freezing damage at − 2 °C, and it will die at -4 °C (Hancock et al., 2014). Low temperature damage is gradually becoming one of the important element affecting the plant development and geographical distribution, to some degree also influence the yield of crops (Sato et al., 2011). There are some researches concentrated on the transgenic potato tolerance to oxidative stress like methyl viologen (MV), drought, and high temperature. Tang et al. (2006) studied the performance of the transgenic potato plants under high temperature. Firstly, they obtained the transgenic potato plant (TPP) overexpressing the *StAPX* and *StSOD* in chloroplast, and then found that the photosynthetic activity of TPP plants decreased by 6%, whereas NT plants decreased by 29% under high temperature. They also found that the TPP plants showed higher tolerance to 250 μM MV compared with NT plants (Tang et al., 2006). Kim et al. (2007) obtained the transgenic potato plants overexpressing *SOD* gene and found that it significantly affected the growth and shape of tubers (Kim et al., 2007). But the research of transgenic potato plants overexpressing *StSOD* tolerant to low temperature have not reported.

In the present study, we constructed the overexpression vector pBI12-SOD1 and interfering-expression vector pHellsgate8-SOD1, respectively, and further obtained the transgenic potato plant lines by *Agrobacterium*-mediated transformation. These plants lines were subjected cold treatment when plants grown to two month in pot to examine whether there is a correlation between the expression of *StSOD1* and cold tolerance. The antioxidant enzymes activity and lipid peroxidation in plants exposed to cold treatment were determined and compared in the transgenic and non-transgenic (NT) potato plants.

2. Materials and methods

2.1. Plant materials and growth conditions

Potato (*Solanum tuberosum* L.) cultivar 'Gannongshu 2' was used as the experiment materials. Potato tuber plantlets, which were provided Gansu Provincial Key Laboratory of Aridland Crop Science of Gansu Agricultural University, were grown on the MS media containing 3% sucrose and 0.45% agar, and cultured under photoperiod 16 h/d, temperature (25 ± 2) °C and 2000 lx light intensity. After a month growth, the plantlets were placed in MS media containing 8% sucrose and 0.45% agar to induce the microtubers under dark conditions at (25 ± 2) °C. The microtubers were used for the further potato transformation experiments.

2.2. Construction of plant expression vectors

The potato SOD1 sequence (GenBank accession ID: AF355460.1) was obtained from NCBI database. The PCR primers was designed based on the *St*SOD1 cDNA sequence using the online program NCBI-primers, then added the appropriate restriction enzyme cutting sites *Xba* I, *Sma* I and protection bases at the 5′ end of the gene cDNA sequence to form the overexpression fragment's primers. Furthermore, added the attb joint sequence at the 5′ end of RNA interference primers to design the interfering-expression fragment's primers (Tabel 1). The primers were synthesized by Shanghai Biological Engineering Co., Ltd. Then constructed expression vector pBI121-StSOD1 and pHellsgate8-StSOD1 respectively. The overexpression and RNA interfering-expression vectors were driven by the constitutive CaMV 35S promoter. The recombinant vectors were transferred into *Escherichia coli* DH5α, and analyzed through double enzymes digestion. Then, the vectors were transformed into *Agrobacterium tumefaciens* LBA4404 using the freeze-thaw method (Raviraja and Sridhar, 2007).

2.3. Potato transformation and identification of the transgenic plants

Potato transformation was performed using the *Agrobacterium*-mediated method (Si et al., 2003). Microtubers were peeled and cut off bud eye totally, then cut into 1–2 mm thin slices. The tuber slices were soaked in *E. coli* broth which contained vectors of pBI121-SOD1 and pHellsgate8-SOD1, for 7 min to make the slices were infected fully; after infection, using the filter paper to suck dry tuber slices and then put it into MS solid media to cultivate 48 h at 28 °C and dark conditions. After co-cultivation, transferred the slices into differentiation media (MS + 0.2 mg/L GA_3 + 0.5 mg/L 6-BA + 1 mg/L IAA + 2 mg/L ZT + 50 mg/L Kan + 500 mg/L carbenicillin) and cultured at 25 °C and 2000 lx light intensity. Taking 7 d for a cycle to replace fresh media for slices until the new buds were grown from the center of tissue slices, then sheared the bud and put it into the rooting medium (MS + 75 mg/L kanamycin + 200 mg/L carbenicillin) for screening. After 2 weeks, some of the buds have taken root and were tentatively considered to be the transgenic seedlings.

DNA was extracted from the transgenic plants using Plant Genomic DNA Kit (Cat. No. DP315, TIANGEN) and the total DNA was used as the template to perform PCR reaction, and the DNA from untransformed potato plants was used as the negative control. Identification of the transgenic plants was performed through a pair of primers designed for neomycin phosphate transferase gene sequence (Table 1). The PCR reaction conditions are as follows: Initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, 60 °C for 45 s, and 72 °C for 1 min, finally at 72 °C for 10 min, the resulting PCR amplicons were resolved through the 1% agarose gel

2.4. Low temperature treatment

Four-week-old potato plants of the transgenic (OE and RNAi lines) and NT plants were transplanted into pot and grown in greenhouse under photoperiod 16 h/d (25 °C days and 22 °C nights). Two-month-old plants were subject to low temperature treatment, namely the plants with a uniform growth status were transferred to a chamber and incubated at 4 °C for 0, 6, 12, 24, 36 and 48 h under weak light. The

Table 1
Primers sequence.

Primer	Primers sequence (5'→3')
StSOD1-F	GCTCTAGACTAAGCCACTCAAGGGTGACC
StSOD1-R	TCCCCCGGGAGACACCACCGTTAGTTGCTG
StSOD1i-F	AAAAAGCAGGCTCACCATCCTCTTCACTCAAGATG
StSOD1i-R	AGAAAGCTGGGTGATCATCAGGATCAGCATGAAC
attb	GGGGACAAGTTTGTACAAAAAAGCAGGCT
NPT II-F:	GCTATGACTGGGCACAACAG
NPT II-R:	ATACCGTAAAGCACGAGGAA

Note: The single underlined section showed enzyme sites; the double underlined section showed the partial sequence of attb joint sequence.

plant leaf samples were collected and quick-frozen in liquid nitrogen as samples for further assays.

2.5. Assay of enzymes activity and MDA content

All the prepared samples were weighed 0.5 g and grinded in 5 ml phosphate buffer in ice bath, then homogenate was poured into centrifuge tube and centrifuged at 10,000 rpm for 20 min under 4 °C. The activity of SOD, CAT and POD were measured by using the T-SOD assay kit (Cat. No. A001-1), POD assay kit (Cat. No. A084-3) and CAT assay kit (Cat. No. A007-1) produced by Nanjing Jianchen Bioengineering Institute according to manufacturer's instruction. Lipid peroxidation in leaf tissues were measured in terms of malondialdehyde (MDA) content in the samples according to method described by Mellacheruvu et al. (2015).

2.6. qRT-PCR analysis of the transgenic potato plants

Relative expression level of *StSOD1* was determined by qRT-PCR assay, which was performed using the SuperReal PreMix Plus PCR Kit (Cat. No. FP205-02, TIANGEN). The 20 μL reaction volume include 100 ng cDNA, 10 μL 2 × SuperReal PreMix Plus, 0.6 μL each primer (Table 2) and 0.4 μL 50 × ROX Reference Dye. The PCR conditions were 10 min at 95 °C, 40 cycles of 95 °C for 10 s, 60 °C for 20 s. Using the *ef1a* gene as the reference gene to normalize the expression level of *StSOD1*, difference between each sample is calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). All samples were analyzed with three biological replicates. Significance was analyzed using the statistics software SPSS version 13.0.

2.7. Statistical analysis

All treatments had three replicates, and the entire experiment was run three times over a period of 18 mo using the same facility with the same methodology. One-way ANOVA was used to analyze using SPSS program (version 17.0; SPSS Inc., 2008). Significant differences were determined using the Duncan multiple range test at the 5% probability level.

3. Results

3.1. Assay of MDA content and phenotype of plants

MDA content usually used as an indicator to reflect the degree of lipid peroxidation of plants, which produced in the process of lipid peroxidation. It can cause cross-linking polymerization of macromolecules such as proteins and nucleic acids, and is cytotoxic. The result from MDA content measurement after 48 h cold treatment showed that there was no significant difference in MDA content between the transgenic and NT lines at untreated control (Fig. 1). However, the NT and RNAi lines showed a sharp increase after 6 h cold treatment, In contrast, OE lines did not increase until 12 h treatment (Fig. 1). Importantly, we found that the OE lines showed an increased MDA content after 48 h cold treatment, but the increase still lower than NT lines. (Fig. 1). In addition, compared to NT and OE lines, RNAi lines had higher MDA content after cold treatment, which may be affected by the repression of SOD expression. Those results revealed that overexpressing *StSOD1* can effectively reduce the MDA content and thereby inhibited lipid peroxidation to protect potato plants form cold stresses.

Table 2
qRT-PCR primers sequence.

Primer	Primers sequence (5'→3')
SOD1-F:	CTGCATGTCAACAGGACCAC
SOD1-R:	CCAATGATGGATTGTGAACC
ef1a-F:	CAAGGATGACCCAGCCAAG
ef1a-R:	TTCCTTACCTGAACGCCTGT

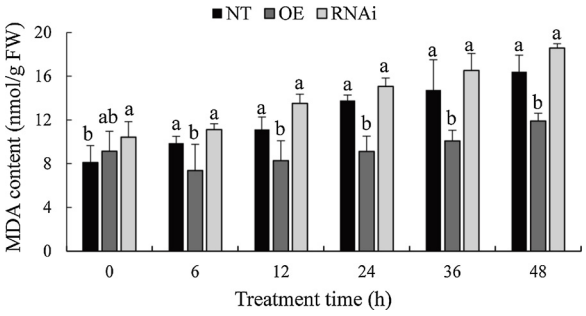

Fig. 1. Changes in MDA content of the transgenic and NT lines during cold treatment. The vertical bars represent the standard deviation of three individual experiments. Data means ± SD of three replicates, means without a common letter indicate significantly difference values ($P < 0.05$).

Two-month-old plants were placed into a growth chamber at 4 °C for 6 d to give a chilling treatment. We found that the leaves of NT and RNAi lines have wilted severely while the OE lines were slightly affected (Fig. 2). In addition, we found that the RNAi lines were heavily affected by cold treatment.

3.2. SOD activity

SOD is an important antioxidant enzyme in organisms and is widely distributed in various organisms such as animals, plants and microorganisms. Usually, the level of SOD in the plants means a visual indicator of aging and death. Thus, it plays a vital role in the plant antioxidant defense system which against ROS produced by various stresses. To test whether the downregulation and overexpression of *StSOD1* transcript have effects on its enzymatic activity under cold treatment, in this study, the SOD activity of OE and RNAi lines were measured and compared to NT lines. The results showed that SOD activity increased during the duration of cold treatment, with peak activity at 48 h, which indicated SOD-mediated plant response against the cold stress. Except for the 48 h timepoint, the SOD activity was significantly higher in the OE lines compared to NT in the whole treatment. (Fig. 3). Interestingly, the activity of SOD also significantly increased in the RNAi lines after 36 h of cold treatment compared to the control. In addition, we also found that there is a little increase in the transgenic lines compared with NT lines at untreated control. But after treatment, the SOD activity of OE lines have increased remarkably and higher than NT lines (increased almost 1.38-fold), while RNAi lines showed a decrease after treatment and lower than NT lines. This indicated that overexpressing *StSOD1* can contribute plants to better response environment stresses (Fig. 3). In all, those results suggested that the SOD activity can elevate by enhancing the expression of *StSOD* using genetic engineering technology.

3.3. POD and CAT activity

CAT, an enzyme that catalyzes the decomposition of hydrogen peroxide into oxygen and water, is present in the peroxisomes of cells. It is a peroxidase-labeled enzyme that accounts for about 40% of the total peroxidase enzyme. POD is widely distributed in plants and is an active enzyme. It is related to respiration, photosynthesis and oxidation of auxin, so it can be used as a physiological indicator of tissue aging. In order to detect the changes of POD and CAT activities in plants of overexpressing *StSOD1*, the activity of POD and CAT were also measured. Like SOD activity, the POD activity increased during the duration of cold treatment, with peak activity at 48 h (Fig. 4). After 48 h cold treatment, it showed increased in both the transgenic lines and NT lines

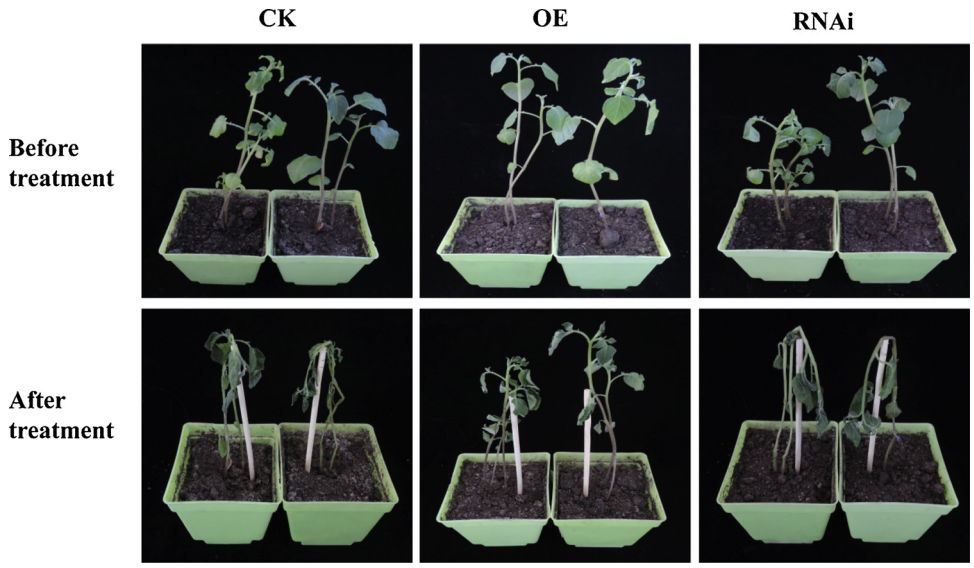

Fig. 2. Phenotypic changes of leaves in low temperature stressed (4 °C) two-month-old potato plants after 6 d treatment. NT: non-transgenic plants; OE: transgenic potato plant of overexpressing *StSOD1*; RNAi: transgenic potato plant of interfering expression of *StSOD1*.

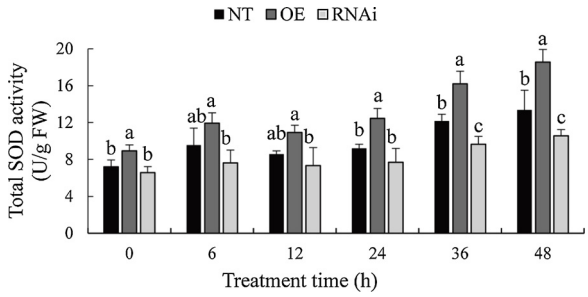

Fig. 3. Changes in SOD activity of OE, RNAi and NT plants during cold treatment. Potato plants were grown one month under pot, then exposed to 4 °C for 0, 6, 12, 24, 36 and 48 h and leaves were taken for determination of SOD activity. NT: non-transgenic plants; OE: transgenic potato plant of overexpressing *StSOD1*; RNAi: transgenic potato plant of interfering expression of *StSOD1*. The vertical bars represent the standard deviation of three individual experiments. Data means ± SD of three replicates, means without a common letter indicate significantly difference values ($P < 0.05$).

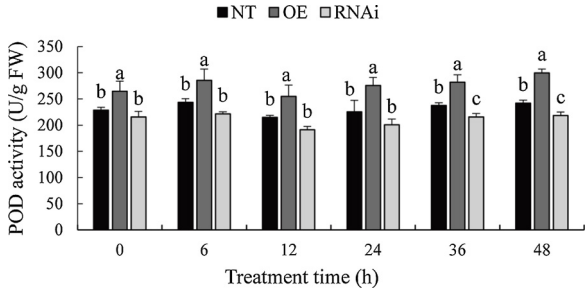

Fig. 4. Changes in POD activity of OE, RNAi and NT plants during cold treatment. One month potato plantlets were exposed 4 °C for five phase then determined POD activity. The vertical bars represent the standard deviation of three individual experiments. Data means ± SD of three replicates, means without a common letter indicate significantly difference values ($P < 0.05$).

compared to untreated control. Furthermore, OE lines showed remarkable higher activity (almost 1.24-fold) compared to NT lines after treatment, while RNAi lines lower than NT lines (Fig. 4). Similarly, we also found that CAT activity increased when subjected to cold treatment, whether the transgenic lines or the NT lines. But after 48 h, OE lines have much higher CAT activity and evaluate 1.37 fold compared

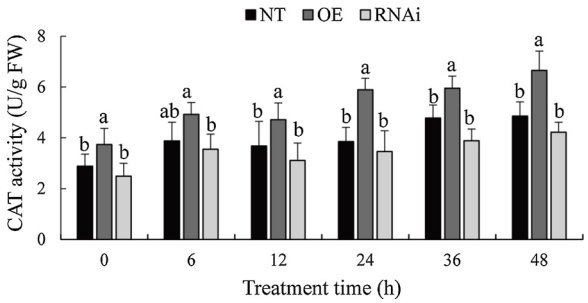

Fig. 5. Changes in CAT activity of OE, RNAi and NT lines during cold treatment. The vertical bars represent the standard deviation of three individual experiments. Data means ± SD of three replicates, means without a common letter indicate significantly difference values ($P < 0.05$).

with NT lines, while RNAi lines decreased a little than NT lines (Fig. 5). Those results maybe indicate that other anti-oxidant enzymes activity will also enhance to response abiotic stresses by cooperating with SOD under low temperature treatment.

3.4. Relative expressional levels of StSOD1 gene of the transgenic plants under cold stress

To analysis the changes of the transgenic plants against low temperature treatment at the level of gene, in this study, qRT-PCR was performed to determine the relative expression level of *StSOD1* gene under cold treatment, and further analyze the correlation between ROS produce and expression of *StSOD1* gene. The result of qRT-PCR analysis demonstrated that the OE lines was up-regulated (1.38-fold) compared to NT lines under cold treatment, while RNAi lines was down-regulated. Moreover, Compared to untreated control, the relative expression of *StSOD1* gene was enhanced in both the transgenic lines and NT plants. Interestingly, in the process of 48 h cold treatment, the expression of *StSOD1* gene was first decreased after 12 h cold treatment and then enhanced gradually (Fig. 6), which coincided with changes of SOD enzyme and further demonstrated that plants will regulate transcriptional levels when subjected to cold stress, then further eliminate the damages of ROS through enhancing antioxidant enzymes.

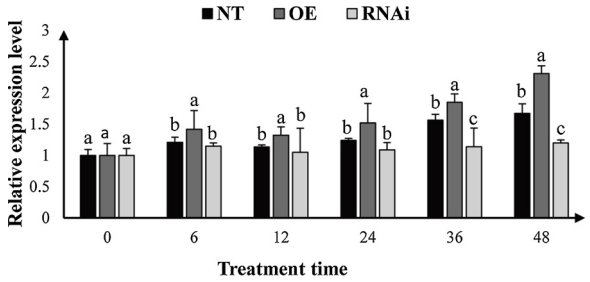

Fig. 6. Expressional levels of *StSOD1* gene between the transgenic and non-transgenic potato plants under low temperature stress. NT: non-transgenic potato as negative control; OE: transgenic plants of overexpression *StSOD1*; RNAi: transgenic plants of interfering expression *StSOD1*. Data are means ± SD of three biological replicates, means without a common letter indicate significantly difference values ($P < 0.05$).

4. Discussion

ROS was implicated in abiotic and biotic stresses, and the electron transfer chain in chloroplast of plants has been considered the place of producing considerable ROS (Prasad and Stewart, 2001). When subjected to stress, the plant will produce a large amount of ROS, which causes serious damage to the plant cells. Once the concentration of ROS exceeds the threshold, the plant begins to activate the antioxidant enzyme system. First of all, the ROS is decomposed into hydrogen peroxide and oxygen by SOD, and then the hydrogen peroxide is decomposed into water and oxygen by CAT and POD, thereby eliminating the toxic ROS. In this study, the *StSOD1* was over-expressed through genetic engineering technology to study the ability of transgenic potato to resist low temperature stress. There is clear evidence that ROS will result in lipid peroxidation. Okuda et al. reported that chilling stress double increased MDA content in wheat leaves (Tohru et al., 1991). Similarly, there was four-fold increase in maize seedlings during 4 °C treatment (Prasad et al., 1994). In our study, the NT lines showed a 2.02-fold elevation of MDA content while OE lines increased 1.3-fold (Fig. 1). This indicated that cold stress would lead to lipid peroxidation intensified but the transgenic plants can reduce the lipid peroxidation and protect the cell form ROS damage. It is known that ROS can trigger lipid peroxidation in cellular membrane (Cadenas, 1989; Halliwell and Gutteridge, 1986). We found that the leaves of NT and RNAi lines were wilted severely compared to OE lines (Fig. 6). All of these results indicated that ROS-induced lipid peroxidation was, at least in part, responsible for increased cold sensitivity of crop plants.

There is clear evidence indicated that the total SOD activity was no change in tolerant cultivars of tobacco and rice, whereas it significantly decreased in the shoots or leaves of sensitive type of banana, tobacco and rice (Mutlu et al., 2013; Sheng-chun et al., 2010). But in our study, the total SOD activity was increased in potato plants under cold stress and elevated 1.85-fold after 48 h cold treatment compared to untreated control (Fig. 3).

It is interesting to ask the question of whether an increase of antioxidant ability was contribute plants to withstand the low temperature (Hodges, 2001). There was increasing evidence of a relationship between cold stress and antioxidant enzymes (Duan et al., 2012; Payton et al., 2001; Wang et al., 2005). Cu/Zn SOD was strongly induced by cold stress in cassava plants (Jia et al., 2014). In this study, we found that the total SOD activities in plants was enhanced 1.39 fold in OE lines compared to NT lines after 48 h at 4 °C treatment (Fig. 3). Obviously, it is only a moderate increase, which is consistent with the studies of Sen Gupta et al (Ashima et al., 1993). They found that moderate increase of SOD activity can provide more effective protection from MV damage than high SOD levels. In fact, Elroy-Stein et al. (1986) has already reported that moderate increase of Cu/Zn SOD activity can resist MV stress in human and mouse cells but large increase do not (Elroy-Stein et al., 1986). Since Cu/Zn SOD are sensitive to the end-product (H_2O_2), it is possible that, when cells enhanced the Cu/Zn SOD activity to response MV treatment, it will produce a burst of H_2O_2 that would deactivate the enzymes. Therefore, the elimination of end-product H_2O_2 was very important to plants to resist stresses.

CAT and POD can decompose the H_2O_2 into water (H_2O) and oxygen (O_2) (Asada, 1999). In our study, we found that both CAT and POD activity were also enhanced under low temperature treatment (Fig. 4,5). The results indicated that except SOD, the other antioxidant enzymes like POD and CAT also plays positive effect to cooperate with SOD to eliminate ROS to improve the plants cold resistance, which is consistent with Xu et al. studies (Jia et al., 2014). They found that upon chilling, the enzymatic activity of MDHAR, DHAR and GR in the ASC-GSH cycle in the transgenic cassava was significantly increased. Furthermore, in previous cassava cold transcriptome studies, the ROS scavenging enzymes, especially SOD, CAT and glutathione-S-transferases (GSTs), were upregulated during 7 °C treatment in greenhouse (An et al., 2012). It can be concluded, hence, that the antioxidant enzymes interact and cooperate with each other to reduce damages of ROS caused by abiotic stress.

Development of genetic engineering plants by introducing or over-expressing selective genes was seems the feasibility selection to produce tolerant abiotic stress plants (Bhatnagar-Mathur et al., 2008). It has been concerned a key role in protecting plants from various of oxidative stresses that direct modification of SOD expression (Ghazi Hamid et al., 2004; W. Van et al., 1997). The temperature stresses have different effects on expression of SOD genes of wheat. Both low temperature and high temperature can induce expression of Mn-SOD genes, but Cu/Zn-SOD was only induced by low temperature (Guohai et al., 1999). In this study, though qRT-PCR analysis, we found that the relative expression of *StSOD1* was significantly evaluated under low temperature and nearly a 1.38-fold increase in OE lines compared to NT plants (Fig. 2). The results confirmed that the SOD activity was regulated by the expression of SOD genes at the transcription level to a large extent.

To further analysis the correlation between SOD activity and expression of *Cu/Zn SOD* gene, we inhibited the expression of *StSOD1* though the RNA interference technology. During cold treatment, we observed that RNAi lines SOD activity gradually increased with the treatment time increasing (Fig. 3), meanwhile, the relative expressional level of *StSOD1* gene of RNAi lines was not significantly reduced (Fig. 6), indicating that the expression of *StSOD* gene in RNAi lines is not completely restrained. In addition, POD and CAT activity were also decreased in RNAi lines compared with NT plants during cold treatment (Fig. 4,5). This result further suggested that the expression of *StSOD1* had a remarkable effect on the activity of antioxidant enzymes under response oxidative stress.

In conclusion, we successfully developed the transgenic potato plants overexpressing and interfering-expressing *StSOD1* under the low temperature. The OE lines evaluated the activity of antioxidant enzyme, especially SOD activity, and exhibited strong tolerance to low temperature while RNAi and NT lines showed a decrease of SOD activity and performed weak in response to low temperature. The result confirmed that the manipulation of ROS scavenging enzymes by genetic engineering and consequently control the equilibrium of ROS producing and eliminating is an extremely effective means to obtain abiotic stresses tolerant plants.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledges

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References

Ajay, A., Sairam, R.K., Srivastava, a.G.C., 2002. Oxidative stress and antioxidative system in plants. Curr. Sci. India 82, 1227–1238.

Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E., 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ. 24, 1337–1344. https://doi.org/10.1046/j.1365-3040.2001.00778.x.

An, D., Yang, J., Zhang, P., 2012. Transcriptome profiling of low temperature-treated cassava apical shoots showed dynamic responses of tropical plant to cold stress. BMC Genomics 13, 64. https://doi.org/10.1186/1471-2164-13-64.

Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701.

Asada, K., 1999. The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 601–639. https://doi.org/10.1146/annurev.arplant.50.1.601.

Ashima, S.G., Jeanie, L.H.A., Scorr, H., Burket, J.J., Allen, a.R.D., 1993. Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc. Natl. Acad. Sci. U. S A. 90, 1629–1633. https://doi.org/10.1073/pnas.90.4.1629.

Azra, Y., Basra, S.M.A., Muhammad, F., Rehman, Hu., Nazim, H., Athar, Hu.R., 2013. Exogenous application of moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. Plant Growth Regul. 69, 225–233. https://doi.org/10.1007/s10725-012-9764-5.

Bhatnagar-Mathur, P., Vadez, V., Sharma, K.K., 2008. Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. Plant Cell Rep. 27 (3), 411–424. https://doi.org/10.1007/s00299-007-0474-9.

Bor, M., zdemir, F.Ö., Türkan, I., 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. And wild beet *Beta maritima* L. Plant Sci. 164, 77–84. https://doi.org/10.1016/S0168-9452(02)00338-2.

Cadenas, E., 1989. Biochemistry of oxygen toxicity. Annu. Rev. Biochem. 58, 79–110. https://doi.org/10.1146/annurev.bi.58.070189.000455.

Deeba, F., Pandey, A.K., Ranjan, S., Mishra, A., Singh, R., Sharma, Y.K., Shirke, P.A., Pandey, V., 2012.). Physiological and proteomic responses of cotton (*Gossypium herbaceum* L.) to drought stress. Plant Physiol. Biochem. 53, 6–18. https://doi.org/10.1016/j.plaphy.2012.01.002.

Doupis, G., Bertaki, M., Psarras, G., Kasapakis, I., Chartzoulakis, K., 2013. Water relations, physiological behavior and antioxidant defence mechanism of olive plants subjected to different irrigation regimes. Sci. Hortic-Amsterdam 153, 150–156. https://doi.org/10.1016/j.scienta.2013.02.010.

Duan, M., Feng, H.L., Wang, L.Y., Li, D., Meng, Q.W., 2012. Overexpression of thylakoidal ascorbate peroxidase shows enhanced resistance to chilling stress in tomato. J. Plant Physiol. 169 (9), 867–877. https://doi.org/10.1016/j.jplph.2012.02.012.

Elroy-Stein, O., Bernstein, Y., Groner, Y., 1986. Overproduction of human Cu/Zn-superoxide dismutase in transfected cells: extenuation of paraquat-mediated cytotoxicity and enhancement of lipid peroxidation. EMBO J. 5 (3), 615–622. https://doi.org/10.1002/j.1460-2075.1986.tb04255.x.

Ghazi Hamid, B., Naoyoshi, K., Yasuo, Y., Emi, S., Ryozo, S., Akihiro, K., Kiyoshi, T., 2004. Over-expression of ascorbate peroxidase in tobacco chloroplasts enhances the tolerance to salt stress and water deficit. Physiol. Plant. 121, 1231–1238. https://doi.org/10.1111/j.1399-3054.2004.00308.x.

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48 (12), 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016.

Guohai, W., Ronald, W.W., Albert, J.R., Lawrence, V.G., 1999. Isolation, chromosomal localization, and differential expression of mitochondrial manganese superoxide dismutase and chloroplastic copper/zinc superoxide dismutase genes in wheat. Plant Physiol. 120, 513–520. https://doi.org/10.2307/4278826.

Halliwell, B., Gutteridge, J., 1986. Oxigen free radicals and ion in relation to biology and medicine: some problems and concepts. Arch. Biochem. Biophys. 246, 501–514.

Hancock, R.D., Morris, W.L., Ducreux, L.J., Morris, J.A., Usman, M., Verrall, S.R., Fuller, J., Simpson, C.G., Zhang, R., Hedley, P.E., Taylor, M.A., 2014. Physiological, biochemical and molecular responses of the potato (*Solanum tuberosum* L.) plant to moderately elevated temperature. Plant Cell Environ. 37 (2), 439–450. https://doi.org/10.1111/pce.12168.

Hernandez, J.A., Del Río, L.A., Sevilla, F., 2006. Salt stress-induced changes in superoxide dismutase isozymes in leaves and mesophyll protoplasts from *Vigna unguiculata* (L.). Walp. New Phytol 126, 37–44. https://doi.org/10.1111/j.1469-8137.1994.tb07527.x.

Hodges, D., 2001. Chilling effects on active oxygen species and their scavenging systems in plant. In: Basra, A.S. (Ed.), Crop Responses and Adaptations to Temperature Stress. Food products press, pp. 53–76.

Igor, N.Z., Thomas, J.M., Rodney, J.F., 2002. Superoxide dismutase multigene family: a comparision of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expressioon. Free Radic. Biol. Med. 33, 337–349. https://doi.org/10.1016/s0891-5849(02)00905-x.

Jahnke, L.S., Hull, M.R., Long, S.P., 1999. Chilling stress and active oxygen metabolizing enzymes in Zea mays and Zea diploperennis. Plant Cell Environ. 14, 97–104. https://doi.org/10.1111/j.1365-3040.1991.tb01375.x.

Janas, K.M., Zielinska-Tomaszewska, J., Rybaczek, D., Maszewski, J., Posmyk, M.M., Amarowicz, R., Kosinska, A., 2010. The impact of copper ions on growth, lipid peroxidation, and phenolic compound accumulation and localization in lentil (Lens culinaris Medic.) seedlings. J. Plant Physiol. 167 (4), 270–276. https://doi.org/10.1016/j.jplph.2009.09.016.

Jia, X., Jun, Y., Xiaoguang, D., Yueming, J., Peng, Z., 2014. Increased expression of native cytosolic Cu/Zn superoxide dismutase and ascorbate peroxidase improves tolerance to oxidative and chilling stresses in cassava (Manihot esculenta Crantz). BMC Plant Biol. 14, 208. https://doi.org/10.1186/s12870-014-0208-4.

Kim, M.S., Kim, H.S., Kim, Y.S., Baek, K.H., Oh, H.W., Hahn, K.W., Bae, R.N., Lee, I.J., Joung, H., Jeon, J.H., 2007. Superoxide anion regulates plant growth and tuber development of potato. Plant Cell Rep. 26 (10), 1717–1725. https://doi.org/10.1007/s00299-007-0380-1.

Kumar, D., Yusuf, M.A., Singh, P., Sardar, M., Sarin, N.B., 2013. Modulation of antioxidant machinery in alpha-tocopherol-enriched transgenic Brassica juncea plants tolerant to abiotic stress conditions. Protoplasma 250 (5), 1079–1089. https://doi.org/10.1007/s00709-013-0484-0.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 25 (4), 402–408. https://doi.org/10.1006/meth.2001.1262.

Mahanty, S., Kaul, T., Pandey, P., Reddy, R.A., Mallikarjuna, G., Reddy, C.S., Sopory, S.K., Reddy, M.K., 2012. Biochemical and molecular analyses of copper-zinc superoxide dismutase from a C4 plant Pennisetum glaucum reveals an adaptive role in response to oxidative stress. Gene 505 (2), 309–317. https://doi.org/10.1016/j.gene.2012.06.001.

Maksymiec, W., 2007. Signaling responses in plants to heavy metal stress. Acta Physiol. Plant. 29 (3), 177–187. https://doi.org/10.1007/s11738-007-0036-3.

Masaru, N., Tadahiko, S., Yayoi, W., Toshio, S., 2002. Salt stress-induced dissociation from cells of a germin-like protein with Mn-SOD activity and an increase in its mRNA in a moss, barbula unguiculata. Plant Cell Physiol. 43, 1568–1574.

Mellacheruvu, S., Tamirisa, S., Vudem, D.R., Khareedu, V.R., 2015. Pigeonpea Hybrid-Proline-Rich Protein (CcHyPRP) confers biotic and abiotic stress tolerance in transgenic rice. Front. Plant Sci. 6, 1167. https://doi.org/10.3389/fpls.2015.01167.

Mutlu, S., Karadağoğlu, Ö., Atici, Ö., Nalbantoğlu, B., 2013. Protective role of salicylic acid applied before cold stress on antioxidative system and protein patterns in barley apoplast. Biol. Plant. 57 (3), 507–513. https://doi.org/10.1007/s10535-013-0322-4.

Payton, P., Webb, R., Kornyeyev, D., Allen, R., Holaday, A., 2001. Protecting cotton photosynthesis during moderate chilling at high light intensity by increasing chloroplastic antioxidant enzyme activity. J. Exp. Bot. 52 (365), 2345–2354. https://doi.org/10.1093/jexbot/52.365.2345.

Prasad, T., Anderson, M., Martin, B., Stewart, C., 1994. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. Plant Cell 6, 65–74. https://doi.org/10.2307/3869675.

Prasad, T., Stewart, C., 2001.). Mechanisms of chilling injury and tolerance. In: Basra, A.S. (Ed.), Crop Responses and Adaptations to Temperature Stress. Food products press, pp. 1–52.

Rasool, S., Ahmad, A., Siddiqi, T.O., Ahmad, P., 2012. Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. Acta Physiol. Plant. 35 (4), 1039–1050. https://doi.org/10.1007/s11738-012-1142-4.

Raviraja, N.S., Sridhar, K.R., 2007. A modified freeze–thaw method for efficient transformation of Agrobacterium tumefaciens. Curr. Sci. India 93, 769–770.

Ron, M., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410. https://doi.org/10.1016/S1360-1385(02)02312-9.

Saed-Moucheshi, A., Shekoofa, A., Pessarakli, M., 2014. Reactive oxygen species (ROS) generation and detoxifying in plants. J. Plant Nutr. 37 (10), 1573–1585. https://doi.org/10.1080/01904167.2013.868483.

Sato, Y., Masuta, Y., Saito, K., Murayama, S., Ozawa, K., 2011. Enhanced chilling tolerance at the booting stage in rice by transgenic overexpression of the ascorbate peroxidase gene. OsAPXa Plant Cell Rep. 30 (3), 399–406. https://doi.org/10.1007/s00299-010-0985-7.

Sheng-chun, X., Yong-ping, L., Jin, H., Ya-jing, G., Wen-guang, M., Yun-ye, Z., Shui-jin, Z., 2010. Responses of antioxidant enzymes to chilling stress in tobacco seedlings. Agric. Sci. China 9 (11), 1594–1601. https://doi.org/10.1016/s1671-2927(09)60256-x.

Si, H.J., Xie, C.H., Liu, J., 2003. An efficient protocol for Agrobacterium-mediated transformation of microtuber and the introduction of antisense class I patatin gene into potato. Acta Agron. Sin. 29, 801–805.

Sunita, S., Anita, K., Veena, S., 2008. Effect of nitric oxide and putrescine on antioxidative responses under NaCl stress in chickpea plants. Physiol. Mol. Biol. Plants 14, 355–362. https://doi.org/10.1007/s12298-008-0034-y.

Tang, L., Kwon, S.Y., Kim, S.H., Kim, J.S., Choi, J.S., Cho, K.Y., Sung, C.K., Kwak, S.S., Lee, H.S., 2006. Enhanced tolerance of transgenic potato plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against oxidative stress and high temperature. Plant Cell Rep. 25 (12), 1380–1386. https://doi.org/10.1007/s00299-006-0199-1.

Tohru, O., Yusuke, M., Akira, Y., Shonosuke, S., 1991. Abrupt increase in the level of hydrogen peroxide in leaves of winter wheat is caused by cold treatment. Plant Physiol. 97, 1265–1267. https://doi.org/10.2307/4273980.

Van, W., Inzé, C., D, Van M, M., 1997. The regulation and function of tobacco superoxide dismutases. Free Radic. Biol. Med. 23, 515–520. https://doi.org/10.1016/s0891-5849(97)00112-3.

Wang, Y., Wisniewski, M., Meilan, R., Cui, M., Webb, R., Fuchigami, L., 2005. Overexpression of cytosolic ascorbate peroxidase in tomato confers tolerance to chilling and salt stress. J. Am. Soc. Hortic. Sci. 130 (2), 167–173.

Yousuf, P.Y., Hakeem, K.U.R., Chandna, R., Ahmad, P., 2012. Role of glutathione reductase in plant abiotic stress. Abiotic Stress Responses in Plants. pp. 149–158.

Yu, Q., Rengel, Z., 1999. Drought and salinity differentially influence activities of superoxide dismutases in narrow-leafed lupins. Plant Sci. 142, 1–11. https://doi.org/10.1016/S0168-9452(98)00246-5.

Zambounis, A., Nianiou, O., Tsaftaris, A., 2002. Cloning of superoxide dismutase (Cu/Zn SOD) gene in peppers for stress tolerance. Acta Hortic. 579, 101–106.

Zhang, S., Lu, S., Xu, X., Korpelainen, H., Li, C., 2010. Changes in antioxidant enzyme activities and isozyme profiles in leaves of male and female Populus cathayana infected with Melampsora larici-populina. Tree Physiol. 30 (1), 116–128. https://doi.org/10.1093/treephys/tpp094.