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Tetraploidization of diploid *Dioscorea* results in activation of the antioxidant defense system and increased heat tolerance

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ABSTRACT

Polyploidy is reported to show increased tolerance to environmental stress. In this work, tetraploid plants of *Dioscorea zingiberensis* were obtained by colchicine treatment of shoots propagated *in vitro*. The highest tetraploid induction rate was achieved by treatment with 0.15% colchicine for 24 h. Diploid and tetraploid plants were exposed to normal (28 °C) and high temperature (42 °C) for 5 d during which physiological indices were measured. Compared with diploid plants, relative electrolyte leakage and contents of malondialdehyde, superoxide anions and hydrogen peroxide were lower in tetraploids, while activities of antioxidant enzymes, such as superoxide dismutase, peroxidase, catalase, ascorbate peroxidase and glutathione reductase, were stimulated and antioxidants (ascorbic acid and glutathione) were maintained at high concentrations. These results indicate that tetraploid plants possess a stronger antioxidant defense system and increased heat tolerance.

Introduction

High temperature is one of the major abiotic stresses depressing plant growth and productivity (Huang and Xu, 2008). High-temperature stress can result in inhibition of photosynthesis, cell membrane damage, premature senescence and even cell death (Xu et al., 2006). One mechanism of injury involves the generation and reactions of reactive oxygen species (ROS), such as superoxide anions ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Liu and Huang, 2000). The accumulation of ROS can lead to, for example, lipid peroxidation, denaturation of proteins, breakage of DNA strands, and metabolic disorders. However, plants protect cells from ROS injury by means of an antioxidant enzyme system (superoxide dismutase [SOD], peroxidase [POD], catalase [CAT], ascorbate peroxidase [APX] and glutathione reductase [GR]) and antioxidant materials (ascorbic acid [AsA] and glutathione [GSH]) to scavenge ROS. Thus, these antioxidant compounds and enzyme activities can be used as physiological indices to assess the thermotolerance of a plant (Mittler, 2002; Sairam et al., 2000).

Dioscorea zingiberensis belongs to the family Dioscoreaceae (Monocotyledonae). The rhizome of *D. zingiberensis* contains a high concentration of diosgenin, which is one of the important raw materials for the synthesis of steroid hormone drugs. In addition, *D. zingiberensis* is also a traditional Chinese medicine with a variety of effects, such as alleviating the symptoms of coughing and pneumonia, subsidence of swelling and reducing the cholesterol level in the body. Because *D. zingiberensis* has been over-intensively harvested in the past, few wild populations can be found and current production of this medicinal plant comes from cultivation (Chen et al., 2003; Yuan et al., 2005). In agricultural production, improved varieties of *D. zingiberensis* are often propagated in nursery beds under controlled conditions to promote their germination and enhance their quality. When seedlings are transplanted to the field, the sudden rise in temperature to about 30 °C injures the plants. Exposure of *D. zingiberensis* seedlings to high temperature for a long period may lead to stagnation of growth or abortion, which will reduce rhizome production (Zhang et al., 2005). Thus investigation of the heat tolerance of *D. zingiberensis* is of profound significance.

Polyploidy is reported to have the potential to enhance accumulation of secondary metabolites (Berkow, 2001; Jesus-Gonzalez and Weathers, 2003) and confer increased tolerance to a number of abiotic stress forms (Liu et al., 2002; Shang et al., 2003). Therefore, polyploidy may be a useful tool for obtaining new cultivars of medicinal plants. For example, tetraploid seeds of *Datura innoxia* and *D. stramonium* have about twofold higher alkaloid contents than their diploid counterparts (Berkow, 2001), while tetraploid *Artemisia annua* produces sixfold more artemisinin than diploid plants (Jesus-Gonzalez and Weathers, 2003). Additionally, tetraploid *Zingiber officinale* shows higher heat and

Abbreviations: APX, ascorbate peroxidase; AsA, ascorbic acid; CAT, catalase; GR, glutathione reductase; GSH, glutathione; MDA, malondialdehyde; $O_2^{\bullet -}$, superoxide radical; POD, peroxidase; REL, relative electrolyte leakage; ROS, reactive oxygen species; SOD, superoxide dismutase

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cold resistance than diploid genotypes (Shang et al., 2003), and tetraploid Chinese cabbages possess higher nutritional quality and heat resistance than their corresponding diploids (Liu et al., 2002). *D. zingiberensis* is a diploid cross-pollinating vine, and occasionally triploid, tetraploid and mixoploid genotypes can be found in nature (Huang et al., 2002). However, their performance under environmental stress has not been investigated. Therefore, the objective of this study was to induce autotetraploids from diploids of *D. zingiberensis* by colchicine treatment and to evaluate contents of antioxidant compounds and activities of antioxidant enzymes in tetraploids under high temperature.

Materials and methods

Plant material and in vitro multiplication

Diploid *Dioscorea zingiberensis* (peltate yam) plants were grown in the experimental field in Huazhong Agricultural University. For *in vitro* culture, shoot apices were treated with 0.1% mercuric chloride for 8 min, washed three times (2–3 min) with sterile water and placed in shoot multiplication medium, which consisted of MS medium (Murashige and Skoog, 1962) supplemented with 2.0 mg L^{-1} benzyladenine and 0.2 mg L^{-1} naphthalene acetic acid (NAA). Cultures were maintained at 25 ± 1 °C, under a 16-h photoperiod and a light intensity of 60 μM m^{-2} s^{-1}.

Tetraploid induction

Liquid MS medium supplemented with 2% dimethyl sulfoxide and filter-sterilized colchicine (0.1%, 0.2%, or 0.3%) was used for tetraploid induction. Apical buds (3–5 mm length), excised from *in vitro* cultures, were placed in MS liquid medium containing the respective concentration of colchicine described above or in colchicine-free MS medium, and incubated at 25 °C on an orbital shaker (100 rpm) for 24, 36, or 48 h. Shoot apices were washed three times (2–3 min) with sterile water and transferred to shoot multiplication medium for 2 weeks. All of the induced shoots were placed in rooting medium (solid MS medium containing half-strength macronutrient concentration, supplemented with 2.0 mg L^{-1} indole butyric acid and 0.2 mg L^{-1} NAA) to form roots.

Analysis of ploidy level

The relative ploidy of the colchicine-treated plants was estimated with a flow cytometer (PA-I, Partec, Germany) according to the protocol of Yokoya et al. (2000). A leaf sample from a known diploid plant of *D. zingiberensis* was used as a control to measure the *C*-value of colchicine-treated plants. Chromosome counting was also used to estimate the efficiency of chromosome doubling. Root tips of 3–5 mm length were excised from the rooted plantlets induced by the method described above and were treated following the method of Huang et al. (2002).

High-temperature stress treatment

Rooted tetraploid and diploid plantlets (Fig. 1A) were grown in a growth chamber at 25 ± 2 °C under a 16-h photoperiod at a light intensity of 200 μM m^{-2} s^{-1} for 2 months. Healthy seedlings of uniform size were selected for further temperature treatment.

Prior to the formal experiment, a preliminary test incorporating treatment with 38/23, 42/27, 45/30 or 48/33 °C day/night temperature regimes for 5 d, was performed to determine the optimal treatment temperature. The results indicated that temperatures of 42/27 °C caused visible injury, whereas 38/23 °C failed to induce noticeable symptoms and 45/30 °C caused lethal injuries. Thus, a day/night temperature regime of 42/27 °C was chosen as the high-temperature stress treatment.

The collected plantlets, with six plants per treatment, were exposed to 42/27 °C for 5 d, then moved to 28/18 °C for recovery for 2 d. Control plantlets were exposed to 28/18 °C continuously for 7 d. The third to fifth leaves from the shoot apex were sampled after 0, 1, 3, 5 and 7 d (7 d = 5 d in 42/27 °C+2 d in 28/18 °C). The leaves were frozen in liquid nitrogen and stored at −80 °C prior to assay of physiological and biochemical indices. Each

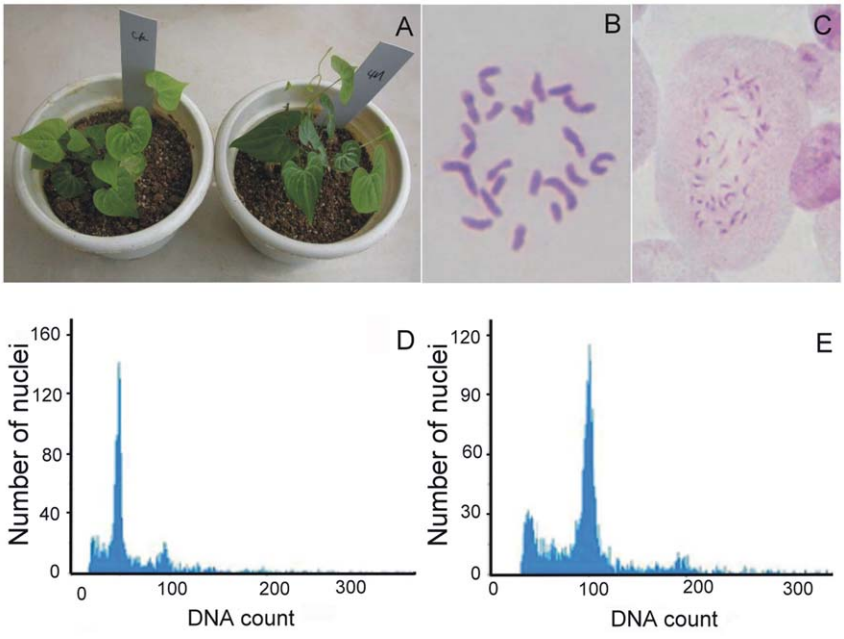

Fig. 1. Diploid (2*x*) and tetraploid (4*x*) potted plants (40 d after potting) (A). Chromosomes of a root tip cell from diploid control (B) and induced tetraploid (C) *D. zingiberensis* plants. Flow cytometric histograms of nuclei from leaves of control diploid (D) and tetraploid (E) plants. In the analysis, a control diploid plant was set at channel 50. Tetraploids were then predicted to display peaks around channel 100.

measurement was replicated four times except relative electrolyte leakage (REL; two measurements).

Relative electrolyte leakage and lipid peroxidation index

REL was determined according to the method of Sairam and Srivastava (2002). Lipid peroxidation was measured in terms of malondialdehyde (MDA) content following the method of Heath and Packer (1968). Each frozen sample (0.8 g) was homogenized with a mortar and pestle in 8 mL ice-cold extraction buffer (50 mM potassium phosphate buffer [PBS], pH 7.0, containing 1% soluble polyvinyl pyrrolidone). The homogenate was centrifuged at 12,000*g* for 15 min at 4 °C and the supernatant was used for the MDA, $O_2^{\bullet -}$, SOD, POD, CAT, APX and GR assays. Absorption values were measured with an ultraviolet spectrophotometer (U-1800 spectrophotometer, Hitachi, Japan).

Rate of $O_2^{\bullet -}$ production and H_2O_2 concentration

The rate of $O_2^{\bullet -}$ production was assayed according to the method of Ke et al. (2002) with slight modification. The supernatant was mixed with 50 mM PBS (pH 7.8), 1 mM hydroxylammonium chloride, 17 mM 4-aminobenzenesulfonic acid and 7 mM α-naphthylamine and incubated for 30 min at 30 °C. The specific absorption was determined at 530 nm. The H_2O_2 concentration was measured by monitoring the absorbance of the titanium-peroxide complex at 415 nm, following the method of Patterson et al. (1984).

Enzyme activities

The activity of SOD was determined according to the method of Dhindsa et al. (1981). One unit of enzyme activity was taken as the activity to cause 50% inhibition. The nitroblue tetrazolium reduction rate was measured by monitoring the absorbance at 560 nm.

The activity of POD was determined in a reaction solution composed of 50 mM PBS (pH 7.0), 2 mM H_2O_2, 2.7 mM guaiacol and 0.05 mL enzyme extract by monitoring the increase in absorbance at 470 nm due to guaiacol oxidation (Polle et al., 1994).

The activities of CAT, APX and GR were assayed in accordance with the method of Jiang and Huang (2001a) with some modification. Activity of CAT was determined by a decline in absorbance at 240 nm from the decomposition of H_2O_2 at 25 °C. The assay mixture contained 50 mM PBS (pH 7.0), 15 mM H_2O_2 and 0.05 mL enzyme extract. Activity of APX was measured by monitoring the decrease in absorbance of AsA at 290 nm. The activity was assayed using a reaction mixture containing 50 mM PBS (pH 7.0), 1 mM AsA, 0.3 mM H_2O_2 and 0.1 mL enzyme extract. Activity of GR was assayed as the decline in the absorbance of NADPH at 340 nm. The reaction mixture consisted of 50 mM PBS (pH 7.0), 0.5 mM oxidized GSH, 0.1 mM NADPH and 0.1 mL enzyme extract.

AsA and GSH concentrations

The concentrations of AsA and GSH were measured according to the methods of Yin et al. (2008) and Griffith (1980), respectively. Each frozen sample (0.5 g) was extracted with 5% trichloroacetic acid and centrifuged at 10,000*g* for 10 min. The AsA concentration was determined by changes in absorption at 525 nm. The GSH concentration was assayed following changes in absorption at 412 nm after addition of 5,5′-dithio-bis-(2-nitrobenzoic acid).

Statistical analysis

Analysis of variance was performed using SAS version 6.12 software (SAS Institute, Inc., Cary, USA). Tetraploid induction rate and the sets of means for each temperature treatment were analyzed with Duncan's multiple range test. The mean values for diploids and tetraploids were compared using the least significant difference test at the 5% significance level.

Results

Induction of tetraploids

Colchicine-treated explants were subjected to flow cytometry to determine their ploidy. The gain value was adjusted so that the peak of nuclei isolated from a control diploid plant was set at about channel 50 (Fig. 1D), while peaks of putative tetraploids had nuclei at about channel 100 (Fig. 1E). Suspected tetraploids were further examined by chromosome counting. The chromosome number of the diploid control was 20 (Fig. 1B), whereas that of tetraploid plants was 40 (Fig. 1C).

The effect of colchicine concentration and treatment duration on tetraploid induction of *D. zingiberensis* was assessed after culture for 40 d (Table 1). To a certain extent, the tetraploid induction rate rose with the increase of colchicine concentration and treatment duration. The optimal treatment was 0.15% colchicine for 24 h, above which the increased colchicine concentration and treatment duration led to increased mortality.

Table 1
Rate of tetraploids and dead shoots after colchicine treatment in *D. zingiberensis*.

Colchicine concentration (%)	Duration (h)	Dead shoots	Shoot death rate (%)	Tetraploid shoots	Rate of tetraploids (%)
0.1	24	6.0±1.4	16.7±3.9	7.0±1.4	19.4±3.9^{abc}
	36	7.5±0.7	20.8±2.0	8.5±2.1	23.6±5.9^{ab}
	48	12.0±2.8	33.3±7.9	5.5±0.7	15.3±2.0^{bcd}
0.15	24	8.5±0.7	23.6±2.0	9.5±2.1	26.4±5.9^{a}
	36	13.0±1.4	36.1±3.9	8.0±0.0	22.2±0.0^{ab}
	48	17.5±2.1	48.6±5.9	4.0±1.4	11.1±3.9^{cd}
0.2	24	11.5±2.1	31.9±5.9	6.0±1.4	16.7±3.9^{bcd}
	36	18.5±4.9	51.4±13.7	4.5±3.5	12.5±9.8^{cd}
	48	25.5±3.5	70.8±9.8	3.0±1.4	8.3±3.9^{d}
0	48	0.5±0.7	1.4±2.0	0.0±0.0	0.0±0.0^{e}

Each value represents the mean±SE of two repeat experiments with 36 replicates each.
^{a,b,c,d,e}Different letters indicate significant difference among treatments at $P<0.05$ according to LSD test.

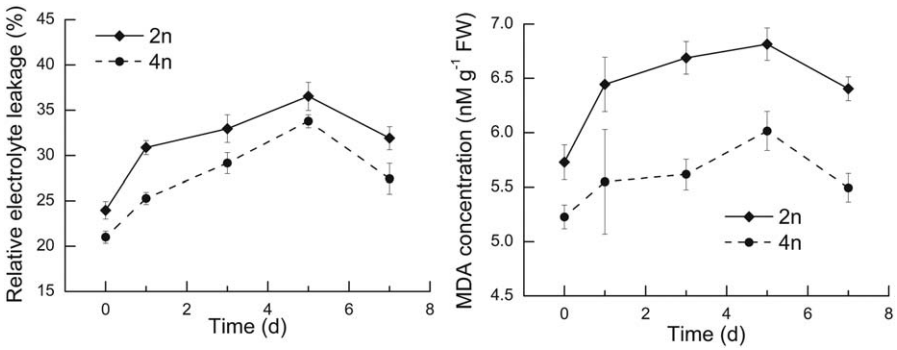

Fig. 2. Effect of high-temperature stress (42/28 °C day/night) on REL and MDA concentration in leaves of diploid and tetraploid *D. zingiberensis* after treatment for 0 (28/18 °C, normal condition), 1, 3, 5 and 7 d (placed in the normal condition for 2 d after treatment at 42/28 °C). Each value represents the mean ± SE of two or four replicates (REL or MDA).

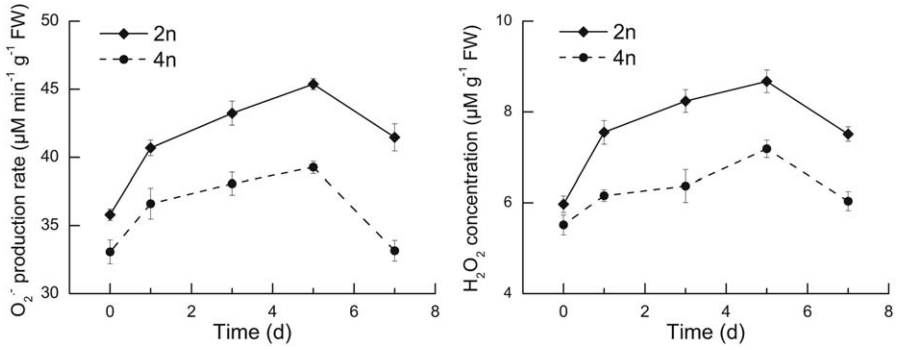

Fig. 3. Effect of high-temperature stress (42/28 °C day/night) on $O_2^{\bullet-}$ production rate and H_2O_2 concentration in leaves of diploid and tetraploid *D. zingiberensis* after 0 (28/18 °C, normal condition), 1, 3, 5 and 7 d (displaced in normal condition) treatments. Each value represents the mean ± SE of four replicates.

In this experiment, the tetraploid induction rate in the optimal treatment was as high as 26.4%. When the explants were treated with 0.2% colchicine for 48 h, the mortality rate was up to 70%.

REL and MDA concentration

The REL and MDA concentration in leaves of diploid and tetraploid *D. zingiberensis* gradually increased after exposure to 42 °C for 0, 1, 3 and 5 d. After the plants were removed from high temperature, the REL and MDA concentration decreased gradually. The REL ($P<0.01$) and MDA ($P<0.01$) concentration in tetraploids were significantly lower at all treatments than those of diploids (Fig. 2). These results indicate that diploid plants suffered stronger membrane damage than tetraploid plants under high-temperature stress.

Rate of $O_2^{\bullet-}$ production and H_2O_2 concentration

Under normal conditions, the $O_2^{\bullet-}$ production rate and H_2O_2 concentration in leaves of diploid and tetraploid *D. zingiberensis* exhibited only slight differences (Fig. 3). With exposure of *D. zingiberensis* to high temperature, the $O_2^{\bullet-}$ production rate and H_2O_2 concentration increased gradually and the difference between the diploids and tetraploids was enlarged significantly as the exposure time lengthened. After recovery at 28/18 °C for 2 d, the $O_2^{\bullet-}$ production rate and H_2O_2 concentration returned to normal levels immediately in tetraploids.

Antioxidant enzyme activities

The changes in antioxidant enzyme activities after exposure to 42 °C stress were shown in Fig. 4. The activities of SOD and GR displayed similar trends. In diploids they slightly increased after 1 d and thereafter declined gradually; in tetraploids the activities kept rising within the first 3 days and thereafter started to decrease, which indicated that the activities of SOD and GR in tetraploids could be tolerated for a longer period under high-temperature stress. The changes of POD, CAT and APX activities in diploids and tetraploids showed a similar trend; these indices declined with the continuance of treatment time, and then rose after recovery at 28 °C for 2 d, but the tetraploid plants exhibited higher antioxidant enzyme activities than diploid plants under the same conditions. The activities of SOD ($P<0.05$), POD ($P<0.05$), CAT ($P<0.01$), APX ($P<0.05$) and GR ($P<0.01$) were significantly higher in tetraploids than in diploids.

AsA and GSH concentrations

Under high-temperature stress, the AsA and GSH concentrations showed a decreasing trend with increasing treatment time (Fig. 5). The GSH concentration significantly declined in diploids after 1 d treatment, while in tetraploids the decrease was less pronounced. Additionally, in all treatments the tetraploids had significantly higher AsA ($P<0.01$) and GSH ($P<0.01$) concentrations than the diploids.

Discussion

Tetraploid induction

The technique of *in vitro* polyploid induction with colchicine has been employed in many plants, such as *Scutellaria baicalensis* (Gao et al., 2002), *Punica granatum* (Shao et al., 2003) and

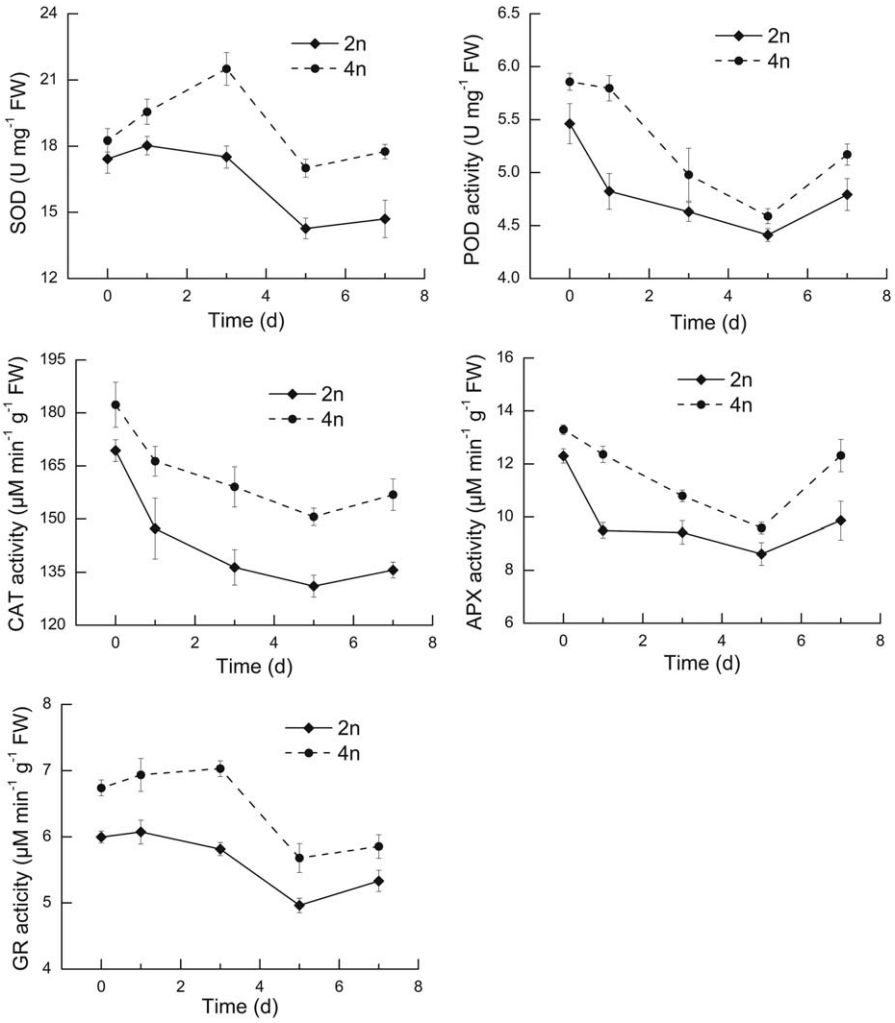

Fig. 4. Effect of high-temperature stress (42/28 °C day/night) on SOD, POD, CAT, APX and GR activities in leaves of diploid and tetraploid *D. zingiberensis* after 0 (28/18 °C, normal condition), 1, 3, 5 and 7 d (displaced in normal condition) treatments. Each value represents the mean ± SE of four replicates.

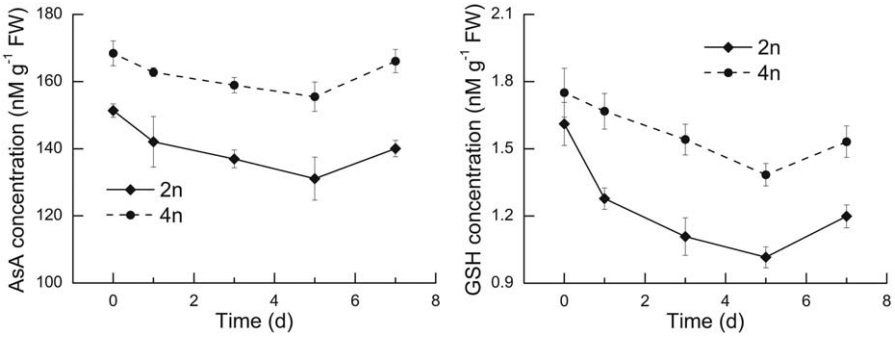

Fig. 5. Effect of high-temperature stress (42/28 °C day/night) on AsA and GSH contents in leaves of diploid and tetraploid *D. zingiberensis* after 0 (28/18 °C, normal condition), 1, 3, 5 and 7 d (displaced in normal condition) treatments. Each value represents the mean ± SE of four replicates.

Lavandula angustifolia (Urwin et al., 2007). The combination of colchicine concentration and treatment duration is the key factor affecting tetraploid induction efficiency. Early studies showed that induction of tetraploid banana (Hamill et al., 1992) needs a high concentration of colchicine (0.5%) for 2 h; in other words, the combination of high colchicine concentration and short treatment duration is appropriate for tetraploid induction. However, tetraploid jujubes (Gu et al., 2005) are obtained with 0.15% colchicine and 24 h treatment. In our study, 0.15% colchicine treatment for 24 h proved to be the optimum combination to induce tetraploidization in *D. zingiberensis*. Therefore, selection of a suitable colchicine concentration and treatment duration is necessary to obtain a high tetraploid induction rate.

Evaluation of heat stress tolerance in diploid and tetraploid D. zingiberensis

Heat stress can induce the accumulation of ROS, which results in membrane peroxidation and cellular membrane disruption

(Jiang and Huang, 2001b; Liu and Huang, 2000). Therefore, membrane thermostability and ROS can be used to assess the heat injury of plants. In this study, REL and MDA content showed positive correlations with the concentrations of $O_2^{\bullet-}$ (REL: $P<0.01$, $2x$; MDA: $P<0.01$, $2x$) and H_2O_2 (REL: $P<0.01/P<0.01$, $2x/4x$; MDA: $P<0.01/P<0.01$, $2x/4x$) under high temperature, and they rose with increasing treatment duration. Therefore, the increases of REL and MDA concentration indicate that the plasmalemma injury is caused by ROS production. The result is consistent with a previous study on *Lilium longiflorum* (Yin et al., 2008). In our study, the diploid and tetraploid plants exhibited different changes in REL, MDA, $O_2^{\bullet-}$ and H_2O_2 concentrations, which indicated that they had different tolerance to high-temperature stress. Under normal conditions, the differences of REL, MDA, $O_2^{\bullet-}$ and H_2O_2 concentrations between diploid and tetraploid plants were small. Under high-temperature stress, however, the differences were significantly increased. The results indicated that greater accumulation of REL, MDA, $O_2^{\bullet-}$ and H_2O_2 occurred in diploids than in tetraploids, demonstrating that tetraploid plants are relatively thermotolerant in comparison with diploids.

On the other hand, activities of antioxidases (POD, CAT and APX) and contents of antioxidants (ASA and GSH) showed a decreasing trend with continuance of heat treatment. In diploids, the activities of POD, CAT, APX and GSH significantly decreased under 42 °C for 1 d, but in tetraploids the decrease of antioxidase activities appeared to be light under high temperature, suggesting that the tetraploids have a stronger capacity to maintain the activities of antioxidases (Shang et al., 2003; Tang et al., 2006). Interestingly, in our experiment, the activities of SOD and GR in tetraploid plants were elevated after a few days under heat stress. Some papers (Almeselmani et al., 2006; Wang and Li, 2006) have documented that SOD is involved in temperature stress tolerance. Higher SOD activity can efficiently remove $O_2^{\bullet-}$, which leads to the production of H_2O_2, and H_2O_2 can be scavenged by GR in the Halliwell–Asada pathway (Pan et al., 2004). Increased GR activity results in enhancement of heat-tolerance in grape seedlings (Wang and Li, 2006). Therefore, tetraploid plants show a stronger capability to scavenge ROS and higher resistance to heat stress than diploid plants.

Polyploidization is a major driving force in eukaryote evolution. In plants, genetic and epigenetic changes occur rapidly after formation of allopolyploids. Hybridization is considered as the main cause for the resulting differential gene expression, while little is known about the genetic changes imposed by autopolyploidization (Stupar et al., 2007; Warren et al., 2005). In this study, the tetraploids were autotetraploid derived from chromosome doubling by colchicine treatment and were not subjected to genomic changes nor selective forces. The increased heat tolerance in tetraploids compared with diploids can thus be more likely attributed to ploidy *per se* and the factors therein, such as nuclear dosage and ploidy-driven cellular modifications. Lu et al. (2006) used an *Arabidopsis thaliana* whole genome Affymetrix gene chip (ATH1) to quantify the variation in transcript abundance of 22,810 genes between autotetraploid and diploid *Isatis indigotica*. Their results revealed a coordinated induction and suppression of 715 and 251 ploidy-responsive genes, respectively, in autotetraploid *I. indigotica*. Their study also showed the functional classification of some of these up-regulated genes involved in various metabolic, signal transduction, transcriptional regulation, and developmental pathways. In autopolyploid *Paspalum notatum*, there are significant gene expression alterations after polyploidization (Martelotto et al., 2005). However, some studies showed that there are few differences in gene expression alteration between diploids and autopolyploids. Stupar et al. (2007) argued that, in autopolyploid potato, few genes' expression is linearly correlated with the ploidy and can be dramatically changed because of ploidy alteration. Autopolyploidy in cabbages also does not alter significantly the proteomes of green tissues (Warren et al., 2005). In our study, tetraploid plants have higher activities of APX and GR and greater contents of AsA and GSH than those of diploids under normal conditions and heat stress. We speculate that the changes might be driven by expression of a duplicated gene. In addition, the activities of SOD and CAT in diploid and tetraploid plants showed slight differences at 28 °C, but under high-temperature stress the differences were significantly increased. It indicated that ROS accumulation can be induced by heat stress, which may result in injury to the plant, whereas moderate elevation of ROS could be a signal to induce expression of those genes related to stress resistance. Therefore, we speculate that genome-cached information could be reinstated with polyploidization by an uncharacterized mechanism, possibly activated by stress.

In conclusion, our results indicate that tetraploid *D. zingiberensis* plants display a stronger tolerance to heat stress than diploid plants. Tetraploidization might be responsible for the greater stress resistance, which results in enhanced activation of antioxidant enzymes and increased concentration of antioxidant materials. The precise mechanisms that produce certain interesting traits in polyploids, such as resistance to abiotic stress, are still little understood. Future research should explore these mechanisms and attempt to improve vigor, yield or diosgenin content through tetraploidization in breeding programs of *D. zingiberensis*.

References

Almeselmani M, Deshmukh PS, Sairam RK, Kushwaha SR, Singh TP. Protective role of antioxidant enzymes under high temperature stress. Plant Sci 2006; 171:382–8.

Berkow S. Size and alkaloid content of seeds in induced autotetraploids of *Datura innoxia*, *Datura stramonium* and *Hyoscyamus niger*. Pharm Biol 2001;39:329–31.

Chen Y, Fan J, Yi F, Luo Z, Fu Y. Rapid clonal propagation of *Dioscorea zingiberensis*. Plant Cell Tissue Organ Cult 2003;73:75–80.

Dhindsa RS, Plumb-Dhindsa P, Throne TA. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. J Exp Bot 1981;32:93–101.

Gao S, Chen B, Zhu D. In vitro production and identification of autotetraploids of *Scutellaria baicalensis*. Plant Cell Tissue Organ Cult 2002;70:289–93.

Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 1980;106:207–12.

Gu X, Yang A, Meng H, Zhang J. *In vitro* induction of tetraploid plants from diploid *Zizyphus jujuba* Mill. cv. Zhanhua. Plant Cell Rep 2005;24:671–6.

Hamill SD, Smith MK, Dodd WA. *In vitro* induction of banana autotetraploidy by colchicine treatment of micropropagated diploids. Aust J Bot 1992;40:887–96.

Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I: Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 1968; 125:189–98.

Huang B, Xu C. Identification and characterization of proteins associated with plant tolerance to heat stress. J Integr Plant Biol 2008;50:1230–7.

Huang T, Zhang Y, Zhang J, Qing T. Variation of chromosome number in *Dioscorea zingiberensis*. J Huazhong Agric Univ 2002;21:158–60.

Jesus-Gonzalez LD, Weathers PJ. Tetraploid *Artemisia annua* hairy roots produce more artemisinin than diploids. Plant Cell Rep 2003;21:809–13.

Jiang Y, Huang B. Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. J Exp Bot 2001a;52:341–9.

Jiang Y, Huang B. Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. Crop Sci 2001b;41:436–42.

Ke DS, Wang A, Sun G, Dong L. The effect of active oxygen on the activity of ACC synthase induced by exogenous IAA. Acta Bot Sin 2002;44:551–6.

Liu X, Huang B. Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. Crop Sci 2000;40:503–10.

Liu H, Zhang S, Wang H. Breeding an autotetraploid hybrid non-heading Chinese cabbage cultivar Shuyou No. 11 with green stalk, high quality and heat-resistance. J Nanjing Agric Univ 2002;25:22–6.

Lu B, Pan X, Zhang L, Huang B, Sun L, Li B, et al. A genome-wide comparison of genes responsive to autopolyploidy in *Isatis indigotica* using *Arabidopsis thaliana* affymetrix genechips. Plant Mol Biol Rep 2006;24:197–204.

Martelotto LG, Ortiz JPA, Stein J, Espinoza F, Quarin CL, Pessino SC. A comprehensive analysis of gene expression alterations in a newly synthesized *Paspalum notatum* autotetraploid. Plant Sci 2005;169:211–20.

Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 2002;7:405–10.

Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 1962;15:473–97.

Pan R, Wang X, Li N. Plant Physiology. High Education Press; 2004. p. 280–1.

Patterson BD, Macrae EA, Ferguson IB. Estimation of hydrogen peroxide in plant extracts using titanium(IV). Anal Biochem 1984;134:487–92.

Polle A, Otter T, Seifert F. Apoplastic peroxidases and lignification in needles of Norway spruce (*Picea-abies* L.). Plant Physiol 1994;106:53–60.

Sairam RK, Srivastava GC. Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Sci 2002;162:897–904.

Sairam RK, Srivastava GC, Saxena DC. Increased antioxidant activity under elevated temperature: a mechanism of heat stress tolerance in wheat genotypes. Biol Plant 2000;43:245–51.

Shang H, Gao Q, Song M, Liu H, Wang Q, Chen L, et al. A preliminary study of heat- and cold-resistance of tetraploid ginger (*Zingiber officinale*). J Southwest Agric Univ 2003;25:210–33.

Shao J, Chen C, Deng X. *In vitro* induction of tetraploid in pomegranate (*Punica granatum*). Plant Cell Tissue Organ Cult 2003;75:241–6.

Stupar RM, Bhaskar PB, Yandell BS, Rensink WA, Hart AL, Ouyang S, et al. Phenotypic and transcriptomic changes associated with potato autopolyploidization. Genetics 2007;176:2055–67.

Tang N, Wang F, Gao S. Studies on agronomic characteristics and anti-oxygen activities of *Atractylodes macrocephala* Koidz. Pharm Biol 2006;13:442–5.

Urwin NAR, Horsnell J, Moon T. Generation and characterisation of colchicine-induced autotetraploid *Lavandula angustifolia*. Euphytica 2007;156:257–66.

Wang L, Li S. Thermotolerance and related antioxidant enzyme activities induced by heat acclimation and salicylic acid in grape (*Vitis vinifera* L.) leaves. Plant Growth Regul 2006;48:137–44.

Warren A, Philippe B, Olivier C, Frédérique E, Eric J, Anne-Marie C, et al. Autopolyploidy in cabbage (*Brassica oleracea* L.) does not alter significantly the proteomes of green tissues. Proteomics 2005;5:2131–9.

Xu S, Li J, Zhang X, Wei H, Cui L. Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplast in two cool-season turgrass species under heat stress. Environ Exp Bot 2006;56:274–85.

Yin H, Chen Q, Yi M. Effects of short-term heat stress on oxidative damage and responses of antioxidant system in *Lilium longiflorum*. Plant Growth Regul 2008;54:45–54.

Yokoya K, Roberts AV, Mottley J, Lewis R, Brandham PE. Nuclear DNA amounts in roses. Ann Bot 2000;85:557–61.

Yuan S, Yan Y, Lin H. Plant regeneration through somatic embryogenesis from callus cultures of *Dioscorea zingiberensis*. Plant Cell Tissue Organ Cult 2005;80:157–61.

Zhang Y, Zhang J, Yan J., Huang T. High-yield cultivation techniques of *Dioscorea zingiberensis* C. H. Wright. Golden Shield Press; 2005. p. 96–7.