VERSITA

Biologia **67**/4: 681—688, 2012
Section Botany
DOI: 10.2478/s11756-012-0048-3

Differential expression of gibberellin 20 oxidase gene induced by abiotic stresses in Zoysiagrass (*Zoysia japonica*)

Xiaomei Dai[1], Xiaoxia Cheng[1], Yunxia Li[1], Wei Tang[2]* & Liebao Han[1,3]*

[1] *Institute of Turfgrass Science, 116 Box, Beijing Forestry University, Beijing 100084, China; e-mail: hanliebao@163.com*
[2] *Institute for Genome Sciences and Policy, Duke University, Durham 27708, USA; e-mail: wei.tang@duke.edu*
[3] *College of Horticulture and Gardening, Yangtze University, Jingzhou, Hubei Province 434025, China*

Abstract: The gibberellin 20-oxidase gene (*GA20*) plays an important role in plant growth and development. Differential expression of Zoysiagrass (*Zoysia japonica* Steud.) gibberellin 20 oxidase gene (*ZjGA20*) induced by abiotic stresses has not been reported. In this investigation, we first reported the differential expression of *ZjGA20* in different *Z. japonica* tissues including root, young leaf, senescent leaf, blade, sheath, and stolon, as well as differential expression induced by abiotic stresses including low temperature (4 ℃), H_2O_2(8 μM), salt stress (250 mM NaCl), 25% PEG_{6000}, and high temperature (42 ℃) by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Higher expression of *ZjGA20* was observed in young leaf and sheath, compared to root, senescent leaf, blade, and stolon. Among different abiotic stresses, expression of *ZjGA20* decreased under low temperature, 25% PEG_{6000}, and high temperature. The highest expression of *ZjGA20* was obtained when plants were treated with 8 μM H_2O_2 for 10 h and with 250 mM NaCl for 5 h. The analysis of the MDA content, POD activity, and permeability of the plasma membrane demonstrated that application of exogenous GA_3 recovered tissue damage derived from low temperature treatment. In addition, the expression of *ZjGA20* increased under low temperature stress. These results demonstrated that expression of *ZjGA20* was regulated by abiotic stresses and the damage derived from abiotic stresses could be rescued by exogenously applied plant growth regulator GA_3. Further more, exogenous gibberellin and salicylic acid (SA) alleviated the growth inhibition and death of the seedlings under stresses. The SA content in the seedlings treated with 80 μM GA_3 was far greater than the control (with H_2O) and plants under stress treatments. These data suggest that exogenous addition of GA_3 is able to counteract the inhibitory effects of these adverse environmental conditions in Zoysiagrass growth through modulation of SA biosynthesis. This is the first study of differential expression of gibberellin 20 oxidase gene and growth regulation of GA_3 in Zoysiagrass under stresses.

Key words: *Zoysia japonica*; abiotic stresses; gibberellin 20-oxidase gene; gene expression; salicylic acid

Abbreviations: GA20, gibberellin 20-oxidase gene; SA, salicylic acid; RT-PCR, Reverse Transcriptase- Polymerase Chain Reaction; MDA, malondialdehyde; POD, peroxidase.

Introduction

Gibberellins have been characterized as important plant hormones that control many aspects of plant development (Xu et al. 1995; Phillips 1998; Olszewski et al. 2002). Gibberellins participate in light signaling (Chen et al. 2007), promote the accumulation of protein and non-structural carbohydrate (Wasilewska et al. 1987), induce the expression of the calcium-dependent protein kinase gene (Yang et al. 2003), revert floral primordia to vegetative growth (Bhaskaran & Smith 1990), control α-amylase II-4 expression in germinating seeds (Nanjo et al. 2004), regulate expression of neutral and vacuolar invertase genes in petioles of plants (Gonzalez & Ceju 2007), and influence the expression of genes for vacuolar H^+ -inorganic pyrophosphatase, H^+-ATPase subunit A, and Na^+/H^+ antiporter (Fukuda et al. 2006). The biosynthesis of gibberellins is regulated by different factors. High temperature has been shown to change gibberellins levels in the flowering shoot of *Phalaenopsis* hybrid (Su et al. 2001). It was also reported that DREB1A and ZmOPR1 regulate the expression of GA dioxygenases in Arabidopsis (Cong et al. 2008; Gu et al. 2008). On the other hand; the sensitivity of plant to gibberellins could be elevated by vernalization (Oka et al. 2001).

The gibberellins biosynthetic pathway includes three sequential steps according to the nature of the enzymes involved (Goldman et al. 1994; Kim et al. 1995; Phillips 1998). The last step includes oxidation and elimination of C-20 to yield the C19-GAs, which are the biologically active plant hormones (Phillips 1998). This step is catalyzed by a GA 20-oxidase, which is considered as a site of regulation (Yamaguchi & Kamiya 2000;

* Corresponding author

Kusaba et al. 2001). GA20 gene has been reported to be involved in different GA-regulated plant development, such as stem elongation, root growth, flower formation, and fruit growth (Kusaba et al. 2001). GA20 oxidase gene transcripts accumulate in germinating seeds, expanding leaves, inflorescence, actively growing shoots and internodes (Phillips 1998; Valkonen et al. 1999; Xu et al. 2002). The multifunctional gibberellins 20-oxidase gene has been cloned and characterized from perennial ryegrass (*Lolium perenne* L.) and from several other plant species (Phillips 1998; Kusaba et al. 2001; Xu et al. 2002; Sakamoto et al. 2004).

Zoysiagrass (*Zoysia japonica* Steud.), a native grass in the east Asia, is a widely planted warm-season turfgrass for utility turf, home lawns, sports fields, and golf fairways and tees. Differential expression of Zoysiagrass gibberellin 20 oxidase gene (*ZjGA20*, GenBank accession For # DQ645453) induced by abiotic stresses has not been reported. In this study, we reported the differential expression of *ZjGA20* in different *Z. japonica* tissues including root, young leaf, senescent leaf, blade, sheath, and stolon, as well as differential expression induced by abiotic stresses including low temperature (4℃), H_2O_2(8 μM), salt stress (250 mM NaCl), 25% PEG_{6000}, and high temperature (42℃) by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Our results demonstrated that expression of ZjGA20 was regulated by abiotic stresses and could be rescued by exogenously applied plant growth regulator GA_3. These results may be useful in exploring the potentials for growth enhancement of Zoysiagrass. To our knowledge, this is the first study on the differential expression of *ZjGA20* in a species of turfgrass and it could be useful in stress biology of turfgrass.

Material and methods

Plant materials

Mature seeds of *Zoysia japonica* Steud. were purchased from International Seeds, Inc. (USA). The seeds were stored in plastic bags at 4℃ before they were used for germination. The seeds of zoysiagrass were germinated at 1/2 MS medium with 200 mg/L kinetin in a greenhouse at 25℃ in 16-h light/8-h dark photoperiod. After 15 days, the seedlings were transferred to 1/2 MS mediums and allowed to grow for 2 months. Then the sterile plants were transplanted to 11cm diameter plastic pots filled with a perlite/peatmoss/vermiculite (1:1:1 v/v) mixture in the same greenhouse. The fifteen-day-old seedlings, five-month-old plants and ten-month-old plants were used in our study.

Treatment of abiotic stresses for ZjGA20 gene expression

Five different abiotic stresses including low temperature (4℃), H_2O_2(8 μM), salt stress (250 mM NaCl), 25% PEG_{6000}, and high temperature (42℃) were applied to five-month-old *Z. japonica* plants. Abiotic stress experiments were carried out as described by Sugano et al. (2003) and by Yi et al. (2004). The plants were grown in plastic pots filled with a perlite/peatmoss/vermiculite (1:1:1 v/v) mixture located in a greenhouse. They were grown at 25℃ with 16 h light and 8 h dark periods before abiotic stress treatments. After abiotic stress treatment for 0 h, 1 h, 2 h, 5 h, 10 h, 24 h, 36 h plants were allow to grow under normal conditions for recovery.

Semi-quantitative PCR analysis of the ZjGA20 gene expression under abitoic stresses

The total leaves of five-month-old Zoysiagrass at different sampling times under each stress were homogenized in liquid nitrogen followed by RNA extraction with the TRNzol kit (Tiangen, China), according to the protocol of the manufacturer. cDNA was synthesized from 0.5 mg total RNA, which was obtained from treated materials, using oligo (dT) as a primer and reverse transcriptase M-MLV reverse transcript (Promega, Madison, WI, USA). Genes-specific RT-PCR primers were designed according to the full-length nucleotide sequence of *Zj*GA20 oxidase (GeneBank: DQ645453) cDNA sequences and were synthesized by synthesizing services (AuGCT). According to the released sequence of barley, wheat, and rice, the following degenerate primers were designed and synthesized: forward primer (*ZjGA20*-F) 5'-GCCCACCGCTGCATGGACAACTTC-3' and reverse primer (*ZjGA20*-R) 5'-CGGTGAAGGTGTCGCCGATGTTG-3', (*ZjActin*-F) 5'-GTGCTTGACTCTGGTGATGGT-3' and reverse primer (*ZjActin-R*) 5'-GAACCACCAATCCAGACGCTG-3' to isolate the middle part of the cDNA sequence of the Zoysiagrass *ZjActin* gene and GA 20–oxidase gene. As a control, the *ZjActin* transcripts were analyzed. The program was denaturation at 94℃ for 5 min, and followed by PCR cycles at 94℃ for 30 s, 60/56℃ for 30 s, and 72℃ for 1 min, and then a final extension at 72℃ for 10 min. The PCR products (5μL) were analyzed by electrophoresis in 1.2% agarose gels.

Semi-quantitative PCR analysis of the ZjGA20 gene expression at different developmental stages and different types of Zoysiagrass tissues

The roots and total leaves of fifteen-day-old seedlings, five-month-old plants and ten-month-old plants were homogenized respectively in liquid nitrogen. The young leaves, senescent leaves, blades, sheaths of five-month-old plants, and stolons of ten-month-old plants were homogenized respectively in liquid nitrogen, followed by RNA extraction and Semi-quantitative PCR analysis as same as the *ZjGA20* gene expression under abiotic stresses.

Physiological detecting of Zoysiagrass plants under low temperature stress

Five-month-old Zoysiagrass plants treated with H_2O, 80 μM GA_3 or 200 mM paclobutrazol were subjected to low temperature stress at 4℃ for 72 h. Physiological changes were analyzed by measuring the relative permeability of the plasma membrane, the content of malondialdehyde (MDA) (μmol/g fresh weight), and the activity of peroxidase (POD) (unit/g fresh weight). Permeability of the plasma membrane by electrolyte leakage measurement was conducted essentially as described elsewhere (Yi et al. 2004; Warren et al. 1996) with minor modifications. The plants were incubated in growth chambers at either 25℃ (for non-acclimated plants) or 4℃ (for cold-acclimated plants). The tissues were harvested, washed, and placed in 5 mL aliquots of 0.4 M sorbitol (Sigma, St. Louis, MO, USA). Tubes were equilibrated to either 4℃ in an 818-low temperature incubator (Precision Scientific, Winchester, VA, USA), and allowed to remain there for 24 h. The cold-treated tubes were held at 4℃ for different periods of time and then warmed to room temperature. Electrical conductivity was measured (model 455C, Istek conductivity meter; Seoul, Korea), after which

Table 1. Primer sequence, annealing temperature, and cycles used in RT-PCR.

Primer name	Primer sequence (5'→ 3')	Tm (℃)	Cycles
*Zj*Actin	GTGCTTGACTCTGGTGATGGT GAACCACCAATCCAGACGCTG	56	25
*Zj*GA20	GCCCACCGCTGCATGGACAACTTC CGGTGAAGGTGTCGCCGATGTTG	60	30

the tubes were autoclaved to release all electrolytes for the second determination of the total content of electrolytes in each sample. The content of malondialdehyde (MDA) was measured using 0.25 g fresh leaves skived with 5% (v/v) TCA (trichloroacetic acid). The even mixture of 2 mL 0.67% TBA (2-thiobarbituric acid) with the same volume clear extract after centrifuging at 1000 *g* for 10 min was heated in a boiling water bath for 30 min. After cooled to room temperature and centrifuged at 10000 *g* for 10 min, the supernatant was read for absorbance at 450, 532 and 600 nm. The amount of MDA was calculated as Zhang described (2001). Absorbance of the supernatant at 663 and 645 nm was measured with a spectrophotometer (UV-7504c, Shanghai). Peroxidase (POD) activity was measured following the description of Gong (2001). The experiment was repeated three times. After stress, the five-month-old Zoysiagrass plants were returned to normal growth conditions for one week.

Survival rate of Zoysiagrass seedlings under abiotic stresses with exogenous GA_3 (80uM) and SA (50uM) treatments
With applying H_2O (Ck), GA_3 (80 μM) and SA(50 μM), 15-day-old Zoysiagrass seedlings were used for survival rate analysis under various abiotic stresses [H_2O_2(8 μM), salt stress (250 mM NaCl), 25% PEG_{6000}, and high temperature (42℃)]. Each of the four kinds of stresses lasted for 0 day, 2 days, 4 days, 6days, 8 days, 10 days and 12 day. The experiments were repeated three times.

SA quantification
Plant hormones were analyzed by HPLC coupled to tandem mass spectrometry as described in Durgbanshi et al (2005). Plant tissue was extracted in ultrapure water using a tissue homogenizer (Ultra-Turrax, Ika-Werke, Staufen, Germany). Before extraction, samples were spiked with deuterated standards of every compound. After extraction and centrifugation, the pH of the supernatant was adjusted to 3.0 and partitioned twice against diethyl ether. The organic layers were combined and evaporated in a centrifuge vacuum evaporator (Jouan, Saint-Herblain, France). The dry residue was thereafter resuspended in a water : methanol (9 : 1) solution, filtered, and injected in a HPLC system (Alliance 2695, Waters Corp., Milford, MA). Hormones were separated in a reversed-phase C18 column using methanol and 0.01% acetic acid in water as solvents. The mass spectrometer, a triple quadrupole (Quattro LC, Micromass Ltd., Manchester, UK), was operated in negative ionization electrospray mode and the different plant hormones were detected according to their specific transitions using a multiresidue mass spectrometric method. Further details on the determination procedure are given in Durgbanshi et al (2005).

Statistical analyses
The results were presented as mean values ± standard errors. Comparisons between means were made using the Fisher's exact test at $p \leq 0.05$ using SPSS-10 statistical software (SPSS Inc., Chicago, IL).

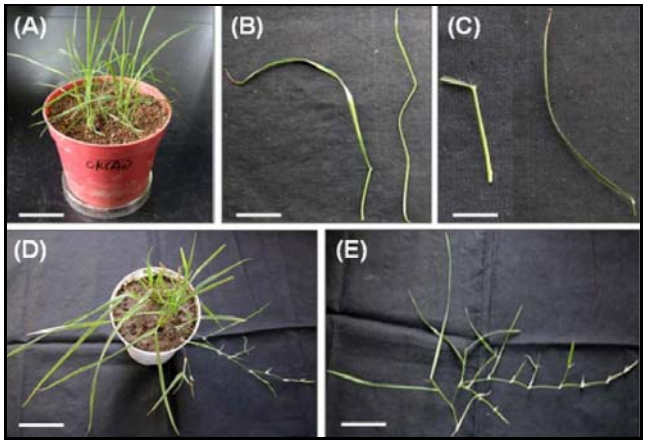

Fig. 1. Different developmental stages and different types of tissues of Zoysiagrass. A – 5-month-old Zoysiagrass plants; B – young leaf (left) and senescent leaf (light) of 5–month-old Zoysiagrass; C – blade (left) and sheath (right) of 5-month-old Zoysiagrass; D – 10-month-old Zoysiagrass plants; E – stolon of 10-month-old Zoysiagrass plant; A and B bars = 0.5 cm, C–E bars = 1cm.

Results

Different expression of ZjGA20 at different developmental stages and different types of Zoysiagrass tissues
Phenotype of five-month-old Zoysiagrass plants, young leaf, senescent leaf, blade and sheath of five-month-old Zoysiagrass plant, ten-month-old Zoysiagrass plants, stolon of ten-month-old Zoysiagrass plant were shown in Fig. 1A-E. Expression of *ZjGA20* at different developmental stages and different types of tissues by RT-PCR demonstrated that expression of *ZjGA20* was observed in leaves but not roots of fifteen-day-old, five-day-old, and 10-month-old Zoysiagrass plants (Fig. 2A). Among different tissues of five-month-old Zoysiagrass plants, higher expression of *ZjGA20* was observed in young leaf and sheath, compared to root, senescent leaf, blade (Fig. 2B). Expression of *ZjGA20* was not observed in stolons of 10-month-old Zoysiagrass plants (Fig. 2B).

Different expression of ZjGA20 under abiotic stresses
The five-month-old Zoysiagrass plants were used to investigate the different expression of *ZjGA20* under abiotic stresses including low temperature (4℃), H_2O_2 (8 μM), salt stress (250 mM NaCl), 25% PEG_{6000}, and high temperature (42℃) by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Expression of *ZjGA20* in five-month-old Zoysiagrass plants decreased sharply under stress of 4℃ (Fig. 3A), stress of 25%

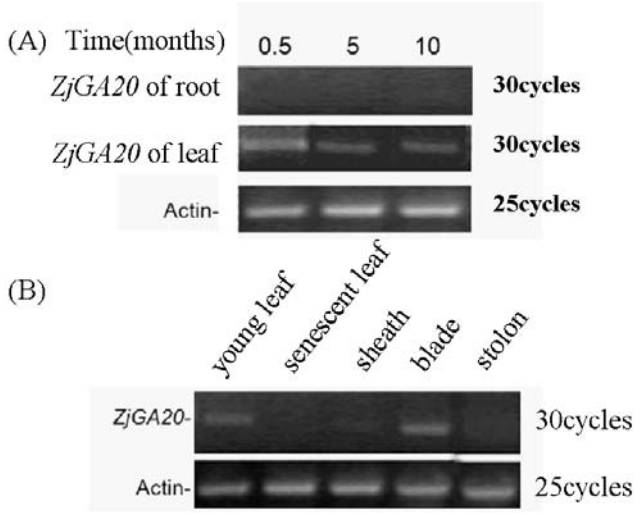

Fig. 2. Expression of *ZjGA20* at different developmental stages and different types of tissues. A – expression of *ZjGA20* in leaves and roots of 0.5, 5 and 10-month-old Zoysiagrass plant; B – expression of *ZjGA20* at different tissues of 5-month-old Zoysiagrass plant and stolon of 10-month-old Zoysiagrass plant.

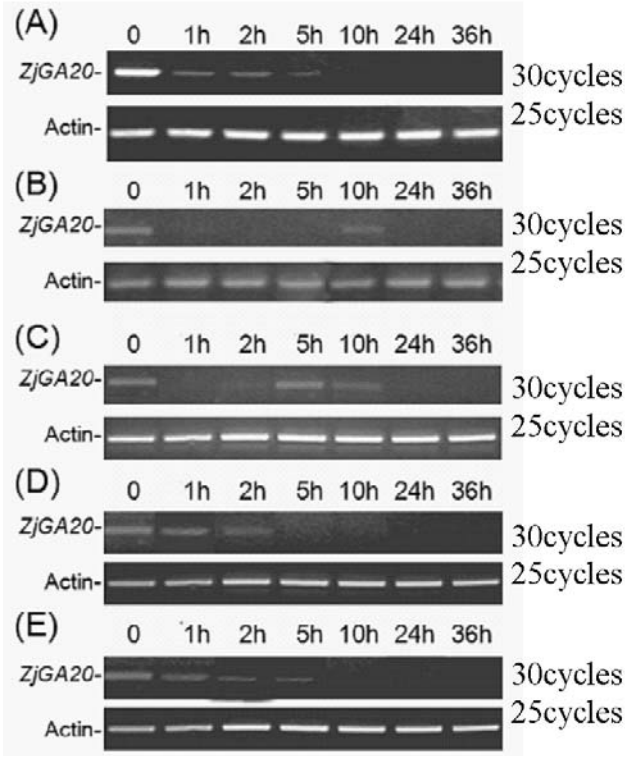

Fig. 3. Expression of ZjGA20 induced by abiotic stresses. A – expression of ZjGA20 in 3-month-old Zoysiagrass induced by 4 ℃ for 0,1,2,5,10,24,36 h; B – expression of ZjGA20 in 3-month-old Zoysiagrass induced by 8 μM H_2O_2 for 0, 1, 2, 5, 10, 24, 36 h; C – expression of ZjGA20 in 5-month-old zoysiagrass induced by 250 mM NaCl for 0, 1, 2, 5, 10, 24, 36 h; D – expression of ZjGA20 in 3-month-old Zoysiagrass induced by 25% PEG6000 for 0, 1, 2, 5, 10, 24, 36 h; E – expression of ZjGA20 in 3-month-old Zoysiagrass induced by 42 ℃ for 0, 1, 2, 5, 10, 24, 36 h.

PEG_{6000} (Fig. 3D), and treatment of 42 ℃ for 1, 2, 5, 10, 24, and 36 h (Fig. 3E). Among different treatment times with 8μM H_2O_2, the plants treated for 10 h had the highest expression of *ZjGA20* (Fig. 3B). Among different treatment times with 250 mM NaCl, the plants treated for 5 h had the highest expression of *ZjGA20* (Fig. 3C).

Physiological changes of Zoysiagrass plants as influenced by GA_3 and paclobutrazol under low temperature stress

Low temperature stress caused an increase in RP in the plants treated with GA_3 as measured at 24 h and in those treated with H_2O at 48 h (Fig. 4A) (note, pleae comfirm this because your stastical analysis did not compare the times). The GA_3 and paclobutrazol treatments reduced RP as measured at 48 h of stress (Fig. 4A). The content of malondialdehyde (MDA) decreased at 24 h and then increased thereafter (Fig 4B). The GA_3 and paclobutrazol did not impact MDA content when compared to H_2O treatment, except for paclobutrazol which incresaed MDA at 48 h. The activity of peroxidase (POD) increased in response to low temperature stress. The GA_3 and paclobutrzol increased POD activity relative to H_2O treatment as measured at 24 h of stress (Fig. 4C).

Exogenously applied GA_3 induced expression of ZjGA20 under low temperature stress

After being treated at 4 ℃ for 72 h, the 5-month-old Zoysiagrass plants were allowed to grow under normal condition for one week. The plants treated with GA_3 had better recovery (Fig. 5B) relative to those treated with H_2O (Fig. 5A) or paclobutrazol (Fig. 5C). RT-PCR analysis demonstrated that expression of *ZjGA20* increased in five-month-old Zoysiagrass treated with 80 μM GA_3, but not with paclobutrazol (Fig. 5D).

Exogenous application of GA_3 and SA increased the survival rate of Zoysiagrass seedlings under different stresses

The GA_3 (80 μM) or SA (50 μM) treatment alleviated decline of survival rate of *Zoysia japonica* seedlings under stresses (Fig. 6). In addition, seedlings with gibberellin grew faster than others and plant height increased significantly under salt stress and oxidation stress, compared with drought stresses and heat stress.

Exogenous application of GA_3 increased the content of endogenous salicylic acid

SA content (ng g^{-1} fresh weight) was measured in 15-day-old *Zoysia japonica* seedlings treated with water or GA_3 for 24 h (Table 2). The SA content of seedlings treated with 80 μM GA_3 was far greater than the control (with H_2O) and plants under stress treatments. This result demonstrated that maybe GA_3 alleviated the growth inhibition and death of the seedlings under stresses through increasing the content of endogenous salicylic acid.

Discussion

Gibberellin biosynthesis genes have been isolated and

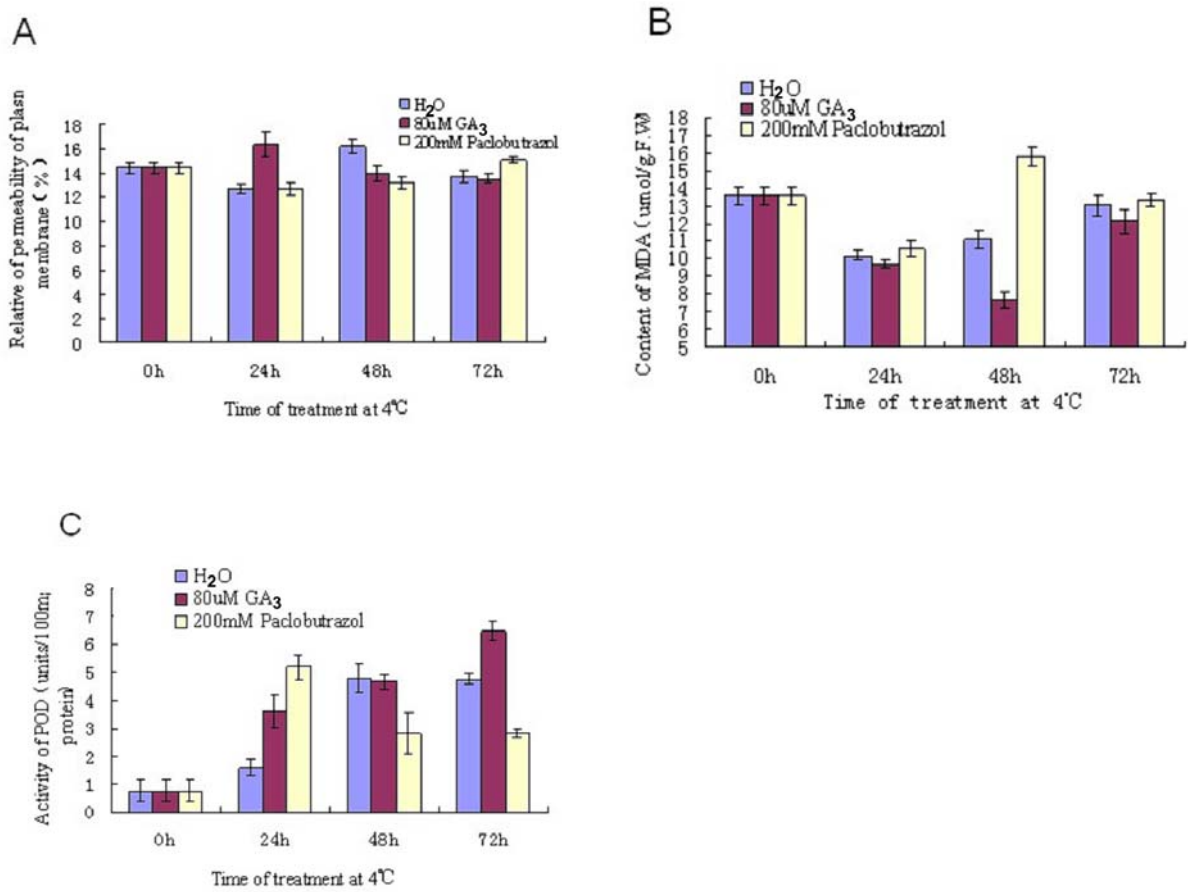

Fig. 4. Physiological changes of 3-month-old Zoysiagrass plants as influenced by GA_3 and paclobutrazol under low temperature stress (4 ℃, 72 h). A – relative permeability of the plasma membrane (%); B – content of malondialdehyde (MDA) (μmol/g fresh weight); C – activity of peroxidase (POD) (unit/g fresh weight). The values were the average of three independent experiments. Experiments were repeated three times. Data represent the mean ± SD.

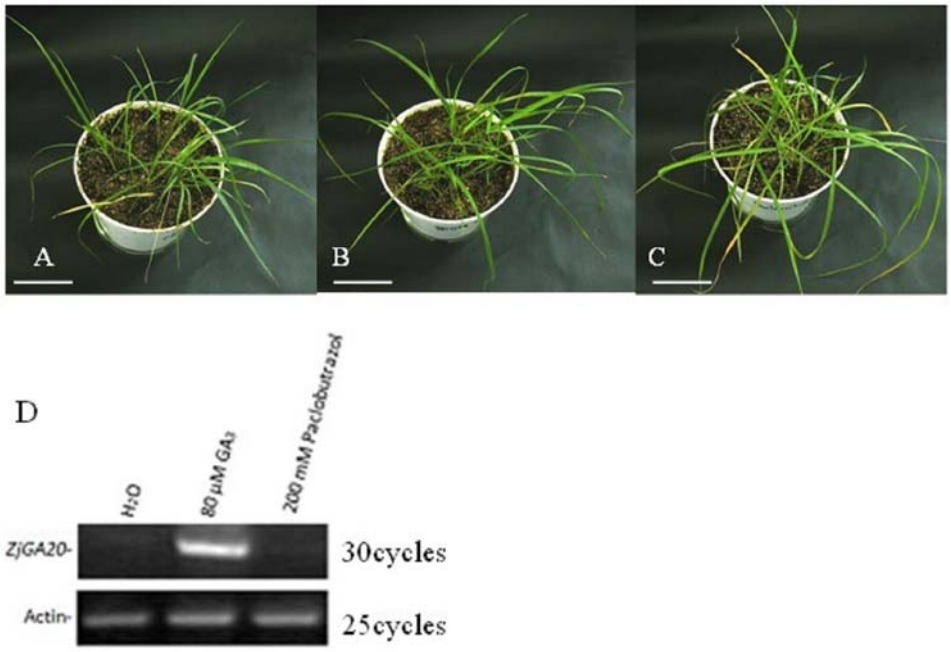

Fig. 5. Physiological changes of 5-month-old Zoysiagrass after treatment at 4 ℃ for 72 h. A – three-month-old Zoysiagrass plants after treatment at 4 ℃ for 72 h, then applied H_2O; B – 3-month-old Zoysiagrass plants after treatment at 4 ℃ for 72 h, then applied 80 μM GA_3; C – 3-month-old Zoysiagrass plants after treatment at 4 ℃ for 72 h, then applied 200 mM paclobutrazol; D – expression of *ZjGA20* in 5-month-old Zoysiagrass after treatment at 80 μM GA_3 and 200 mM paclobutrazol. Bars = 0.5 cm.

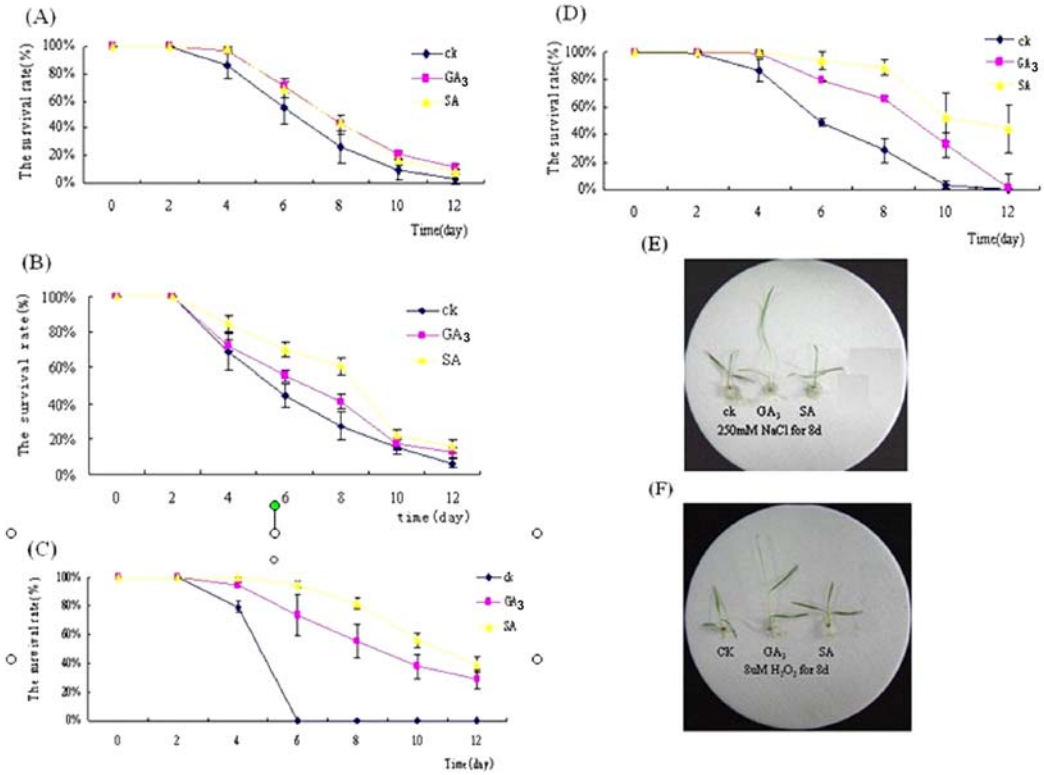

Fig. 6. The survival rate of *Zoysia japonica* seedling under stresses: (A) – 250 mM NaCl stress; (B) – 8 μM H_2O_2 oxidative stress; (C) – 25% PEG drought stress; (D) – 42 ℃ high temperature stress; (E) – the growth of *Zoysia japonica* seedling under 250mM NaCl salt stress; (F) – the growth of *Zoysia japonica* seedling under 8 μM H_2O_2 oxidative stress. The values were the average of three independent experiments. Experiments were repeated three times. Data represent the mean ± SD.

Table 2. SA content (ng g^{-1} fresh weight) in 15-day-old *Zoysia japonica* seedlings treated with water or GA_3 for 24 h. Values are means of two replicates ± SD. Statistically significance respect in the Fisher's exact test ($p < 0.05$) are represented by an asterisk.

Treated for 24 h	CK	4 ℃	250 mM NaCl	8 μM H_2O_2	25% PEG	42 ℃
H_2O	750 ± 10	385 ± 12*	407 ± 2	375 ± 14*	493 ± 7	365 ± 9*
80 μM GA_3	1769 ± 8*	1578 ± 20*	849 ± 5	905 ± 15	1438 ± 11*	1311 ± 20*

analyzed in pea and bean (Garcia-Martinez et al. 1997), hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) (Eriksson & Moritz 2002), developing pumpkin seeds (Frisse et al. 2003; Lange et al. 2005), spinach (Lee & Zeevaart 2007), and in tomato (Serrani et al. 2007). Regulation of gibberellin biosynthesis and gene expression have been investigated in *Pisum sativum* L (Martin et al. 1996), tomato (Rebers et al. 1999), potato (Carrera et al. 2000), citrus (Vidal et al. 2003), and in tobacco (Gallego-Giraldo et al. 2008). An overview of gibberellin metabolism enzyme genes and their related mutants in rice has been reported (Kusaba et al. 1998; Sakamoto et al. 2004). However, no differential expression of *ZjGA20* induced by abiotic stresses including low temperature (4 ℃), H_2O_2, salt stress (250mM NaCl), 25% PEG_{6000}, and high temperature (42 ℃) by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) has been reported in zoysiagrass.

GA 20-oxidase (GA20ox) is a regulatory enzyme for the syntheses of biologically active GAs in plants. GA20–oxidase catalyzes the conversion of gibberellin precursor GA_{12}- to GA_9 via GA_{15} and GA_{24}. GA_9 is a direct precursor of the GA-plant hormone GA_4. In this study, we have investigated the differential expression of *ZjGA20* in different *Z. japonica* tissues including root, young leaf, senescent leaf, blade, sheath, and stolon, as well as differential expression induced by abiotic stresses including low temperature (4 ℃), H_2O_2, salt stress (250 mM NaCl), 25% PEG_{6000}, and high temperature (42 ℃) by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). Higher expression of *ZjGA20* was observed in young leaf and sheath, compared to root, senescent leaf, blade, and stolon. In Arabidopsis, the enzyme was encoded by a gene family of at least five members (AtGA20ox1, AtGA20ox2, AtGA20ox3, AtGA20ox4 and AtGA20ox5) with differential patterns of expression (Shinjiro Yamaguchi, 2008); while in this research, only one gene *ZjGA20* (GeneBank: DQ645453) has been investigated. So far, only this one gene has been cloned. Maybe there are any other members of GA20 gene family involved in these abiotic stresses.

The results of this study indicated that among different abiotic stresses, expression of *ZjGA20* decreased

under low temperature, 25% PEG_{6000}, and high temperature. The highest expression of ZjGA20 was obtained when plants were treated with 8 μM H_2O_2 for 10 h and by 250 mM NaCl for 5 h. Tissue damage derived from low temperature treatment was recovered through application of GA_3 by increasing the expression of *ZjGA20* and regulating MDA content, POD activity, and permeability of the plasma membrane. These results demonstrated that expression of *ZjGA20* was regulated by abiotic stresses and could be rescued by exogenously applied plant growth regulator GA_3. This is the first study of differential expression of gibberellin 20 oxidase gene induced by abiotic stresses in Zoysiagrass. However, further studies are needed to test whether the different expression of the zoysiagrass GA20 oxidase may have a different physiological effect on growth and development in comparison with other plant species.

Low-temperature stress includes two different processes, chilling and freezing. Chilling stress (0–10℃) causes membrane leakiness as a result of an inability to increase membrane fluidity and inhibition of photosythetic processes, whereas freezing stress (below 0℃) leads to cellular dehydration caused by the formation of ice crystals in the extracellular space (Zhang et al., 2004; Ensminger et al., 2006; Verslues et al., 2006). Low temperature treatment, such as Zoysiagrass plants were treated at 4℃ for 72 h, caused physiological changes including the relative permeability of the plasma membrane, the content of malondialdehyde, and the activity of peroxidase. Normal growth was recovered under treatment of 4℃ for 72 h with 80 μM GA_3 (Fig. 5B), but not recovered with H_2O and 200 mM paclobutrazol (Fig. 5A and C). RT-PCR analysis demonstrated that expression of *ZjGA20* increased in five-month-old Zoysiagrass after spraying with 80 μM GA_3, but not increased after spraying 200 mM paclobutrazol (Fig. 5B). Our results demonstrated that expression of the Zoysiagrass GA20 gene was regulated by abiotic stresses, but damage derived from abiotic stress could be recovered by spraying with 80 μM GA_3. This is the first study on the differential expression of *ZjGA20* in a species of turfgrass and it could be useful in stress biology of turfgrass.

GAs have a role in SA biosynthesis and/or action. GA-responsive gene and exogenous addition of gibberellins are able to counteract the inhibitory effects of these adverse environmental conditions in seed germination and seedling growth through modulation of SA biosynthesis. Furthermore, this hypothesis is supported by the fact that sid2 mutants, impaired in SA biosynthesis, are more sensitive to salt stress than wild type and are not affected by exogenous application of GA_3. (Alonso-Ramírez et al. 2009). In order to study the regulation of exogenous GA_3 (80 μM) on the growth of *Zoysia japonica* under abiotic stresses, the survival rate of *Zoysia japonica* seedlings was statisticsed in different stress time. The survival rate of *Zoysia japonica* seedlings with GA_3 (80 μM), SA (50 μM) decreased more slowly than the control, indicating that exogenous gibberellin and salicylic acid alleviated the growth inhibition and death of the seedlings under stresses. In addition, data showed that the SA amount of seedlings treated with 80 μM GA_3 was far greater than the control (with H_2O) and plants under stress treatments (Table 2). This result demonstrated that maybe exogenously GA_3 recovered tissue damage derived from low temperature treatment and alleviated the growth inhibition and death of the seedlings under stresses through increasing the content of endogenous salicylic acid.

Acknowledgements

This work was supported by a grant from the Chinese National Research Program (2006AA10Z132), a grant from National Transgenic Plant Research and Application Program (J2002-B-006), and grants from China Scientific Research Program (2006BAD01A19) and (2006BAC18B04-1).

References

Alonso-Ramírez A., Rodríguez D., Reyes D., Jiménez J.A., Nicolás G., López-Climent M., Gómez-Cadenas A. & Nicolás C. 2009. Evidence for a role of gibberellins in salicylic acid modulated early plant responses to abiotic stress in *Arabidopsis thaliana* seeds. Plant Physiology Preview. DOI:10.1104/pp.109.139352

Bhaskaran S., Smith R.H. & Frederiksen R.A. 1990. Gibberellin A_3 reverts floral primordia to vegetative growth in sorghum. Plant Sci. **71:** 113–118.

Carrera E., Bou J., Garcia-Martinez J.L. & Prat S. 2000. Changes in GA 20–oxidase gene expression strongly affect stem length, tuber induction and tuber yield of potato plants. Plant J. **22:** 247–256.

Chen I.C., Lee S.C., Pan S.M. & Hsieh H.L. 2007. GASA4, a GA-stimulated gene, participates in light signaling in *Arabidopsis*. Plant Sci. **172:** 1062–1071.

Cong L., Zheng H.C., Zhang Y.X. & Chai T.Y. 2008. *Arabidopsis* DREB1A confers high salinity tolerance and regulates the expression of GA dioxygenases in tobacco. Plant Sci. **174:** 156–164.

Durgbanshi A., Arbona V., Pozo O., Miersch O., Sancho J.V. & Gómez-Cadenas A. 2005. Simultaneous determination of multiple phytohormones in plant extracts by liquid chromatography-electrospray tandem mass spectrometry. J. Agric. Food Chem. **53:** 8437–8442.

Eriksson M.E. & Moritz T. 2002. Daylength and spatial expression of a gibberellin 20–oxidase isolated from hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.). Planta **214:** 920–930.

Ensminger I., Busch F. & Huner N.P.A. 2006. Photostasis and cold acclimation:sensing low temperature through photosynthesis. Physiol. Plant. **126:** 28–44.

Frisse A., Pimenta M.J. & Lange T. 2003. Expression studies of gibberellin oxidases in developing pumpkin seeds. Plant Physiol. **131:** 1220–1227.

Fukuda A. & Tanaka Y. 2006. Effects of ABA, auxin, and gibberellin on the expression of genes for vacuolar H^+ -inorganic pyrophosphatase, H^+-ATPase subunit A, and Na^+/H^+ antiporter in barley. Plant Physiol. Biochem. **44:** 351–358.

Gallego-Giraldo L., Ubeda-Tomas S., Gisbert C., Garcia-Martinez J.L., Moritz T. & Lopez-Diaz I. 2008. Gibberellin homeostasis in tobacco is regulated by gibberellin metabolism genes with different gibberellin sensitivity. Plant Cell Physiol. **49:** 679–690.

Garcia-Martinez J.L., Lopez-Diaz I., Sanchez-Beltran M.J., Phillips A.L., Ward D.A., Gaskin P. & Hedden P. 1997. Isolation and transcript analysis of gibberellin 20–oxidase genes in pea and bean in relation to fruit development. Plant Mol. Biol. **33:** 1073–1084.

Goldman S., Mawal Y.R., Tanida I. & Wu R. 1994. Studies of a gibberellin-dependent DNA-binding protein related to the expression of a rice α-amylase gene. Plant Sci. **99:** 75–88.

Gong P.B. 2001. Principles and techniques of plant physiological biochemical experiment, Beijing, China, pp. 164–165.

González M.C. & Ceju F.J. 2007. Gibberellin-regulated expression of neutral and vacuolar invertase genes in petioles of sugar beet plants. Plant Sci. **172:** 839–846.

Gu D., Liu X., Wang M., Zheng J., Hou W., Wang G. & Wang J. 2008. Overexpression of ZmOPR1 in *Arabidopsis* enhanced the tolerance to osmotic and salt stress during seed germination. Plant Sci. **174:** 124–130.

Huang S., Anuradha S.R., Ream J.E., Fujiwara H., Cerny R.E. & Brown S.M. 1998. Overexpression of 20–oxidase confers a gibberellin-overproduction phenotype in *Arabidopsis*. Plant Physiol. **118:** 773–781.

Kim J.K., Chang M.C., Nahm B.H., Hwang Y.S. & Wu R. 1995. Isolation and characterization of a gibberellin-stimulated rice (*Oryza sativa* L.) gene encoding a protein that resembles a tumor suppressor. Plant Sci. **112:** 75–84.

Kusaba S., Fukumoto M., Honda C., Yamaguchi I., Sakamoto T. & Kano-Murakami Y. 1998. Decreased GA1 content caused by the overexpression of OSH1 is accompanied by suppression of GA 20–oxidase gene expression. Plant Physiol. **117:** 1179–1184.

Kusaba S., Honda C. & Kano-Murakami Y. 2001. Isolation and expression analysis of gibberellin 20–oxidase homologous gene in apple. J. Exp. Bot. **52:** 375–376.

Lange T., Kappler J., Fischer A., Frisse A., Padeffke T., Schmidtke S. & Lange M.J. 2005. Gibberellin biosynthesis in developing pumpkin seedlings. Plant Physiol. **139:** 213–223.

Lee D.J. & Zeevaart J.A. 2007. Regulation of gibberellin 20–oxidase1 expression in spinach by photoperiod. Planta **226:** 35–44.

Martin D.N., Proebsting W.M., Parks T.D., Dougherty W.G., Lange T., Lewis M.J., Gaskin P. & Hedden P 1996. Feed-back regulation of gibberellin biosynthesis and gene expression in *Pisum sativum* L. Planta **200:** 159–166.

Nanjo Y., Asatsuma S., Itoh K., Hori H., Mitsui T. & Fujisawa Y. .2004. Posttranscriptional regulation of α-amylase II-4 expression by gibberellin in germinating rice seeds. Plant Physiol. Biochem. **42:** 477–484.

Olszewski N., Sun T.P. & Gubler F. 2002. Gibberellin signaling: Biosynthesis, catabolism, and response pathways. Plant Cell. **14:** S61– 80.

Oka M., Tasaka Y., Iwabuchi M. & Mino M. 2001. Elevated sensitivity to gibberellin by vernalization in the vegetative rosette plants of *Eustoma grandiflorum* and *Arabidopsis thaliana*. Plant Sci. **160:** 1237–1245.

Phillips A.L. 1998. Gibberellins in*Arabidopsis*. Plant Physiol. Biochem. **36:** 115–124.

Rebers M., Kaneta T., Kawaide H., Yamaguchi S., Yang Y.Y., Imai R., Sekimoto H. & Kamiya Y. 1999. Regulation of gibberellin biosynthesis genes during flower and early fruit development of tomato. Plant J. **17:** 241–250.

Sakamoto T., Miura K., Itoh H., Tatsumi T., Ueguchi-Tanaka M., Ishiyama K., Kobayashi M., Agrawal G.K., Takeda S., Abe K., Miyao A., Hirochika H., Kitano H., Ashikari M. & Matsuoka M. 2004. An overview of gibberellin metabolism enzyme genes and their related mutants in rice. Plant Physiol. **134:** 1642–1653.

Sakamoto T., Koutarou M., Hironori I., Tomoko T., Miyako T.U., Masatomo K., Ganesh K.A., Shin T., Kiyomi A., Akio M., Hirohiko H., Hidemi K., Motoyuki A. & Makoto M. 2004. An Overview of gibberellin metabolism enzyme genes and their related mutants in rice. Plant Physiol. **134:** 1642–1653.

Serrani J.C., Sanjuan R., Ruiz-Rivero O., Fos M. & Garcia-Martinez J.L. 2007. Gibberellin regulation of fruit set and growth in tomato. Plant Physiol. **145:** 246–257.

Su W.R., Chen W.S., Koshioka M., Mander L.N., Hung L.S., Chen W.H., Fu Y.M. & Huang K.L. 2001. Changes in gibberellin levels in the flowering shoot of *Phalaenopsis* hybrida under high temperature conditions when flower development is blocked. Plant Physiol. Biochem. **39:** 45–50.

Valkonen J.P.T., Moritz T., Watanabe K.N. & Rokka V.M. 1999. Dwarf (di)haploid pito mutants obtained from a tetraploid potato cultivar (*Solanum tuberosum* subsp. *tuberosum*) via anther culture are defective in gibberellin biosynthesis. Plant Sci. **149:** 51–57.

Verslues P.E., Agarwal M., Katiyar-Agarwal S., Zhu J.H. & Zhu J.K. 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J. **45:** 523–539.

Vidal A.M., Ben-Cheikh W., Talon M. & Garcia-Martinez J.L. 2003. Regulation of gibberellin 20–oxidase gene expression and gibberellin content in citrus by temperature and citrus exocortis Fo viroid. Planta **217:** 442–448.

Warren G., McKown R., Marin A.L. & Teutonico R. 1996. Isolation of mutations affecting the development of freezing tolerance in *Arabidopsis thaliana* (L.) Heynh. Plant Physiol. **111:** 1011–1019.

Wasilewska L.D., Bralczyk J. & Szczegielniak J. 1987. The role of gibberellin in regulation of dwarf plants development. Plant Sci. **53:** 11–19.

Xu J., Lange T. & Altpeter F. 2002. Cloning and characterization of a cDNA encoding a multifunctional gibberellin 20–oxidase from perennial ryegrass (*Lolium perenne* L.). Plant Sci **163:** 147–155.

Yamaguchi S. 2008. Gibberellin Metabolism and its Regulation. Annu. Rev. Plant Biol. **59:** 225–251.

Yamaguchi S. & Kamiya Y. 2000. Gibberellin biosynthesis: Its regulation by endogenous and environmental signals. Plant Cell Physiol. **41:** 251–257.

Yang G., Shen S., Yang S. & Komatsu S. 2003. OsCDPK13, a calcium-dependent protein kinase gene from rice, is induced in response to cold and gibberellins. Plant Physiol. Biochem. **41:** 369–374.

Yi S.Y., Kim J.H., Joung Y.H., Lee S., Kim W.T., Yu S.H. & Choi D. 2004. The pepper transcription factor CaPF1 confers pathogen and freezing tolerance in *Arabidopsis*. Plant Physiol. **136:** 2862–2874.

Zhang W.H. 2001. Principles and techniques of plant physiological biochemical experiment. Beijing, China, pp. 260–261.

Zhang X., Fowler S.G., Cheng H., Lou Y., Rhee S.Y., Stockinger E.J. & Thomashow M.F. 2004. Freezing-sensitive tomato has a functional CBF cold response pathway, but a CBF regulon that differs from that of freezing-tolerant *Arabidopsis*. Plant J. **39:** 905–919.

Received June 10, 2011
Accepted September 29, 2011